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Use of cannabis derivatives in veterinary medicine

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Use of cannabis derivatives in veterinary medicine

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Robin Temmerman is an employee of Zoetis. James Baumgartner is an employee of Panacea Life Sciences that performs cannabinoid research and produces commercial products in the human and veterinarian wellbeing markets.

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Editorial: Use of cannabis derivatives in veterinary medicine

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Editorial on the Research Topic
Use of cannabis derivatives in veterinary medicine

Introduction

Cannabis species (*Cannabis* spp.) are pharmacologically diverse plants containing myriad distinct compounds, with the phytocannabinoids (pCBs) tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) and their derivatives Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) as prime examples.

The main pharmacodynamic target of these compounds is the endocannabinoid system (ECS), which comprises of endocannabinoids (eCBs) such as anandamide (AEA) and 2-Arachidonoylglycerol (2-AG), cannabinoid receptors (CB) 1 and 2 and their anabolic/catabolic enzymes (Di Salvo et al.) (1–3). Inclusion of several eCB-like lipid mediators, their metabolic enzymes and their molecular targets forms the endocannabinoidome (2).

In human medicine, the use of cannabis-derived products is increasing globally for a variety of indications, such as post-injury and back pain, chronic and neuropathic pain, sleeping disorders, multiple sclerosis, epilepsy and others (Klatzkow et al.) (4, 5). Veterinary medicine is dovetailing this trend with growing interest from clients and veterinarians for treating medical conditions in animals with these molecules. In general, CBD is the primary entity of interest in veterinary medicine, but other molecules are being investigated, as exemplified in this Research Topic (e.g. studies with CBDA) (Klatzkow et al.; Johns et al.; Thomson et al.).

While cannabinoids show considerable veterinary therapeutic potential in the management of osteoarthritis, epilepsy, pain and other conditions, there is currently a paucity in adequately controlled studies and data to confirm the safe and effective use in these indications. Considering the current knowledge and research gap, the goal of this Research Topic was to consolidate recent findings and results of high-quality research on the pharmacokinetics and the safety and efficacy of cannabis-derivatives in animal species. This in turn will serve as a basis for further discussions and investigations in this growing therapeutic area. This editorial synthesizes findings from 16 recent studies, published in this Research Topic, highlighting the diverse applications and potential benefits of CBD and cannabis derivatives in veterinary practice.

Pharmacokinetics and bioavailability

Understanding the pharmacokinetics (PK) of CBD and other cannabinoids is crucial for determining appropriate dosing regimens and ensuring therapeutic efficacy. Several

studies in this Research Topic have focused on the pharmacokinetics of CBD in different formulations and species:

- Dogs: research comparing four CBD preparations (three liquid ones: oil-based, nanoemulsion-based and water-soluble and semi-solid formulation) in dogs revealed that liquid forms provided higher bioavailability and faster absorption compared to semi-solid forms (Limsuwan et al.). The concentration-time profiles of CBD were comparable between the oil- and water-based formulations. The CBD in all preparations reached the maximum plasma concentration within 3 h post-dose, with an average range of 92–314 μg/L, which aligns well with other Cmax values reported in CBD PK studies in dogs using same or similar dose levels (Di Salvo et al.).

  Another study demonstrated that there were no difference in PK parameters of CBD when administered either orally or transmucosally, indicating that CBD is not readily adsorbed by the oral mucosa and that CBD is probably swallowed and absorbed in the gastrointestinal tract (della Rocca et al.).

  In addition, subcutaneous injection of a liposomal-CBD formulation produced detectable CBD plasma concentrations for 6 weeks and the following PK parameters (median and range): C_{max} 45.2 (17.8–72.5) ng/ml, T_{max} 4 (2–14) days and half-life12.4 (7.7–42.6) days (Shilo-Benjamini et al.). The long-acting properties of the formulation could offer an advantage for patient and owner compliance. Finally, the study of Corsato Alvarenga et al. shows that long-term supplementation of a broad-spectrum hemp oil leads to dose-proportional accumulation in the canine body.
- Cats: a study on the oral administration of a 1:20 THC:CBD cannabis herbal extract in cats has shown that CBD and THC are quickly absorbed, with peak plasma concentrations occurring within 2–3 h post-dose (Lyons et al.). The plasma concentrations also increased dose-proportionally. Importantly, the bioavailability of CBD in cats appears to be lower than in dogs administered the same extract (6), suggesting potentially species-specific differences in absorption and metabolism. Another explanation could be the more general issue of the difficulty of oral administration to cats (Lyons et al.).
- Horses: research on horses by Thomson et al. using a cross-over design and nasogastric tube dosing (2 mg/kg and 8 mg/kg) with a CBD/CBDA-rich hemp oil product indicated that CBD levels were lower than CBDA and therefore that CBDA showed a higher bioavailability. Additionally, it was shown that CBDA was absorbed with a biphasic pattern. Reported Cmax values were: CBD and CBDA 2 mg/kg-−5.2 and 36.95 ng/mL; CBD and CBDA 8 mg/kg-−40.35 and 353.56 ng/mL. The elimination half-life of CBD and CBDA in horses was found to be relatively short or not possible to calculate due to lack of quantifiable timepoints, suggesting the need for frequent dosing to maintain therapeutic levels. Also, the product did not appear to impact the horses on neurological, behavioral and gastrointestinal levels (see below). A study from Eichler, Poźniak, et al. reported a mean Cmax of 12.17 ng/ml when CBD was dosed at 3 mg/kg. In that study, leveraging PK data from a single dose escalation study and a multiple dosing study (also discussed below), a 3-compartmental population pharmacokinetic (popPK) was built to describe and predict CBD and metabolite concentration-time profiles. Also, urine samples were analyzed, with higher CBD and metabolite concentrations (7-OH-CBD) compared to plasma. The study showed CBD is extensively metabolized and showed high volumes of tissue distribution (not corrected for bioavailability) with a resulting extended elimination phase (Eichler, Poźniak, et al.).
- Cynomolgus macaques (nonhuman primate): a study by Johns et al. investigated the PK of a CBD/CBDA-rich hemp oil (CBD/ArHO), orally administered at two dose levels (4 and 8 mg/kg) in cynomolgus macaques, a species of nonhuman primates. Mean Cmax of CBDA was around 30 times higher than CBD (456.75 ng/ml vs. 15.98 ng/ml). The PK data suggests that once daily dosing and the chosen dosing levels are insufficient in maintaining serum CBD concentrations (Johns et al.).

Therapeutic applications

CBD has shown promise in managing various conditions in veterinary patients, including pain, inflammation, epilepsy, and anxiety, although the strength of the scientific evidence for these indications can fluctuate:

- Pain and Inflammation: the study from Klatzkow et al. investigated the therapeutic efficacy on post-operative pain following tibial plateau leveling osteotomy (TPLO) of a CBD/CBDA product (dosed at 2–2.5 mg/kg PO every 12 h for 4 weeks) in a randomized, placebo controlled, blinded clinical trial with client-owned dogs. Variables investigated included serum biochemistry, standardized veterinary assessments for pain score, weight-bearing, and lameness and the Canine Brief Pain Inventory. The study did not show a significant impact on pain or early bone healing. CBA/CBDA hemp extract administration was associated with an increase in alkaline phosphatase (ALP) and a decrease in eosinophils (see also below). Finally, there was a potential association of CBD/CBDA and reduced post-operative anxiety. The study of Shilo-Benjamini et al. has demonstrated the efficacy of a long-acting (liposomal) CBD formulation in alleviating pain and improving the quality of life in dogs with osteoarthritis with minimal adverse events. However, the results should be interpreted cautiously due to non-blinding and lack of placebo.
- Epilepsy: clinical trials have shown that CBD can reduce the frequency and severity of seizures in dogs with intractable idiopathic epilepsy (Di Salvo et al.). These findings suggest that CBD could be a valuable adjunctive treatment for epilepsy in veterinary patients.
- Dermatological conditions: a case-report in a 2-year old mixed breed dog has show therapeutic efficacy of a CBD-rich full spectrum Cannabis oil for the management of the autoimmune skin disorder discoid lupus erythematosus

(DLE) (da Silva et al.). CBD has also been used to manage pruritus and atopic dermatitis in dogs. The study of Mariga et al. evaluated the effectiveness of a full-spectrum high cannabidiol oil in canine atopic dermatitis (CAD) compared to a negative control (olive oil) based on the degree of pruritus, dermatological evaluation (CADESI-4 scoring) and skin histopathology. The study however could not show a therapeutic advantage of the CBD oil compared to the olive oil group (Mariga et al.).

- Behavioral disorders: CBD has been explored as a treatment for anxiety and stress-related behaviors in dogs, with some studies indicating positive outcomes (Di Salvo et al.).

Safety and tolerability

The safety profile of CBD is a critical consideration for its use in veterinary medicine. Most studies have reported that CBD is well-tolerated in animals, with few adverse effects:

- Dogs: CBD/CBDA administered for the management of post-operative pain following TPLO did result in increased blood levels of ALP and a decrease in eosinophils and warrants caution (Klatzkow et al.). Another study reported no serious adverse events following single-dose administration of various CBD formulations (Limsuwan et al.). The study of Bookout et al. investigated the general safety of different cannabinoids in healthy beagle dogs in a randomized, non-blinded, negatively-controlled, parallel design 90-day repeat dose study with an additional 14-day recovery period. The authors report no somnolence, adverse events (AE) or serious adverse events (SAE). There were some significant changes in clinical pathology parameters (e.g. ALT, ALP and GGT), but were not considered clinically relevant. It is noted that the study from Klatzow et al. also reported an increase in ALP. The authors also highlight the low AE and SAE incidence in the US National Animal Supplement Council (NASC) Adverse Event Reporting System (NAERS).
- Cats: no significant adverse effects were observed in cats following single-dose administration of a 1:20 THC:CBD cannabis herbal extract with dose levels 2 and 5 mg/kg CBD (Lyons et al.). Coltherd et al. conducted a long-term (6 month) tolerance study with daily dosing (4 mg/kg) of a THC-free, CBD distillate in healthy cats with a negative (placebo) control group. The product was well tolerated and no clinically significant differences were found between biochemistry and hematology data.
- Horses: the study of Thomson et al. has shown that single-dose administration of CBD/CBDA-rich hemp extract is well-tolerated in horses, with no significant neurologic, behavioral, or gastrointestinal effects. Moreover, Eichler, Ehrle, Machnik, et al. and Eichler, Ehrle, Jensen, et al. found that a CBD paste administered orally (TAMACAN XL 55%®) was well tolerated and adverse events-free. However, both the conducted single dose escalation study (0.2, 1.0 and 3.0 mg/kg) and the multiple dosing study (CBD paste every 12 h for 15 days) did not significantly impact parameters such as heart rate, sedation level, behavioral observations or morning blood cortisol levels in healthy horses when compared to placebo.

Future directions

While the current body of research, and this Research Topic specifically, provides valuable insights into the pharmacokinetics, safety and efficacy of CBD and other cannabinoids in veterinary medicine, several areas warrant further investigation:

- Long-term safety: more studies are needed to assess the long-term safety and potential cumulative effects of cannabinoids in various animal species and several dose levels.
- Dosing regimen and formulation optimization: future research should focus on optimizing dosing regimens (posology and duration) and formulations to maximize exposure and subsequently therapeutic benefits while minimizing adverse effects.
- Mechanisms of action: understanding the pharmacodynamics and mode of action by which cannabinoids exert their therapeutic and other (off-target) effects will help in developing targeted treatments for specific conditions.
- Comparative studies: comparative studies across different species and formulations will provide a clearer understanding of the interspecies differences inherent to veterinary medicine and the underlying physiological mechanisms in cannabinoid pharmacokinetics, safety and efficacy.

In conclusion, a growing body of evidence highlights the potential of cannabinoids as a versatile therapeutic agent in veterinary medicine, although not all claimed indications are supported robustly and the PK is showing high intra- and interspecies variability. Subsequently, the medicalization of cannabinoids presents several opportunities as well as challenges for veterinary medical professionals, making continued research essential to fully elucidate its benefits in adequately supported indications and to ensure its safe and effective use in animal health care.

Author contributions

RT: Writing – original draft, Writing – review & editing.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The author(s) declare that Gen AI was used in the creation of this manuscript. Summary of the studies and overall structure of the editorial.

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References

1. Di Salvo A, Chiaradia E, Sforna M, Della Rocca G. Endocannabinoid system and phytocannabinoids in the main species of veterinary interest: a comparative review. *Vet Res Commun*. (2024) 48:2915–41. doi: 10.1007/s11259-024-10509-7

2. Di Marzo V. The endocannabinoidome as a substrate for noneuphoric phytocannabinoid action and gut microbiome dysfunction in neuropsychiatric disorders. *Dialogues Clin Neurosci*. (2020) 22:259–69. doi: 10.31887/DCNS.2020.22.3/vdimarzo

3. Lutz B. Neurobiology of cannabinoid receptor signaling. *Dialogues in Clinical Neuroscience*. (2020) 22:207–22. doi: 10.31887/DCNS.2020.22.3/blutz

4. Levinsohn EA, Hill KP. Clinical uses of cannabis and cannabinoids in the United States. *J Neurol Sci*. (2020) 411:116717. doi: 10.1016/j.jns.2020.116717

5. Leinen ZJ, Mohan R, Premadasa LS, Acharya A, Mohan M, Byrareddy SN. Therapeutic potential of cannabis: a comprehensive review of current and future applications. *Biomedicines*. (2023) 11:2630. doi: 10.3390/biomedicines11102630

6. Chicoine A, Illing K, Vuong S, Pinto KR, Alcorn J, Cosford K. Pharmacokinetic and safety evaluation of various oral doses of a novel 1:20 THC:CBD Cannabis herbal extract in dogs. *Front Vet Sci*. (2020) 7:583404. doi: 10.3389/fvets.2020.583404

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Evaluation of the efficacy of a cannabidiol and cannabidiolic acid rich hemp extract for pain in dogs following a tibial plateau leveling osteotomy

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Objective: To determine the impact of a cannabidiol (CBD) and cannabidiolic acid (CBDA) rich hemp product on acute post-operative pain in dogs following a tibial plateau leveling osteotomy (TPLO), and to evaluate for changes in early bone healing, serum chemistry profiles, and complete blood counts.

Methods: In this randomized, placebo controlled, blinded clinical trial, 44 client-owned dogs were assigned to receive either a CBD/CBDA product dosed at 2–2.5 mg/kg PO every 12 h or a placebo for 4 weeks following a TPLO. Variables evaluated before (week 0), and at 2 and 4 weeks post-operatively included standardized veterinary assessments for pain score, weight-bearing, and lameness, the Canine Brief Pain Inventory (pain interference score–PIS, pain severity score–PSS), and serum biochemistry. Complete blood counts were performed at weeks 0 and 4. Additionally, orthogonal radiographs evaluating the degree of healing were taken at week 4. A mixed model analysis, analyzing changes of variables of interest from enrollment baseline to all other time points was utilized, with a p-value ≤ 0.05 considered significant.

Results: Of the 44 enrolled patients, 3 were lost to follow up and excluded from analysis. No significant differences were noted between placebo ($n = 19$) and CBD/CBDA ($n = 22$) groups at any point in pain score, degree of lameness, degree of weight-bearing, PIS, PSS, or radiographic healing of the osteotomy. A significant finding of elevation of ALP above normal reference range in the treatment group was identified ($p = 0.02$) and eosinophil count was affected by treatment ($p = 0.01$), increasing from baseline in placebo and decreasing in treatment groups. Finally, a significant difference ($p = 0.03$) was noted at 2 weeks post-operatively where 4 patients in the placebo group and no treatment patients received trazodone to facilitate activity restrictions.

Clinical significance: Use of a CBD/CBDA rich hemp product dosed at 2–2.5 mg/kg PO every 12 h did not have a significant impact on pain or delay early bone healing. A statistically significant increase in ALP, decrease in eosinophils, and reduced use of trazodone was identified in the treatment group.

KEYWORDS

cannabidiol, CBD, cannabidiolic acid, hemp, canine

Introduction

Cannabinoids have been used therapeutically in human medicine for a variety of ailments including epilepsy, anxiety, depression, sleep disorders, nausea, glaucoma, Multiple Sclerosis, Parkinson's, Alzheimer's, and chronic or neuropathic pain (1). Use of cannabinoids has quickly gained traction in veterinary medicine, fueled by its myriad of uses in human medicine and changes in federal and state regulations resulting in the legal sale and use of these products (2, 3).

Endocannabinoids occur naturally in mammals, maintaining homeostasis by acting on cannabinoid receptors throughout the body, involving neuronal pathways and potentially the immune system to help modulate pain and inflammation. The 2 main receptors of this endocannabinoid system are cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). CB1 is primarily located within the central nervous system, and plays a role in neuropathic pain modulation, movement, and memory processing. CB2 is predominantly located within cells related to the immune function such as B-cells and natural killer cells, where it can modulate the inflammatory response. This role is complex and characterized by release of anti-inflammatory cytokines combined with inhibition of pro-inflammatory mediators, through inhibition of inflammatory cell migration and T cell proliferation, and modulation of the production and signaling of cytokines and chemokines, including TNF-α, IFN-γ, IL-6, and IL-10 (4–11). CB2 receptors have been identified in murine and human bones, with the endocannabinoid system playing a role in regulation of bony mass and remodeling (12). Phytocannabinoids are plant produced cannabinoids which influence the same receptors in mammals and are the therapeutic basis of cannabinoid rich hemp products.

Cannabis sativa L. is the strain of hemp plant from which therapeutic cannabinoids are derived. The plant contains over 100 cannabinoids, with the most abundant being cannabidiolic acid (CBDA) and tetrahydrocannabinolic acid (THCA). Better known and marketed compounds are cannabidiol (CBD) and tetrahydrocannabinol (THC) which are the decarboxylated form of the prior molecules produced during heat extraction when processing hemp products (13–18). THC is responsible for the psychotropic activity in cannabis primarily through interactions at the CB1 receptor in the central nervous system, while CBD, CBDA and THCA have no psychotropic effects and are widely regarded as being highly tolerable with minimal reported adverse side effects (5, 7, 8, 14, 15, 19–22).

More importantly, since CBD, CBDA and THCA are not known agonists of the CB1 and are weak agonists of the CB2 receptors at very high concentrations, much of the mechanism for nociception appears to be through other receptors involved in pain perception that are considered part of the endocannabinoid system and their interaction with the endogenous ligands anandamide and 2-arachidonyoylglycerol. At the level of CB receptors, CBD can inhibit the reuptake of anandamide, resulting in increased levels. The interaction of CBD with these receptor systems includes modulation of the transient receptor potential channels (TRPV1) through increase of endogenous ligand anandamide, agonist for glycine receptor activity, serotonin release through direct and indirect activation of the 5-hydroxytryptophan receptor (5HT1A) by CBD and anandamide, and direct agonist of CBD on peroxisomal proliferation activation receptor gamma (PPAR -γ) (4, 5, 7, 15, 23).

Despite the increasing popularity, there remains limited literature on the use of CBD rich hemp-based products in veterinary medicine, with the current knowledge of the full extent of the impact on pain being limited, particularly as it relates to acute surgical pain (11). Disagreement exists among reports regarding the efficacy of CBD for use in veterinary patients, with multiple studies reporting benefits in chronic osteoarthritis pain and epilepsy (6, 9, 24, 25) and a single report suggesting no significant improvement in pain (26). To date, there has been no formal study on the use of CBD in post-operative patients. The primary objective of this study was to determine the impact of a CBD/CBDA-rich hemp product on acute post-operative pain in dogs associated with a tibial plateau leveling osteotomy (TPLO) when administered for 4 weeks post-operatively. The hypothesis was that a CBD/CBDA-rich hemp product would reduce acute pain scores as compared to a placebo control. Secondary objectives were to evaluate radiographs at week 4 post-operatively to assess for changes in early bone healing and to examine serum chemistry profiles and complete blood count for changes throughout the study period.

Materials and methods

Forty-four dogs were enrolled from a large private practice specialty hospital and veterinary university hospital from August 2019 to January 2021 in a randomized, placebo-controlled, blinded clinical trial. Enrollment was based on a power analysis (UBC Power/Sample Size Calculator, β- 0.80, α-0.05) expecting a reduction of approximately 20 points on the Canine Brief Pain Inventory with a standard deviation of approximately 20 points based on Gamble et al. (9) resulting on a need for minimally 16 dogs per group. It was assumed that there could be dropouts due to the nature of the clinical trial; hence the goal for enrollment was 22 per group, allowing for a 25% dropout rate. Patients were eligible for inclusion in the study if there was a diagnosed unilateral cranial cruciate ligament rupture with no significant concurrent orthopedic, neurologic, or systemic disease. A tibial plateau leveling osteotomy (TPLO) was recommended as the treatment of choice and surgery was performed by a board-certified surgical specialist. All owners were informed of the study and consented to have their dogs enrolled. With the exception of non-steroidal anti-inflammatory drugs, medications and supplements outside the study design were discouraged for at least 2 weeks prior to the surgery, and for 4 weeks following surgery. All patients were treated with a NSAID for 5 days following surgery. Each owner agreed to have their dog evaluated at time zero, 2 weeks and 4 weeks following surgery. Owners were informed that throughout the study period, the use of new medications, supplements, or dose changes should be minimized and reported to investigators and may result in exclusion from the study. Throughout the study, all assigned capsules, bloodwork, radiographs, and sedation performed at the designated time points were provided at no cost to the owner. No direct compensation or waived fees were provided for the surgery, medications, or visits outside the scope of this trial.

All dogs were anesthetized and received intra-operative and immediate post-operative pain control including injectable opiates and ultrasound guided nerve blocks at the discretion of the attending anesthesiologist and surgeons managing the case. All TPLOs were unilateral and performed by a single board-certified surgeon at each facility using standard accepted surgical techniques. Partial medial meniscectomy was performed in all cases of meniscal tears and recorded. Dogs were hospitalized overnight and were treated for immediate post-operative pain at the discretion of the attending surgeon. The day following surgery, all dogs were initiated on a 5-day course of a NSAID and a 28-day course of either CBD/CBDA-rich hemp oil or placebo capsules. Carprofen was administered in all but 3 patients (all in the treatment group) who pre-operatively were receiving meloxicam, firocoxib, or grapiprant. The use of antibiotics was at the discretion of the treating veterinarian. Use of sedatives such as trazodone was discouraged by clinicians and only prescribed if requested by the owner to help minimize the dog's physical activity. Use of trazodone or other sedatives was recorded. All patients were discharged from hospital the day following surgery.

TABLE 1 Standardized veterinary assessment scoring system used to evaluate all dogs in the study, before and after a tibial plateau leveling osteotomy (TPLO).

Criterion	Grade	Clinical evaluation
Lameness	1	Walks normally
	2	Slightly lame when walking
	3	Moderately lame when walking
	4	Severely lame when walking
	5	Reluctant to rise, recumbent
Pain	1	No pain elicited
	2	Mild, turns head
	3	Moderate, resists and vocalizes
	4	Severe, growls and shows teeth
	5	Will not allow manipulation
Weight-bearing	1	Equal on all limbs standing and walking
	2	Normal standing, favors limb when walking
	3	Partially weight-bearing walking and standing
	4	Partial weight-bearing walking, sits immediately when not walking
	5	Non-weight-bearing at stand or walk

Patients were evaluated at time intervals of T0 (initial evaluation at week 0), T1 (2 weeks post-operatively), and T2 (4 weeks post-operatively). Pain was evaluated on both palpation and range of motion of the affected stifle.

Each dog was randomly assigned into the CBD/CBDA treatment or placebo group using a random number generator (Randomizer iPhone application) for a total of 22 dogs in each group. The treatment consisted of a hydrocarbon extracted, hemp derived cannabinoid product emulsified in sesame seed oil (ElleVet Sciences, Portland, ME, USA) from a United States Department of Agriculture hemp facility that is certified and audited annually for good manufacturing practices in compliance with the 2018 Farm Bill. The oil suspension was utilized to make 10 mg, 25 mg, and 50 mg CBD/CBDA containing capsules to be dispensed. A certificate of analysis of the batch of product used in this study was performed by an ISO 17025 accredited third-party laboratory (ProVerde Laboratories, Milford MA, USA) and was approximately 30 mg/mL of CBD, 31 mg/mL of CBDA, 1.2 mg/mL THC, 1.3 mg/mL THCA, 1 mg/mL of cannabichromene and 1.2 mg/mL of cannabichromenic acid. The certification of the hemp product passed all quality control measures regarding microbial, mycotoxin, pesticide, heavy metal and solvent contamination. The placebo was formulated utilizing the same volume of sesame seed oil in similar capsules. Patients were dosed with variations in numbers of CBD/CBDA capsules at 2–2.5 mg/kg body weight orally every 12 h. Containers holding the capsules were labeled

as A or B, based on placebo or treatment, to keep owners and clinicians blind to treatment.

Time zero was defined as a point no > 2 weeks prior to a scheduled surgery date. At this evaluation, clients were educated as to what was involved with the study, including the potential that their dogs may be placed in a placebo group, and provided informed consent to participate in the study under an approved IACUC from the University of Florida and compliance with institutional guidelines. Patients presented to one of 2 sites (Red Bank Veterinary Hospital, Red Bank NJ [RB]; University of Florida Veterinary Hospitals, Gainesville, FL [FL]). An initial client survey asked owners to report any travel plans or guests in the home anticipated during the study period, quantify the number of episodes of vomiting, diarrhea, and lethargy over the previous 2 weeks, and to document dosages of any medications or supplements the patient had received over the previous 2 weeks.

At initial evaluation (T0 or week 0), 2 weeks (+/− 2 days) post-operative (T1), and 4 weeks (+/− 2 days) post-operative (T2), all patients were examined by a single participating investigator at each hospital. At each evaluation, patients underwent a standardized veterinary assessment performed by either investigator which evaluated patient lameness, pain, and weight-bearing on scales of 1-5 based on standard descriptors as summarized in Table 1. Each dog had a complete blood count performed at T0 and T2, and chemistry performed at all 3 time points. Bloodwork was performed at either Antech Diagnostics or the University of Florida Veterinary Hospital's Diagnostic Clinical Pathology Laboratory. At each time point, the owners completed a Canine Brief Pain Inventory (CBPI). For the purpose of this study, the overall quality of life index was not considered as dogs were expected to score better after surgical intervention regardless of group. Additionally, at T2, standardized orthogonal TPLO radiographs, including a 90/90 flexed lateral stifle and a craniocaudal stifle projections, were obtained. Sedation could be performed as needed to facilitate the acquisition of well positioned radiographs at the safety of patients and staff. A schematic of the study design is provided in Figure 1.

All radiographs from T2 were evaluated by a single board-certified radiologist who was blinded to group assignments. Radiographs were evaluated for the degree of callus formation, distinctness of the osteotomy gap line, stage of union, and assigned a healing score based on a system proposed by Hammer et al. (27).

Statistical analysis

Statistical analysis was performed with a commercially available software package (JMP 10.0; Cary NC, USA). Demographics (sex, age, body condition score [BCS] and body weight in kilograms) were assessed across the treatment groups using Student's *t*-test (age, BCS and weight) or Fisher's exact testing (sex) to assess group differences. Veterinary assessment scores (pain, lameness, and weight-bearing) and CBPI were assessed using a Wilcoxon Signed rank test to compare placebo and treatment groups. All data were assessed utilizing a Shapiro-Wilks test for normality and residual plots were examined to determine normality, and when normality was rejected the data was log transformed and visually inspected for normal distribution before analysis utilizing a two-way analysis of variance with repeated measures for serum chemistry, and without repeated measures for complete blood count assessments. Variables included in the model were: fixed effects of treatment, time, treatment x time. Tukey's tests were performed *post hoc* on any significant effects of time, or time x treatment to assess differences found. Due to different clinic sites, a similar mixed model was utilized for CBPI and veterinary scores (pain, lameness, gait) with the added variable of trial site (FL or RB). Bone healing parameters were assessed using a Wilcoxon Signed rank test to compare bone healing between placebo and treatment groups for the 4 week follow up radiographs. As NSAIDs (pain relief) and trazodone (sedative) were used in some patients at baseline and continued throughout the trial period, a Fisher's exact test was performed between groups at T1 and T2 to assess whether there were differences in use of these drugs at these time points compared to baseline. A p-value of < 0.05 was determined to be significant for all analyses.

Results

Forty-one patients met the inclusion criteria and completed the study of the 44 originally enrolled. Of the dogs completing the study, 15 dogs were male; 2 were intact and 13 neutered. Twenty-six were female and all were spayed. The median age of patients was 7 years (range 1–13 years, mean 6.5 years). Median weight of patients was 31 kg (range 20.3–53.4 kg, mean 33.3 kg). Median body condition score graded on a scale of 1–9 was 6/9 (range 4-9/9, mean 6.05/9). During the trial 3 patients in the placebo group were lost to follow up leaving only 19 dogs in the placebo group with 22 dogs in the treatment group that completed the trial. No significant differences were noted between the 2 groups.

Veterinary assessments

The veterinary assessment evaluated a patient's lameness, pain score, and degree of weight-bearing on a scale of 1–5 at all 3 time points. Improvement was determined as a decrease in score on each of these scales.

The median and range of scores are summarized (Table 2). For lameness assessment there was an effect of time across

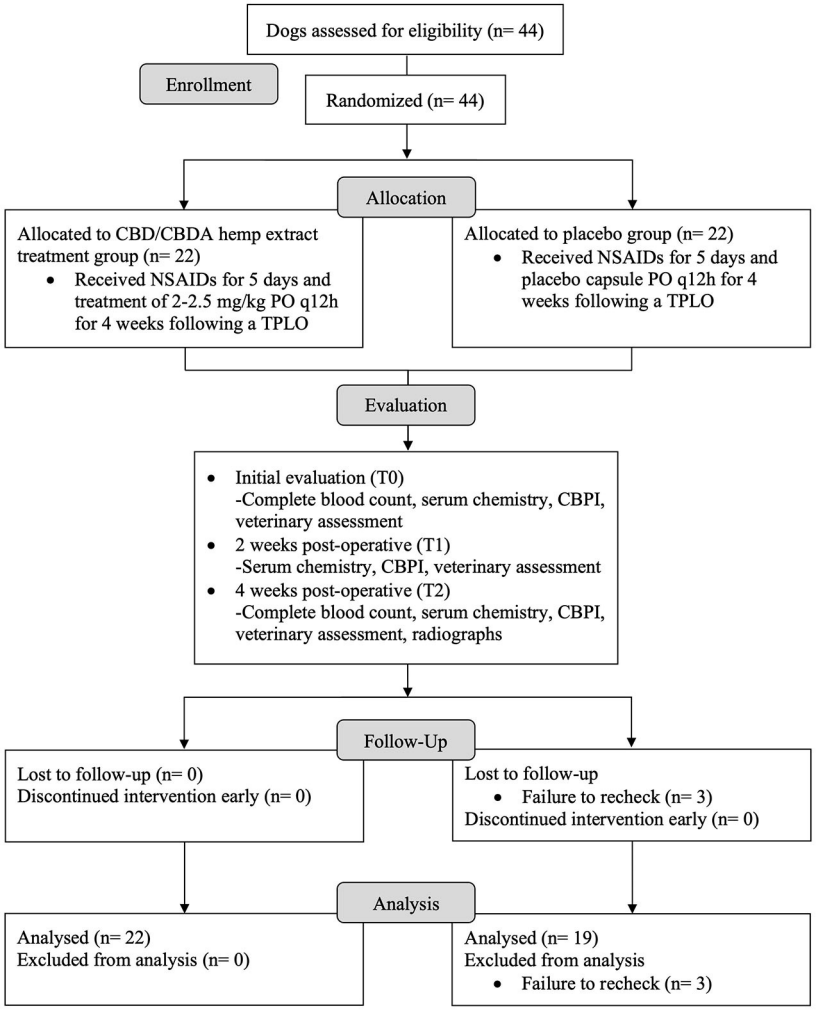

FIGURE 1
Flow diagram for a randomized, blinded, placebo-controlled design study to evaluate the effects of a cannabidiol (CBD) and cannabidiolic acid (CBDA) rich hemp product on post-operative pain, complete blood count, serum biochemistry, and early bone healing in dogs following a tibial plateau leveling osteotomy (TPLO).

all time points regardless of treatment group ($P < 0.001$). No treatment or treatment over time effect was found. Similarly, pain scores were found to decrease over the time of the trial ($P < 0.001$) with no effect of treatment or treatment over time. Finally, for degree of weight-bearing, effect of time was also found to be significant ($P < 0.01$); however, an effect of treatment or treatment over time could not be found. In addition, pain scoring appeared to differ across the 2 sites used in this study with RB pain scoring suggesting lower pain scores compared to FL ($P < 0.001$).

Canine brief pain inventory

The Canine Brief Pain Inventory was categorized as pain severity score (PSS) and pain interference score (PIS and measured at all 3 time points. Mean and standard deviation are summarized (Table 3). For the PSS and PIS there was significant effect of time observed ($P < 0.001$); however, a treatment effect or treatment over time effect were not observed.

Clinical pathology

All clinicopathologic findings were summarized (Table 4). Complete blood counts assessed at the beginning and end of the trial showed that only eosinophil counts were affected by treatment ($P = 0.01$), as eosinophil counts increased in the placebo group and decreased in the treatment group. A treatment over time effect was not observed. Serum chemistry data was normally distributed for all parameters except for serum ALT and serum ALP, which were log transformed before

TABLE 2 Results of lameness, pain, and weight-bearing scores graded on a 1–5 scale (1 = best; 5 = worst) in dogs treated with either a cannabidiol (CBD) and cannabidiolic (CBDA) rich hemp product (2–2.5 mg/kg orally every 12 h; $n = 22$) or a placebo (sesame seed oil every 12 h, $n = 19$) for 4 weeks after a tibial plateau leveling osteotomy surgery.

Score	Placebo T0	Placebo T1	Placebo T2	Tx T0	Tx T1	Tx T2	P site	P_{Tx}	P_{Time}	$P_{Tx*time}$
Lameness	3 (1–4)	2.5 (2–3)	2 (1–3)	3 (1–4)	3 (2–4)	2 (1–3)	0.92	0.25	<0.001	0.98
Pain	3 (1–3)	1 (1–3)	1 (1–3)	3 (2–3)	2 (1–2)	1 (1–3)	<0.001	0.91	<0.001	0.66
Weight-bearing	3 (1–4)	3 (2–3)	2 (1–3)	3 (1–5)	3 (2–3)	2.5 (1–3)	0.28	0.17	<0.001	0.70

Results reported as median (range) at week 0 (T0; initial evaluation before surgery) and at 2 and 4 weeks post-operatively (T1 and T2, respectively). *P*-values < 0.05 considered significant and evaluated effect of site (FL vs. RB) treatment (P_{Tx}), effect of time (P_{Time}), and effect of treatment over time ($P_{Tx*time}$) by a mixed model analysis of Wilcoxon Signed rank test and Tukey's tests.

TABLE 3 Results of pain severity score (PSS) and pain interference scores (PIS) in dogs treated with either a cannabidiol (CBD) and cannabidiolic (CBDA) rich hemp product (2–2.5 mg/kg orally every 12 h; $n = 22$) or a placebo (sesame seed oil every 12 h, $n = 19$) for 4 weeks after a tibial plateau leveling osteotomy surgery.

Score	Placebo T0	Placebo T1	Placebo T2	Tx T0	Tx T1	Tx T2	P site	P_{Tx}	P_{Time}	$P_{Tx*time}$
PSS	19 ± 7	12 ± 6	6 ± 6	20 ± 9	12 ± 8	6 ± 6	0.37	0.88	<0.001	0.77
PIS	30 ± 12	21 ± 12	10 ± 8	38 ± 15	23 ± 15	11 ± 11	0.78	0.24	<0.001	0.29

Results reported as mean ± SD at week 0 (T0; initial evaluation before surgery) and at 2 and 4 weeks post-operatively (T1 and T2, respectively). *P*-values < 0.05 considered significant and evaluated effect of site (FL vs. RB) treatment (P_{Tx}), effect of time (P_{Time}), and effect of treatment over time ($P_{Tx*time}$) by a mixed model analysis of Wilcoxon Signed rank test and Tukey's tests.

analysis. Serum chemistry evaluations across the entire cohort revealed an increase in potassium ($P < 0.01$), a decrease in glucose ($P < 0.02$), a decrease in ALT ($P = 0.03$), and a decrease in AST ($P = 0.05$) from baseline regardless of treatment over time. For ALP, an effect of treatment was noted whereby the treatment group showed rises above reference ranges for the respective lab regardless of time, while the placebo group showed decreases in ALP from baseline over time ($P = 0.02$).

Radiographic assessment

Radiographs were submitted for the 41 patients and reviewed by a board-certified radiologist for assessment of callus formation and healing scores according to methods described (28). There was a median callus formation score of 1 (range 0-3) in the placebo and median score of 1 (range 0–4) in the treatment group. There was a median healing score of 1 (range 0-3) in the placebo and 1 (range 0–4) in the treatment group. There were no significant differences between the degree of callus formation ($P = 0.67$) and subjective healing scores ($P = 0.53$) for either group.

Additional medications

Carprofen remained the NSAID of choice for this study; however, 3 of the treatment patients received alternate NSAIDs as they historically were administered them, 1 each of meloxicam, firocoxib, and grapiprant. No difference was found between these patients and the remainder of the treatment group based on Fisher's exact test. Additionally, despite being prescribed for a 5 day course, owners continued to administer NSAIDs in a group of patients based on discretion and report of pain. At T1, there were 4 placebo and 5 treatment patients, while at T2 there were 1 placebo and 2 treatment patients still receiving NSIADs. There was no difference in pain scores identified between groups at either timepoint. Finally, although use of trazodone or other sedative was discouraged, there were 8 placebo and 9 treatment patients receiving trazodone at the time of surgery. At T1, 4 placebo and 0 treatment patients remained on trazodone, the difference was found to be significant on a Fisher's exact test ($P = 0.03$).

Discussion

There is paucity information on the use of cannabinoid-rich hemp products to control post-operative pain. This study was conducted to determine the clinical effect of a CBD/CBDA-rich hemp product on acute pain in canine patients treated for a cranial cruciate ligament rupture with a routine, commonly practice TPLO surgical procedure with established outcomes. Clinical metrology instruments (veterinary assessments and CBPI) were used to assess pain for up to 4 weeks post-operatively. In this study, it was found that the only difference between groups were veterinary assessed pain scores between sites over time, leading the authors to reject the initial hypothesis that a CBD/CBDA rich hemp product would reduce acute post-operative pain scores compared to the control in this cohort.

TABLE 4 Clinicopathologic values of complete blood count and serum biochemistry in dogs treated with either a cannabidiol (CBD) and cannabidiolic (CBDA) rich hemp product (2–2.5 mg/kg orally every 12 h; $n = 22$) or a placebo (sesame seed oil every 12 h, $n = 19$) for 4 weeks after a tibial plateau leveling osteotomy surgery.

Variable	Placebo T0	Placebo T1	Placebo T2	Tx T0	Tx T1	Tx T2	P_{Tx}	P_{Time}	$P_{Tx*time}$
Total Protein (g/dL)	6.4 ± 0.7	6.4 ± 0.6	6.4 ± 0.6	6.2 ± 0.5	6.4 ± 0.5	6.4 ± 0.5	0.58	0.61	0.45
Albumin (g/dL)	3.5 ± 0.4	3.4 ± 0.4	3.4 ± 0.4	3.3 ± 0.4	3.3 ± 0.3	3.3 ± 0.4	0.22	0.11	0.83
Globulin (g/dL)	2.9 ± 0.5	2.9 ± 0.5	2.9 ± 0.4	2.9 ± 0.3	3.1 ± 0.4	3.1 ± 0.4	0.59	0.13	0.41
AST (U/L)	35 ± 15	30 ± 13	28 ± 7	35 ± 30	28 ± 8	25 ± 6	0.72	0.05	0.41
ALT (U/L)	59 ± 49	43 ± 40	51 ± 50	45 ± 43	34 ± 27	36 ± 23	0.23	0.03	0.06
ALP (U/L)	101 ± 292	88 ± 178	70 ± 113	71 ± 129	192 ± 345	253 ± 477	0.02	0.03	0.46
T bili. (mg/dL)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.44	0.18	0.61
BUN (mg/dL)	16 ± 4	18 ± 5	17 ± 4	14.0 ± 4	16 ± 4	16 ± 5	0.28	0.21	0.88
Creatinine (mg/dL)	1.0 ± 0.2	1.1 ± 0.2	1.1 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.2	0.64	0.16	0.88
Phosphorus (mg/dL)	3.8 ± 1.0	4.0 ± 0.7	3.7 ± 0.8	3.7 ± 1.1	3.8 ± 0.9	3.5 ± 0.9	0.75	0.13	0.84
Glucose (mg/dL)	99 ± 19	97 ± 14	97 ± 10	97 ± 25	86 ± 25	92 ± 26	0.89	0.02	0.19
Calcium (mg/dL)	10.0 ± 0.5	10.0 ± 3.3	10.0 ± 0.4	9.8 ± 0.6	10.0 ± 3.8	9.9 ± 0.4	0.29	0.06	0.58
Sodium (mEq/L)	147 ± 3	146 ± 2	147 ± 2	147 ± 3	147 ± 2	146 ± 3	0.95	0.44	0.02
Potassium (mEq/L)	4.4 ± 0.2	4.5 ± 0.3	4.5 ± 0.4	4.3 ± 0.3	4.5 ± 0.4	4.5 ± 0.6	0.77	<0.01	0.8
Chloride (mEq/L)	112 ± 3	119 ± 32	112 ± 2	113 ± 4	111 ± 4	112 ± 5	0.27	0.06	0.8
Cholesterol (mg/dL)	247 ± 91	261 ± 101	247 ± 87	271 ± 110	298 ± 116	288 ± 90	0.32	0.12	0.67
WBC (thous/uL)	9.81 ± 4.87	–	8.53 ± 2.09	9.72 ± 5.13	–	8.20 ± 2.27	0.85	0.08	0.89
RBC (mill/uL)	7.21 ± 0.87	–	7.42 ± 0.61	7.05 ± 0.91	–	7.32 ± 0.62	0.67	0.04	0.76
HGB (g/dL)	17.0 ± 2.0	–	17.5 ± 1.5	16.9 ± 2.1	–	17.6 ± 1.51	0.83	0.02	0.65
Hct (%)	50.1 ± 5.8	–	50.9 ± 4.4	49.8 ± 5.9	–	51.6 ± 4.9	0.82	0.1	0.49
Platelet (thous/uL)	281 ± 82	–	300 ± 89	265 ± 96	–	315 ± 97	0.08	0.01	0.03
Neutrophil (abs)	7240 ± 4612	–	5833 ± 4213	7167 ± 4913	–	5598 ± 1752	0.6	0.06	0.91
Lymphocyte (abs)	1743 ± 657	–	1848 ± 660	1505 ± 537	–	1691 ± 624	0.51	0.09	0.63
Monocyte (abs)	422 ± 285	–	473 ± 348	404 ± 216	–	370 ± 179	0.26	0.86	0.43
Eosinophils (abs)	381 ± 265	–	391 ± 238	619 ± 603	–	556 ± 323	0.01	0.67	0.56

Results reported as mean ± SD at week 0 (T0; initial evaluation before surgery) and at 2 and 4 weeks post-operatively (T1 and T2, respectively). Serum biochemistry was performed at all timepoints while complete blood count at T0 and T2. P-values < 0.05 considered significant and evaluated effect of treatment (P_{Tx}), effect of time (P_{Time}), and effect of treatment over time (P_{Tx^*time}) by a mixed model two–way analysis of variance and Tukey's test.
AST, aspartate animotransferase; ALT, alanine animotranferase; ALP, alkaline phosphatase; T bili, total bilirubin; BUN, blood urea nitrogen; WBC, white blood cells; RBC, red blood cell; HGB, hemoglobin; Hct, hematocrit; abs, absolute.

Currently in the veterinary literature, clinical effects in osteoarthritis and refractory seizures suggests that a dose of approximately 2–2.5 mg/kg every 12 to 24 h shows clinical efficacy which were the tenants of dosing in our study (6, 8, 9, 15, 19, 26, 28, 29). While no benefit was identified in this present study, interestingly a recent abstract using the same product used in this study at a 5 mg/kg dose every 8 h may decrease pain scores in patients undergoing intervertebral disk disease surgery (30). Currently, no toxic dose has been established with reports of 20 mg/kg orally every 12 h for 6 weeks, 4 mg/kg once daily for 6 months, and lower doses of 2 mg/kg every 12 h for 12 weeks being tolerated in dogs (19, 28, 31).

CBDA is less well studied yet recent studies show that both CBDA and THCA demonstrate superior absorption in dogs than CBD. While the understanding of CBDA pharmacology remains lacking, CBDA has been shown to increase serum CBD concentrations with lower CBD dosing due to improved absorption and retention of CBD and CBDA (16, 17, 32). This process has been described as an "entourage effect," by which CBDA and THCA work synergistically with CBD, lowering the dose of CBD/CBDA-rich hemp product required to meet similar therapeutic levels when compared to a purified CBD product (16). It may have been useful to assess serum steady state concentrations at the 4-week visit to better understand the effects of serum concentrations; however, at the time of study initiation, no commercial laboratories were assessing CBDA serum concentrations.

In this cohort, only two significant clinicopathologic changes were noted. The only treatment effects observed was a serum chemistry elevation in ALP. The elevation of liver values in this cohort, particularly ALP, is consistent with previous reports in human, murine, and canine literature, and thought to be due to induction of cytochrome p450 mediated oxidative metabolism (9, 14, 15, 19, 24–26). In a recent trial in dogs receiving 4 mg/kg daily of CBD for 6 months, similar rises in ALP were identified which returned to baseline within 4 weeks of cessation of CBD (31). However, elevation of liver enzymes is inconsistent in the literature, with multiple reports in canine patients having

no identified changes, particularly in young healthy cohorts (6, 8, 15).

Additionally, there was a slight increase in eosinophils in the placebo group and a mild decrease identified in our treatment group, though still within reference ranges. Human eosinophils exposed *in vitro* to high concentrations of THC or CBD respond with increased expression of macrophage inflammatory protein-1β (MIP-1β), suggesting that THC or CBD may exacerbate pre-existing eosinophilic inflammatory disease (33). Studies in dogs have mixed results, suggesting mild anti-inflammatory effect when used at very high concentrations, with a more recent report in which oral CBD at typical dosing had no influence on immune cell regulation (34). To the authors knowledge, a relative eosinopenia associated with CBD use has not been reported in the literature and further studies are necessary to understand the significance particularly in light of the rise observed in the placebo group.

The most interesting finding in this study was the use of trazodone to limit activity and mildly sedate dogs throughout the study period. The use of sedative was discouraged and implemented based on owner insistence for the safety of the patient and implants. At the initial evaluation, 8 dogs in the placebo group and 9 dogs in the CBD/CBDA received trazodone. At the second visit at 2 weeks post-operatively, 4 placebo dogs remained on trazodone while 0 required trazodone in the treatment group, suggesting a potentially sedating effect of CBD/CBDA. In human and veterinary studies adverse effects of somnolence and lethargy have been noted with using CBD-rich hemp extracts (35, 36). Direct comparison of a CBD-rich hemp extract in a treat format given as approximately 0.7 mg/kg orally every 12 h was assessed in dogs identified with noise phobias showing that treatment with CBD did not impact behavioral anxiety scoring, while trazodone was mildly effective for some behavioral parameters (37). However, our dosing was significantly higher and contained a mix of CBD/CBDA rich hemp. Though not the primary outcome and a small sample size, these data suggest future study of CBD rich hemp products for agitation and anxiety are warranted in the post-surgical period for activity restriction.

CBD has been shown to increase the recruitment of mesenchymal stem cells and subsequent differentiation to osteoblastic lineage in experimental models (12). Additionally, CBD enhances mechanical properties of callus formation through expression of procollagen-lysine 2-oxoglutarate 5-dioxygenase, a collagen cross linking enzyme. When administered in murine studies, radiographic evidence of CBD stimulated callus formation was seen after week 6 (12, 38, 39). In the present study, no impact on bone healing from the treatment was observed.

Overall, this study had several limitations. While a control population was used, a confounding placebo effect or regression of the mean cannot be ruled out. A small population size was investigated in this study, though this is thought to have had minimal impact on the results as the sample size was determined by a power analysis. As this was multi-institutional, 2 principal examiners were involved and despite using a standardized veterinary assessment scoring system, pain scores differed between the 2 sites. Additionally, given that 2 clinical pathology laboratories were used, values were evaluated individually and in terms of the respective lab reference ranges, rather than being combined as means. An additional limitation is that a complete blood count was only performed at 2 time points as it was not anticipated to see change in eosinophil based on prior literature. While the use of additional medications (NSAIDs after 5 days or trazodone) was discouraged, owner insistence on the use remained an unavoidable factor. While prolonged use of NSAIDs or use of NSAIDs other than carprofen had no impact on results, there was a significant difference between groups with long term trazodone administration. Stricter exclusion criteria in regard to medications may be considered in future studies. Finally, there was a lack of standardization between anesthetic protocols which included the use of local nerve blocks. While this may have impacted immediate post-operative pain, it is not suspected to have a significant effect given an overall lack of efficacy of CBD/CBDA in the study.

In conclusion, the results of the current study indicate that when administered at a dose of 2–2.5 mg/kg twice daily for 4 weeks following a TPLO, the CBD/CBDA hemp extract had no effect on measures of pain or early bone healing. Administration was associated with an increase in ALP and a relative eosinopenia compared to a relative eosinophilia in the placebo group. Finally, there was a possible association of CBD/CBDA and reduced post-operative anxiety. Further investigation is warranted for this use, although possible negative effects on ALP and eosinophils should be considered.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by University of Florida IACUC and in compliance with Red Bank Veterinary Hospital Institutional Guidelines. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

SK was responsible for data interpretation, drafting of the manuscript revision, and approval of the submitted manuscript. GD, JS, EM, LE, and MJ were responsible for acquisition of data

and manuscript revision. AG was responsible for interpretation, assessment of radiographic images, and manuscript revision. JT was responsible for acquisition of data, data entry, and manuscript revision. KL was responsible for data entry and manuscript revision. JW was responsible for the conception of the study, statistical analysis, and manuscript revision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Levinsohn EA, Hill KP. Clinical uses of cannabis and cannabinoids in the United States. *J Neurol.* (2020) 411:1–6. doi: 10.1016/j.jns.2020.116717

2. Alharbi YN. Current legal status of medical marijuana and cannabidiol in the United States. *Epilepsy Behav.* (2020) 112:1–3. doi: 10.1016/j.yebeh.2020.107452

3. Kogan LR, Hellyer PW, Schoenfeld-Tacher R. Dog owner's use and perceptions of cannabis products. *J Am Holist Vet Med Assoc*. (2018) 51:26–33.

4. Peng J, Fan M, An C, Feng N, Huang W, Juo J, et al. narrative review of molecular mechanisms and therapeutic effect of cannabidiol (CBD). *Basic Clin Pharmacol Toxicol.* (2022) 130:439–56. doi: 10.1111/bcpt.13710

5. Pacher P, Bátkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev.* (2006) 58:389–462. doi: 10.1124/pr.58.3.2

6. Verrico CD, Wesson S, Konduri V, Hofferek CJ, Vazquez-Perez J, Blair E, et al. A randomized, double-blind, placebo-controlled study of daily cannabidiol for the treatment of canine osteoarthritis pain. *Pain.* (2020) 161:2191–202. doi: 10.1097/j.pain.0000000000001896

7. Philpott HT, O'Brien M, McDougall JJ. Attenuation of early phase inflammation by cannabidiol prevents pain and nerve damage in rat osteoarthritis. *Pain.* (2017) 158:2442–51. doi: 10.1097/j.pain.0000000000001052

8. Brioschi FA, Di Cesare F, Gioeni D, Rabbogliatti V, Ferrari F, D'Urso ES, et al. Oral transmucosal cannabidiol oil formulation as part of a multimodal analgesic regimen: effects on pain relief and quality of life improvement in dogs affected by spontaneous osteoarthritis. *Animals.* (2020) 10:1505. doi: 10.3390/ani10091505

9. Gamble LJ, Boesch JM, Frye CW, Schwark WS, Mann S, Wolfe L, et al. Pharmacokinetics, safety, and clinical efficacy of cannabidiol treatment in osteoarthritic dogs. *Front Vet Sci.* (2018) 5:165. doi: 10.3389/fvets.2018.00165

10. Di Marzo V, Bifulco M, De Petrocellis L. The endocannabinoid system and its therapeutic exploitation. *Nat Rev Drug Discov.* (2004) 3:771–84. doi: 10.1038/nrd1495

11. Bouchet CA, Ingram SL. Cannabinoids in the descending pain modulatory circuit: role in inflammation. *Pharmacol Ther.* (2020) 209:107495. doi: 10.1016/j.pharmthera.2020.107495

12. Kamali A, Oryan A, Hosseini S, Ghanian MH, Alizadeh M, Eslaminejad MB, et al. Cannabidiol-loaded microspheres incorporated into osteoconductive scaffold enhance mesenchymal stem cell recruitment and regeneration of critical-sized bone defects. *Mater Sci Eng C Mater Biol Appl.* (2019) 101:64–75. doi: 10.1016/j.msec.2019.03.070

13. Corsetti S, Borruso S, Malandrucco L, Spallucci V, Maragliano L, Perino R, et al. Cannabis sativa L. may reduce aggressive behaviour towards humans in shelter dogs. *Sci Rep.* (2021) 11:2773. doi: 10.1038/s41598-021-82439-2

14. Ewing LE, Skinner CM, Quick CM, Kennon-McGill S, McGill MR, Walker LA, et al. Hepatotoxicity of a cannabidiol-rich Cannabis extract in the mouse model. *Molecules.* (2019) 24:1694. doi: 10.3390/molecules24091694

15. Deabold KA, Schwark WS, Wolf L, Wakshlag JJ. Single-dose pharmacokinetics and preliminary safety assessment with use of CBD-rich hemp nutraceutical in healthy dogs and cats. *Animals.* (2019) 9:832. doi: 10.3390/ani9100832

16. Wakshlag JJ, Schwark WS, Deabold KA, Talsma BN, Cital S, Lyubimov A, et al. Pharmacokinetics of cannabidiol, cannabidiolic acid, Δ9-tetrahydrocannabinol, tetrahydrocannabinolic acid and related metabolites in canine serum after dosing with three oral forms of hemp extract. *Front Vet Sci.* (2020) 7:505. doi: 10.3389/fvets.2020.00505

17. Eichler M, Spinedi L, Unfer-Grauwiler S, Bodmer M, Surber C, Luedi M, et al. Heat exposure of cannabis sativa extracts affects the pharmacokinetic and metabolic profile in healthy male subjects. *Planta Med.* (2012) 78:686–91. doi: 10.1055/s-0031-1298334

18. Wang M, Wang YH, Avula B, Radwan MM, Wanas AS, Antwerp J, et al. Decarboxylation study of acidic cannabinoids: a novel approach using ultra-high-performance supercritical fluid chromatography/photodiode array-mass spectrometry. *Cannabis Cannabinoid Res.* (2016) 1:262–71. doi: 10.1089/can.2016.0020

19. McGrath S, Bartner LR, Rao S, Kogan LR, Hellyer PW. A report of adverse effects associated with the administration of cannabidiol in healthy dogs. *J Am Holist Vet Med Assoc.* (2018) 52:34–8.

20. De Briyne N, Holmes D, Sandler I, Stiles E, Szymanski D, Moody S, et al. Cannabis, cannabidiol oils and tetrahydrocannabinol-what do veterinarians need to know? *Animals.* (2021) 11:892. doi: 10.3390/ani11030892

21. Bartner LR, McGrath S, Rao S, Hyatt LK, Wittenburg LA. Pharmacokinetics of cannabidiol administered by 3 delivery methods at 2 different dosages to healthy dogs. *Can J Vet Res.* (2018) 82:178–83.

22. Mlost J, Bryk M, Starowicz K. Cannabidiol for pain treatment: focus on pharmacology and mechanism of action. *Int J Mol Sci.* (2020) 21:8870. doi: 10.3390/ijms21228870

23. Landa L, Sulcova A, Gbelec P. The use of cannabinoids in animals and therapeutic implications for veterinary medicine: a review. *Vet Med (Praha).* (2016) 61:111–22. doi: 10.17221/8762-VETMED

24. McGrath S, Bartner LR, Rao S, Packer RA, Gustafson DL. Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. *J Am Vet Med Assoc.* (2019) 254:1301–8. doi: 10.2460/javma.254.11.1301

25. Kogan L, Hellyer P, Downing R. The use of cannabidiol-rich hemp oil extract to treat canine osteoarthritis-related pain: a pilot study. *J Am Holist Vet Med Assoc*. (2020) 58:1–10.

26. Mejia S, Duerr FM, Griffenhagen G, McGrath S. Evaluation of the effect of cannabidiol on naturally occurring osteoarthritis-associated pain: a pilot study in dogs. *J Am Anim Hosp Assoc.* (2021) 57:81–90. doi: 10.5326/JAAHA-MS-7119

27. Hammer RR, Hammerby S, Lindholm B. Accuracy of radiologic assessment of tibial shaft fracture union in humans. *Clin Orthop Relat Res.* (1985) (199):233–8. doi: 10.1097/00003086-198510000-00033

28. Vaughn D, Kulpa J, Paulionis L. Preliminary investigation of the safety of escalating cannabinoid doses in healthy dogs. *Front Vet Sci.* (2020) 7:51. doi: 10.3389/fvets.2020.00051

29. Morris EM, Kitts-Morgan SE, Spangler DM, Gebert J, Vanzant ES, McLeod KR, et al. Feeding cannabidiol (CBD)-containing treats did not affect canine daily voluntary activity. *Front Vet Sci.* (2021) 8:645667. doi: 10.3389/fvets.2021.645667

30. Wright JL. Evaluating the benefits of cannabidiol for analgesia following surgery for intervertebral disc herniation in dogs. In: *Proceedings of the American College of Veterinary Internal Medicine Conference*. Austin, TX: Journal of Veterinary Internal Medicine (2022). p. 427.

31. Bradley S, Young S, Bakke AM, Holcombe L, Waller D, Hunt A, et al. Long-term daily feeding of cannabidiol is well-tolerated by healthy dogs. *Front Vet Sci.* (2022) 9:97. doi: 10.3389/fvets.2022.977457

32. Anderson LL, Etchart MG, Bahceci D, Golembiewski TA, Arnold JC. Cannabis constituents interact at the drug efflux pump BCRP to markedly increase plasma cannabidiolic acid concentrations. *Sci Rep.* (2021) 11:14948. doi: 10.1038/s41598-021-94212-6

33. Srivastava MD, Srivastava BI, Brouhard B. Delta 9 tetrahydrocannabinol and cannabidiol alter cytokine production by human immune cells. *Immunopharmacology.* (1998) 40:179–85. doi: 10.1016/S0162-3109(98)00041-1

34. Morris EH, Kitts-Morgan SE, Spangler DM, McLeod KR, Suckow MA, Harmon DL. Feeding treats containing cannabidiol (CBD) did not alter canine immune response to immunization with a novel antigen. *Res Vet Sci.* (2022) 143:13–9. doi: 10.1016/j.rvsc.2021.12.012

35. Loewinger M, Wakshlag JJ, Bowden D, Peters-Kennedy J, Rosenberg A. The effect of a mixed cannabidiol and cannabidiolic acid based oil on client-owned dogs with atopic dermatitis. *Vet Dermatol.* (2022) 33:329–e77. doi: 10.1111/vde.13077

36. Lattanzi S, Trinka E, Striano P, Rocchi C, Salvemini S, Silvestrini M, et al. Highly purified cannabidiol for epilepsy treatment: a systematic review of epileptic conditions beyond Dravet Syndrome and Lennox-Gastaut Syndrome. *CNS Drugs.* (2021) 35:265–81. doi: 10.1007/s40263-021-00807-y

37. Morris EM, Kitts-Morgan SE, Spangler DM, McLeod KR, Costa JHC. and Harmon DL. The impact of feeding cannabidiol (CBD) containing treats on canine response to a noise-induced fear response test. *Front Vet Sci.* (2020) 7:569565. doi: 10.3389/fvets.2020.569565

38. Raphael-Mizrahi B, Gabet Y. The cannabinoids effect on bone formation and bone healing. *Curr Osteoporos Rep.* (2020) 18:433–8. doi: 10.1007/s11914-020-00607-1

39. Kogan NM, Melamed E, Wasserman E, Raphael B, Breuer A, Stok KS, et al. Cannabidiol, a major non-psychotropic cannabis constituent enhances fracture healing and stimulates lysyl hydroxylase activity in osteoblasts. *J Bone Miner Res.* (2015) 30:1905–13. doi: 10.1002/jbmr.2513

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Pharmacokinetics of cannabidiol following single oral and oral transmucosal administration in dogs

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Introduction: In the last few years, different formulations containing cannabidiol (CBD) were tested with regard to its efficacy on chronic pain, refractory epilepsy, anxiety, aggressive behavior and atopic dermatitis in dogs. CBD is generally administered orally, but its low bioavailability, probably due to a first-pass metabolism, represents a great limitation. The aim of this study was to evaluate if CBD bioavailability increases after oral transmucosal administration (OTM) compared to oral treatment.

Methods: Twelve dogs diagnosed with mild chronic pain were enrolled in the study and treated once orally or OTM (6 dogs/group) with a pure CBD in oil formulation at a dosing rate of 1 mg/kg b.w. At prefixed time points, blood samples were collected to define CBD plasma concentrations vs. time profiles, and the main pharmacokinetics parameters were obtained by non-compartmental model.

Results: CBD Cmax, Tmax, terminal half-life and AUC_{0-t} were 206.77 ± 167 and 200.33 ± 158.33 ng/mL, 2.17 ± 0.98 and 1.92 ± 1.11 h, 2.67 ± 0.53 and 2.62 ± 0.64 h, 647.51 ± 453.17, and 536.05 ± 370.21 h*ng/mL, following oral and OTM administration, respectively. No significant difference in pharmacokinetic parameters were observed between treatments.

Discussion: The OTM administration did not increase cannabidiol bioavailability compared to oral treatment. The almost perfectly superimposable mean plasma concentrations of cannabidiol following the two treatments suggests that CBD is not able to be adsorbed by the oral mucosa or that its absorption is very scarce, and that CBD is swallowed and absorbed in the gastrointestinal tract.

KEYWORDS

cannabidiol, CBD, dog, oral administration, oral transmucosal administration, OTM, pharmacokinetics

Introduction

In the recent years, a growing interest has raised toward the use of Cannabis sativa extracts in veterinary medicine for the treatment of several type of pain, refractory epilepsy, anxiety, aggressive behavior or atopic dermatitis (1, 2). An online anonymous survey conducted by Kogan et al. (3) outlined that several pet owners were inclined to administer cannabis products to their animals due to the feeling that the efficacy was comparable to that obtained with conventional drugs. For this reason and due to the involvement of the endocannabinoid system in the pain pathways, several clinical studies investigated the efficacy of cannabis derivatives, in particular cannabidiol (CBD), on osteoarthritic chronic pain in dog. The oral administration of different CBD oils (as a sole treatment or as add-on to other analgesic drugs), generally at doses ranging between 1 and 2 mg/kg every 12 h for at least 4 weeks, resulted in a significant reduction of pain scores, an improvement of mobility and of quality of life as well as a decrease of inflammatory serum biomarkers (4–7).

Cannabidiol is generally administered orally, but its low bioavailability, lesser than 19% in dog (8), is a great limitation. One factor that influences the CBD concentration in the systemic circulation following oral administration is its formulation. Several studies have evaluated the pharmacokinetics of CBD after its oral administration as dry raw material in gelatin capsules, microencapsulated oil beads, soft chews, hemp extracts mixed with different oil types and CBD enriched cannabis herbal extracts. The CBD oil-based formulations and soft chews resulted in higher plasma concentrations (4, 8–12), indicating that the type of formulation largely influences the oral absorption of CBD.

Due to its lipophilic nature, CBD undergoes to extended metabolism as proved by Samara et al. (13) which identified several CBD metabolites in dog urine following its intravenous administration. The first-pass metabolism is believed to be one of the most plausible causes of the scarce oral CBD bioavailability (2).

An alternative route of administration that might improve the bioavailability of CBD bypassing the first-pass metabolism is the oral transmucosal (OTM). This route of drug's administration does not require particular restriction of the animal or specific skills of the owner when compared to parenteral administrations, it is painless and non-invasive and it is successfully applied in veterinary medicine to manage pain or sedate animals (14, 15). Indeed, it was recently used in a clinical study on efficacy of CBD in dogs affected by osteoarthritis, resulting in an improvement of pain scores and quality of life (16). The OTM administration could also minimize the great individual absorption variability usually observed following oral administration (4, 9, 10). The cause of this variability may be due to gastric pH, emptying time, differences between young and old in gastrointestinal anatomy and eventual presence of food and its composition in the gastrointestinal tract (17), all factors not influencing the OTM administration. An increase in CBD blood concentrations following OTM route could also allow the reduction of the administered dose with consequent containment of the cost of therapy.

The aim of this study was to evaluate the pharmacokinetics of a CBD oil-based formulation following single oral and oral transmucosal administration in the canine species, hypothesizing that CBD bioavailability was increased after OTM with respect to oral treatment.

Materials and methods

Animals and treatment

Twelve dogs (4 females and 8 males) of various breeds, weighing 24.4 ± 9.4 kg (mean ± standard deviation), and of 8.4 ± 4.7 years of age, were enrolled in the study (Table 1). The animals were referred to the Veterinary Teaching Hospital of the University of Perugia (Italy) and diagnosed by the veterinary clinician with mild chronic pain due to osteoarthritis (10 subjects) and Inflammatory Bowel Disease (2 subjects); no other concomitant pathologies were detected by physical exam; the hematological and biochemical parameters related to the liver and kidney functions were in the normal range. At the time of enrollment, dogs were not receiving any pharmacological treatment.

All dog's owners were interested to administer CBD as an alternative to traditional treatments and gave their written consent to participate to the study, previously approved by the Bioethical Committee of the University of Perugia (on 2nd September 2019 with protocol number: 2019 14/R).

Before the CBD treatment, food and water were withdrawn for 12 and 2 h, respectively, and an IV catheter was aseptically inserted into the right cephalic vein. This was considered as the most appropriate site for collecting blood sample after OTM administration, as the jugular vein, usually used in pharmacokinetic study, collects buccal veins, thus overestimating drugs' plasma concentrations (18, 19).

A 10% CBD oil-based formulation was prepared by an authorized pharmacy using synthetic CBD crystals of pharmaceutical grade (Cannabidiol Pharma, purity grade 100.7%; Metapharmaceutical Industrial SL, Barcelona, Spain) in medium-chain triglycerides (MCT) oil. Dogs were randomly assigned to the oral or OTM treatment group (6 dogs/group) and administered with 1 mg/kg b.w. of CBD. The 10% CBD oil allowed to administer a limited number of drops (range: 4–11 drops) to all animals, favoring an appreciable dosage

TABLE 1 Age, weight, sex, breed, disease of recruited animals, and pharmacological treatments.

Age (years)	Weight (kg)	Sex	Breed	Disease	Drops administered	Dose (mg/kg)
Oral						
9	36.5	F	Labrador	OA	10	0.93
2.5	36	M	Mixed	OA	10	0.94
11	22	M	Mixed	OA	7	1.08
13	12.3	M	Mixed	OA	4	1.10
1.5	20.7	M	Mixed	OA	6	0.98
8	23.3	M	Mixed	IBD	7	1.02
OTM						
4	17.9	M	Breton	OA	5	0.95
12	19.2	F	Border collie	OA	6	1.06
4	36	M	Dobermann	IBD	11	1.04
17	12.4	M	Mixed	OA	4	1.09
10	20	F	Border collie	OA	6	1.02
9	36.6	F	Labrador	OA	10	0.93

OA, osteoarthritis; IBD, Inflammatory Bowel Disease.

correctness (Table 1). When given orally, the CBD oil was added to a small amount of commercial dry food, while for the OTM administration the CBD oil was instilled along the lateral gingiva and a gentle massage was applied to the dog's cheek to promote the transmucosal absorption of the drug. Two hours after treatment, dogs were allowed to eat their meal.

Before treatment and at prefixed post-administration time-points (0.25, 0.5, 1, 1.5, 2, 4, 6, 8, and 10 h) blood sample were collected from the cephalic vein in tubes containing sodium citrate as anticoagulant and centrifuged at 3,500 rpm; the obtained plasma samples were then stored at −80°C pending analytical determinations.

Quantification of CBD in plasma

Chemicals and reagents

Cannabidiol (CBD, cod C-045-1ML) and its deuterated internal standard cannabinol-d3 (CBD-d3, cod C-084-1ML) were purchased from Sigma-Aldrich (St. Louis, MO, USA) as methanolic solutions at concentrations of 1,000 and 100 μg/mL, respectively. Working solutions were then prepared diluting the commercial products with MeOH.

MeOH, acetonitrile, n-hexane (all LC–MS grade) were obtained from Honeywell (Charlotte, NC, USA), while water and formic acid were purchased from VWR International (Radnor, PA, USA).

Analytical determination of CBD in canine plasma

CBD was extracted from canine plasma using the protocol suggested by Zgair et al. (20). Briefly, 0.3 mL of canine plasma was added into a 15 mL Falcon tube with 30 μL of a solution of CBD-d3 (0.1 μg/mL) in MeOH. The samples were subjected to protein precipitation with 1.2 mL of acetonitrile and left at −20°C for 5 min. Water (1.2 mL) was added to each sample prior to the addition of 6 mL of n-hexane performing liquid-liquid phase extraction. The n-hexane layer was collected and then evaporated at 30°C under nitrogen stream. Finally, the dry residue was resuspended with 0.3 mL of MeOH/H_2O 80/20 (v/v) with 0.1% of formic acid and, after centrifugation, the sample was transferred into a glass vials and injected. LC-MS/MS measurements were performed by a Surveyor LC pump, coupled with a triple quadrupole mass spectrometer (TSQ Quantum Ultra, Thermo Fisher, San Jose, CA, USA), equipped with an electrospray source operating in positive ionization mode. Separation was achieved on a Kinetex C8 column (100 × 2.1 mm, 2.6 μm) which was connected to a guard column Kinetex C8 (2.1 × 3 mm), both from Phenomenex (Torrance, CA, USA). The mobile phases were water (A) and MeOH (B) both containing HCOOH 0.1%. The gradient profile was as follows: (1) 0–1 min, 60% B; (2) 1–7 min, to 80% B; (3) 7–9 min, to 100% B; (4) 9–14 min, 100% B; (5) 14–15 min, to 60% B, and (6) 15–22 min, 60% B. The total run time was 22 min. The column temperature was set at 40°C, the flow rate at 0.25 mL/min and the injection volume was 5 μL. Analytes were

detected using multiple reaction monitoring (MRM) selecting the following transitions: CBD 315.2 m/z → 193.1 m/z, 315.2 m/z → 123.0 m/z and 315.2 m/z → 259.2 m/z; CBD-d3 (IS) 318.2 m/z → 196.1 m/z. In each analytical batch, eight concentration points (0, 2.5, 5, 10, 50, 75, 100, 150, and 200 ng/mL in MeOH) were injected as calibration curve. CBD was quantified applying the isotopic dilution technique.

Five replicates of canine plasma samples were analyzed at five spiking concentrations (1, 2.5, 10, 75, and 150 ng/mL) on two different days. Within-run and between-run precision were in the range 2.3–7.0% and 4.9–10.4%, respectively. Accuracy was always from 85 to 115%. The lower (LLOQ) and upper (ULOQ) limit of quantification were 1 and 150 ng/mL, respectively. Samples with concentrations higher than 150 ng/mL were afresh extracted, introducing a dilution factor of 10 fold, and reanalyzed.

Pharmacokinetic and statistical analysis

The homogeneity of groups with respect to age and weight was verified by Kruskal–Wallis test while that with respect to sex by exact Fisher test.

The time/concentration curves obtained by each dog were analyzed by a non-compartmental model using the PK-Solver programme (21). The areas under the concentration-time curves from 0 to the last time (AUC_{0-t}) were calculated using the trapezoidal method.

The non-parametric Kruskal–Wallis test was applied to statistically compare the pharmacokinetic parameters between the two groups of treatment. All statistical analyses were conducted by Statistics for Data Analysis powered by SPSS version 25 (SPS srl, Italy). Differences were considered significant when $p < 0.05$.

Results

At the first experimental time point (15 min) CBD was detectable in plasma of 2 and 4 subjects following oral and OTM treatment, respectively. Thirty minutes after OTM administration, CBD was detected in all dogs, while only in five out of 6 subjects in the orally treated group, where CBD was detectable in all subjects 1 h post administration. The CBD plasma peak (Cmax) was achieved between 1 and 4 h (Tmax) in both treatment groups and ranged between 73 and 526 and 67 and 451 ng/mL following oral and OTM administration, respectively. At the last experimental time-point (10 h after the administration), CBD was detectable in all subjects in variable concentrations ranging from 5 to 26 and from 3 to 12 ng/mL after oral and OTM treatment, respectively. A large intersubjective variation in CBD blood concentrations was obtained at almost all the scheduled sample times as shown in Figure 1 in which CBD plasma concentrations vs. time plots of the two groups of treatment are represented.

Following non-compartmental analysis, the extrapolated percentage of the area under the curve (AUC) of one dog in the oral group was >20% (26.8%), therefore the pharmacokinetic parameters depending on terminal rate constant of this subject were considered unreliable and excluded, while parameters such as Cmax, Tmax and AUC_{0-t} were maintained.

Table 2 shows the main pharmacokinetic parameters obtained after oral and OTM treatment with CBD. No significant differences in pharmacokinetic data resulted between the two routes of administration.

Discussion

Previous studies evaluating the pharmacokinetics of CBD in dogs employed dosages higher than those usually applied in clinical practice (9, 11, 22). From anecdotal data and published studies on the efficacy of CBD in dogs in the treatment of osteoarthritis and epilepsy, oral doses between 1 and 2 mg/kg every 12 h are generally used successfully (2). According to the aphorism "Start low, go slow, stay low" (23), a dose of 1 mg/kg of CBD was chosen by the clinician responsible for the enrollment of animals in the present study.

A 10% formulation of CBD in MTC oil was used in the present study to permit the administration of reduced volumes of solution considering the dogs' weight range, thus avoiding losses outside the mouth when given OTM (16). Moreover, besides preventing the oxidative degradation and the decrease of cannabinoid's concentration better than other oils (24), MTC oil is flavorless, limiting ptyalism and vomiting (16). When given orally, CBD oil was mixed with a small amount of dry food to facilitate the administration of the drug and as a food bolus is reputed to enhance the gastrointestinal absorption of very lipophilic substances such as CBD (2). In a human study, the administration of CBD with a high-fat meal, resulted in Cmax and AUC over 4 times greater than in fasted condition (25). It is believed that food enhances the absorption of lipophilic drugs by increasing their permanence in the gastrointestinal tract, their solubilization and their lymphatic transport by lymph lipoproteins (26). Deabold et al. (10), suggested that the same phenomena might incur in dogs, where the administration of CBD formulated as soft chew, considered a food matrix, resulted in Cmax and AUC about 3 times greater than that observed in a previous published study performed with CBD oil (4). On the other hand, a study where the pharmacokinetic of Bedrocan®, a cannabis oil extract, was performed in fasting and fed dogs, the latter showed a longer Tmax and a lower Cmax compared with the fasted group, and a relative oral bioavailability of THC of 48.22% (27). The Authors speculated that being THC a lipophilic compound, it should have increased bioavailability in the fed condition. However, the lipophilicity of the olive oil formulation

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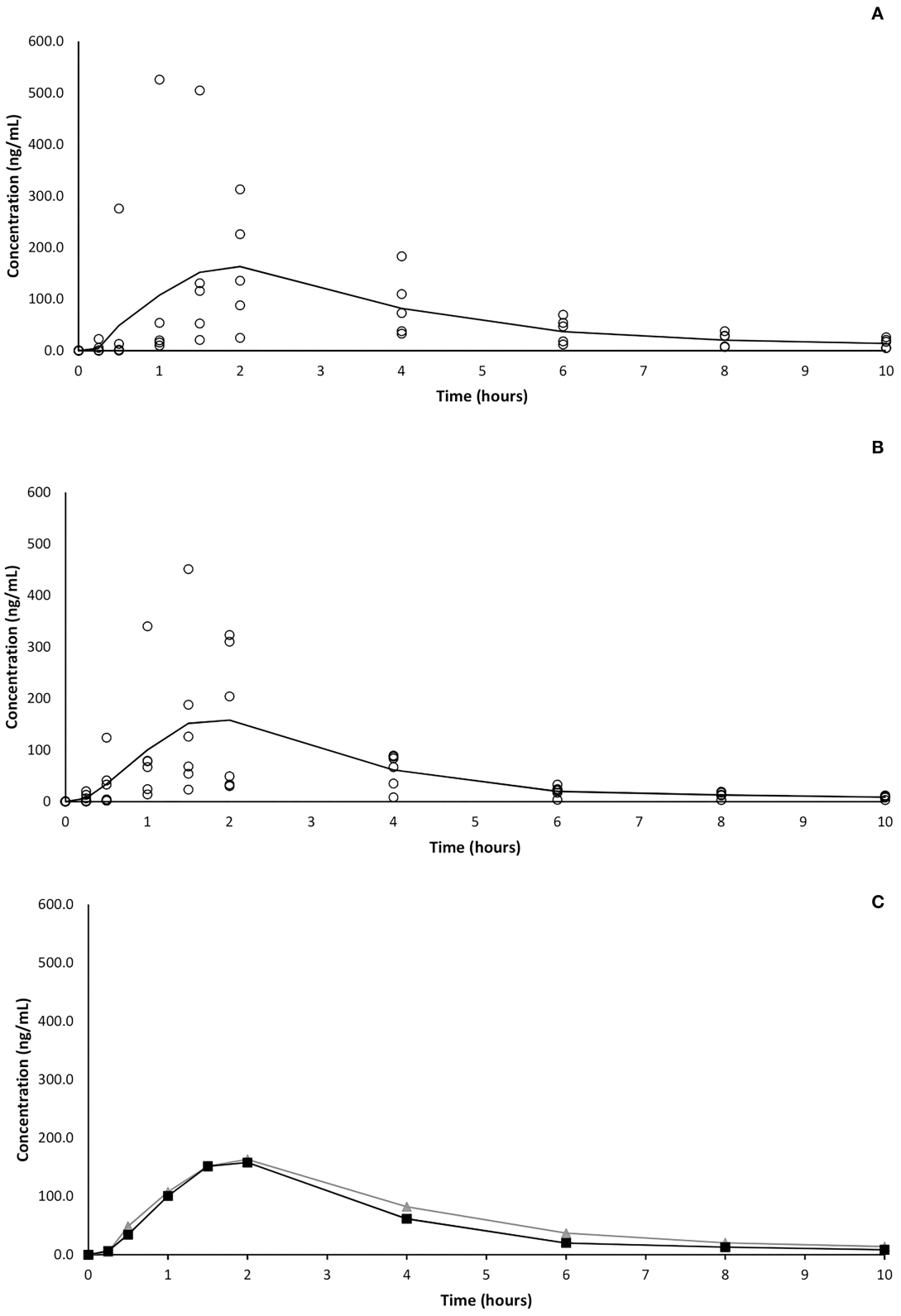

FIGURE 1
Average (solid line) and single (circles) CBD plasma concentrations vs. time following single oral **(A)** and OTM **(B)** treatment and comparison **(C)** of mean concentrations of the two different route of administration (oral in gray and OTM in black).

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TABLE 2 Main pharmacokinetic parameters obtained following oral and OTM administration of CBD at 1 mg/kg in dogs (6 dogs/group).

Parameter Unit	λz 1/h	$t_{1/2}$ h	T_{max} h	C_{max} ng/mL	AUC_{0-t} h∗ng/mL	$AUC_{0-\infty}$ h∗ng/mL	AUC_{Estr} %	$AUMC_{0-\infty}$ h^2∗ng/mL	MRT h
Oral									
Dog 1	0.33	2.09	2	135.60	394.33	410.57	4.12	1371.46	3.34
Dog 2	0.21	3.27	2	192.00	482.00	524.49	8.81	2177.71	4.15
Dog 3	0.22	3.17	2	88.00	251.00	273.84	9.10	1167.83	4.26
Dog 4	0.29	2.40	1	526.00	1419.00	1477.84	4.15	4431.41	3.00
Dog 5	0.25	2.80	2	226.00	971.00	1076.01	10.81	5324.44	4.95
Dog 6	n.a.	n.a.	4	73.00	367.75	n.a.	n.a.	n.a.	n.a.
Mean	**0.25**	**2.67***	**2.17**	**206.77**	**647.51**	**752.55**	**7.40**	**2894.57**	**3.94**
(S.D)	**(0.05)**	**(0.53)§**	**(0.98)**	**(167.07)**	**(453.17)**	**(507.15)**	**(3.08)**	**(1876.27)**	**(0.78)**
OTM									
Dog 1	0.25	2.74	1.5	451.00	1128.50	1175.95	4.20	3501.87	2.98
Dog 2	0.21	3.27	2	323.00	783.10	825.59	5.44	2853.82	3.46
Dog 3	0.38	1.83	1	79.00	150.08	158.00	5.28	447.32	2.83
Dog 4	0.27	2.52	4	67.00	275.75	304.88	10.56	1601.08	5.25
Dog 5	0.25	2.74	2	204.00	577.00	608.63	5.48	2282.41	3.75
Dog 6	0.21	3.24	1	78.00	301.88	353.22	17.01	1840.16	5.21
Mean	**0.26**	**2.62***	**1.92**	**200.33**	**536.05**	**571.04**	**8.00**	**2087.78**	**3.91**
(S.D)	**(0.06)**	**(0.64)§**	**(1.11)**	**(158.34)**	**(370.21)**	**(379.74)**	**(4.95)**	**(1059.59)**	**(1.07)**

AUC_{0-t}, area under serum concentration-time curve; $AUC_{0-\infty}$, area under serum concentration-time curve from time 0 to infinity; AUC_{extr}, area under the concentration-time curve extrapolated from t_{last} to ∞ in % of the total AUC; $AUMC_{0-\infty}$, area under moment curve; C_{max}, maximum concentration observed; λz, terminal rate constant; MRT, mean residence time; T_{max}, time of maximum concentration observed; $t_{½}$, terminal half-time; *, Harmonic mean; §, pseudo-standard deviation; n.a, not available.

might have increased the THC absorption in the fasting dogs, while, in fed status, THC might have been adsorbed by food showing a longer Tmax (27). This speculation could also apply to CBD. It should be highlighted that studies where the relative bioavailability of CBD orally administrated to fed and fasted dogs is compared are not available.

In the present study, after oral administration of CBD mean Cmax and AUC values were higher than those obtained in previous published pharmacokinetic studies where CBD oil was orally administered to fed or fasted dogs, when normalized for the dose (4, 9, 11, 12). This difference could be due to the great individual variability in CBD plasma concentrations observed in the present and previous studies, but also to the different administered oral formulations. Indeed, while other Authors used formulations containing also other cannabinoids, in the present study a pure CBD in MTC oil formulation was employed. In a study where CBD was orally administered both as a full-spectrum extract or as a pure molecule to mice, higher mean peak plasma (304 ± 28 vs. 60 ± 6 ng/mL) and AUC value (104 vs. 43 μg*min/mL) were observed following treatment with pure CBD (28). In the same study, a shorter half-life (217 vs. 484 min) after treatment with pure CBD was also observed, so the Authors speculated that the presence of other cannabinoids in the formulation might influence the rate of CBD biotransformation (28). Similarly, in the present study the terminal half-life (2.67 h) was shorter than that obtained by Gamble et al. (4) and Wakshlag et al. (12) (more than 4 h) when a CBD:CBDA (1:1)- predominant hemp oil was used. On the other hand, it was similar to that obtained following oral administration of a CBD infused oil (9) or CBD enriched cannabis herbal extract (11). As the concentration of CBDA in these last two formulations was not declared, it is possible to hypothesize that the presence of CBDA may be responsible for a slower clearance of CBD. It is important to underline that the Tmax obtained in the present study was quite close to that observed in the above cited studies (4, 11, 12) and consequently that the differences in terminal half-life values are not due to different absorption rates due to the formulation' differences.

Even if it is rather complicated to compare the pharmacokinetic of CBD following oral administration from different studies, because of several factors that might influence the plasma concentrations of the drug, it is generally believed that CBD has a low oral bioavailability due to a first-pass metabolism and a scarce absorption (29). Furthermore,

also an absorption rate slower than the elimination rate could be as responsible for the reduced CBD plasma concentrations (22, 24). More studies exploring the influence of formulation (i.e., pure CBD, hemp extract or CBD enriched hemp extracts) and "food effect" on oral pharmacokinetics of CBD in dogs are warranted.

To the authors' knowledge, this is the first pharmacokinetic study comparing OTM vs. oral administration of CBD in dogs. We hypothesized that CBD bioavailability could increase after OTM with respect to oral treatment. Indeed, the drug absorption by the OTM route should allow its rapid uptake across the oral mucosa and avoid its first-pass metabolism and any other problems related to its absorption in the gastrointestinal tract, consequently increasing its bioavailability and allowing to a dose reduction (30, 31).

Contrarily to our hypothesis, the OTM administration of CBD did not improve its bioavailability. The possibility that salivation and subsequent swallowing could have affected the drug's transmucosal absorption cannot be ruled out (30). However, in this case a secondary drug plasma peak should have been observed, while in the present study no double peaks resulted following OTM administration. Even if we cannot exclude to have missed the sample time point of the secondary peak, the almost perfectly superimposable mean plasma concentrations of CBD following OTM and oral administration (as shown in Figure 1C), suggests the inability, or a reduced ability, of CBD to be absorbed through the oral mucosa and that probably it was swallowed and absorbed at the gastrointestinal tract level. Comparing some pharmacokinetic studies on CBD following its administration as an oral mucosal spray in fed and fasted humans, Itin et al. (32) supposed the presence of a "food effect." However, the presence of food in gastrointestinal tract should not influence the plasma profile of a drug following its transmucosal application, letting these Authors to hypothesize that the majority of CBD was swallowed instead of passing through the oral mucosa. A possible explanation of the low OTM absorption of CBD could lie in the fact that while a good candidate for OTM delivery should have a log P above 2.0, a higher lipophilicity, as that of CBD, which has a Log P of 5.91, could be an obstacle to its diffusion in the cell cytoplasm (30, 33).

The hypothesis of a lacked or reduced absorption of CBD through the canine mucosa is reinforced by the results obtained by Polidoro et al. (22) who administered CBD by intranasal (IN), intrarectal and oral route in dogs. As the IN and rectal route are alternative administration routes able to avoid or partial avoid the first-pass metabolism in the liver, an increase of CBD plasma concentrations was expected compared to oral administration. However, following rectal treatment, CBD plasma concentrations were not quantifiable and no significant differences between oral and IN administration were observed regarding plasma peaks and AUCs (when normalized for the dose) (22). The Authors concluded that even if the eventual presence of sneeze, nasal congestion and mucous could have reduced the absorption of CBD, it was possible that CBD was largely swallowed (22).

A limitation of the present study is that it did not detect the metabolite (7-COOH derivative of CBD) that is known to be produced in dogs (12). The quantification of CBD metabolites in canine blood after OTM concentration could be important in order to better understand the pharmacokinetics of CBD and fully attribute the results of future pharmacodynamic studies.

Conclusions

Due to its multiple biological effects, various health benefits and lack of psychoactive properties, CBD is becoming of great interest in veterinary medicine. To better take advantage of the therapeutic effects of CBD it is important to assure that the necessary plasma concentrations to obtain therapeutic effects are achieved.

Contrarily to our expectations, the OTM administration of a pure CBD oil did not increase its bioavailability compared to oral administration. The development of innovative formulations able to enhance a fast penetration of CBD in the systemic circulation through the oral mucosa is therefore desirable.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by BioEthical Committee of the University of Perugia. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

GR contributed in the study design, sample collection, and revising the final manuscript. FP and RG performed the method validation and quantification of CBD in plasma samples. MC participated in the conceptualization of the study, coordinated the clinical phase, and revised the manuscript. EC, MS, CD, and AP participated in the study design and revised the manuscript for intellectual content. AD contributed in the study design, performed the analysis

and interpretation of data, drafted the manuscript, and supervised the whole work. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Della Rocca G, Di Salvo A. Hemp in veterinary medicine: from feed to drug. *Front Vet Sci.* (2020) 7:387. doi: 10.3389/fvets.2020.00387

2. Coelho MPRC, Leme FDOP, Moreira FA, Branco SEMT, Melo MM, de Melo EG. Current review of hemp-based medicines in dogs. *J Vet Pharmacol Ther.* (2021) 44:870–82. doi: 10.1111/jvp.13016

3. Kogan LR, Hellyer PW, Silcox S, Schoenfeld-Tacher R. Canadian dog owners' use and perceptions of cannabis products. *Can Vet J.* (2019) 60:749–755.

4. Gamble L-J, Boesch JM, Frye CW, Schwark WS, Mann S, Wolfe L, et al. Pharmacokinetics, safety, and clinical efficacy of cannabidiol treatment in osteoarthritic dogs. *Front Vet Sci.* (2018) 5:165. doi: 10.3389/fvets.2018.00165

5. Kogan LR, Hellyer PW, Downing R. The use of cannabidiol-rich hemp oil extract to treat canine osteoarthritis-related pain: a pilot study. *J Am Holistic Vet Med Assoc.* (2020) 58:1–10.

6. Martello E, Biasibetti E, Bigliati M, Meineri G, Bruni N. Preliminary results on the efficacy of a dietary supplement combined with physiotherapy in dogs with osteoarthritis on biomarkers of oxidative stress and inflammation. *Ital J Anim Sci.* (2021) 20:2131–3. doi: 10.1080/1828051X.2021.2004249

7. Verrico CD, Wesson S, Konduri V, Hofferek CJ, Vazquez-Perez J, Blair E, et al. A randomized, double-blind, placebo-controlled study of daily cannabidiol for the treatment of canine osteoarthritis pain. *Pain.* (2020) 161:2191–202. doi: 10.1097/j.pain.0000000000001896

8. Samara E, Bialer M, Mechoulam R. Pharmacokinetics of cannabidiol in dogs. *Drug Metab Dispos.* (1988) 16:469–72.

9. Bartner LR, McGrath S, Rao S, Hyatt LK, Wittenburg LA. Pharmacokinetics of cannabidiol administered by 3 delivery methods at 2 different dosages to healthy dogs. *Can J Vet Res.* (2018) 3:178–83.

10. Deabold KA, Schwark WS, Wolf L, Wakshlag JJ. Single-dose pharmacokinetics and preliminary safety assessment with use of CBD-rich hemp nutraceutical in healthy dogs and cats. *Animals.* (2019) 9:832. doi: 10.3390/ani9100832

11. Chicoine A, Illing K, Vuong S, Pinto KR, Alcorn J, Cosford K. Pharmacokinetic and safety evaluation of various oral doses of a novel 1:20 THC:CBD cannabis herbal extract in dogs. *Front Vet Sci.* (2020) 7:583404. doi: 10.3389/fvets.2020.583404

12. Wakshlag JJ, Schwark WS, Deabold KA, Talsma BN, Cital S, Lyubimov A, et al. Pharmacokinetics of cannabidiol, cannabidiolic acid, Δ9-tetrahydrocannabinol, tetrahydrocannabinolic acid and related metabolites in canine serum after dosing with three oral forms of hemp extract. *Front Vet Sci.* (2020) 7:505. doi: 10.3389/fvets.2020.00505

13. Samara E, Bialer M, Harvey DJ. Pharmacokinetics of urinary metabolites of cannabidiol in the dog. *Biopharm Drug Dispos.* (1990) 11:785–95. doi: 10.1002/bdd.2510110906

14. Ko JC, Freeman LJ, Barletta M, Weil AB, Payton ME, Johnson BM, et al. Efficacy of oral transmucosal and intravenous administration of buprenorphine before surgery for postoperative analgesia in dogs undergoing ovariohysterectomy. *J Am Vet Med Assoc.* (2011) 238:318–28. doi: 10.2460/javma.238.3.318

15. Slingsby LS, Taylor PM, Monroe T. Thermal antinociception after dexmedetomidine administration in cats: a comparison between intramuscular and oral transmucosal administration. *J Feline Med Surg.* (2009) 11:829–34. doi: 10.1016/j.jfms.2009.03.009

16. Brioschi FA, Di Cesare F, Gioeni D, Rabbogliatti V, Ferrari F, D'Urso ES, et al. Oral transmucosal cannabidiol oil formulation as part of a multimodal analgesic regimen: effects on pain relief and quality of life improvement in dogs affected by spontaneous osteoarthritis. *Animals.* (2020) 10:1505. doi: 10.3390/ani10091505

17. Vinarov Z, Abdallah M, Agundez JAG, Allegaert K, Basit AW, Braeckmans M, et al. Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: an UNGAP review. *Eur J Pharm Sci.* (2021) 162:105812. doi: 10.1016/j.ejps.2021.105812

18. Hedges AR, Pypendop BH, Shilo Y, Stanley SD, Ilkiw JE. Impact of the blood sampling site on time-concentration drug profiles following intravenous or buccal drug administration. *J Vet Pharmacol Ther.* (2014) 37:145–50. doi: 10.1111/jvp.12075

19. Messenger KM, Davis JL, LaFevers DH, Barlow BM, Posner LP. Intravenous and sublingual buprenorphine in horses: pharmacokinetics and influence of sampling site. *Vet Anaesth Analg.* (2011) 38:374–84. doi: 10.1111/j.1467-2995.2011.00613.x

20. Zgair A, Wong JC, Sabri A, Fischer PM, Barrett DA, Constantinescu CS, et al. Development of a simple and sensitive HPLC-UV method for the simultaneous determination of cannabidiol and Δ(9)-tetrahydrocannabinol in rat plasma. *J Pharm Biomed Anal.* (2015) 114:145–51. doi: 10.1016/j.jpba.2015.05.019

21. Zhang Y, Huo M, Zhou J, Xie S. PKSolver: an add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Comput Methods Programs Biomed.* (2010) 99:306–14. doi: 10.1016/j.cmpb.2010.01.007

22. Polidoro D, Temmerman R, Devreese M, Charalambous M, Ham LV, Cornelis I, et al. Pharmacokinetics of cannabidiol following intranasal, intrarectal, and oral administration in healthy. *Dogs Front Vet Sci.* (2022) 9:899940. doi: 10.3389/fvets.2022.899940

23. Hartsel JA, Boyar K, Pham A, Silver RJ, Makriyannis A. Cannabis in veterinary medicine: cannabinoid therapies for animals. In: Gupta R, Srivastava A, Lall R. Editors. *Nutraceuticals in Veterinary Medicine*. Cham, Switzerland: Springer Nature (2019). p. 121–55. doi: 10.1007/978-3-030-04624-8_10

24. Millar SA, Maguire RF, Yates AS, O'Sullivan SE. Towards better delivery of cannabidiol (CBD). *Pharmaceuticals.* (2020) 13:219. doi: 10.3390/ph13090219

25. Taylor L, Gidal B, Blakey G, Tayo B, Morrison G. A phase I, randomized, double-blind, placebo-controlled, single ascending dose, multiple dose, and food effect trial of the safety, tolerability and pharmacokinetics of highly purified cannabidiol in healthy subjects. *CNS Drugs.* (2018) 32:1053–67. doi: 10.1007/s40263-018-0578-5

26. Stott CG, White L, Wright S, Wilbraham D, Guy GW. A phase I study to assess the effect of food on the single dose bioavailability of the THC/CBD oromucosal spray. *Eur J Clin Pharmacol*. (2013) 69:825–34. doi: 10.1007/s00228-012-1393-4

27. Łebkowska-Wieruszewska B, Stefanelli F, Chericoni S, Owen H, Poapolathep A, Lisowski A, et al. Pharmacokinetics of Bedrocan®, a cannabis oil extract, in fasting and fed dogs: an explorative study. *Res Vet Sci*. (2019) 123:26–8. doi: 10.1016/j.rvsc.2018.12.003

28. Anderson LL, Etchart MG, Bahceci D, Golembiewski TA, Arnold JC. Cannabis constituents interact at the drug efflux pump BCRP to markedly increase plasma cannabidiolic acid concentrations. *Sci Rep*. (2021) 11:14948. doi: 10.1038/s41598-021-94212-6

29. Perucca E, Bialer M. Critical aspects affecting cannabidiol oral bioavailability and metabolic elimination, and related clinical implications. *CNS Drugs*. (2020) 34:795–800. doi: 10.1007/s40263-020-00741-5

30. Lam JK, Xu Y, Worsley A, Wong IC. Oral transmucosal drug delivery for pediatric use. *Adv Drug Deliv Rev*. (2014) 73:50–62. doi: 10.1016/j.addr.2013.08.011

31. Abhang P, Momin M, Inamdar M, Kar S. Transmucosal drug delivery- an overview. *Drug Deliv Lett*. (2014) 4:26–37. doi: 10.2174/2210303111303999011

32. Itin C, Domb AJ, Hoffman A. A meta-opinion: cannabinoids delivered to oral mucosa by a spray for systemic absorption are rather ingested into gastrointestinal tract: the influences of fed / fasting states. *Expert Opin Drug Deliv*. (2019) 16:1031–5. doi: 10.1080/17425247.2019.1653852

33. Nelson KM, Bisson J, Singh G, Graham JG, Chen SN, Friesen JB, et al. The essential medicinal chemistry of cannabidiol (CBD). *J Med Chem*. (2020) 63:12137–55. doi: 10.1021/acs.jmedchem.0c00724

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Pharmacokinetics, efficacy, and safety of cannabidiol in dogs: an update of current knowledge

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In the last 5 years, interest has grown in using phytocannabinoids, particularly cannabidiol (CBD), in veterinary medicine to treat several pathologies, including pain, epilepsy, anxiety, nausea, anorexia, skin lesions, and even some types of cancer, among others. Indeed, due to a positive perception of CBD use, many pet owners are increasingly requesting this option to relieve their pets, and many veterinarians are exploring this possibility for their patients. Besides the widespread empiric use of CBD in pets, the research is trying to obtain proof of its efficacy and lack of adverse effects and to know its pharmacokinetics to define an appropriate posology. This review summarizes all data published so far about the canine pharmacokinetics, efficacy, and tolerability of CBD and cannabidiolic acid (CBDA). Despite a certain number of available pharmacokinetic studies, the kinetic profile of CBD has yet to be fully known, probably because of the very different experimental conditions. In terms of efficacy, most studies have tested CBD' ability to relieve osteoarthritic pain. In contrast, few studies have evaluated its role in epilepsy, behavioral disorders, and skin lesions. From obtained results, some evidence exists supporting the beneficial role of CBD. Nevertheless, the limited number of published studies and the occurrence of bias in almost all require caution in interpreting findings. From tolerability studies, CBD' side effects can be classified as mild or unremarkable. However, studies were prevalently focused on short- to medium-term treatment, while CBD is usually employed for long-term treatment. Further studies are warranted to define better whether CBD could be a valid adjunct in canine treatment.

KEYWORDS

phytocannabinoids, cannabidiol, CBD, dog, pharmacokinetics, efficacy, tolerability

1. Introduction

In the last 30 years, research has made considerable strides in studying and understanding the endocannabinoid system (ECS) and its bodily functions.

The ECS can be synthetically defined as the set of cannabinoid receptors [such as Type 1 cannabinoid receptor (CB1), Type 2 cannabinoid receptor (CB2), G protein-coupled receptor 55 and 119 (GPR55, GPR119), transient receptor potential vanilloid (TRPV) and peroxisome proliferator-activated receptor (PPAR)], endocannabinoids [compounds produced by the body that bind to cannabinoid receptors, such as anandamide (AEA) and 2-Arachidonoylglycerol (2-AG)], enzymes responsible for their synthesis and their catabolism and genes that code for these proteins. The term "endocannabinoidome" has recently been coined for this set (1). This system is of great importance for the organism's normal functioning as it underlies numerous

homeostatic functions, exerting an antioxidant, hypotensive, immunosuppressive, anti-inflammatory and pain-relieving action. Furthermore, the distribution of cannabinoid receptors in the brain also suggests a physiological role for endocannabinoids in the control of movement and perception, regulation of sleep and appetite, inhibition of learning and memory processes, regulation of emotional states (such as pleasure and aggression), neuroprotection, as well as in enhancing the action of opioids. Various observations also suggest a role of the ECS in the control of vasomotor functions and fertility, as well as of tumor cell proliferation (2).

The discovery of a pre-established endogenous cannabinoid system has led researchers to hypothesize that the active ingredients, mainly phytocannabinoids, contained in *Cannabis sativa* (both medical and industrial cultivar – this last also known as hemp), could interact with this system, producing both the therapeutic and psychotropic effects of the plant.

Cannabidiol (CBD) is an abundant non-psychoactive phytocannabinoid which has affinity on a series of receptors, including CB1, CB2, GPR55, GPR119, TRPV and PPAR. By modulating the activities of these receptors, CBD exhibits multiple therapeutic effects, including neuroprotective, antiepileptic, anxiolytic, antipsychotic, anti-inflammatory, analgesic and anticancer properties (3).

In veterinary medicine, the use of *Cannabis* derivatives as a therapeutic approach started to be considered a few years ago. The first studies were devoted to establishing the presence of the ECS in animal species. With specific regard to the canine species, the presence of cannabinoid receptors or their ligands has been identified in skin and skin appendages of healthy dogs and dogs with atopic dermatitis (AD) (4–8), gastrointestinal tract (9, 10), peripheral and central nervous system (11–13), joints (14) and embryo (15).

Among the possible use of *Cannabis* in animals, several areas of interest have been considered, such as pain management (acute and chronic pain) (16), neurological conditions (seizures, neuroinflammation, degenerative diseases, brain tumors) (17), well-being (anxiety disorders) (18), gastrointestinal health (appetite modulation, nausea and vomiting, visceral pain/hypersensitivity, esophageal reflux, diarrhea/peristalsis) (19), dermatologic diseases (skin inflammation, wound healings, skin allergies, pruritus) (20), oncology and immune response (21).

Due to increased knowledge regarding the potential therapeutic role of *Cannabis* derivatives, especially cannabidiol (CBD), in veterinary medicine, and the recent legalization of cannabinoids in some states, more veterinarians and pet owners are exploring options for providing cannabinoid products for their patients/pets. Pet owners' and veterinarians' perceptions of CBD use are generally positive, although many veterinarians do not feel knowledgeable enough about the therapeutic and toxic effects of cannabinoid products (22, 23).

This review aims to summarize all data published so far about the pharmacokinetics, efficacy, and tolerability of *Cannabis* derivatives, specifically CBD and cannabidiolic acid (CBDA), in the canine species.

2. Pharmacokinetics of CBD

Cannabidiol is a high lipophilic molecule. In veterinary practice, it is generally administered orally (24). In the last few years, several pharmacokinetic studies on CBD were conducted in dogs (25–35). However, its kinetic behavior has yet to be fully elucidated.

The gastrointestinal absorption of CBD seems very low. Indeed, the only study where oral bioavailability was evaluated, it resulted lesser than 19% (36); however, it is essential to underline that the tested oral form was a capsule containing CBD as raw material. This low bioavailability, associated with a large individual variability observed in almost all conducted studies, is a challenge in identifying an appropriate dosing regimen.

Some studies have investigated the influence of CBD pharmaceutical formulation on its oral absorption in dogs; microencapsulated CBD oil beads resulted in a lower Cmax and AUC when compared with CBD-infused oil (26). Soft gel capsules containing CBD-rich hemp extract showed a significant increase in mean Cmax value, but not in that of AUC, compared to the administration of the same extract in sesame oil (33). A similar result was obtained by Wakshlag et al. (2020) comparing soft chews containing a CBD/CBDA predominant extract with the same extract diluted in an oil consisting of 75% of organic sesame oil and 25% of sunflower lecithin, while no significant difference was observed when the soft chews were compared to the extract solubilized in an oil mixture of 75:25 of organic sesame and medium-chain triglycerides (MCT) (34).

The presence of an eventual "food effect" was also hypothesized as a factor conditioning the absorption of CBD: indeed, as a lipophilic substance, CBD is thought to be more absorbable if administered with a fat meal, but in the only study that directly compared the kinetics of CBD orally administered to fed and fasted dogs, the results were not entirely conclusive. Indeed, even if the Cmax observed in fed condition was significantly higher than in fasted condition, no significant difference was observed in AUC values (30). However, in this study only 3 dogs/group were tested, and in the two groups, respectively, treated with 5 and 20 mg/kg, a greater Cmax and AUC were obtained in one fasted dog.

Cannabidiol is subject to a sizeable hepatic metabolism, witnessed by the identification of several metabolites in canine urine (37). Thus, to avoid or at least reduce the first-pass metabolism and increase its plasma concentrations, some alternative routes of CBD administration were tested, albeit without satisfactory results. In fact, following rectal administration of a suppository containing 100 mg of CBD, corresponding to a dose between 6.9–13.7 mg/kg to six dogs, the plasma concentration resulted below the lower limit of quantification (31). After application of CBD-infused transdermal cream at the dose of ~5 and ~10 mg/kg to dogs' pinnae, Cmax and AUCs resulted smaller than those obtained with the oral administration of CBD-infused oil and microencapsulated CBD oil beads formulations at the same doses (26). Again, intranasal (IN) administration of a formulation containing pure synthetic CBD did not show any significant difference with the oral administration of pure CBD in MCT oil when normalized for the dose, except for Tmax, which was significantly shorter following IN treatment (0.49 vs. 3.50 h for IN and oral administration, respectively) (31). Finally, oral trans-mucosal (OTM) administration was also tested, resulting in a mean plasma CBD concentrations vs. time trend almost superimposable to the oral administration (29). The possibility that salivation and subsequent swallowing could have affected the drug's transmucosal absorption cannot be ruled out (38).

In humans, two main products of CBD biotransformation were identified: a hydroxy- and a carboxy-derivate (7-OH-CBD and 7-COOH-CBD, respectively), and their eventual presence in canine

plasma following oral administration of CBD was thus investigated (27, 34, 35). Following oral administration of soft chews containing CBD/CBDA-predominant extract or the same extract in oil (dose: 1 mg/kg), the observed levels of 7-COOH-CBD was 1–2% of that observed in humans treated with a comparable dose. In the same study, the 7-OH-CBD was not detected (34). This last was observed following oral administration of CBD-purified *Cannabis* extract diluted in MCT oil, but, in any case, the carboxy-metabolite resulted produced in a greater quantity (35). The 7-OH-CBD was detected albeit intermittently in the dog's plasma even after oral treatment with a *Cannabis* herbal extract. In the same study, the 6-OH-CBD was identified up to 48 h following oral administration of CBD at 10 mg/kg (27). The more outstanding production of this latter metabolite compared to the 7-OH-CBD underlines the species/specific difference between dogs and humans in the CBD metabolism (27).

Table 1 resumes the data obtained from the pharmacokinetic studies published so far. The CBD pharmacokinetic parameters, such as terminal half-life, AUC and MRT, are sometimes quite different in average values following oral administration of oily solutions, even when normalized for the given dose. These differences can be attributable to a too small sample size, different experimental sampling times applied in the various studies and a large individual variability (i.e., breed, age and sex differences). Indeed, age may cause physiological and anatomical changes that can modify the drug pharmacokinetics due to a different water/adipose ratio of the body and a possible reduction in renal and hepatic function (39). Similarly, sex was observed to affect metabolism of some drugs (40). Also, the type of CBD used (pure or co-extracted with other phytocannabinoids) can have influenced the pharmacokinetic results. Relatively to this last issue, higher Cmax and AUC values and a shorter half-life were observed in mice when CBD was orally administered as a pure molecule compared to a full-spectrum extract (41). Likewise, della Rocca et al. (2023), comparing the mean value of the terminal half-life of pure CBD orally administered in dogs with that obtained in studies in which equal concentrations of CBD and CBDA were used, hypothesized that the absence of CBDA in their formulation may have played a role for the shorter half-life observed (29).

3. Clinical efficacy of CBD

3.1. Pain

The empiric use of *Cannabis* as an analgesic goes back more than 1,500 years. The discovery of cannabinoid receptors, the identification of endocannabinoids and their biosynthetic and degradation pathways, and the understanding of signal transduction mechanisms paved the road for scientific research in this area. It was soon recognized that one of the main physiological roles of the endocannabinoid system (ECS) is the modulation of pain (42).

An essential basis for concluding that endocannabinoids modulate pain was provided by preclinical studies, which demonstrated the presence of endocannabinoid receptors, endogenous cannabinoids and enzymatic machinery for endocannabinoid biosynthesis and degradation in peripheral and central structures devoted to pain modulation, and their antinociceptive and antihyperalgesic effects in models of transient (physiological) and inflammatory and neuropathic pain, respectively (42–47).

Endogenous cannabinoids produce antinociceptive and antihyperalgesic effects at peripheral, spinal and supraspinal levels (48). Peripherally, endocannabinoids inhibit primary afferent fibers depolarization and modulate mast cells degranulation by interacting with CB1 and CB2 receptors and other receptor types, such as TRPV1, GPR55, GPR119, and PPAR-α. These interactions lead to a decreased firing of the nociceptive fibers and a reduced release of pro-inflammatory and pro-pain mediators, followed by a reduction of the inflammatory and pain response (44, 48–50). In the spinal cord, experimental data suggest that cannabinoids increase the nociceptive threshold and reduce the wide dynamic range neurons' firing by interacting with spinal CB1 receptors. Furthermore, it appears that cannabinoids may modulate the activity of the noradrenergic and opioid spinal systems (44, 46, 48, 49). At the supraspinal level, cannabinoids could act through the activation of the descending inhibitory control and consequent modulation of the spinal cord neurons' activity. This action is probably mediated by CB1 receptors localized in several areas involved in pain control, such as periaqueductal grey matter, rostroventromedial medulla, some areas of the thalamus and amygdala, and A5 noradrenergic nucleus (44, 48, 49). It has also been hypothesized that the ECS exerts a tonic activity able to modulate the nociceptive threshold in basal conditions and hyperalgesia and that cannabinoids and opioids can mutually potentiate each other (44).

Studies conducted in animal models have paid particular attention to verifying the role of the ECS in neuropathic, cancer and osteoarthritic (OA) pain: in all cases, the "endocannabinoid machine" is present and able to modulate the excitability of nociceptors and spinal neurons (51–53).

Several preclinical studies have investigated phytocannabinoids' efficacy in animal OA pain models. Overall, data indicate that the activation of the ECS by exogenous cannabinoids proves effective in limiting joint pain both centrally and peripherally (53).

As regards the clinical efficacy of CBD in the treatment of OA pain in dogs, six scientific studies have been published so far (four of them being randomized, double-blind, placebo-controlled clinical trials, and the remaining two being a case report and a non-blinded observational study, respectively), whose study design, treatments and results are summarized in Table 2. Five studies (24, 25, 54–56) indicated that CBD significantly reduced pain and increased the activity of dogs, thus improving their quality of life. Indeed, Gamble et al. (2018) revealed a significant decrease in pain scores, as measured by the Canine Brief Pain Inventory (CBPI), and an increase in activity, as measured by the Hudson activity scale, at week 2 and 4 during CBD treatment (2 mg/kg twice daily for 4 weeks) when compared to baseline (week 0) (25). In 2019, De Álava (Cited by Coelho, 2021) described a case report of a dog with chronic osteoarthritis that was treated with CBD (1 mg/kg twice daily for 30 days): the treatment showed analgesic effect with consequent improvement of mobility and quality of life of the dog (24). Kogan et al. (2020) assessed the impact of CBD (0.3–4.12 mg/kg twice daily for 90 days) in association with the previous multimodal analgesic therapy (acupuncture, laser, nutraceuticals, polysulfated glycosaminoglycan, and/or gabapentin), and found that 30 out of 32 dogs showed pain relief and 21 out of 23 dogs could reduce or discontinue the administration of gabapentin (54). Verrico and co-workers (2020) evaluated the effect of two different CBD formulations (naked 20 and 50 mg/day, and liposomal 20 mg/day, for 4 weeks): owner assessment of animal pain by means of

TABLE 1 Main pharmacokinetic parameters following single administration of different CBD formulations in dogs.

CBD formulation (dose)	Administration route	n.dogs, sex, age, and fed/fasted status	PK Parameters						References
			$t_{1/2}$ (h)	Tmax (h)	Cmax (ng/mL)	$AUC_{0\text{-}12h}$ (h*ng / mL)	$AUC_{0-\infty}$ (h*ng / mL)	MRT (h)	
CBD in 70% alcohol solution (45 mg/dog equal to a range of ~1.9–2.8 mg/kg)	Intravenous	3F + 3 M, n.d.	6.8 (2.7)	----	----	n.a.	2,706 (519)	7.0 (3.5)	(36)
CBD in 70% alcohol solution (90 mg/dog equal to a range of ~3.8–5.6 mg/kg)	Intravenous	3F + 3 M, n.d.	9.3 (3.3)	----	----	n.a.	6,095 (1741)	7.5 (2.7)	(36)
CBD/CBDA -predominant hemp oil[1] (1 mg/kg CBD + 1 mg/kg CBDA)	Oral	4 MN, 3.5–7 y fasted	4.73 (1.41)	1.5 (0.58)	99.00 (29.13)	338.25 (109.44)	n.a.	6.1 (2.13)	(25)
CBD/CBDA -predominant hemp oil[1] (4 mg/kg CBD + 4 mg/kg CBDA)	Oral	4 MN, 3.5–7 y fasted	4.22 (0.42)	1.75 (0.5)	618.75 (225.88)	2529.5 (591.7)	n.a.	5.75 (0.87)	(25)
CBD-infused oil (75 mg/dog equal to ~5 mg/kg of CBD)	Oral	5 M, 4-5y fed	3.33[§] (0.93)*	n.a.	625.3 (164.3)	2305.2 (787.1)	2500.7 (834.7)	3.62 (0.77)	(26)
CBD-infused oil (150 mg/dog equal to ~10 mg/kg of CBD)	Oral	5 M, 4-5y fed	2.12[§] (0.54)*	n.a.	845.5 (262.2)	5059.2 (1917.6)	5395.8 (1999.2)	4.97 (0.72)	(26)
Microencapsulated CBD oil beads (75 mg/dog equal to ~5 mg/kg of CBD)	Oral	5 M, 4-5y fed	1.59[§] (0.49)*	n.a.	346.3 (158.7)	1666.0 (736.1)	1759.5 (790.5)	5.88 (0.8)	(26)
Microencapsulated CBD oil beads (150 mg/dog equal to ~10 mg/kg of CBD)	Oral	5 M, 4-5y fed	1.93[§] (1.48)*	n.a.	578.1 (287.1)	2767.6 (1040.4)	3014.1 (994.5)	5.53 (1.22)	(26)
CBD enriched Cannabis extract[2] (2 mg/kg)	Oral	6, mixed gender, ~2y, fasted	2.5[#] (0.5)	2.1 (1)	213 (49)	692 (292)	n.a.	n.a	(27)
CBD enriched Cannabis extract[2] (5 mg/kg)	Oral	6, mixed gender, ~2y, fasted	2.6[#] (0.4)	1.9 (0.6)	838 (304)	2,433 (911)	n.a.	n.a.	(27)
CBD enriched Cannabis extract[2] (10 mg/kg)	Oral	6, mixed gender, ~2y, fasted	2.3[#] (0.2)	2.3 (0.5)	1868 (698)	5.883 (2181)	n.a.	n.a	(27)
CBD enriched soft chews (1 mg/kg CBD + 1 mg/kg CBDA)	Oral	5, ~1–5 y fasted	1.0 (0.5)	1.4 (0.55)	301 (141.69)	1297[a] (469.53)	n.a.	1.44 (0.72)	(28)
CBD pure in MCT oil (1 mg/kg)	Oral	5 M + 1F, 1.5-13y fasted°	2.67[§] (0.53)*	2.17 (0.98)	206.77 (167.07)	647.51 [b] (453.17)	752.55 (507.15)	3.94 (0.78)	(29)
CBD pure in MCT oil (5 mg/kg)	Oral	3F, 4–5 y fasted	13.4 (4.4)	n.a.	143.0 (112.1)	1130.1[a] (712.1)	n.a.	n.a.	(30)

(Continued)

TABLE 1 (Continued)

CBD formulation (dose)	Administration route	n.dogs, sex, age, and fed/fasted status	PK Parameters						References
			$t_{1/2}$ (h)	Tmax (h)	Cmax (ng/mL)	$AUC_{0\text{-}12h}$ (h*ng / mL)	$AUC_{0-\infty}$ (h*ng / mL)	MRT (h)	
CBD pure in MCT oil. (5 mg/kg)	Oral	3F, 4–5 y fed	19.3 (7.7)	n.a.	581.0 (400.9)	1977.1 [a] (1389.4)	n.a.	n.a.	(30)
CBD pure in MCT oil (10 mg/kg)	Oral	3F, 4–5 y fasted	6.5 (2.2)	n.a.	231.2 (222.6)	1370.5[a] (671.4)	n.a.	n.a.	(30)
CBD pure in MCT oil (10 mg/kg)	Oral	3F, 4–5 y fed	7.5 (3.5)	n.a.	579.0 (150.0)	3215.9[a] (1196.0)	n.a.	n.a.	(30)
CBD pure in MCT oil (20 mg/kg)	Oral	3F, 4–5 y fasted	8.8 (2.2)	n.a.	155.4 (78)	1289.0[a] (638.1)	n.a.	n.a.	(30)
CBD pure in MCT oil (20 mg/kg)	Oral	3F, 4–5 y fed	11.0 (2.1)	n.a.	288.5 (359.6)	4247.8 [a] (6203.8)	n.a.	n.a.	(30)
CBD-purified cannabis extract[3] diluted in MCT oil (1 mg/kg)	Oral	4, 1.75 y fasted	5.6 (1.0)	4.5 (1.0)	30 (7)	183[a] (143)	n.a.	7.9 (1.6)	(35)
CBD-purified cannabis extract[3] diluted in MCT oil (2 mg/kg)	Oral	4, 1.75 y fasted	9.3 (6.6)	3.5 (1)	46 (23)	287[a] (178)	n.a.	11.9 (6.4)	(35)
CBD-purified cannabis extract[3] diluted in MCT oil (4mg/kg)	Oral	4, 1.75 y fasted	5.4 (1.4)	3.5 (0.5)	130 (47)	859[a] (475)	n.a.	8.0 (0.8)	(35)
CBD-purified cannabis extract [3] diluted in MCT oil (12 mg/kg)	Oral	4, 1.75 y fasted	7.2	4.5 (1.7)	201 (55)	1,430[a] (610)	n.a.	10.4	(35)
CBD rich hemp extract [4] in soft gel capsules (1 mg/kg CBD + 1 mg/kg CBDA)	Oral	7F + 1 M, 1-7y n.d. [‡]	2.2 (1.7)	1.1 (0.4)	267.6 (98.9)	n.a.	693.2 (191.4)	3.4 (1.7)	(33)
CBD rich hemp extract [4] in sesame oil (1 mg/kg CBD + 1 mg/kg CBDA)	Oral	7F + 1 M, 1-7y n.d. [‡]	3.4 (1.4)	1.4 (0.5)	184.5 (55.8)	n.a.	687.8 (218.2)	4.4 (1.6)	(33)
CBD/CBDA-predominant extract in a mix of MCT: organic sesame oil (25:75) [5] (1 mg/kg CBD + 1 mg/kg CBDA)	Oral	6F, ~1–1.5y n.d. [‡]	4.1 (0.7) [†]	1.5 (0.5) [†]	145 (69) [†]	635[a] (399) [†]	656 (414) [†]	5.2 (1.4) [†]	(34)
CBD/CBDA-predominant extract in a mix of sunflower lecithin: organic sesame oil (25:75) [5] (1 mg/kg CBD + 1 mg/kg CBDA)	Oral	6F, ~1–1.5y n.d. [‡]	4.4 (1.4) [†]	2 (1.1) [†]	124 (62) [†]	683[a] (146) [†]	707 (144) [†]	6.5 (2.1) [†]	(34)

(Continued)

TABLE 1 (Continued)

CBD formulation (dose)	Administration route	n.dogs, sex, age, and fed/fasted status	PK Parameters						References
			$t_{1/2}$ (h)	Tmax (h)	Cmax (ng/mL)	$AUC_{0\text{-}12h}$ (h*ng / mL)	$AUC_{0-\infty}$ (h*ng / mL)	MRT (h)	
soft chew containing CBD/CBDA-predominant extract [6] (1 mg/kg CBD +1 mg/kg CBDA)	Oral	6F, ~1–1.5y n.d. [‡]	3.8 (0.3) [†]	2.5 (1.2) [†]	226 (89) [†]	826[a] (74) [†]	845 (74) [†]	5.3 (1.4) [†]	(34)
tablets containing 100 mg of CBD pure (1 tablet/dog equal to a range of ~6.9–13.7 mg/kg)	Oral	4FN + 2MN, 3-8y, fasted	15.65 (2.82)	3.50 (0.56)	216.76 (108.51)	n.a.	1376.03 (828.95)	13.07 (3.61)	(31)
CBD pure in (MCT) oil (1 mg/kg)	Oral trans-mucosal	3F + 3 M, 4-17y fasted	2.62[§] (0.64)*	1.92 (1.11)	200.33 (158.34)	536.05[b] (370.21)	571.04 (379.74)	3.91 (1.07)	(29)
3 consecutive sprays of Sativex (8.1 mg of Δ^9-THC + 7.5 mg of CBD/dog equal to a range of ~0.58–0.68 mg/kg)	Sublingual	3F + 3 M, 0.67 ± 0.067y, fasted	2	2	10.5	60.4[a]	n.a.	n.a.	(32)
CBD pure in PEG: NaCl 0.9% (50:50) solution (20 mg/dog equal to a range of ~1.39–2.74 mg/kg)	Intranasal	4FN + 2MN, 3-8y	7.02 (7.97)	0.49 (0.29)	27.96 (25.29)	n.a.	61.31 (88.22)	10.30 (14.04)	(31)
suppositories containing 100 mg of CBD pure (1 suppository/dog equal to a range of ~6.9–13.7 mg/kg of CBD)	Intrarectal	4FN + 2MN, 3-8y	n.a.	n.a.	<LOQ = 1 ng/mL	n.a.	n.a.	n.a	(31)
CBD-infused oil cream (75 mg/dog equal to ~5 mg/kg of CBD)	Transdermal	5 M, 4-5y	n.a	n.a.	74.3 (127.2)	198.9 (321.3)	n.a.	8.17 (1.23)	(26)
CBD-infused oil cream (150 mg/dog equal to ~10 mg/kg of CBD)	Transdermal	5 M, 4-5y	n.a.	n.a.	277.6 (476)	504.9 (503.2)	n.a.	7.73 (2.05)	(26)

All parameters are expressed as mean with standard deviation in brackets (To better compare the data, the values, where necessary, have been converted into the same measurement unit or calculated from single data when available). AUC_{0-12}, area under serum concentration–time curve from zero to 12 h; $AUC_{0-\infty}$, area under serum concentration–time curve from time zero to infinity; C_{max}, maximum concentration observed; MRT, mean residence time; T_{max}, time of maximum concentration observed; $t_{½}$, terminal half-life; F, female; FN, female neutered; M, male; MCT, medium-chain triglycerides; MN, male neutered; PEG, polyethylene glycol; y, years; n.a., not available; n.d., not declared. [§]Harmonic mean. *Pseudo standard deviation. [#]$t_{1/2\beta}$ phase from 4 to 12 h post-dose. [†]Standard error of the mean. [°]Administered with a small amount of feed. [‡]Wet food was offered following administration. [a]AUC area under serum concentration–time curve from zero to 24 h. [b]AUC area under serum concentration–time curve from zero to 10 h. [1]~5 mg/mL CBD, ~5 mg/mL CBDA, 0.24 mg/mL THC, 0.27 mg/mL cannabichromene (CBC), 0.11 mg/mL cannabigerol (CBG); other cannabinoids < 0.01 mg/mL. [2]19.7–19.9 mg CBD, 1.0–1.1 mg THC, 3.6–4.3 mg CBC, and 0.2 mg CBG. [3]Other cannabinoids < lower limit of quantification. [4]32 mg/mL of CBD, 35 mg/mL CBDA, 1.3 mg/mLTHC, 1.4 mg/mL tetrahydrocannabinolic acid (THCA), 0.9 mg/mL cannabigerolic acid (CBGA), 1.3 mg/mL (CBC), 0.5 mg/mL (CBG). [5]28 mg/mL CBD, 29 mg/mLCBDA, 1 mg/mL THC, 0.8 mg/mL THCA, 0.7 mg/mL CBGA, 1.3 mg/mL (CBC). [6]same herbal extract of (5) to contain ~5 mg CBD.

the Helsinki Chronic Pain Index (HPCI),as well as veterinary clinical examination, were not significantly altered by administration of placebo or 20 mg/day naked CBD, while the administration of 50 mg/day naked CBD or 20 mg/day liposomal CBD generated statistically significant reductions in pain scores (55). Finally, Brioschi et al. (2021) evaluated the efficacy of oral transmucosal (OTM) CBD (2 mg/kg

twice daily for 12 weeks), in addition to a multimodal pharmacological treatment (firocoxib or prednisone, gabapentin and amitriptyline) for chronic osteoarthritis-related pain and found that, when evaluated by owners based on the CBPI scoring system, scores were significantly decreased when compared with dogs that did not receive CBD (56). Conversely, in the study by Mejia et al. (2021), no difference was observed with the use of CBD (2.5 mg/kg twice daily for 6 weeks) at any time for any of the recorded outcome measures (activity count, clinical metrology instruments, and objective gait analysis) (57).

In the only published randomized, placebo controlled, blinded clinical trial where the role of CBD/CBDA (2–2.5 mg/kg twice daily for 4 weeks) in acute postoperative pain following a tibial plateau leveling osteotomy (TPLO) was investigated, no significant differences were noted between placebo and CBD/CBDA groups at any point in pain score (CBPI), degree of lameness, degree of weight-bearing, or radiographic healing of the osteotomy (58) (Table 2). However, a recent abstract suggested lower postsurgical pain scoring based on blinded veterinary assessment compared to placebo in postsurgical intervertebral disc disease with the same product (CBD/CBDA) at a higher dose (5 mg/kg) (59).

3.2. Epilepsy

In recent years, phytocannabinoids have been emphasized in treating various neurological disorders, including epilepsy (60). Data obtained so far allow hypothesizing that the ECS plays a crucial role in modulating the brain activities in brain areas directly or indirectly affected in patients with epilepsy. This hypothesis is supported by numerous anatomical, electrophysiological, biochemical and pharmacological findings (61).

The molecular mechanisms underlying the antiepileptic action of endocannabinoids are still largely unclear. Numerous researchers are carrying out studies to elucidate the role of the ECS in controlling epileptic seizures. The CB1 receptor is thought to play a critical role. Indeed, the activation of the CB1 receptor:

- Modulates N- and Q-type calcium channels, reducing the calcium influx and the consequent calcium-dependent release of glutamate (Glu); since this mediator is the primary excitatory neurotransmitter of the CNS and epilepsy is related to excess glutamatergic transmission, the cannabinoid-induced reduction of its release would induce an anticonvulsant effect;
- Improves the presynaptic conductance of internally rectified potassium channels; the activation of potassium channels reduces neuronal excitability through the stabilization of both membrane potentials and other factors involved in the reduction of epileptiform discharge;
- Reduces the GABAergic release and function in the hippocampus; since GABA, which usually is an inhibitory neurotransmitter, can nevertheless induce a depolarization leading to abnormal electrical activity in human epileptic temporal lobe slices, the cannabinoid-mediated decrease of the GABAergic tone would therefore justify, at least in part, the anticonvulsant effect of cannabinoids (61).

Although the association between epilepsy and the ECS has not been fully elucidated, the complex relationship between brain excitability and the ECS suggests that phytocannabinoids may induce beneficial effects on epilepsy, paving the way for the possibility of developing new treatments involving the use of compounds, especially CBD, that selectively target individual elements of the endocannabinoid signaling system (61).

It has been proposed that CBD acts through polypharmacological interactions leading to modulation or prevention of neuronal hyperexcitability. Multiple putative mechanisms of action of CBD have been discussed, which include (a) interactions with different receptors, such as GRP55, vanilloid (TRPV), serotonergic (5HT1α) and glycinergic receptors; (b) regulation of sodium and calcium currents; (c) enhancement of synaptic signaling mediated by adenosine and other mediators; (d) enhancement of GABAergic activity (62–64). It has been hypothesized that CBD may limit neuronal hyperexcitability through the following mechanisms:

- Reduction of presynaptic intracellular calcium concentrations (which prevents excessive glutamate release), mediated by a functional antagonism at GPR55 and desensitization of TRPV1 (65).
- Adenosine reuptake inhibition, with an increase of its extracellular concentrations (65) and the consequent impact on calcium and potassium fluxes, which affect presynaptic neurotransmitter release and contribute to postsynaptic hyperpolarization resulting in reduced activation of glutamatergic NMDA receptors (66);
- Activation of 5-HT1α receptors;
- Activation of the ankyrin receptor type 1 (TRPA1);
- Inhibition of the reuptake of norepinephrine, GABA and dopamine;
- Stimulation of the activity of glycine α1 and α3 receptors (60).

The antiepileptic properties of CBD have been studied in various animal models of acute epilepsy. The obtained data support the anticonvulsant role of CBD administered both as a pre-treatment and after causing the onset of epileptic seizures (60).

Cannabidiol's clinical efficacy in treating idiopathic epilepsy in dogs has been investigated so far in only three scientific studies (Table 3), only two of which were randomized controlled clinical trials. McGrath et al. (2019) showed that CBD (2.5 mg/kg twice daily for 12 weeks) in association with the previous antiepileptic therapy (phenobarbital, potassium bromide, levetiracetam, and/or zonisamide) significantly reduced the frequency of seizures (median change, 33%) compared with the placebo group. However, the proportion of dogs with a response to treatment (a ≥ 50% reduction in mean monthly seizure frequency from before the study began to when the study concluded) was statistically similar between CBD and placebo groups (67). Garcia et al. (2023) reported a significant reduction in epileptic seizure frequency as well as the number of epileptic seizure days in dogs receiving an equal mix of CBD/CBDA (2 mg/kg twice daily for 12 weeks) when compared with the placebo group. More in details, epileptic seizure frequency decreased from a mean of 8.0 ± 4.8 during placebo treatment to 5.0 ± 3.6 with CBD/CBDA-rich hemp extract, and epileptic seizure event days of CBD/CBDA-rich hemp treatment decreased from a mean of 5.8 ± 3.1 during placebo treatment to 4.1 ± 3.4 in treated dogs. The number of dogs with a 50% reduction in epileptic activity while on the placebo were 0/14, whereas while on treatment were 6/14 (68). In a

TABLE 2 Studies on clinical efficacy of CBD-based products in the treatment of pain in dogs.

Study design	Treatment	Results	References
Randomized, placebo-controlled, double-blind, crossover clinical trial to evaluate analgesic efficacy of a CBD-dominant hemp oil (equal mix of CBD and CBDA) on OA-related pain relief in 16 dogs.	2 mg/kg CBD (CBD + CBDA) orally twice daily for 4 weeks.	CBD produced a significant decrease in pain scores (measured by the Canine Brief Pain Inventory) and an increase in activity levels (measured by the Hudson activity scale).	(25)
Case report of one dog with chronic osteoarthritis treated with a CBD-purified hemp oil to improve analgesia, mobility, and quality of life.	1 mg/kg of CBD given orally with food twice daily for 30 days.	CBD produced analgesia with consequent improvement of mobility and quality of life of the dog.	(24)
Non-blinded observational study to evaluate the impact of using a CBD-dominant full-spectrum hemp oil-based product as adjunctive therapy on OA-related pain in 32 dogs.	0.3–4.12 mg/kg CBD (individually adjusted dose based on pain assessment) orally twice daily for 90 days.	30 out of 32 dogs showed pain relief (measured using a 0 to10 scale, with 10 representing the worst possible pain) and 21 out of 23 dogs were able to reduce or stop gabapentin after adding the CBD-dominant oil.	(54)
Randomized, placebo-controlled, double-blind clinical trial to evaluate the safety and therapeutic potential of different doses and formulations of hemp-derived CBD oil for OA pain relief in 20 dogs.	20 mg/day of naked CBD, 50 mg/day of naked CBD, 20 mg/day of liposomal CBD orally for 4 weeks.	CBD significantly reduced pain (measured by the Helsinki Chronic Pain Index) and increased mobility in a dose-dependent manner. Liposomal CBD (20 mg/day) was as effective as the highest dose of non-liposomal CBD (50 mg/day) in improving clinical outcomes.	(55)
Randomized placebo-controlled study to evaluate the efficacy of a pure CBD oil formulation, included in a multimodal drug regimen, in relieving pain in 9 dogs with spontaneous OA.	2 mg/kg of CBD administered orally transmucosally (OTM) twice daily for 12 weeks, added to the multimodal drug protocol.	Adding oral OTM CBD to a multimodal pharmacological treatment for canine OA improved owner-reported pain scores and quality of life of dogs (measured by the Canine Brief Pain Inventory).	(56)
Double-blind, randomized, placebo-controlled, cross-over clinical study to evaluate the efficacy of a CBD-dominant hemp oil on OA-related pain relief in 23 dogs.	2.5 mg/kg of CBD orally twice daily for 6 weeks.	No differences were observed between groups at any time point for any of the recorded outcome measures (objective gait analysis, activity counts - *via* accelerometry - and clinical metrology instruments - Liverpool Osteoarthritis in Dogs and Canine Brief Pain Inventory).	(57)
Randomized, placebo controlled, blinded clinical trial to determine the impact of capsules containing a CBD/CBDA rich hemp oil on acute post-operative pain in dogs following a tibial plateau leveling osteotomy (TPLO).	2–2.5 mg/kg of CBD/CBDA orally twice daily for 4 weeks following a TPLO.	No significant differences were noted between placebo and CBD/CBDA groups at any point in Canine Brief Pain Inventory scores, degree of lameness, and degree of weight-bearing.	(58)

case series, Mogi and Fukuyama (2019) reported different and sometimes contradictory results in the three evaluated dogs treated with CBD (0.51 mg/kg twice daily, 1.24–1.25 mg/kg twice daily, 5.00 mg/kg twice daily, respectively, for 8 weeks), with considerable reduction, slight reduction, and no reduction in the epileptic seizures, respectively (69).

3.3. Behavioral disorders

Emotional behavior is also included among the many physiological functions modulated by the ECS. This system is essential in promoting synaptic plasticity responsible for learning and the ability to respond to emotionally impacting adverse events (70).

The hypothesis that the ECS plays a role in the modulation of emotional behavior is supported by the demonstration that CB1 receptors and Fatty Acid Amide hydrolase (FAAH - the enzyme responsible for the degradation of endocannabinoids), as well as the endocannabinoids AEA and 2-AG, are expressed and produced in brain areas (such as the amygdala, nucleus accumbens, hippocampus and prefrontal cortex) involved in stress, fear, emotions and reward mechanisms (71). However, the effects of the ECS in the modulation of anxious states are not unique: the complexity of the ECS is probably responsible for the various anxiolytic and anxiety-producing effects manifested by agonists interacting with CB1 receptors, but also TRPV1 and 5 -HT1A (72, 73).

As for CBD, this compound has been studied in a wide range of animal models, such as the stress-induced anxiety model, the panic

disorders and compulsive behavior model, the fear conditioning test, the fear extinction test and the reconsolidation blockade test. These studies have demonstrated CBD's therapeutic potential in treating anxiety disorders. Indeed, CBD exhibited a wide range of activities, including anxiolytic, panicolytic, and anticompulsive actions, as well as decreased autonomic arousal, decreased conditioned fear expression, increased fear extinction, reconsolidation block, and prevention of the long-term anxiety-provoking effects of stress (70).

The anxiolytic and panicolytic effects and reduced fear conditioned expression produced by CBD could be due to the activation of 5-HT1A receptors, although CB1 receptors may also play a limited role. By contrast, the activation of CB1 receptors mediates the anticompulsive effects, the enhancement of fear extinction, the blockade of reconsolidation and the ability to prevent the long-term anxiety-producing consequences of stress. Furthermore, CBD, even at high doses, does not produce anxiety-producing effects (70).

As regards the clinical efficacy of CBD in treating behavioral disorders in dogs, only three scientific studies (a replicated 4×4 Latin square design; a placebo controlled study; a blinded, placebo-controlled, parallel design study) are currently published (Table 4). Morris et al., 2020 reported a lack of an anxiolytic effect of CBD (1.4 mg/kg 4–6 h prior the test) on behavioral responses to fear-inducing stimuli (74). The study by Corsetti et al. (2021) was aimed to determine if CBD (~ 1.25 mg/kg once a day for 45 days) could affect stress related behavior in shelter dogs and reported that aggressive behavior toward human were decreased over time in the CBD group, albeit not statistically significant; other behaviors indicative of stress, such as displacing activities and stereotypes, did not decrease (75). Hunt et al. demonstrated an anxiolytic effect of CBD (~ 4 mg/kg 2 h prior the test, dose which is much higher than the previous anxiety study) in dogs experiencing a separation event or a car travel (76).

3.4. Skin diseases

The ECS (with its receptors, mediators, and regulatory molecules produced/expressed by most skin cellular elements) is an emerging key player in skin homeostasis. Indeed, it was proposed that it exerts a protective role against skin inflammation, itch and pain, thanks to the involvement of the endocannabinoid palmitoylethanolamide (PEA) and its ALIA (Autacoid Local Injury Antagonism) effects (77, 78).

The CB1 and CB2 cannabinoid receptors are expressed in canine keratinocytes (5, 7, 8), with higher immunoreactivity to CB1 and CB2 in atopic dogs than in healthy dogs (5). Canine keratinocytes also express TRPV1 receptors (6).

Most papers published on cannabinoids in pruritus deal with ALIAmides (i.e., Adelmidrol®), with PEA being considered one of the most promising compounds in this respect. It has been demonstrated that cannabinoid receptor agonists (i.e., PEA) attenuated inflammation in the skin of mice in a model of allergic contact dermatitis (79, 80) and reduced skin lesions and pruritus in atopic dogs during a comprehensive open label study (81). These effects seem mainly dependent upon mast cell down-modulation, but other cell types (i.e., macrophages and T cells) seem down-regulated by PEA. Moreover, PEA also acts indirectly by elevating the levels, reducing the degradation, and increasing the affinity of endocannabinoids for their receptors (20).

Cannabidiol does not appear to interact with CB1 or CB2 receptors directly, yet it has been implicated in altering endogenous levels of endocannabinoids such as AEA. CBD also may interact with other receptor systems (such as the TRPV, adenosine reuptake inhibitor, and PPAR) in the inflammatory cells or neurons, based on *in vitro* and *in vivo* assessments in humans and rodents (82).

To the best of the authors' knowledge, only two studies (an open-label case series study and a randomized placebo-controlled study, respectively) have been conducted investigating the efficacy of CBD and CBD/CBDA as a treatment for canine atopic dermatitis (Table 5). In both studies, phytocannabinoids [0.07 to 0.25 mg/kg of CBD twice daily for at list 8 weeks (83), 2 mg/kg of CBD/CBDA twice daily for 28 days (82)] decreased the occurrence of pruritus in dogs with canine atopic dermatitis. A third study (a randomized complete block design, placebo controlled study) was not conducted on atopic dogs, being intended to determine the influence of CBD (1.25 mg/kg or 2.5 mg/kg twice daily for 7 days before and another 14-day during collection of activity) on the dogs' daily activity (measured by an activity tracker, including activity points, activity duration, resting, running, walking, head shaking, and sleep quality, among others): among the checked activities, scratching tended to be reduced compared to control, albeit not statistically, leading the Authors to hypothesize that CBD could maybe exert an antipruritic effect (84) (Table 5).

4. Tolerability of CBD

When based on CBD containing less than 0.3% of THC, formulations are devoid of psychoactive properties (85). In men, it is therefore unlikely that they give rise to abuse, although they are not entirely free of side effects. Indeed, somnolence, loss of appetite and diarrhea have been reported as common signs in human clinical trials during treatment with CBD (86, 87).

In dogs, there are some studies concerning tolerability not only when CBD is administered as a single dose (25, 35, 88) but, above all, for prolonged use over time, when the accumulation of such lipophilic compounds is possible. However, to the best of Authors' knowledge, these studies were prevalently focused on short- to medium-term treatment (4–6 weeks), with only two studies assessing CBD tolerability after long-term treatments, i.e., over 12 weeks (28) and over 6 months (89).

While the oral lethal dose of THC in dogs is estimated as more than 3,000 mg/kg (90), an oral lethal dose of CBD is still undetermined. CBD has low acute intravenous (i.v.) toxicity with a lethal dose for 50% of the exposed dogs of >254 mg/kg (91). Preclinical safety studies performed in dogs prior to FDA approval of Epidiolex® (a purified CBD extract to be used as adjunctive treatment of seizures in children with Lennox Gastaut or Dravet Syndrome) indicate a no observable adverse effects level (NOAEL) of 100 mg/kg BW of CBD (92). CBD-based products usually contain a dose of CBD significantly lower than the lethal i.v. dose as well as the NOAEL, making the formulations relatively safe. Regardless of this consideration, an oral dose of 2 mg/kg once a day and up to 20 mg/kg/ twice daily seemed well-tolerated and associated with mild side effects, both in healthy and diseased animals (Table 6). A similar favorable safety profile has been further confirmed by a study by Vaughn et al. (2020), where escalating doses of CBD up to 62 mg/kg were used (88).

TABLE 3 Studies on clinical efficacy of CBD-based products in the treatment of epilepsy in dogs.

Study design	Treatment	Results	References
Randomized placebo-controlled, double-blinded clinical trial to assess the effect of using a CBD-infused hemp oil in addition to conventional antiepileptic treatment on seizure frequency in 26 dogs with idiopathic epilepsy.	2.5 mg/kg of CBD oil orally twice daily for 12 weeks.	Compared with the placebo group, dogs in the CBD group had a significant reduction in seizure frequency (median change, 33%). However, the proportion of dogs considered responders to treatment (≥ 50% decrease in seizure activity) was similar between groups.	(67)
Case report of three dogs with suspected epilepsy, each one treated with a different dose of a CBD-predominant full-spectrum hemp oil.	0.51 mg/kg of CBD for the first dog, 1.24–1.25 mg/kg for the second dog, and 5 mg/kg for the third dog, given orally twice daily for 8 weeks.	Considerable reduction in epileptic seizures frequency and improvement of other signs (i.e., undesirable behavior) in one dog, slight improvement of seizure intensity in another, and no response to therapy in the third, as reported by the owners.	(69)
Randomized, controlled-placebo, cross-over study to examine the efficacy of a CBD and CBDA-rich hemp product for the treatment of refractory epileptic seizures in 14 dogs.	2 mg/kg of CBD orally twice daily for 12 weeks.	Statistically significant reduction in epileptic seizure frequency, as well as number of epileptic seizure days (the number of dogs with a 50% reduction in epileptic activity while on treatment were 6/14, whereas 0/14 had reductions of 50% or greater while on the placebo).	(68)

Although the side effects of CBD are classified as mild or unremarkable, the reported clinical trials showed that various adverse clinical signs might occur following the administration of CBD, primarily indicative of gastrointestinal upset, such as nausea, ptyalism, loss of appetite, vomiting and loose stools (28, 30, 33, 35, 56, 57, 68, 75, 82, 93). These could be partly related to the CBD-based products' and oil vehicle's disgusting taste. Different oral formulations are likely to reduce the incidence of these signs. Indeed, the liposomal packaging of CBD oil seems the best option, as no clinical side effects were noted (55). Similarly, only a mild ptyalism was observed when CBD was compounded in a flavorless oil (56). Furthermore, in a pilot study on eight dogs (94), a 99 + % pure CBD crystalline powdered in tablets, added to a purified mixture of terpenes acids from the *Boswellia serrata Roxb*. and powdered melon fruit pulp and juice extract, demonstrated good palatability and no adverse clinical signs.

Other adverse signs noted in dogs, even if less frequently, were somnolence and lethargy (56, 69, 82) as well as ataxia (56, 67, 68). Neurological signs such as head bobbing, hyperesthesia, ataxia or swaying, among others, have been reported in the study by Chicoine and co-workers (2020), where six dogs were treated with 1:20 THC:CBD *Cannabis* herbal extract (10 mg/kg of CBD and 0.5 mg/kg of THC). However, data in humans suggest that these neurological signs in the dogs are attributable to effects of THC and not CBD (27).

All these findings were generally self-limiting without discontinuing the administration, and they seemed dose-dependent, as their incidence increased for dosage over 10 mg/kg. It is interesting to note that a particular adverse sign, i.e., erythematous pinnae, which may be observed during treatment with CBD compounded in a transdermal cream, is likely to occur also during oral administration of doses over 10 mg/kg (93). Furthermore, it is also noteworthy a case report (95) where a dog manifested widespread cutaneous erythema and ulceration associated with anorexia and diarrhea 5 days after receiving an oral hemp oil formulation (CBD 0.3 mg/kg, once daily) for anxiety. The absence of a history of cutaneous or systemic disease, the histopathological findings, and the remission after symptomatic treatment and discontinuation of the CBD product allowed the Authors to consider a possible CADR (cutaneous adverse reaction to drugs) to CBD, as described in men (96), or to additional substances in the vehicle.

Besides the described signs, a common finding during prolonged treatment with CBD in some but not all dogs across clinical studies is the elevation of alkaline phosphatase enzyme (ALP) activity, which generally return to baseline values after a washout period (25, 35, 54, 56, 58, 67, 82, 88, 89, 93). This alteration is usually attributed to a reversible upregulation of cytochrome p450-mediated oxidative metabolism of the liver (97, 98). The clinical importance of such finding is still unknown, and without the results of other investigations to assess liver function, such as biliary acids and histopathologic exams could be irrelevant: in this sense, it is interesting to report the study of Bradley et al. (2022), where the Authors identified a strong positive correlation between the elevations of total ALP and that of the bone-specific ALP (BALP), suggesting that ALP isoenzymes of different origin may be overproduced during CBD treatments (89).

Besides clinical trials, a preclinical/preregistration study was conducted in healthy Beagles dogs to evaluate the toxicology of Epidiolex. Given by gavage up to 100 mg/kg for 39 weeks, CBD showed only mild gastrointestinal signs, a dose-dependent decrease in body weight, an increase in ALT (up to 1.5X) and in ALP (up to 8X), increased liver weight and hepatocyte hypertrophy (92) (Table 6).

5. Discussion and conclusion

An appropriate drug dose at specific time intervals needs to be administered to obtain an adequate pharmacological response. Knowledge of a drug's pharmacokinetic profile is essential to define the dosing regimen (99).

Currently, the CBD doses used in veterinary medicine are variable and empirical. Indeed, although several studies on the pharmacokinetics of CBD in dogs have been conducted, the kinetic

TABLE 4 Studies on clinical efficacy of CBD-based products in the treatment of behavioral disorders in dogs.

Study design	Treatment	Results	References
Replicated 4×4 Latin square design experiment to evaluate the influence of a CBD industrial hemp extract incorporated into treats on behavioral responses to fear-inducing stimuli in 16 dogs.	1.4 mg/kg of CBD orally 4–6 h prior the test.	The results of the current study did not provide strong support of an anxiolytic effect of CBD in dogs.	(74)
Placebo controlled study design to determine if a 5% CBD based oil affects stress related behavior in 12 shelter dogs.	1 drop of oil/2 kg (~1.25 mg/kg) of CBD orally once a day for 45 days.	Aggressive behavior toward humans decreased significantly over time in CBD treatment group. However, in the pairwise comparisons, only the T0-T2 (45th day) comparison was significant.	(75)
Blinded, placebo-controlled, parallel design study to determine the anxiolytic effect of a CBD based hemp derived distillate incorporated into soft gel capsules in dogs experiencing a separation event (n. = 21) or a car travel (n. = 19).	~ 4 mg/kg of CBD orally 2 h prior the test.	The mitigating effect of CBD treatment varied by outcome measures and tests, with some indicating a significant reduction in canine stress compared to the placebo group.	(76)

TABLE 5 Studies on clinical efficacy of CBD-based products in the treatment of skin diseases in dogs.

Study design	Treatment	Results	References
Retrospective study to examine the effect of a 10% CBD-containing broad-spectrum hemp oil as a supplemental treatment for canine atopic dermatitis in 8 dogs.	Initial dose: 0.07 to 0.25 mg/kg of CBD orally twice daily. The dose was increased depending on the skin condition of each dog and the observed response at 0.125 mg/kg. Administration for at list 8 weeks.	CBD decreased the occurrence of pruritus in dogs with canine atopic dermatitis.	(83)
Randomized, double-blinded and placebo-controlled trial to determine if CBD/CBDA-rich hemp extract (in gelatin capsules) decreased pruritus and cutaneous lesions in 17 dogs with atopic dermatitis.	2 mg/kg of CBD/CBDA twice daily orally for 28 days.	CBD/CBDA does not affect lesion severity yet does have a positive effect on pruritus as an adjunct therapy in some dogs with atopic dermatitis.	(82)
Randomized complete block design, placebo controlled, to determine the influence of CBD treats on the daily activity in adult dogs.	2.5 mg/kg (LOW) and or 5.0 mg/kg (HIGH) of CBD per day (split in 2 administrations) orally for 7 days before and another 14-day during collection of activity.	CBD (LOW and HIGH) did not alter the total daily activity points or activity duration but tended ($p = 0.071$) to reduce total daily scratching compared with the control.	(84)

profile of CBD is not yet fully known, probably because of the very different experimental conditions used, such as different oily vehicles (sunflower lecithin, MCT, and sesame oil), pharmaceutical forms (tablets, chews, microencapsulated oil beads, or drops), type of CBD (synthetic and purified or full spectrum extract) and route of administration (oral, rectal, intranasal or oral transmucosal) (Table 1). Moreover, these studies differ in sample times, number of withdrawals and number of treated animals (from 3 to 8), all influencing the pharmacokinetic parameters. Lastly, the large individual variability in the plasma concentrations observed in all studies further weakens the interpretation of obtained data. Therefore, to define a rational regimen of dosing and avoid the empirical use of CBD, more studies are necessary to elucidate the pharmacokinetics and pharmacodynamics of CBD in light of inter-individual CYPp450 expression and polymorphisms leading to metabolism differences across dog populations.

In terms of efficacy, most studies have been conducted to test the ability of CBD to relieve pain in dogs with osteoarthritis. Albeit in one study no differences were noted between groups for any of the recorded outcome measures (57), from results obtained in all other studies CBD seemed able to significantly reduce pain and increase the activity of dogs, thus improving their quality of life (24, 25, 54–56). The only study where the role of CBD in acute postoperative pain following a TPLO was investigated did not give satisfactory results (58). Although future studies could disprove this result, it is possible to hypothesize that CBD is effective in chronic but not in acute pain. Regarding the possible efficacy of CBD in treating epilepsy, the results obtained in the two randomized controlled clinical trials (67, 68) are promising, as both McGrath and Garcia data show a reduction in seizures in 33 and 42% of treated dogs, respectively. However, the study by Mogi and Fukuyama (2019) (69) reported different and sometimes contradictory results in the three evaluated dogs. The only three scientific studies currently published on the efficacy of CBD in behavioral disorders reported a lack of an anxiolytic effect of CBD on behavioral responses to fear-inducing stimuli (74), but a decrease in aggressive behavior toward humans (75), and a reduction in canine stress (76). As per the efficacy of CBD in skin diseases, from the three published studies, it appears that CBD can decrease the occurrence of pruritus in healthy and atopic dogs (82, 84).

TABLE 6 Studies on tolerability of CBD-based products.

Formulation and dose of CBD, route of administration and treatment' duration	Dogs (n°)	Side effects (n° of involved dogs/recruited dogs)	References
1 mg/kg CBD + 1 mg/kg CDBA hemp oil twice a day orally for 4 weeks	16	Increase in ALP activity (9/16, CBD group)	(25)
CBD oil, CBD microencapsulated and CBD cream, 10 mg/kg or 20 mg/kg twice daily orally or transdermally for 6 weeks	30	Diarrhea (30/30), vomiting (6/30), erythematous pinnae (11/30). Other signs: nasal discharge, salivary staining, lameness, prolapsed nictitans, hyperthermia. Transient isosthenuria, hyposthenuria or proteinuria (15/30). Increased ALP activity (11/30).	(93)
CBD hemp oil, 0.51–5 mg/kg/day for 8 weeks	3	Somnolence (2/3)	(69)
1 mg/kg of CBD +1 mg/kg of CBDA oil in soft chew, orally twice daily for 12 weeks	8	Loose stool, vomiting (food or bile products)	(29)
CBD-industrial hemp extract oil, *Casperome*® and powdered melon fruit pulp and juice extract, 2,4 mg/per 15 kg of BW for 4 weeks	8	None	(94)
CBD-infused oil, 2.5 mg/kg orally twice daily for 12 weeks	16	Increased ALP activity	(67)
CBD oil, 2 mg/kg twice daily orally transmucosally (OTM) for 12 weeks	24	Minimal ptyalism (2/24, only in CBD group), somnolence and mild ataxia (3/24, 1 dog of CBD group, 2 dogs of control group). No relevant changes in the blood cell count and serum biochemical analysis.	(56)
CBD hemp oil, 0.3–4.12 mg/kg daily twice for 12 weeks	30	Increase in ALP activity	(54)
CBD oil and CBD oil liposomally encapsulated, 20 mg/day of naked CBD oil, 50 mg/day of naked CBD oil, 20 mg/day of liposomal CBD oil or placebo orally for 4 weeks	20	No relevant changes in cell blood counts and biochemical profile	(55)
CBD hemp oil, 1 drop/2 kg (~1.25 mg/kg) orally once a day for 45 days	24	One-day duration diarrhea (1/24)	(75)
CBD hemp oil, 2.5 mg/kg orally twice daily for 6 weeks	24	Vomiting (1/24), mild elevation in liver enzymes (14/24)	(57)
CBD hemp oil, 1, 2, 4, or 12 mg CBD/kg once daily for 4 weeks	20	Mild and self-limiting gastrointestinal signs (mainly hypersalivation), more incident at the dosage of 12 mg/kg. Transient increase of ALP	(88)
CBD hemp oil, 4 mg/kg PO daily for 26 weeks	40	Increased ALP activity	(89)
CBD-CBDA hemp oil, 2 mg/kg PO twice daily for 12 weeks	10	Mild and self-limiting gastrointestinal signs (2/10), somnolence (3/10) and mild worsening of ataxia (4/10)	(68)
CBD/CBDA in sesame oil, 2 mg/kg twice daily for 4 weeks	29	Lethargy (2/29), somnolence and sleepiness (2/29), decreased aggression (1/29) and increased calmness (3/29), regurgitation (1/29), increased flatulence (1/29), loss of appetite (1/29), increased energy/mobility (2/29). Elevation of ALP activity. Placebo group: diarrhea and regurgitation (1/29). 1 dog excluded for lethargy and behavioral changes.	(82)
CBD/CBDA-rich soft gel and hemp oil, 2 mg/kg daily twice orally for 4 weeks	8	Soft gel: vomiting (2/8), loose stools (6/8) Oil: vomiting (1/8) and occasional episodes of licking, grimacing and chomping	(33)
5, 10 or 20 mg/kg of pure CBD in (MCT) oil twice daily for 2 weeks	9 (3x dosage)	Vomiting, hyporexia, anorexia (5/9) and an increase in serum ALP activity	(30)
CBD/CBDA rich hemp oil, 2–2.5 mg/kg twice daily for 4 weeks	44	Increased ALP activity	(58)
Purified CBD (Epidiolex™) 0 mg/kg/day (control group, C), 10 mg/kg/day (Low dose, LD), 50 mg/kg/day (Medium dose, MD), 100 mg/kg/day (High Dose, HD) over 39 weeks	4/sex/group +2/sex for C and HD	• Soft/liquid/mucoid feces at all doses • Reduced body weight observed at all doses in males (5, 15, and 12% at LD, MD, and HD, respectively) and females (22, 29, and 32% at LD, MD, and HD, respectively) • Consistent decreases in heart rate in HD males but no drug-related cardiac rhythm disturbances • Marked increases in ALP (up to 8-fold compared to C) at all doses. • Liver changes: hepatocyte hypertrophy associated with increased liver weight, macroscopic enlargement at all doses (dose-related only in males)	(92)

Therefore, some evidence exists supporting the beneficial role of CBD for adverse conditions, including OA, seizures, behavioral and skin problems in dogs. However, when considering all the published studies, results are not always consistent. Many reasons can account for the evidenced discrepancies, often declared among the studies' limitations: the small sample size, short study duration, heterogeneity of clinical signs, different outcomes, concomitant administration of other drugs, subjective evaluations by owners and veterinarians, caregiver placebo effects. Moreover, it must be emphasized that, as shown on Tables 2–5, some studies published on hemp-based medicines in dogs are not randomized, placebo-controlled, double-blinded, and differ in dose, duration, and, last but not list, type of used product (pure CBD or hemp extracts containing different amounts of other *Cannabis* components – see later the discussion about the entourage effect). In 2022, Lima and co-workers published a systematic review to summarize the evidence of efficacy and safety of the use of *Cannabis* for treating animal disease obtained so far, and to assess the risk of bias in each study (100). The bias assessment accounted for randomization process, deviation from intended interventions, missing outcome data, measurement of the outcome, selection of the reported results; and was classified as low risk, some concern and high risk. Among the six studies that met the inclusion criteria for this review, being randomized clinical trials (RCTs) that described the efficacy or safety of cannabis in monotherapy or as an adjuvant in naturally diseased animals (25, 55–57, 67, 75), four of them (25, 55, 57, 67) were classified as having some concerns in the overall bias assessment using the Revised Cochrane Risk of Bias Tool for Randomized Trials (RoB 2). All studies were judged to have a low risk of bias from the "deviations from intended interventions" and "missing outcome data," as well as some bias concerns from the "selection of the reported result." Five studies (25, 55, 57, 67, 75) were judged to have a low risk of bias from "measurement of the outcome." Two studies (55, 56) were judged to have some bias concerns from the "randomization process," while one study (75) was judged to have a high risk of bias in the same domain. Finally, one study (56) was judged to have a high risk of bias from the "measurement of the outcome" and considered at high overall risk of bias. Overall, this systematic review suggests that the results of published studies, albeit randomized and/or double-blinded and/or placebo-controlled, need to be carefully interpreted and that greater attention to study design and definition and measurement of outcomes should be considered in future studies to strengthen the evidence regarding the benefits of the therapeutic use of CBD in dogs.

Regarding tolerability, the reported studies allow considering two primary limits: the relatively small sample size and the paucity of long-term studies.

As regards the increase of ALP, it would be desirable to carry out further investigations concerning the relationship between CBD and liver function, as in men the impairment of the cytochrome p450 is suspected to affect the metabolism of drugs concomitantly administered (101), particularly antiepileptic ones. However, no significant pharmacokinetic interactions were found between CBD and phenobarbital when simultaneously administered to healthy dogs (30).

One last consideration deserves to be made. In the studies cited in this review, the CBD formulations used were all different and described either as CBD hemp oil (88, 89), CBD-predominant full-spectrum hemp oil (54, 69), hemp-derived CBD oil (55), CBD-purified hemp oil (24), CBD-purified *Cannabis* extract (35), CBD-infused hemp oil (26, 67), CBD enriched *Cannabis* extract (27), CBD based oil (75), CBD-containing broad-spectrum hemp oil (83), galenic CBD (29, 30, 56), CBD/CBDA-predominant hemp oil (25, 34, 57), CBD/CBDA rich hemp product (58, 68), CBD industrial hemp extract incorporated into treats (74, 84), CBD/CBDA oil in soft chew (28), CBD/CBDA-rich hemp extract in gelatine capsules (33, 82), pure CBD in capsules (31), microencapsulated CBD oil beads (26), CBD-infused oil cream (26). Besides the large variability of formulations, in some cases the presence of trace amounts of other cannabinoids was specified, while most studies did not report whether other phytocannabinoids (such as cannabichromene, cannabigerol, and cannabinol, among others) or other chemical components of hemp (such as terpenes, triterpenes, and flavonoids) were present. Because the entourage effect can impact the pharmacokinetic, effectiveness and safety of the *Cannabis*-based product (102), differences in CBD formulation observed among the included studies could have influenced the obtained results, that, again, should be interpreted with caution.

Author contributions

GD, MC, and AD wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Di Marzo V, Wang J. *The endocannabinoidome: the world of endocannabinoids and related mediators*. Cambridge, MA: Academic Press (2015), 1–187.

2. Fraguas-Sánchez AI, Martín-Sabroso C, Torres-Suárez AI. Insights into the effects of the endocannabinoid system in cancer: a review. *Br J Pharmacol*. (2018) 175:2566–80. doi: 10.1111/bph.14331

3. Peng J, Fan M, An C, Ni F, Huang W, Luo J. A narrative review of molecular mechanism and therapeutic effect of cannabidiol (CBD). *Basic Clin Pharmacol Toxicol*. (2022) 130:439–56. doi: 10.1111/bcpt.13710

4. Mercati F, Dall'Aglio C, Pascucci L, Boiti C, Ceccarelli P. Identification of cannabinoid type 1 receptor in dog hair follicles. *Acta Histochem*. (2012) 114:68–71. doi: 10.1016/j.acthis.2011.01.003

5. Campora L, Miragliotta V, Ricci E, Cristino L, Di Marzo V, Albanese F, et al. Cannabinoid receptor type 1 and 2 expression in the skin of healthy dogs and dogs with atopic dermatitis. *Am J Vet Res*. (2012) 73:988–95. doi: 10.2460/ajvr.73.7.988

6. Barbero R, Vercelli C, Cuniberti B, Della Valle MF, Martano M, Re G. Expression of functional TRPV1 receptor in primary culture of

canine keratinocytes. *J Vet Pharmacol Ther*. (2018) 41:795–804. doi: 10.1111/jvp.12694

7. Chiocchetti R, De Silva M, Aspidi F, Zamith Cunha R, Gobbo F, Tagliavia C, et al. Distribution of cannabinoid receptors in keratinocytes of healthy dogs and dogs with atopic dermatitis. *Front Vet Sci*. (2022) 9:915896. doi: 10.3389/fvets.2022.915896

8. Chiocchetti R, Salamanca G, De Silva M, Gobbo F, Aspidi F, Zamith Cunha R, et al. Cannabinoid receptors in the inflammatory cells of canine atopic dermatitis. *Front Vet Sci*. (2022) 9:987132. doi: 10.3389/fvets.2022.987132

9. Dall'Aglio C, Mercati F, Pascucci L, Boiti C, Pedini V, Ceccarelli P. Immunohistochemical localization of CB1 receptor in canine salivary glands. *Vet Res Commun*. (2010) 34:9–12. doi: 10.1007/s11259-010-9379-0

10. Galiazzo G, Giancola F, Stanzani A, Fracassi F, Bernardini C, Forni M, et al. Localization of cannabinoid receptors CB1, CB2, GPR55, and PPARα in the canine gastrointestinal tract. *Histochem Cell Biol*. (2018) 150:187–205. doi: 10.1007/s00418-018-1684-7

11. Freundt-Revilla J, Kegler K, Baumgärtner W, Tipold A. Spatial distribution of cannabinoid receptor type1 (CB1) in normal canine central and peripheral nervous system. *PLoS One*. (2017) 12:e0181064. doi: 10.1371/journal.pone.0181064

12. Pirone A, Cantile C, Miragliotta V, Lenzi C, Giannessi E, Cozzi B. Immunohistochemical distribution of the cannabinoid receptor 1 and fatty acid amide hydrolase in the dog claustrum. *J Chem Neuroanat*. (2016) 74:21–7. doi: 10.1016/j.jchemneu.2016.02.002

13. Chiocchetti R, Galiazzo G, Tagliavia C, Stanzani A, Giancola F, Menchetti M, et al. Cellular distribution of canonical and putative cannabinoid receptors in canine cervical dorsal root ganglia. *Front Vet Sci*. (2019) 6:313. doi: 10.3389/fvets.2019.00313

14. Valastro C, Campanile D, Marinaro M, Franchini D, Piscitelli F, Verde R, et al. Characterization of endocannabinoids and related acylethanolamides in the synovial fluid of dogs with osteoarthritis: a pilot study. *BMC Vet Res*. (2017) 13:309. doi: 10.1186/s12917-017-1245-7

15. Pirone A, Lenzi C, Coli A, Giannessi E, Stornelli MR, Miragliotta V. Preferential epithelial expression of type-1 cannabinoid receptor (CB1R) in the developing canine embryo. *Springerplus*. (2015) 4:804. doi: 10.1186/s40064-015-1616-0

16. Mosley C, Gaynor J, Cital S, Brassard J. Cannabinoids for pain management In: S Cital, K Kramer, L Hughston and JS Gaynor, editors. *Cannabis therapy in veterinary medicine*. New York: Springer Cham (2021). 117–41.

17. Williamson BG, Jarboe J, Weaver C. Cannabinoids for neurological conditions In: S Cital, K Kramer, L Hughston and JS Gaynor, editors. *Cannabis therapy in veterinary medicine*. New York: Springer Cham (2021). 143–70.

18. Peyton J, Kramer K, Quesnell B, Cital S. Well Being In: S Cital, K Kramer, L Hughston and JS Gaynor, editors. *Cannabis therapy in veterinary medicine*. New York: Springer Cham (2021). 171–92.

19. McCabe M, Cital S. Cannabinoids for gastrointestinal health In: S Cital, K Kramer, L Hughston and JS Gaynor, editors. *Cannabis therapy in veterinary medicine*. New York: Springer Cham (2021). 193–205.

20. Miragliotta V, Noli C. Dermatology: endocannabinoids and related N-Acylethanolamines in the skin In: S Cital, K Kramer, L Hughston and JS Gaynor, editors. *Cannabis therapy in veterinary medicine*. New York: Springer Cham (2021). 207–30.

21. de Lorimier LP, Hazzah T, Amazonas E, Cital S. Cannabinoids in oncology and immune response In: S Cital, K Kramer, L Hughston and JS Gaynor, editors. *Cannabis therapy in veterinary medicine*. New York: Springer Cham (2021). 231–69.

22. Kogan LR, Hellyer PW, Silcox S, Schoenfeld-Tacher R. Canadian dog owners' use and perceptions of cannabis products. *Can Vet J*. (2019) 60:749–55.

23. Kogan L, Schoenfeld-Tacher R, Hellyer P, Rishniw M. US Veterinarians' knowledge, experience, and perception regarding the use of Cannabidiol for canine medical conditions. *Front Vet Sci*. (2019) 5:338. doi: 10.3389/fvets.2018.00338

24. Coelho MPRC, Leme FDOP, Moreira FA, Branco SEMT, Melo MM, de Melo EG. Current review of hemp-based medicines in dogs. *J Vet Pharmacol Ther*. (2021) 44:870–82. doi: 10.1111/jvp.13016

25. Gamble L-J, Boesch JM, Frye CW, Schwark WS, Mann S, Wolfe L, et al. Pharmacokinetics, safety, and clinical efficacy of Cannabidiol treatment in osteoarthritic dogs. *Front Vet Sci*. (2018) 5:165. doi: 10.3389/fvets.2018.00165

26. Bartner LR, McGrath S, Rao S, Hyatt LK, Wittenburg LA. Pharmacokinetics of cannabidiol administered by 3 delivery methods at 2 different dosages to healthy dogs. *Can J Vet Res*. (2018) 3:178–83.

27. Chicoine A, Illing K, Vuong S, Pinto KR, Alcorn J, Cosford K. Pharmacokinetic and safety evaluation of various Oral doses of a novel 1:20 THC:CBD Cannabis herbal extract in dogs. *Front Vet Sci*. (2020) 7:583404. doi: 10.3389/fvets.2020.583404

28. Deabold KA, Schwark WS, Wolf L, Wakshlag JJ. Single-dose pharmacokinetics and preliminary safety assessment with use of CBD-rich hemp nutraceutical in healthy dogs and cats. *Animals*. (2019) 9:832. doi: 10.3390/ani9100832

29. della Rocca G, Paoletti F, Conti MB, Galarini R, Chiaradia E, Sforna M, et al. Pharmacokinetics of cannabidiol following single oral and oral transmucosal administration in dogs. *Front Vet Sci*. (2023) 9:1104152. doi: 10.3389/fvets.2022.1104152

30. Doran CE, McGrath S, Bartner LR, Thomas B, Cribb AE, Gustafson DL. Drug-drug interaction between cannabidiol and phenobarbital in healthy dogs. *Am J Vet Res*. (2021) 83:86–94. doi: 10.2460/ajvr.21.08.0120

31. Polidoro D, Temmerman R, Devreese M, Charalambous M, Ham LV, Cornelis I, et al. Pharmacokinetics of cannabidiol following intranasal, intrarectal, and oral administration in healthy dogs. *Front Vet Sci*. (2022) 9:899940. doi: 10.3389/fvets.2022.899940

32. Fernández-Trapero M, Pérez-Díaz C, Espejo-Porras F, de Lago E, Fernández-Ruiz J. Pharmacokinetics of Sativex® in dogs: towards a potential cannabinoid-based therapy for canine disorders. *Biomol Ther*. (2020) 10:279. doi: 10.3390/biom10020279

33. Tittle DJ, Wakshlag JJ, Schwark WS, Lyubimov A, Zakharov A, Gomez B. Twenty-four hour and one-week steady state pharmacokinetics of cannabinoids in two formulations of Cannabidiol and Cannabidiolic acid rich hemp in dogs. *Med Res Archiv*. (2022) 10:7. doi: 10.18103/mra.v10i7.2907

34. Wakshlag JJ, Schwark WS, Deabold KA, Talsma BN, Cital S, Lyubimov A, et al. Pharmacokinetics of Cannabidiol, Cannabidiolic acid, Δ9-tetrahydrocannabinol, Tetrahydrocannabinolic acid and related metabolites in canine serum after dosing with three Oral forms of hemp extract. *Front Vet Sci*. (2020) 7:505. doi: 10.3389/fvets.2020.00505

35. Vaughn DM, Paulionis LJ, Kulpa JE. Randomized, placebo-controlled, 28-day safety and pharmacokinetics evaluation of repeated oral cannabidiol administration in healthy dogs. *Am J Vet Res*. (2021) 82:405–16. doi: 10.2460/ajvr.82.5.405

36. Samara E, Bialer M, Mechoulam R. Pharmacokinetics of cannabidiol in dogs. *Drug Metab Dispos*. (1988) 16:469–72.

37. Samara E, Bialer M, Harvey DJ. Pharmacokinetics of urinary metabolites of cannabidiol in the dog. *Biopharm Drug Dispos*. (1990) 11:785–95. doi: 10.1002/bdd.2510110906

38. Lam JK, Xu Y, Worsley A, Wong IC. Oral transmucosal drug delivery for pediatric use. *Adv Drug Deliv Rev*. (2014) 73:50–62. doi: 10.1016/j.addr.2013.08.011

39. Gestrich A, Bedenice D, Ceresia M, Zaghloul I. Pharmacokinetics of intravenous gentamicin in healthy young-adult compared to aged alpacas. *J Vet Pharmacol Ther*. (2018) 41:581–7. doi: 10.1111/jvp.12506

40. Chen W, Xiao Y, Chen J, Liu J, Shao J, Li T, et al. Sex-related pharmacokinetic differences and mechanisms of metapristone (RU486 metabolite). *Sci Rep*. (2017) 7:17190. doi: 10.1038/s41598-017-17225-0

41. Anderson LL, Etchart MG, Bahceci D, Golembiewski TA, Arnold JC. Cannabis constituents interact at the drug efflux pump BCRP to markedly increase plasma cannabidiolic acid concentrations. *Sci Rep*. (2021) 11:14948. doi: 10.1038/s41598-021-94212-6

42. Walker JM, Huang SM. Endocannabinoids in pain modulation. *Prostaglandins Leukot Essent Fatty Acids*. (2002) 66:235–42. doi: 10.1054/plef.2001.0361

43. Miller RJ, Miller RE. Is cannabis an effective treatment for joint pain? *Clin Exp Rheumatol*. (2017) 35:59–67.

44. Richardson JD. Cannabinoids modulate pain by multiple mechanisms of action. *J Pain*. (2000) 1:2–14. doi: 10.1016/S1526-5900(00)90082-8

45. Walker JM, Krey JF, Chu CJ, Huang SM. Endocannabinoids and related fatty acid derivatives in pain modulation. *Chem Phys Lipids*. (2002) 121:159–72. doi: 10.1016/s0009-3084(02)00152-4

46. Pertwee RG. Cannabinoid receptors and pain. *Prog Neurobiol*. (2001) 63:569–611. doi: 10.1016/s0301-0082(00)00031-9

47. Iversen L, Chapman V. Cannabinoids: a real prospect for pain relief? *Curr Opin Pharmacol*. (2002) 2:50–5. doi: 10.1016/s1471-4892(01)00120-5

48. Beaulieu P, Rice ASC. Pharmacologie des dérivés cannabinoides: applications au traitement de la douleur? *Ann Fr Anesth Réanim*. (2002) 21:493–508. doi: 10.1016/S0750-7658(02)00663-9

49. Rice AS, Farquhar-Smith WP, Nagy I. Endocannabinoids and pain: spinal and peripheral analgesia in inflammation and neuropathy. *Prostaglandins Leukot Essent Fatty Acids*. (2002) 66:243–56. doi: 10.1054/plef.2001.0362

50. Malan TP Jr, Ibrahim MM, Vanderah TW, Makriyannis A, Porreca F. Inhibition of pain responses by activation of CB(2) cannabinoid receptors. *Chem Phys Lipids*. (2002) 121:191–200. doi: 10.1016/s0009-3084(02)00155-x

51. Casey SL, Vaughan CW. Plant-based cannabinoids for the treatment of chronic neuropathic pain. *Medicines*. (2018) 5:67. doi: 10.3390/medicines5030067

52. Brown MRD, Farquhar-Smith WP. Cannabinoids and cancer pain: a new hope or a false dawn? *Eur J Intern Med*. (2018) 49:30–6. doi: 10.1016/j.ejim.2018.01.020

53. O'Brien M, McDougall JJ. Cannabis and joints: scientific evidence for the alleviation of osteoarthritis pain by cannabinoids. *Curr Opin Pharmacol*. (2018) 40:104–9. doi: 10.1016/j.coph.2018.03.012

54. Kogan L, Hellyer P, Downing R. The use of cannabidiol-rich hemp oil extract to treat canine osteoarthritis-related pain: a pilot study. *AHVMA J*. (2020) 58:35–42.

55. Verrico CD, Wesson S, Konduri V, Hofferek CJ, Vazquez-Perez J, Blair E, et al. A randomized, double-blind, placebo-controlled study of daily cannabidiol for the treatment of canine osteoarthritis pain. *Pain*. (2020) 161:2191–202. doi: 10.1097/j.pain.0000000000001896

56. Brioschi FA, Di Cesare F, Gioeni D, Rabbogliatti V, Ferrari F, D'Urso ES, et al. Oral transmucosal cannabidiol oil formulation as part of a multimodal analgesic regimen: effects on pain relief and quality of life improvement in dogs affected by spontaneous osteoarthritis. *Animals*. (2020) 10:1505. doi: 10.3390/ani10091505

57. Mejia S, Duerr FM, Griffenhagen G, McGrath S. Evaluation of the effect of Cannabidiol on naturally occurring osteoarthritis-associated pain: a pilot study in dogs. *J Am Anim Hosp Assoc*. (2021) 57:81–90. doi: 10.5326/JAAHA-MS-7119

58. Klatzkow S, Davis G, Shmalberg J, Gallastegui A, Miscioscia E, Tarricone J, et al. Evaluation of the efficacy of a cannabidiol and cannabidiolic acid rich hemp extract for pain in dogs following a tibial plateau leveling osteotomy. *Front Vet Sci*. (2023) 9:1036056. doi: 10.3389/fvets.2022.1036056

59. Wright J. Evaluating the benefits of cannabidiol for analgesia following surgery for intervertebral disc herniation in dogs. In: Proceedings of the American College of Veterinary Internal Medicine Forum. American College of Veterinary Internal Medicine. (2022).

60. Leo A, Russo E, Elia M. Cannabidiol and epilepsy: rationale and therapeutic potential. *Pharmacol Res*. (2016) 107:85–92. doi: 10.1016/j.phrs.2016.03.005

61. Capasso A. Do cannabinoids confer neuroprotection against epilepsy? An Overview. *Open Neurol J*. (2017) 11:61–73. doi: 10.2174/1874205X01711010061

62. Szaflarski JP, Bebin EM. Cannabis, cannabidiol, and epilepsy--from receptors to clinical response. *Epilepsy Behav*. (2014) 41:277–82. doi: 10.1016/j.yebeh.2014.08.135

63. Iannotti FA, Hill CL, Leo A, Alhusaini A, Soubrane C, Mazzarella E, et al. Nonpsychotropic plant cannabinoids, cannabidivarin (CBDV) and cannabidiol (CBD), activate and desensitize transient receptor potential vanilloid 1 (TRPV1) channels in vitro: potential for the treatment of neuronal hyperexcitability. *ACS Chem Neurosci*. (2014) 5:1131–41. doi: 10.1021/cn5000524

64. Gaston TE, Szaflarski JP. Cannabis for the treatment of epilepsy: an update. *Curr Neurol Neurosci Rep*. (2018) 18:73. doi: 10.1007/s11910-018-0882-y

65. Gray RA, Whalley BJ. The proposed mechanisms of action of CBD in epilepsy. *Epileptic Disord*. (2020) 22:10–5. doi: 10.1684/epd.2020.1135

66. Purnell B, Murugan M, Jani R, Boison D. The good, the bad, and the deadly: Adenosinergic mechanisms underlying sudden unexpected death in epilepsy. *Front Neurosci*. (2021) 15:708304. doi: 10.3389/fnins.2021.708304

67. McGrath S, Bartner LR, Rao S, Packer RA, Gustafson DL. Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. *J Am Vet Med Assoc*. (2019) 254:1301–8. doi: 10.2460/javma.254.11.1301

68. Garcia GA, Kube S, Carrera-Justiz S, Tittle D, Wakshlag JJ. Safety and efficacy of cannabidiol-cannabidiolic acid rich hemp extract in the treatment of refractory epileptic seizures in dogs. *Front Vet Sci*. (2022) 9:939966. doi: 10.3389/fvets.2022.939966

69. Mogi C, Fukuyama T. Cannabidiol as a potential anti-epileptic dietary supplement in dogs with suspected epilepsy: three case reports. *Pet Behav Sci*. (2019) 7:11–6. doi: 10.21071/pbs.v0i7.11800

70. Blessing EM, Steenkamp MM, Manzanares J, Marmar CR. Cannabidiol as a potential treatment for anxiety disorders. *Neurotherapeutics*. (2015) 12:825–36. doi: 10.1007/s13311-015-0387-1

71. Korem N, Zer-Aviv TM, ElazarEG AH, Akirav I. Targeting the endocannabinoid system to treat anxiety-related disorders. *J Basic Clin Physiol Pharmacol*. (2016) 27:193–202. doi: 10.1515/jbcpp-2015-0058

72. Casarotto PC, Terzian AL, Aguiar DC, Zangrossi H, Guimarães FS, Wotjak CT, et al. Opposing roles for cannabinoid receptor type-1 (CB_1) and transient receptor potential vanilloid type-1 channel (TRPV1) on the modulation of panic-like responses in rats. *Neuropsychopharmacology*. (2012) 37:478–86. doi: 10.1038/npp.2011.207

73. Tambaro S, Bortolato M. Cannabinoid-related agents in the treatment of anxiety disorders: current knowledge and future perspectives. *Recent Pat CNS Drug Discov*. (2012) 7:25–40. doi: 10.2174/157488912798842269

74. Morris EM, Kitts-Morgan SE, Spangler DM, McLeod KR, Costa JHC, Harmon DL. The impact of feeding Cannabidiol (CBD) containing treats on canine response to a noise-induced fear response test. *Front Vet Sci*. (2020) 7:569565. doi: 10.3389/fvets.2020.569565

75. Corsetti S, Borruso S, Malandrucco L, Spallucci V, Maragliano L, Perino R, et al. *Cannabis sativa* L. may reduce aggressive behaviour towards humans in shelter dogs. *Sci Rep*. (2021) 11:2773. doi: 10.1038/s41598-021-82439-2

76. Hunt ABG, Flint HE, Logan DW, King T. A single dose of cannabidiol (CBD) positively influences measures of stress in dogs during separation and car travel. *Front Vet Sci*. (2023) 10:1112604. doi: 10.3389/fvets.2023.1112604

77. Re G, Barbero R, Miolo A, Di Marzo V. Palmitoylethanolamide, endocannabinoids and related cannabimimetic compounds in protection against tissue inflammation and pain: potential use in companion animals. *Vet J*. (2007) 173:21–30. doi: 10.1016/j.tvjl.2005.10.003

78. Della Rocca G, Re G. Palmitoylethanolamide and related ALIAmides for small animal health: state of the art. *Biomol Ther*. (2022) 12:1186. doi: 10.3390/biom12091186

79. Karsak M, Gaffal E, Date R, Wang-Eckhardt L, Rehnelt J, Petrosino S, et al. Attenuation of allergic contact dermatitis through the endocannabinoid system. *Science*. (2007) 316:1494–7. doi: 10.1126/science.1142265

80. Petrosino S, Verde R, Vaia M, Allarà M, Iuvone T, Di Marzo V. Anti-inflammatory properties of Cannabidiol, a nonpsychotropic cannabinoid, in experimental allergic contact dermatitis. *J Pharmacol Exp Ther*. (2018) 365:652–63. doi: 10.1124/jpet.117.244368

81. Noli C, Della Valle MF, Miolo A, Medori C, Schievano CSkinalia Clinical Research Group. Efficacy of ultra-micronized palmitoylethanolamide in canine atopic dermatitis: an open-label multi-Centre study. *Vet Dermatol*. (2015) 26:432–40, e101. doi: 10.1111/vde.12250

82. Loewinger M, Wakshlag JJ, Bowden D, Peters-Kennedy J, Rosenberg A. The effect of a mixed cannabidiol and cannabidiolic acid based oil on client-owned dogs with atopic dermatitis. *Vet Dermatol*. (2022) 33:329–e77. doi: 10.1111/vde.13077

83. Mogi C, Yoshida M, Kawano K, Fukuyama T, Arai T. Effects of cannabidiol without delta-9-tetrahydrocannabinol on canine atopic dermatitis: a retrospective assessment of 8 cases. *Can Vet J*. (2022) 63:423–6.

84. Morris EM, Kitts-Morgan SE, Spangler DM, Gebert J, Vanzant ES, McLeod KR, et al. Feeding Cannabidiol (CBD)-containing treats did not affect canine daily voluntary activity. *Front Vet Sci*. (2021) 8:645667. doi: 10.3389/fvets.2021.645667

85. Shannon S, Lewis N, Lee H, Hughes S. Cannabidiol in anxiety and sleep: a large case series. *Perm J*. (2019) 23:18–041. doi: 10.7812/TPP/18-041

86. Devinsky O, Marsh E, Friedman D, Thiele E, Laux L, Sullivan J, et al. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. *Lancet Neurol*. (2016) 15:270–8. doi: 10.1016/S1474-4422(15)00379-8

87. Thiele EA, Marsh ED, French JA, Mazurkiewicz-Beldzinska M, Benbadis SR, Joshi C, et al. Cannabidiol in patients with seizures associated with Lennox-Gastaut syndrome (GWPCARE4): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet*. (2018) 391:1085–96. doi: 10.1016/S0140-6736(18)30136-3

88. Vaughn D, Kulpa J, Paulionis L. Preliminary investigation of the safety of escalating cannabinoid doses in healthy dogs. *Front Vet Sci*. (2020) 7:51. doi: 10.3389/fvets.2020.00051

89. Bradley S, Young S, Bakke AM, Holcombe L, Waller D, Hunt A, et al. Long-term daily feeding of cannabidiol is well-tolerated by healthy dogs. *Front Vet Sci*. (2022) 9:977457. doi: 10.3389/fvets.2022.977457

90. Corsato Alvarenga I, Panickar KS, Hess H, McGrath S. Scientific validation of Cannabidiol for Management of dog and cat Diseases. *Annu Rev Anim Biosci*. (2023) 11:227–46. doi: 10.1146/annurev-animal-081122-070236

91. EMA. Epydiolex assessment report. (2019) Available at: https://www.ema.europa.eu/en/documents/assessment-report/epidyolex-epar-public-assessment-report_en.pdf (Accessed June 1, 2023).

92. Food and Drug Administration application 210365Orig1s000. (2018). GW Pharmaceuticals. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210365Orig1s000PharmR.pdf and https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210365Orig1s000SumR.pdf (Accessed June 1, 2023).

93. McGrath S, Bartner LR, Rao S, Kogan LR, Hellyer P. A report of adverse effects associated with the Administration of Cannabidiol in healthy dogs. *AHVMA J*. (2018) 52:34–8.

94. Martello E, Bigliati M, Bisanzio D, Biasibetti E, Dosio F, Pastorino D. Effects on pain and mobility of a new diet supplement in dogs with osteoarthritis: a pilot study. *Ann Clin Lab Res*. (2019) 7:304. doi: 10.21767/2386-5180.100304

95. Simpson AC, Bradley CW, Schissler JR. Probable cutaneous adverse drug reaction due to a cannabidiol-containing hemp oil product in a dog. *Vet Dermatol*. (2020) 31:404–e108. doi: 10.1111/vde.12876

96. Yin HY, Hadjokas N, Mirchia K, Swan R, Alpert S. Commercial cannabinoid oil-induced Stevens-Johnson syndrome. *Case Rep Ophthalmol Med*. (2020) 2020:6760272–5. doi: 10.1155/2020/6760272

97. Bornheim LM, Correia MA. Effect of cannabidiol on cytochrome P-450 isozymes. *Biochem Pharmacol*. (1989) 38:2789–94. doi: 10.1016/0006-2952(89)90432-2

98. Yamamoto I, Watanabe K, Narimatsu S, Yoshimura H. Recent advances in the metabolism of cannabinoids. *Int J Biochem Cell Biol*. (1995) 27:741–6. doi: 10.1016/1357-2725(95)00043-o

99. Riviere JE. Dosage Regimens. (1999). In , JE Riviere , editor. *Comparative pharmacokinetics: Principles, techniques, and applications 1st Edn*. Ames: Iowa State University Press. 202–214

100. Lima TM, Santiago NR, Alves ECR, Chaves DSA, Visacri MB. Use of cannabis in the treatment of animals: a systematic review of randomized clinical trials. *Anim Health Res Rev*. (2022) 23:25–38. doi: 10.1017/S1466252321000189

101. Brown JD, Winterstein AG. Potential adverse drug events and drug-drug interactions with medical and consumer Cannabidiol (CBD) use. *J Clin Med*. (2019) 8:989. doi: 10.3390/jcm8070989

102. Pavlovic R, Nenna G, Calvi L, Panseri S, Borgonovo G, Giupponi L, et al. Quality traits of "cannabidiol oils": cannabinoids content, terpene fingerprint and oxidation stability of European commercially available preparations. *Molecules*. (2018) 23:1230. doi: 10.3390/molecules23051230

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Pharmacokinetic modelling of orally administered cannabidiol and implications for medication control in horses

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Cannabidiol (CBD) products gain increasing popularity amongst animal owners and veterinarians as an alternative remedy for treatment of stress, inflammation or pain in horses. Whilst the use of cannabinoids is banned in equine sports, there is limited information available concerning CBD detection times in blood or urine. The aim of this study was to determine the pharmacokinetic properties of CBD following oral administration in the horse to assist doping control laboratories with interpreting CBD analytical results. Part 1: dose escalation study: Single oral administration of three escalating doses of CBD paste (0.2mg/kg, n=3 horses; 1mg/kg, n=3; 3mg/kg, n=5) with >7days wash-out periods in between. Part 2: multiple dose study: oral administration of CBD paste (3mg/kg, n=6) twice daily for 15days. Multiple blood and urine samples were collected daily throughout both studies. Following study part 2, blood and urine samples were collected for 2weeks to observe the elimination phase. Concentrations of CBD, its metabolites and further cannabinoids were evaluated using gas-chromatography/tandem-mass-spectrometry. Pharmacokinetic parameters were assessed via two approaches: population pharmacokinetic analysis using a nonlinear mixed-effects model and non-compartmental analysis. AUC_{0-12h} and C_{max} were tested for dose proportionality. During the elimination phase, the CBD steady-state urine to serum concentration ratio (Rss) was calculated. Oral CBD medication was well-tolerated in horses. Based on population pharmacokinetics, a three-compartment model with zero-order absorption most accurately described the pharmacokinetic properties of CBD. High volumes of distribution into peripheral compartments and high concentrations of 7-carboxy-CBD were observed in serum. Non-compartmental analysis identified a C_{max} of 12.17±2.08ng/mL after single administration of CBD (dose: 3mg/kg). AUC_{0-12h} showed dose proportionality, increase for C_{max} leveled off at higher doses. Following multiple doses, the CBD terminal half-life was 161.29±43.65h in serum. Rss was 4.45±1.04. CBD is extensively metabolized and shows high volumes of tissue distribution with a resulting extended elimination phase. Further investigation of the potential calming and anti-inflammatory effects of CBD are required to determine cut-off values for medication control using the calculated Rss.

KEYWORDS

CBD, cannabinoids, doping, drug control, equine, Monolix, PK, NLME model

1. Introduction

Medical cannabis and its extracted cannabinoids are used for the treatment of chronic pain, spasticity, epilepsy and anxiety in humans, and have been gaining popularity for similar indications in veterinary medicine in recent years (1–5). The cannabinoids most commonly known are cannabidiol (CBD), cannabidiolic acid (CBDA) and Δ9-tetrahydrocannabinol (THC) (6). CBD interacts with the CB_1- and CB_2 receptors of the endogenous endocannabinoid system and is described to have anti-inflammatory, relaxing, anti-convulsant and anxiolytic effects, whilst THC is the main agent responsible for the psychotropic characteristics of cannabis (7–14).

Pharmacokinetic studies in healthy dogs and cats, as well as clinical studies investigating the treatment of osteoarthritis, canine epilepsy and canine atopic dermatitis have confirmed positive outcomes with little side effects following the oral administration of CBD oil or paste (5, 15–23). Initial scientific reports of CBD application in horses described the treatment of mechanical allodynia, second intention wound healing and treatment for stereotypic behavior such as crib-biting (24–27). Subsequent studies started to analyze the pharmacokinetic properties of cannabinoids in horses and some studies reported positive therapeutic effects particularly for the treatment of chronic degenerative pain in horses (28–35).

Due to their potential analgesic and psychotropic properties, natural and synthetic cannabinoids are on the list of banned substances in most national and international equine sports associations including the FEI (Fédération Equestre Internationale) (36, 37). CBD and CBDA were moved to the FEI's list of controlled medications as specified substances in 2022 (36). The lipophilic properties of CBD and other cannabinoids can lead to the accumulation in organs and adipose tissue (5, 10, 38). The detection of synthetic cannabinoids in the context of doping control in horses has been described. There are, however, no further reports for detection times of CBD (36, 37, 39).

The aim of this study was to investigate the pharmacokinetic properties of CBD in horses following oral administration of a CBD containing paste, and to use the results for the interpretation of analytical findings following medication control in equestrian sports. The authors hypothesized that cannabinoids would have long retention times in equine biological matrices.

2. Materials and methods

2.1. Animals

Six Haflinger × Warmblood cross horses, including three mares and three stallions were included in the study. Mares and stallions were stabled in separate barns where the mares were kept in paddock boxes. All horses had *ad libitum* access to water, were fed hay and mineral feed and were led to pasture for 8 hours a day. The study was reviewed and approved by the competent authority for licensing and notification procedures for animal experiments (LAVG) in Brandenburg, Germany (AZ: 2347-12-2021).

2.2. CBD product

A paste containing 55% CBD (2,750 mg) and <0.2% THC (TAMACAN XL 55%®, 5,000 mg, Herosan healthcare GmbH, Austria) was used for oral medication. Further ingredients included naturally occurring phytocannabinoids, medium-chain triglyceride coconut oil, terpenes, flavonoids and beeswax. CBD and THC contents were analyzed and confirmed by an independent and internationally accredited anti-doping laboratory (Institute of Biochemistry, German Sport University Cologne, Cologne, Germany).

2.3. Dose escalation study

Initially, the CBD paste was administered in single escalating doses during three individual trials (trial 1: 0.2 mg/kg BWT, $n = 3$ horses; trial 2: 1 mg/kg, $n = 3$; trial 3: 3 mg/kg, $n = 6$). For better acceptance, the paste was inserted into a treat. There was a minimum washout period of 7 days in between trials. Prior to each trial, a physical examination was performed and a jugular vein catheter was aseptically placed. Blood samples were collected at the time points 0, 0.5, 1, 2, 4 and 12 hours (h) post medication for analysis of cannabinoid concentrations and for complete blood count (CBC; Diatron Abacus Junior 30 hematology analyser). Spontaneous urine samples were additionally collected at 2 and 12 h to be analyzed for cannabinoids. A repeated physical examination was performed between the time points 2–4 h following medication and horses were closely monitored for any signs of adverse reaction.

2.4. Multiple dose study

After a 25-day washout period, horses ($n = 6$) were administered oral CBD paste (3 mg/kg) every 12 hours for 15 days. Physical examinations were performed daily. Blood samples were obtained every day following oral medication at 2 and 11.5 h. CBC was performed daily at 2 h post administration (p.a.), and both the 2 and 11.5 h samples were analyzed for cannabinoid content. One spontaneous urine sample for cannabinoid analysis was collected from each horse between the time points 8–11.5 h. Serum kidney and liver biomarkers [blood urea nitrogen (BUN), creatinine (CREA), gamma-glutamyltransferase (GGT), glutamic oxaloacetic transaminase (GOT)] were assessed once a week (Fujifilm DRI-CHEM NX500i dry-chemistry analyser).

Following the final CBD oral application in the morning of day 15, blood samples were obtained at the time points 0, 0.5, 1, 2, 4 and 12 h and urine samples close to scheduled time points at 2 and 12 h for accurate monitoring of the drug elimination phase. Over the following 4 days (days 16–19), blood and urine samples were taken every 24 h

and subsequently every 36–48 h until day 33. CBC and serum kidney and liver biomarkers were assessed 1 week after trial end.

2.5. Cannabinoid analysis

Serum and urine samples were frozen and stored at −20°C until further processing. Quantitative analysis for cannabinoid concentrations was performed at an independent and internationally accredited anti-doping laboratory (Institute of Biochemistry, German Sport University Cologne, Cologne, Germany). All samples were analyzed by gas chromatography/tandem mass spectrometry (GC/MS/MS) for the presence of CBD, CBDA, cannabidivarin (CBDV), cannabigerol (CBG), THC, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (COOH-THC) and 11-hydroxy-Δ9-tetrahydrocannabinol (OH-THC). 7-carboxy-cannabidiol (COOH-CBD) and 7-hydroxy-cannabidiol (OH-CBD) were additionally assessed in serum and urine, respectively. Additional information on the sample preparation/extraction and instrumental conditions that were used in this study are summarized in the Supplementary material.

For the validation of analytical methods, parameters including precision, accuracy, selectivity, robustness, linearity, the lower limit of detection (LLOD), lower limit of quantification (LLOQ) and stability were determined. For selectivity, product ion scans were compared with spectra from the literature (40) or from spectra libraries. Three diagnostic product ions of each analyte were included in the acquisition method. Ten blank samples of each specimen (serum and urine) were prepared as described above and tested for interfering peaks at the expected retention time of the analytes. The samples showed no significant signals that could be attributed to the analytes. It was therefore concluded that the selectivity criteria of the employed method were met.

To evaluate the robustness of the method, 10 different samples of each specimen were spiked with 5 ng/mL of each cannabinoid, prepared and analyzed on two consecutive days. Potential effects of the different sample matrices (e.g., biological background interferences, specific gravity and pH differences, different horse characteristics like gender, race and age, potential haemolysis and analytical system performance) on the detectability (reproducibility of ion ratios, peak shape, signal intensity, signal-to-noise ratio and retention times) of each cannabinoid were controlled and documented. All samples showed signals for each analyte with reproducible signal intensities and ion ratios. Relative retention time shifts were within acceptable ranges <0.8% for all tested cannabinoids.

Linearity for all tested cannabinoids was examined by a series of spiked samples at 10 different concentrations in serum and urine over a concentration range considering the expected concentrations in p.a. samples. Area ratios of analyte and internal standard (y) were plotted against the analyte concentration (x) and a calibration curve ($y = ax + b$) was generated by linear least square regression with a weighting factor of $1/x$ or $1/x^2$ (Thermo Scientific Excalibur software version 4.0). The spiked concentration (theoretical concentration) was compared to the calculated concentration (measured concentration) of each calibrator. Correlation factors (R^2) were >0.98 for all calibration curves and measured concentrations were within the acceptance range of 85%–115% of the theoretical concentration for all cannabinoids.

A signal-to-noise ratio of ≥3 for the most abundant ion transition (quantifier ion) was used to determine the LLOD and a signal-to-noise ratio of ≥9 for the LLOQ in urine and serum. The LLOQ was verified by a six-fold determination of the estimated level to obtain the respective precision. The requirement for acceptance of the LLOQ was a coefficient of variation (CV) below 20%. Precisions were determined using 18 quality control (QC) samples which were spiked at low, medium and high concentrations quantified within 1 day ($n = 6$) and on three separate occasions ($n = 6 + 6 + 6$). The CV was established by 6 (intra-day precision) and 18 samples (inter-day precision). Respective concentrations of the QC samples and precisions for the four relevant cannabinoids in this study (CBD, CBDA, 7-COOH-CBD and 7-OH-CBD) are listed in Table 1. For the validation of the accuracy, QC samples ($n = 6$) each spiked at low, medium and high concentrations were quantified with a calibration curve. The means of measured values were compared with the theoretical values. Accuracies are expressed as relative errors (RE).

The stability was assessed by means of 12 serum and urine samples, each fortified with the tested cannabinoids at 5 ng/mL. One set of samples (6 serum and 6 urine) were prepared and analyzed on day 1, whereas the other spiked sample sets (6 serum and 6 urine) were stored at −20°C for 100 days and then quantified using freshly prepared calibrators. Stability was expressed as percentage ratio of the mean concentration at day 100 and the mean concentration at day 1.

Table 1 summarizes the resulting LLODs, LLOQs, precisions, accuracies and stabilities that were validated for each matrix and each compound.

TABLE 1 Validation results of the relevant cannabinoids in the present study.

Canna binoid	Matrix	LLOD (ng/mL)	LLOQ (ng/mL)	Intra-day precision CV (%) at 0.5/5.0/50 ng/mL	Inter-day precision CV (%) at 0.5/5.0/50 ng/mL	Accuracy RE (%) at 0.5/5.0/50 ng/mL	Stability [%]
CBD	Serum	0.1	0.2	9.4/3.9/1.6	6.9/3.7/4.1	9.9/1.6/6.5	63
	Urine	0.1	0.2	4.4/5.1/5.4	5.7/4.4/5.0	−0.4/−2.5/−6.6	83
CBDA	Serum	0.1	0.5	22.4/13.6/26.1	25.5/16.7/19.7	−2.8/−15.0/−12.4	51
	Urine	0.1	0.5	19.9/9.3/9.9	20.3/15.5/16.0	−19.4/−12.6/−7.1	45
7-COOH-CBD	Serum	0.1	0.2	12.5/5.8/6.7	12.5/6.1/4.2	1.4/2.7/−2.5	45
7-OH-CBD	Urine	0.1	0.2	10.0/4.9/3.9	9.4/11.4/6.6	2.5/−6.2/−3.7	79

LLOD, lower limit of detection; LLOQ, lower limit of quantification; CV, coefficient of variation; RE, relative error; CBD, cannabidiol; CBDA, cannabidiolic acid; 7-COOH-CBD, 7-carboxy-cannabidiol; 7-OH-CBD, 7-hydroxy-cannabidiol.

2.6. Pharmacokinetic analysis

2.6.1. Non-compartmental analysis

Non-compartmental analysis (NCA) was performed on serum CBD and its metabolites using PKanalix™ 2021R2 (MonolixSuite™ 2021R2, Lixoft, Antony, France). For the dose escalation study, the area under the curve from the first to the last sampling time point (AUC_{0-12h}), and value and time of maximum serum concentration (C_{max} and t_{max}) were calculated for CBD, 7-OH-CBD and 7-COOH-CBD and summarized as means and standard deviations (SD). The ratio of the AUC_{0-12h} for 7-OH-CBD/CBD and 7-COOH-CBD/CBD was additionally calculated. For the multiple dose study, the terminal half-life was determined for CBD and 7-COOH-CBD based on the last six time points.

2.6.2. Population pharmacokinetic analysis via a nonlinear mixed-effects model

To evaluate further pharmacokinetic parameters, serum CBD data was used to build a nonlinear mixed-effects model (NLME) applying the stochastic approximation expectation maximization (SAEM) algorithm with Monolix™ 2021R2. All CBD values from the dose escalation and the multiple dose studies were combined and fed into the software. The mean of the full posterior distribution was used to determine individual pharmacokinetic parameters. A mathematical model was written based on previous descriptions (41) with further refinements for veterinary purposes (42, 43):

$$y_{ij} = F\left(\varphi_i, t_{ij}\right) + G\left(\varphi_i, t_{ij}, \beta\right) \times \varepsilon_{ij}$$

$$\varepsilon_{ij} \sim N\left(0, \sigma^2\right), \varphi_i = h\left(\mu, \eta_i, \beta_i\right)$$

$$\varphi_i = \mu \times e^{\eta_i}, \eta_i \sim N\left(0, \Omega, \omega^2\right)$$

$$i = 1, \ldots, N, j = 1, \ldots, n_i$$

i stands for each single individual with N being the sum of all individuals. Sample times from 1 to n_i are described by j. y_{ij} is the CBD concentration observed per individual at time t_{ij}. The function $F(\varphi_i, t_{ij})$ predicts the individual concentration through parameter vector φ_i at timepoint t_{ij}. The associated residual error model G (φ_i, t_{ij}, β) contains the covariate β and is multiplied by the independent random variable ε_{ij}, which has a standard normal distribution including mean 0 and variance σ^2. The parameter vector φ_i was modelled as a function (h) of the mean population parameter μ with random variable η_i describing the individual variability and individual covariate β_i. A normal distribution of η_i with mean value 0, variance-covariance matrix Ω and variance ω^2 is assumed, leading to a log-normal distribution of individual parameters φ_i.

The final model was described by three compartments and zero-order absorption. The data set included oral administration only; therefore, the assessment of clearance (Cl) and volumes of distribution (V) was biased by the unknown bioavailability (F). Model parameters include the duration of the zero-order absorption (Tk0), systemic clearance (Cl/F), volume of distribution of a central (V1/F) and two peripheral (V2/F, V3/F) compartments, and intercompartmental clearances (Q2, Q3). Predicted C_{max} and t_{max} values were obtained from the tables generated for the individual predicted curves.

C_{max} were used to calculate the accumulation ratio (AR):

$$\mathrm{AR} = \frac{C_{\mathrm{max\_multipledose}}}{C_{\mathrm{max\_singledose}}}$$

2.6.2.1. Parameter correlation estimates

To identify correlations between parameters which could aid model performance, scatterplots of η_i versus η_i-values for pharmacokinetic parameter estimates' pairs and the Pearson's correlation coefficient were evaluated. A t-test was performed to test statistical significance, defined as a p-value of <0.05. The obtained samples from the posterior distribution at the last SAEM iteration and the empirical Bayes estimates (EBEs) were assessed for parameter correlation, with the EBEs considered less relevant (43, 44). Correlations which fitted the defined selection criteria (see section 2.6.2.2 Model evaluation) were added to the final model.

2.6.2.2. Model evaluation

Numerical and graphical outputs (standard goodness-of-fit criteria, GOF) were used to evaluate the quality of the model (43, 44). To assess the SAEM algorithm, the stability of the parameter search and precision of the parameter estimates were examined for convergence through the relative standard error of the estimate (determined in the Fisher information matrix). Overparameterization was checked through the condition number of the eigenvalues. For graphical information, assessments were performed on individual observations vs. predictions, individual weighted residuals (IWRES), normalized predicted distribution errors (NPDE), visual predictive check (VPC) and individual fits. Distribution of the individual parameters and standardized random effects were examined through histograms and quantile-quantile plots. The random effects were evaluated for normal distribution using the Shapiro–Wilk test and the full posterior distribution of random effects and residuals. Models which performed satisfactorily were further inspected for precision of their respective parameter estimates and corrected Bayesian information criterion (BICc), before settling on a final model.

2.6.2.3. Addition of covariates

The horses' bodyweight was considered as a continuous covariate. The impact on model performance was assessed through the Pearson's correlation coefficient, Wald test and analysis of variance (threshold: p-value <0.05).

2.6.3. Dose proportionality

Pharmacokinetic parameters AUC_{0-12h} and C_{max} for CBD were tested for dose proportionality using the individual values

obtained from NCA and population pharmacokinetic analysis during the dose escalation study. Individual values were pooled for each parameter and fitted into a previously described power model (45, 46). Pharmacokinetic parameters (y) were log-transformed to apply a linear regression approach with dose as a covariate:

$$\log(y) = \mu + \beta \times \log(\text{dose})$$

The closer the β value is to 1, the more proportionally doses are aligned.

Additionally, the individual pharmacokinetic parameters were log-transformed and dose-normalized to test for significant differences (defined as p-value <0.05) between each trial using an analysis of variance (ANOVA) with a post-hoc Tukey test (Statistica 13, TIBCO, Palo Alto, CA, United States).

2.7. Application to medication control

Medication control in equestrian sports is either performed in urine or blood samples. To draw conclusions about the levels in urine from an existing blood sample of a medication, Toutain and Lassourd recommend estimating the steady-state urine to serum concentration ratio (Rss) of a potential drug (47). The concentrations of CBD in urine (Css_{urine}) and serum (Css_{serum}) were used to calculate the Rss during the elimination phase of the multiple dose study (pseudo-equilibrium condition) (47, 48):

$$\text{Rss} = \frac{\text{Css}_{\text{urine}}}{\text{Css}_{\text{serum}}}$$

3. Results

3.1. Horses

The horses' ages ranged from 3 to 16 years (median = 11 years) and the body weight was 488 ± 55 kg. One horse developed a jugular vein thrombophlebitis during the third trial of the dose escalation study and was excluded, putting the final number of horses participating in trial three to $n = 5$. As the inflammation subsided over the following days, it was considered safe to include the horse in the subsequent multiple dose study. Oral application of the CBD product was well tolerated. Physical examinations showed no irregularities and mean assessments of CBCs, kidney and liver biomarkers remained within reference range throughout both trials in all horses (Table 2). Maximum white blood cell (WBC) count was 13.15 10^9/L (reference range (RR): 5–10 10^9/L). Values for BUN below RR were between 6.9–9.3 mg/dL (RR: 9.4–23.5 mg/dL) and for CREA between 0.8–0.9 mg/dL (RR: 0.9–1.5 mg/dL). GGT remained within RR in all samples. GOT was 387 IU/L in one horse (RR: 165–358 IU/L) after 7 days of treatment (Table 2).

TABLE 2 Mean ± standard deviation of WBC count, kidney and liver biomarkers during multiple administrations of CBD paste (3 mg/kg po) twice daily over two weeks with subsequent sample collection.

Parameter (RR)	Baseline	Day 7	Day 14	Day 21
WBC (5–10 10^9/L)	9.0 ± 2.2	7.8 ± 1.6	7.9 ± 2.0	7.6 ± 1.9
Number of horses out of RR	$n = 2/6$	$n = 1/6$	$n = 0/6$	$n = 1/6$
BUN (9.4–23.5 mg/dL)	10.1 ± 1.1	11.0 ± 0.9	10.0 ± 1.0	11.3 ± 2.2
Number of horses out of RR	$n = 2/6$	$n = 0/6$	$n = 1/6$	$n = 2/6$
CREA (0.9–1.5 mg/dL)	1.0 ± 0.1	1.1 ± 0.2	1.0 ± 0.1	1.0 ± 0.1
Number of horses out of RR	$n = 0/6$	$n = 1/6$	$n = 1/6$	$n = 1/6$
GGT (10–50 IU/L)	22.3 ± 2.9	23.5 ± 4.8	23.0 ± 2.4	20.5 ± 3.3
Number of horses out of RR	$n = 0/6$	$n = 0/6$	$n = 0/6$	$n = 0/6$
GOT (165–358 IU/L)	290.2 ± 38.6	298.0 ± 47.5	288.8 ± 29.7	295.7 ± 21.8
Number of horses out of RR	$n = 0/6$	$n = 1/6$	$n = 0/6$	$n = 0/6$

The number of horses in each group with serum levels outside of RR are also reported. RR, reference range; WBC, white blood cell; BUN, blood urea nitrogen; CREA, creatinine; GGT, gamma-glutamyltransferase; GOT, glutamic oxaloacetic transaminase.

3.2. Pharmacokinetic analysis

3.2.1. Non-compartmental analysis

3.2.1.1. Dose escalation study

Concentration curves with mean ± standard deviations of CBD and its main metabolites 7-COOH-CBD and 7-OH-CBD in serum and urine are shown in Figure 1. In the first trial (dose: 0.2 mg/kg), CBD and 7-COOH-CBD were found in serum and CBD and 7-OH-CBD were found in urine. In the second trial (dose: 1 mg/kg), CBD, 7-OH-CBD and 7-COOH-CBD were identified in serum, but 7-OH-CBD remained below the LLOQ. CBD, 7-OH-CBD, CBDA, CBDV and CBG were detected in urine with CBDA levels being below the LLOQ (Supplementary Figure S1). In the third trial (dose: 3 mg/kg), CBD, 7-OH-CBD and 7-COOH-CBD were identified in serum. In urine, CBD, 7-OH-CBD, CBDA, CBDV and CBG were detected (Figure 1; Supplementary Figure S1). CBDA levels were again below LLOQ. Table 3 presents the parameters $AUC_{0-12\,h}$, C_{max} and t_{max} assessed in the NCA and the $AUC_{0-12\,h}$ ratio between CBD and its metabolites 7-OH-CBD and 7-COOH-CBD. C_{max} and t_{max} could not be determined for 7-COOH-CBD, as the concentration curves have not decreased sufficiently by time point 12 h (Figure 1).

3.2.1.2. Multiple dose study

CBD, 7-OH-CBD, 7-COOH-CBD, CBDV, THC and OH-THC were identified in serum. 7-OH-CBD concentrations were below the LLOQ from 60 h after last CBD administration onwards (Figure 2). CBDV and THC were detected in concentrations around the LLOQ throughout the trial [C_{max}(CBDV) = 0.39 ng/mL; C_{max}(THC) = 0.70 ng/mL]. CBDV and THC values were below the LLOQ at 4 h and 12 h

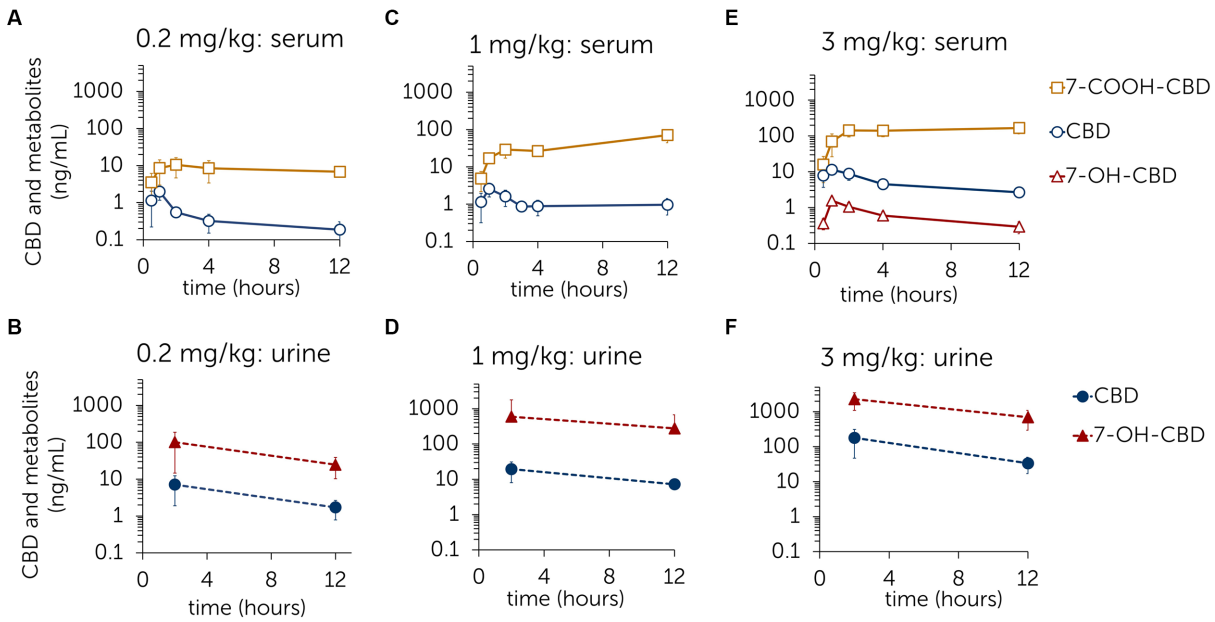

FIGURE 1
Mean ± standard deviation of serum and urine concentrations of cannabidiol (CBD) and the metabolites 7-hydroxy-cannabidiol (7-OH-CBD) and 7-carboxy-cannabidiol (7-COOH-CBD) after single oral administration of CBD paste in three different doses [0.2 mg/kg **(A,B)**; 1 mg/kg **(C,D)**; 3 mg/kg **(E,F)**].

TABLE 3 Mean ± standard deviation of pharmacokinetic parameters for CBD and metabolites following single oral administrations of CBD paste during dose escalation study, derived from NCA.

Parameter	First trial (0.2 mg/kg, *n* = 3)	Second trial (1 mg/kg, *n* = 3)	Third trial (3 mg/kg, *n* = 5)
CBD			
AUC_{0-12h} (h·ng/mL)	4.45 ± 2.52	15.46 ± 6.08	59.53 ± 13.54
C_{max} (ng/mL)	1.98 ± 0.99	2.58 ± 1.25	12.17 ± 2.08
t_{max} (hr)	1 ± 0	1 ± 0	1.1 ± 0.55
7-COOH-CBD			
AUC_{0-12h} (h·ng/mL)	106.95 ± 65.68	571.02 ± 194.33	1768.38 ± 450.86
Ratio: $\frac{AUC_{0-12h}(7-COOH-CBD)}{AUC_{0-12h}(CBD)}$	21.09 ± 3.19 (2109.15%)	38.78 ± 7.82 (3877.88%)	31.02 ± 6.38 (3102.13%)
7-OH-CBD			
AUC_{0-12h} (h·ng/mL)	—	—	6.62 ± 1.86
Ratio: $\frac{AUC_{0-12h}(7-OH-CBD)}{AUC_{0-12h}(CBD)}$	—	—	0.10 ± 0.03 (10.23%)
C_{max} (ng/mL)	—	—	1.42 ± 0.37
t_{max} (hr)	—	—	1.4 ± 0.55

NCA, non-compartmental analysis; CBD, cannabidiol; 7-COOH-CBD, 7-carboxy-cannabidiol; 7-OH-CBD, 7-hydroxy-cannabidiol; AUC_{0-12h}, area under the serum concentration-time curve (from time point 0 to 12 h); C_{max}, maximum concentration; t_{max}, time of maximum concentration.

after last CBD administration. OH-THC concentrations remained mostly below the LLOQ except for the time points 202.5 h (0.26 ng/mL) and 314 h (0.27 ng/mL) (Supplementary Figure S2).

In urine, CBD, 7-OH-CBD, CBDA, CBDV and CBG were identified. CBDA concentrations fell below the LLOQ 36.5 h after the last CBD administration. CBG and CBDV values remained below the LLOQ 131 h and 248 h after the last CBD administration, respectively (Figure 2; Supplementary Figure S2).

The terminal half-life for CBD and 7-COOH-CBD in serum was calculated based on the last six time points (132–360 h) after the last CBD administration. For CBD, the terminal half-life was 161.29 ± 43.65 h and for 7-COOH-CBD, it was 79.85 ± 18.03 h.

3.2.2. Population pharmacokinetic analysis

A three-compartment model best described the pharmacokinetic properties of CBD in horses. Residual error was described through a

combined 1 error model, containing a constant and proportional term. Numerical and graphical outputs were evaluated for GOF and predictive power. Diagnostic plots are shown in Figures 3–6. The visual predictive check (VPC) shows close prediction of median values (Figure 4). Empirical data for the 10th and 90th percentile are deviating from their respective confidence intervals (CI) at around

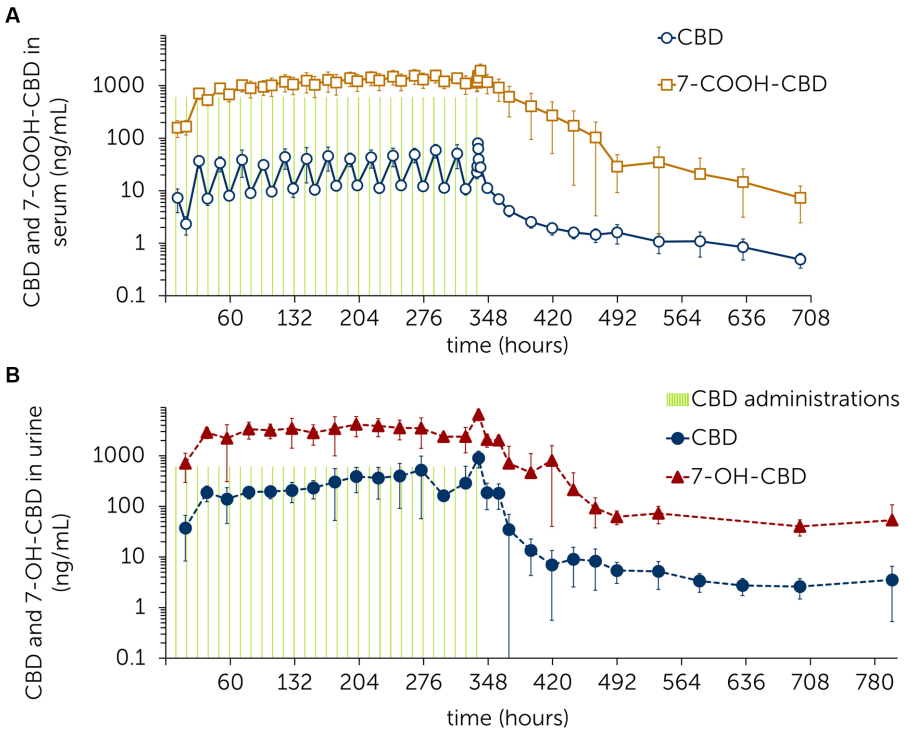

FIGURE 2
Mean ± standard deviation of serum **(A)** and urine **(B)** concentrations of cannabidiol (CBD) and the metabolites 7-hydroxy-cannabidiol (7-OH-CBD) and 7-carboxy-cannabidiol (7-COOH-CBD) following multiple administrations of CBD paste (3 mg/kg po) twice daily over 2 weeks with subsequent sample collection.

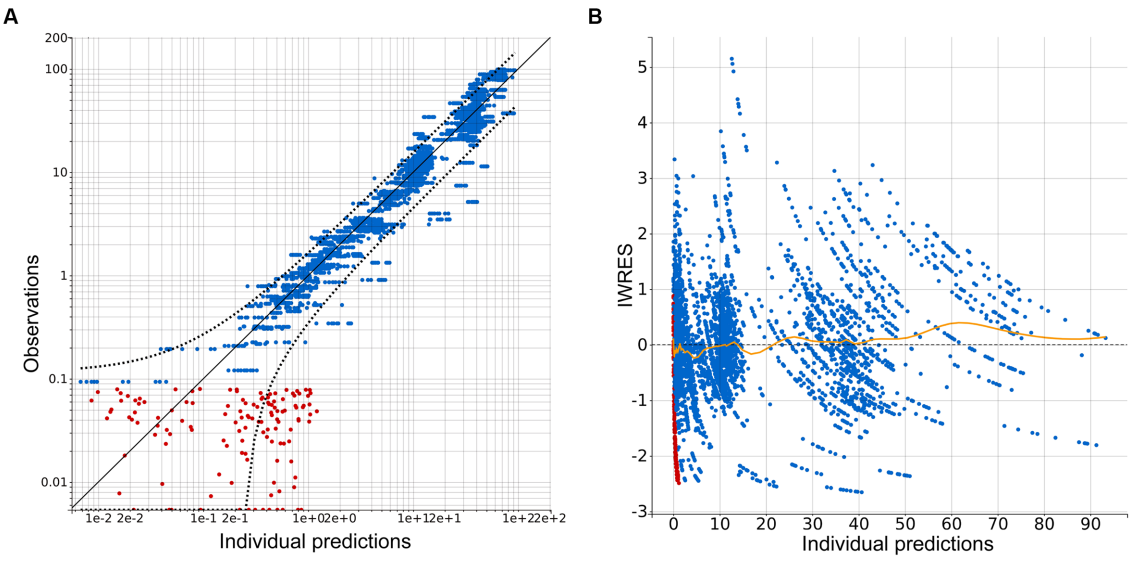

FIGURE 3
Diagnostic plots extracted from the three-compartment model following population pharmacokinetic analysis. **(A)** Plot of observations vs. individual predictions. Blue dots indicate observations, red dots indicate censored data, black line—identity line; dotted black line represents the 90% prediction interval. Outliers proportion was 10.54%. **(B)** Scatterplot of individual weighted residuals (IWRES) vs. individual predictions. Blue dots indicate observations, red dots indicate censored data, spline is marked with a yellow line.

220 h and 350 h, respectively. Exemplary graphs depicting individual predictions are presented in Figure 5.

Inter-occasion variability (IOV) was not included as it was similar to the individual variability and, due to the relatively small number of subjects, led to a low precision of estimates. Profiles were therefore treated as separate individuals. Random effects were estimated for Cl/F, V1/F, Q3 and V3/F. For the other parameters, the population value was used as the random effects were converging to zero and were insufficiently assessed in all individuals. Correlating V3/F and Q3 further improved the fit of the model (Figure 6).

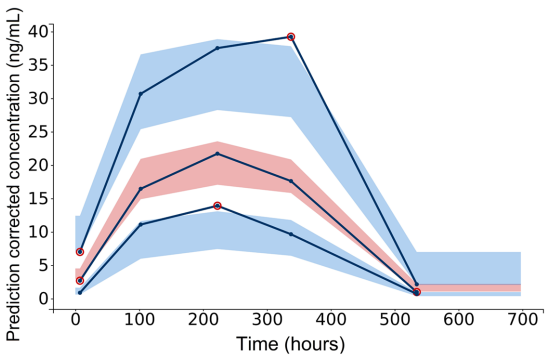

FIGURE 4
Diagnostic plot extracted from the three-compartment model following population pharmacokinetic analysis: visual predictive check for CBD concentrations in serum. Empirical data [10th, 50th (median) and 90th percentile] are marked by solid lines. Outlier dots are circled in red. Shaded areas mark the 90% confidence intervals for corrected prediction of the median (red) and the 10th and 90th percentile (blue).

Table 4 presents the final pharmacokinetic parameters derived through the population pharmacokinetic approach. The low relative standard error (RSE) values confirm accurate assessment for the population parameter estimates. The low eigenvalue ratio (29.07, derived from the Fisher information matrix) and low shrinkage (< 20%, see Table 4) indicate that the model was not over-parameterized. The values for volume of distribution in the central (V1/F) and peripheral compartments (V2/F and V3/F) suggest a very high distribution of CBD as well as retention in tissues. The estimation of convergence accounts for the model's robustness.

Bodyweight as an added covariate did not show any effect on the pharmacokinetic parameters and was excluded from the final model.

AUC_{0-12h} as an additional output and C_{max} and t_{max} (extracted from individual fits) are presented in Table 5. Values are shown in relation to the parameters derived from the NCA (Table 3).

To calculate the accumulation ratio (AR), C_{max} from each day of the multiple dose study was summarized to a mean of 38.39 ± 8.89 ng/mL. Mean C_{max} from trial 3 of the dose escalation study was 14.61 ± 5.08 ng/mL. AR was therefore 2.63.

3.2.3. Dose proportionality

The power model equation revealed the β value for the NCA parameter AUC_{0-12h} to be 0.99 and for C_{max} to be 0.72. For the population pharmacokinetic parameters, the β value for AUC_{0-12h} was 0.93 and 0.80

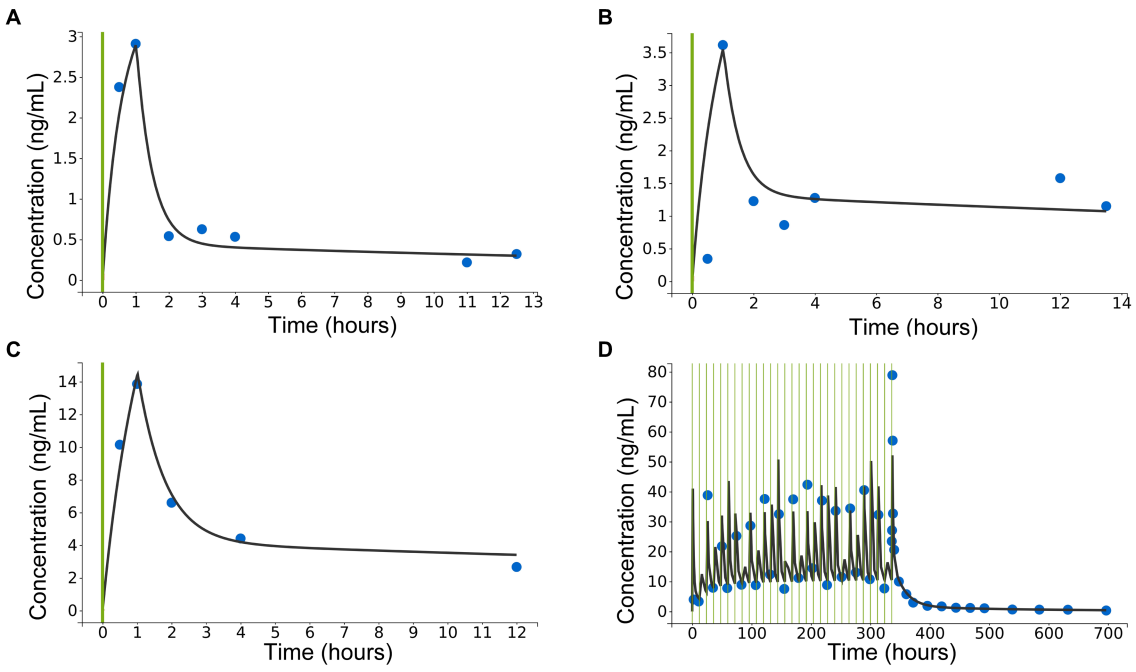

FIGURE 5
Diagnostic plots extracted from the three-compartment model following population pharmacokinetic analysis: exemplary individual predictions for concentrations of cannabidiol (CBD) in serum after single oral administration of CBD paste in three different doses [**(A)**: 0.2 mg/kg po; **(B)**: 1 mg/kg po; **(C)**: 3 mg/kg po], and **(D)**: following multiple administrations of CBD paste (3 mg/kg po) twice daily over 2 weeks with subsequent sample collection. Green lines represent CBD administrations, blue dots are observed data points and black lines are individual fits.

for C_{max}. As the individual values were pooled for this approach, the inter-individual variability through a CI was not determined.

An ANOVA with a post-hoc Tukey test identified a significant difference between the dose-normalized C_{max} obtained from NCA between trial 1 (0.2 mg/kg) and trial 2 (1 mg/kg) ($p = 0.014$). Trials 2 and 3 (3 mg/kg), and trials 1 and 3 showed no statistically significant differences ($p = 0.334$, $p = 0.123$). Similarly, there were no statistically significant differences between the other pharmacokinetic parameters.

3.3. Application to medication control

Between 60 to 360 h after the last CBD administration in the multiple dose study, a pseudo-equilibrium condition was reached (Figure 7) (47, 48). The steady-state urine to serum concentration ratio (Rss) was calculated from the mean concentration values: Rss = 4.45 ± 1.04.

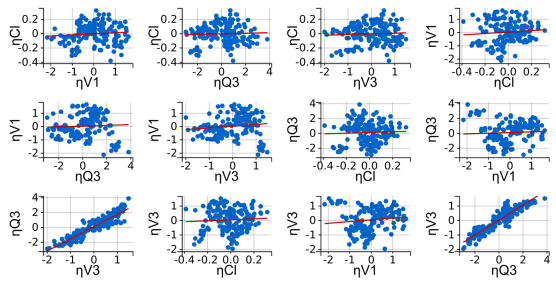

FIGURE 6
Diagnostic plot extracted from the three-compartment model following population pharmacokinetic analysis: Correlation plots of the random effects (η_i). Correlation was applied when correlation coefficients were estimated to be high and met the threshold for inclusion (Pearson's correlation test, $p < 0.05$). Linear regressions are presented as red lines.

4. Discussion

Investigation of the pharmacokinetic properties of CBD following repeated oral administration identified a rapid increase of the CBD serum concentration with an extended elimination phase of CBD and its metabolites. These findings indicate an extensive metabolism of CBD with prolonged tissue retention.

The oral administration of CBD paste was well-tolerated by all horses in the current study and side effects such as gastrointestinal intolerance were not observed. A previous study reported mildly elevated liver enzymes after multiple oral administrations of a CBD-infused oil (1 mg/kg and 3 mg/kg) in horses (30). Another study reported decreased creatinine levels and higher gamma-glutamyltransferase levels, although still within normal reference range (49). In this study, only occasional, slight shifts out of RR without associated clinical signs were observed in WBC count, kidney and liver biomarkers.

Like in other equine and small animal investigations, the pharmacokinetic analysis showed a rapid increase of CBD in serum following oral administration (15, 16, 28, 29, 50–55). The values for C_{max} were similar to those calculated in other studies (28–31, 33). In contrast, the AUC_{0-12h} values obtained here differ significantly. This is caused by the fact that in the previous studies AUC were determined over longer time periods (up to 264 h) (28–31, 33). The AUC_{0-12h} values reported for the single dose part of the current study are much lower as the time dimension of this parameter is terminated at 12 h. It was not possible to credibly determine relative bioavailability for the used formulation. This would require calculating $AUC_{0-\infty}$ and compare it with the results of previously published studies. As for the single dose administration, the terminal portion of the curve was not sufficiently captured to assess $AUC_{0-\infty}$.

A long elimination phase for CBD was shown during the multiple dose study (Figure 2). Based on the visual inspection of the individual log-linear concentration-time profiles, the terminal phase of elimination started approx. 132 h after the last CBD administration. Therefore, only the following data-points were used for the calculation of the elimination half-life. As previous studies have

TABLE 4 Population pharmacokinetic parameters of orally administered CBD paste in four different equine trials.

	Population value	SE	RSE (%)	Omega	SE	RSE (%)	Shrinkage (%)
Population parameter estimates (unit)							
Tk0 (h)	1.02	0.11	10.5	—	—	—	—
Cl/F (L/h/kg)	10.75	0.7	6.53	0.15	0.049	33.6	15.1
V1/F (L/kg)	77.13	20.11	26.1	0.83	0.18	22.1	2.27
Q2 (L/h/kg)	1.35	0.14	10.2	—	—	—	—
V2/F (L/kg)	313.17	50.63	16.2	—	—	—	—
Q3 (L/h/kg)	38.23	15.72	41.1	1.48	0.47	31.8	9.11
V3/F (L/kg)	241.98	67.77	28.0	0.85	0.24	28.0	12.9
Residual error							
a	0.07	0.021	29.8	—	—	—	—
b	0.33	0.016	5.04	—	—	—	—

Data derived from three separate trials with single doses of 0.2 mg/kg (administered to $n = 3$ horses), 1 mg/kg ($n = 3$) and 3 mg/kg ($n = 5$) and a multiple dose study with a dose of 3 mg/kg administered twice daily over 15 days ($n = 6$). CBD, cannabidiol; SE, standard error; RSE, relative standard error, Tk0, duration of the zero-order absorption; Cl/F, total body clearance; V1/F, volume of distribution in the central compartment; V2/F, volume of distribution in the first peripheral compartment; V3/F, volume of distribution in the second peripheral compartment; Q2, clearance between V1 and V2; Q3, clearance between V1 and V3; F, bioavailability.

TABLE 5 Mean ± standard deviation of pharmacokinetic parameters for CBD and metabolites following single oral administrations of CBD paste during the dose escalation study, derived from the individual fits of the population pharmacokinetic model.

	First trial (0.2 mg/kg, n = 3)	Second trial (1 mg/kg, n = 3)	Third trial (3 mg/kg, n = 5)
AUC_{0-12h} (h·ng/mL)	4.99 ± 1.56	13.64 ± 5.33	58.56 ± 12.98
C_{max} (ng/mL)	1.82 ± 0.83	3.10 ± 1.27	14.61 ± 5.08
t_{max} (hr)	1.01 ± 0.01	1.02 ± 0.03	1.02 ± 0.01
Ratio: $\frac{\text{Parameter}(CBD_{Pop\_PK})}{\text{Parameter}(CBD_{NCA})}$			
$\frac{AUC_{0-12h}(CBD_{Pop\_PK})}{AUC_{0-12h}(CBD_{NCA})}$	1.20 ± 0.24	0.86 ± 0.11	0.98 ± 0.09
$\frac{C_{max}(CBD_{Pop\_PK})}{C_{max}(CBD_{NCA})}$	0.92 ± 0.08	1.21 ± 0.21	1.18 ± 0.29
$\frac{t_{max}(CBD_{Pop\_PK})}{t_{max}(CBD_{NCA})}$	1.01 ± 0.01	1.02 ± 0.03	1.12 ± 0.52

Values are presented as ratios to the parameters derived from the non-compartmental analysis (Table 3). CBD, cannabidiol; AUC_{0-12h}, area under the serum concentration-time curve from time point 0 to 12 h; C_{max}, maximum concentration, t_{max}, time of maximum concentration; NCA parameters, parameters derived from non-compartmental analysis; CBD_{Pop_PK}, parameter for CBD derived through population pharmacokinetics; CBD_{NCA}, parameter for CBD derived through non-compartmental analysis.

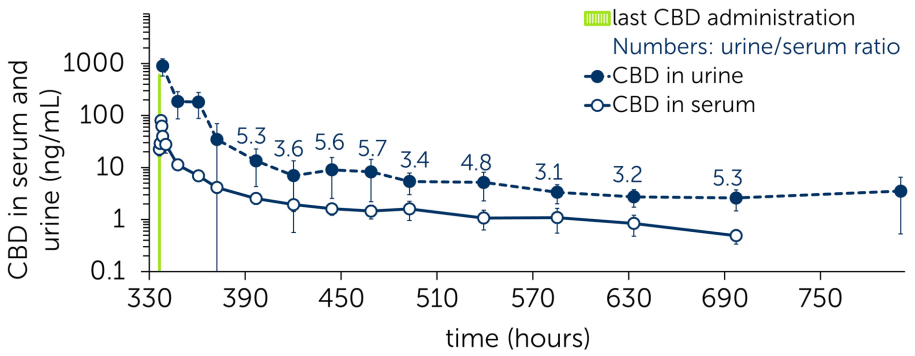

FIGURE 7
Mean ± standard deviation of serum and urine concentrations of cannabidiol (CBD) during the elimination phase. Last CBD administration (dose: 3 mg/kg) to six horses at time point 336 h following multiple administrations of CBD paste (3 mg/kg po) twice daily over 2 weeks. Numbers present the urine/serum ratio between respective time points.

derived the terminal half-life from earlier time points, values are difficult to compare (28–31, 33). The very long elimination phase of CBD suggests a high volume of distribution into different tissue compartments.

Previous studies hypothesized, that CBD is subject to a high first pass effect with a considerable pre-systemic metabolism in the liver (29, 33, 56). The extensive metabolism of CBD into 7-COOH-CBD is mirrored by the high ratio of their AUC_{0-12h} (Table 3). In comparison, the AUC_{0-12h} ratio between CBD and 7-OH-CBD is substantially lower. To the best of the authors knowledge, research detailing the exact steps of CBD metabolism in horses is currently not available. In humans, 7-OH-CBD is further metabolized to 7-COOH-CBD (57, 58). Based on this information, the low serum value of 7-OH-CBD in the current study may be explained by the partial metabolism into 7-COOH-CBD. In line with other reports, higher concentrations of 7-OH-CBD were detected in urine (29). Further research investigating the exact metabolic pathway of CBD in horses following oral administration would be of great interest.

For data derived from the NCA and the population pharmacokinetic approach, CBD ratios for AUC_{0-12h}, C_{max} and t_{max} were close to 1, confirming that the individual fits calculated in the NLME model are close to the actual concentrations measured (Table 5).

Values for volumes of distribution and clearance [both over bioavailability (F)] were derived through the population pharmacokinetic analysis. Although the study design did not

include intravenous administration to precisely estimate the true clearance and volumes of distribution, the application of NLME modelling allowed the pooling of data into a single robust model, despite different study designs (single vs. multiple administrations) and dose levels. Volumes of distribution over F were high in the central and the two peripheral compartments (Table 4). Other studies in horses and dogs describe similar values based on non-compartmental analysis, even though doses and study protocols differ slightly (28, 51). Values are especially high for V2/F and V3/F in the current study, suggesting a very high distribution and tissue retention of CBD. This observation is further supported by the low inter-compartmental clearance value Q2 (1.35 L/h/kg) between V1 and V2. One reason might be the lipophilic properties of CBD, as confirmed by several canine and human studies (5, 10, 38). The high volumes of distribution could however be misleading, as the population pharmacokinetic model does not account for the extensive metabolism of CDB to 7-COOH-CBD. The authors chose to exclude the additional metabolite data out of the NLME modelling, as its inclusion and the subsequent classification of CBD as a parent drug did not produce a satisfying and stable model. The relatively small sample size and the lack of data for intravenous administration necessitated the choice of a simpler but much more stable model that met all the goodness-of-fit criteria.

The estimated clearance value of 10.75 L/h/kg is comparable to one study (33), but lower than the results from other equine studies that were also obtained using oral data with an unknown F (29, 30). Comparing clearance values with those from other species proved to be difficult, as very few reports exist and values are declared in L/h instead of L/h/kg (51, 56). One study reports a very high variance for clearance of CBD and its metabolites in dogs (59).

Considering all species, only few reports compare oral and intravenous administrations of CBD to calculate *F*. *F* has been described to be 7.92% and 14% in horses, putting it in a similar range with findings in humans (6%) and dogs (13%–22.28%) (31, 33, 51, 56, 60). The low *F* values further confirm the high first-pass-effect of CBD with extensive pre-systemic metabolism and a high liver extraction ratio, as described in humans (72%) (29, 56).

The visual predictive check of the population pharmacokinetic analysis shows good agreement with the median values, but there is a noticeable deviation of the 10th and the 90th percentile's empirical data from the 10% and 90% CI at approximately 220 and 350 h after the first CBD administration (Figure 4). These deviations are likely caused by the differing concentration values of CBD in serum in one horse. This particular horse showed consistently higher values than the median. This may have been caused by interindividual variability or over-dosing of the CBD paste due to variation of the horse's bodyweight. The authors decided not to exclude this horse from the dataset, as the other values were not affected by the described deviation. Moreover, such high variability in the internal exposure is not uncommon for drugs with low bioavailability, therefore the authors believe that this dataset may reflect the real-life situation well.

As the CBD product used in this study was extracted from the cannabis plant (*Cannabis sativa*), further phytocannabinoids were identified during the serum and urine analysis. Values for CBDV and THC in serum were very low throughout the study and reached levels just above LLOQ. In urine, CBDV and CBG were detected in higher concentrations. There is very little information available on the potential effects of these phytocannabinoids. One study reports CBDV to have an anti-convulsant effect in mice and rats (61). CBG's influence on pain perception has been tested in mouse models (62, 63) and its pharmacokinetic properties have recently been described in dogs (64). Another study showed that CBG decreases the intraocular pressure in cats (65). The potential therapeutic use of CBG for the treatment of human diseases like multiple sclerosis has additionally been suggested (66).

During the multiple dose study, the steady state for CBD was reached at day 2 (Figure 2). The accumulation ratio (AR) under steady state for CBD in serum was 2.63. In humans, an AR of 2–5 is considered to indicate moderate drug accumulation (67). The time it takes to eliminate CBD from the bloodstream is therefore moderately long compared to the dosing interval (12 h). This observation might be helpful in establishing dosing patterns or time points for maximum efficacy. Concentration values in urine are less stable but are also showing fair consistency from day 2 onwards. As urine samples were collected as spot samples, values must be evaluated with caution.

The dose proportionality evaluated with an ANOVA did not identify any statistically significant differences in the dose-normalized parameters between trials, except for C_{max} obtained from the NCA between trial 1 (dose: 0.2 mg/kg) and 2 (dose: 1 mg/kg). Since C_{max} between trial 1 and trial 3 (dose: 3 mg/kg), and trials 2 and 3 did not differ significantly, this variability might be explained in part by the low bioavailability and small sample size in the dose escalation study. In the power model, C_{max} from the NCA had the lowest β value (0.72), confirming the variability and therefore possible lack of proportionality as seen in the ANOVA. β values for $AUC_{0-12\,h}$ were very close to 1, suggesting that CBD administered as a paste within the studied dose range leads to a dose proportional exposure with the extent of absorption remaining unchanged. On the other hand, the rate of absorption appears to decrease with higher doses as the increase for C_{max} becomes less linear (exemplified by the comparatively small β values). This observation may further support the choice of zero-order absorption as a model parameter in the population pharmacokinetic analysis. However, the small number of individuals within the specific dose groups and the high variability in exposure reduce the statistical significance of these results.

Graphical illustration shows that CBD concentrations in serum and urine achieve a pseudo-equilibrium condition during the elimination phase (Figure 7) (48). The values exemplify that CBD concentrations detected in serum can be translated to residual concentrations in urine by the calculated Rss. Whether these residual concentrations influence a horse's performance and must be subject to medication control, remains unclear. Specific cut-off values for a drug can be defined through a nonexperimental approach, where irrelevant drug plasma concentrations (IPC) and irrelevant drug urine concentrations (IUC) are calculated (47). IPC and IUC are based on the average effective plasma concentration (EPC), which is derived from the standard dose (per dosing interval) and bioavailability. As no standard dose with a proven effect for CBD in horses has been defined so far, EPC, IPC and IUC were not calculated in the current study.

Limitations of the study include the lacking assessment of the inter-occasion variability (IOV) due to the small sample size and testing of only one CBD product through only one route of administration. Further studies may evaluate varying CBD doses administered intravenously to obtain precise estimates for clearance, volumes of distribution and bioavailability, and to gain a better understanding of CBD's metabolism.

5. Conclusion

This study confirms the extensive metabolism of CBD and suggests a prolonged retainment in tissues resulting in the extended elimination phase of CBD and its metabolites. The oral administration of CBD paste proved to be well-tolerated and did not cause any side effects at a maximum dose of 3 mg/kg following oral administrations twice daily over 2 weeks. A population pharmacokinetic model pooling data from both single and multiple dose studies has been successfully developed. Whilst the steady-state urine to serum concentration ratio (Rss) was defined, future research analyzing the effect of CBD on behavioral parameters and anti-inflammatory responses are required. Once an effective therapeutic dose is established, specific cut-off values for medication control may be established further. Until then, the administration of CBD products to sport horses should be treated with caution.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by the competent authority for licensing and notification procedures for animal experiments (LAVG) in Brandenburg, Germany (AZ: 2347-12-2021).

Author contributions

FE and AE were involved in all parts of the project. CL, WB, MM, and IS contributed to study design, planning of the project, and data analysis. FE and NB were responsible for study execution including animal handling and data collection. AW performed the drug assays under supervision of MT, MM, and IS. BP and FE performed the pharmacokinetic analyses. FE wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1234551/full#supplementary-material

References

1. Whiting PF, Wolff RF, Deshpande S, Di Nisio M, Duffy S, Hernandez AV, et al. Cannabinoids for medical use: a systematic review and meta-analysis. *JAMA*. (2015) 313:2456–73. doi: 10.1001/jama.2015.6358

2. Ebbert JO, Scharf EL, Hurt RT. Medical cannabis. *Mayo Clin Proc*. (2018) 93:1842–7. doi: 10.1016/j.mayocp.2018.09.005

3. Kogan L, Schoenfeld-Tacher R, Hellyer P, Rishniw M. US veterinarians' knowledge, experience, and perception regarding the use of cannabidiol for canine medical conditions. *Front Vet Sci*. (2018) 5:338. doi: 10.3389/fvets.2018.00338

4. Kogan LR, Hellyer PW, Silcox S, Schoenfeld-Tacher R. Canadian dog owners' use and perceptions of cannabis products. *Can Vet J*. (2019) 60:749–55.

5. Fernández-Trapero M, Pérez-Díaz C, Espejo-Porras F, de Lago E, Fernández-Ruiz J. Pharmacokinetics of Sativex® in dogs: towards a potential cannabinoid-based therapy for canine disorders. *Biomolecules*. (2020) 10:279. doi: 10.3390/biom10020279

6. Mechoulam R, Hanuš LO, Pertwee R, Howlett AC. Early phytocannabinoid chemistry to endocannabinoids and beyond. *Nat Rev Neurosci*. (2014) 15:757–64. doi: 10.1038/nrn3811

7. Pertwee RG. Cannabinoid pharmacology: the first 66 years. *Br J Pharmacol*. (2006) 147:S163–71. doi: 10.1038/sj.bjp.0706406

8. Mechoulam R, Peters M, Murillo-Rodriguez E, Hanus LO. Cannabidiol-recent advances. *Chem Biodivers*. (2007) 4:1678–92. doi: 10.1002/cbdv.200790147

9. Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol Sci*. (2009) 30:515–27. doi: 10.1016/j.tips.2009.07.006

10. Lucas CJ, Galettis P, Schneider J. The pharmacokinetics and the pharmacodynamics of cannabinoids. *Br J Clin Pharmacol*. (2018) 84:2477–82. doi: 10.1111/bcp.13710

11. Pacher P, Mukhopadhyay P, Mohanraj R, Godlewski G, Bátkai S, Kunos G. Modulation of the endocannabinoid system in cardiovascular disease: therapeutic potential and limitations. *Hypertension*. (2008) 52:601–7. doi: 10.1161/HYPERTENSIONAHA.105.063651

12. Mackie K, Stella N. Cannabinoid receptors and endocannabinoids: evidence for new players. *AAPS J*. (2006) 8:E298–306. doi: 10.1007/BF02854900

13. Genovese T, Cordaro M, Siracusa R, Impellizzeri D, Caudullo S, Raffone E, et al. Molecular and biochemical mechanism of cannabidiol in the management of the inflammatory and oxidative processes associated with endometriosis. *Int J Mol Sci*. (2022) 23:23. doi: 10.3390/ijms23105427

14. Gugliandolo E, Licata P, Peritore AF, Siracusa R, D'Amico R, Cordaro M, et al. Effect of cannabidiol (CBD) on canine inflammatory response: an *ex vivo* study on LPS stimulated whole blood. *Vet Sci*. (2021) 8:185. doi: 10.3390/vetsci8090185

15. Bartner LR, McGrath S, Rao S, Hyatt LK, Wittenburg LA. Pharmacokinetics of cannabidiol administered by 3 delivery methods at 2 different dosages to healthy dogs. *Can J Vet Res*. (2018) 82:178–83.

16. Gamble L-J, Boesch JM, Frye CW, Schwark WS, Mann S, Wolfe L, et al. Pharmacokinetics, safety, and clinical efficacy of cannabidiol treatment in osteoarthritic dogs. *Front Vet Sci*. (2018) 5:165. doi: 10.3389/fvets.2018.00165

17. Vaughn DM, Paulionis LJ, Kulpa JE. Randomized, placebo-controlled, 28-day safety and pharmacokinetics evaluation of repeated oral cannabidiol administration in healthy dogs. *Am J Vet Res*. (2021) 82:405–16. doi: 10.2460/ajvr.82.5.405

18. Potschka H, Bhatti SF, Tipold A, McGrath S. Cannabidiol in canine epilepsy. *Vet J*. (2022) 290:105913. doi: 10.1016/j.tvjl.2022.105913

19. Corsato Alvarenga I, Panickar KS, Hess H, McGrath S. Scientific validation of cannabidiol for management of dog and cat diseases. *Annu Rev Anim Biosci*. (2023) 11:227–46. doi: 10.1146/annurev-animal-081122-070236

20. Miranda-Cortés A, Mota-Rojas D, Crosignani-Outeda N, Casas-Alvarado A, Martínez-Burnes J, Olmos-Hernández A, et al. The role of cannabinoids in pain modulation in companion animals. *Front. Vet. Sci*. (2022) 9:1050884. doi: 10.3389/fvets.2022.1050884

21. Mogi C, Yoshida M, Kawano K, Fukuyama T, Arai T. Effects of cannabidiol without delta-9-tetrahydrocannabinol on canine atopic dermatitis: a retrospective assessment of 8 cases. *Can Vet J*. (2022) 63:423–6.

22. Loewinger M, Wakshlag JJ, Bowden D, Peters-Kennedy J, Rosenberg A. The effect of a mixed cannabidiol and cannabidiolic acid based oil on client-owned dogs with atopic dermatitis. *Vet Dermatol*. (2022) 33:329–e77. doi: 10.1111/vde.13077

23. McGrath S, Bartner LR, Rao S, Packer RA, Gustafson DL. Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. *J Am Vet Med Assoc*. (2019) 254:1301–8. doi: 10.2460/javma.254.11.1301

24. Ellis KL, Contino EK. Treatment using cannabidiol in a horse with mechanical allodynia. *Equine Vet Educ*. (2021) 33:33. doi: 10.1111/eve.13168

25. McIver VC, Tsang AS, Symonds NE, Perkins NR, Uquillas E, Dart CM, et al. Effects of topical treatment of cannabidiol extract in a unique manuka factor 5 manuka honey carrier on second intention wound healing on equine distal limb wounds: a preliminary study. *Aust Vet J*. (2020) 98:250–5. doi: 10.1111/avj.12932

26. Cunha RZ, Felisardo LL, Salamanca G, Marchioni GG, Neto OI, Chiocchetti R. The use of cannabidiol as a novel treatment for oral stereotypic behaviour (crib-biting) in a horse. *Vet Anim Sci*. (2023) 19:100289. doi: 10.1016/j.vas.2023.100289

27. Landa L, Trojan V, Demlova R, Jurica J, Hrib R. Cannabidiol and the possibilities of its use in veterinary medicine of dogs and horses: a brief review. *Vet Med*. (2022) 67:455–62. doi: 10.17221/127/2021-VETMED

28. Williams MR, Holbrook TC, Maxwell L, Croft CH, Ientile MM, Cliburn K. Pharmacokinetic evaluation of a cannabidiol supplement in horses. *J Equine Vet*. (2022) 110:103842. doi: 10.1016/j.jevs.2021.103842

29. Ryan D, McKemie DS, Kass PH, Puschner B, Knych HK. Pharmacokinetics and effects on arachidonic acid metabolism of low doses of cannabidiol following oral administration to horses. *Drug Test Anal*. (2021) 13:1305–17. doi: 10.1002/dta.3028

30. Yocom AF, O'Fallon ES, Gustafson DL, Contino EK. Pharmacokinetics, safety, and synovial fluid concentrations of single- and multiple-dose Oral administration of 1 and 3 mg/kg cannabidiol in horses. *J Equine Vet*. (2022) 113:103933. doi: 10.1016/j.jevs.2022.103933

31. Turner SE, Knych HK, Adams AA. Pharmacokinetics of cannabidiol in a randomized crossover trial in senior horses. *Am J Vet Res*. (2022) 83:83. doi: 10.2460/ajvr.22.02.0028

32. Draeger AL, Hoffman LK, Godwin PR, Davis AJ, Porr SA. Pharmacokinetics of a single feeding of pelleted cannabidiol in horses. *Steeplechase*. (2020):4:1. Available at: https://digitalcommons.murraystate.edu/steeplechase/vol4/iss2/1

33. Sánchez de Medina A, Serrano-Rodríguez JM, Díez de Castro E, García-Valverde MT, Saitua A, Becero M, et al. Pharmacokinetics and oral bioavailability of cannabidiol in horses after intravenous and oral administration with oil and micellar formulations. *Equine Vet J*. (2023). doi: 10.1111/evj.13923

34. Mercer MA, Davis JL. Cannabinoids in veterinary medicine: is there evidence to support the trend? *Equine Vet Educ*. (2021) 33:177–9. doi: 10.1111/eve.13199

35. Turner S, Knych HK, Adams AA. The effects of cannabidiol on immune function and health parameters in senior horses. *Vet Immunol Immunopathol*. (2023) 257:110549. doi: 10.1016/j.vetimm.2023.110549

36. Fédération Equestre Internationale. *FEI clean sport—prohibited substances database* (2022). Available at: https://inside.fei.org/content/anti-doping-rules

37. Deutsche Reiterliche Vereinigung. *Anti-Doping und Medikation im Pferdesport* (2022). Available at: https://www.pferd-aktuell.de/turniersport/anti-doping-und-medikation

38. Johansson E, Norén K, Sjövall J, Halldin MM. Determination of delta 1-tetrahydrocannabinol in human fat biopsies from marihuana users by gas chromatography-mass spectrometry. *Biomed Chromatogr*. (1989) 3:35–8. doi: 10.1002/bmc.1130030109

39. You Y, Proctor RM, Vasilko ED, Robinson MA. Doping control analysis of four JWH-250 metabolites in equine urine by liquid chromatography-tandem mass spectrometry. *Drug Test Anal*. (2019) 11:649–58. doi: 10.1002/dta.2542

40. Mareck U, Fusshöller G, Geyer H, Huestis MA, Scheiff AB, Thevis M. Preliminary data on the potential for unintentional antidoping rule violations by permitted cannabidiol (CBD) use. *Drug Test Anal*. (2021) 13:539–49. doi: 10.1002/dta.2959

41. Sheiner LB, Ludden TM. Population pharmacokinetics/dynamics. *Annu Rev Pharmacol Toxicol*. (1992) 32:185–209. doi: 10.1146/annurev.pa.32.040192.001153

42. Wang J, Schneider BK, Sun P, Gong X, Qiu J, Li J, et al. Nonlinear mixed-effects pharmacokinetic modeling of the novel COX-2 selective inhibitor vitacoxib in dogs. *J Vet Pharmacol Ther*. (2019) 42:530–40. doi: 10.1111/jvp.12802

43. Petkova T, Milanova A, Poźniak B. The effects of cyclosporine a or activated charcoal co-administration on the pharmacokinetics of enrofloxacin in chickens. *Poult Sci*. (2022) 102:102225. doi: 10.1016/j.psj.2022.102225

44. Pelligand L, Soubret A, King JN, Elliott J, Mochel JP. Modeling of large pharmacokinetic data using nonlinear mixed-effects: a paradigm shift in veterinary pharmacology. A case study with robenacoxib in cats. *CPT Pharmacometrics Syst Pharmacol*. (2016) 5:625–35. doi: 10.1002/psp4.12141

45. Gough K, Hutchison M, Keene O, Byrom B, Ellis S, Lacey L, et al. Assessment of dose proportionality: report from the statisticians in the pharmaceutical industry/pharmacokinetics UK joint working party. *Drug Inf J*. (1995) 29:1039–48. doi: 10.1177/009286159502900324

46. Hummel J, McKendrick S, Brindley C, French R. Exploratory assessment of dose proportionality: review of current approaches and proposal for a practical criterion. *Pharm Stat*. (2009) 8:38–49. doi: 10.1002/pst.326

47. Toutain PL, Lassourd V. Pharmacokinetic/pharmacodynamic approach to assess irrelevant plasma or urine drug concentrations in post competition samples for drug control in the horse. *Equine Vet J*. (2002) 34:242–9. doi: 10.2746/042516402776185985

48. Machnik M, Kaiser S, Koppe S, Kietzmann M, Schenk I, Düe M, et al. Control of methylxanthines in the competition horse: pharmacokinetic/pharmacodynamic studies on caffeine, theobromine and theophylline for the assessment of irrelevant concentrations. *Drug Test Anal*. (2017) 9:1372–84. doi: 10.1002/dta.2097

49. Leise JM, Leatherwood JL, Paris BL, Walter KW, George JM, Martinez RE, et al. Evaluation of an oral supplemental cannabidiol product for acceptability and performance in mature horses. *Animals*. (2023) 13:13. doi: 10.3390/ani13020245

50. Deabold KA, Schwark WS, Wolf L, Wakshlag JJ. Single-dose pharmacokinetics and preliminary safety assessment with use of CBD-rich hemp nutraceutical in healthy dogs and cats. *Animals*. (2019) 9:9. doi: 10.3390/ani9100832

51. Samara E, Bialer M, Mechoulam R. Pharmacokinetics of cannabidiol in dogs. *Drug Metab Dispos*. (1988) 16:469–72.

52. Tittle D, Wakshlag J, Schwark W, Lyubimov A, Zakharov A, Gomez B. Twenty-four hour and one-week steady state pharmacokinetics of cannabinoids in two formulations of cannabidiol and xannabidiolic acid rich hemp in dogs. *Med Res Arch*. (2022) 10:10. doi: 10.18103/mra.v10i7.2907

53. Wang T, Zakharov A, Gomez B, Lyubimov A, Trottier NL, Schwark WS, et al. Serum cannabinoid 24 h and 1 week steady state pharmacokinetic assessment in cats using a CBD/CBDA rich hemp paste. *Front. Vet. Sci*. (2022) 9:895368. doi: 10.3389/fvets.2022.895368

54. Della Rocca G, Paoletti F, Conti MB, Galarini R, Chiaradia E, Sforna M, et al. Pharmacokinetics of cannabidiol following single oral and oral transmucosal administration in dogs. *Front Vet Sci*. (2022) 9:1104152. doi: 10.3389/fvets.2022.1104152

55. Wakshlag JJ, Schwark WS, Deabold KA, Talsma BN, Cital S, Lyubimov A, et al. Pharmacokinetics of cannabidiol, cannabidiolic acid, Δ9-tetrahydrocannabinol, tetrahydrocannabinolic acid and related metabolites in canine serum after dosing with three oral forms of hemp extract. *Front. Vet. Sci*. (2020) 7:505. doi: 10.3389/fvets.2020.00505

56. Perucca E, Bialer M. Critical aspects affecting cannabidiol oral bioavailability and metabolic elimination, and related clinical implications. *CNS Drugs*. (2020) 34:795–800. doi: 10.1007/s40263-020-00741-5

57. Kicman A, Toczek M. The effects of cannabidiol, a non-intoxicating compound of cannabis, on the cardiovascular system in health and disease. *Int J Mol Sci*. (2020) 21:21. doi: 10.3390/ijms21186740

58. Beers JL, Fu D, Jackson KD. Cytochrome P450-catalyzed metabolism of cannabidiol to the active metabolite 7-hydroxy-cannabidiol. *Drug Metab Dispos*. (2021) 49:882–91. doi: 10.1124/dmd.120.000350

59. Chicoine A, Illing K, Vuong S, Pinto KR, Alcorn J, Cosford K. Pharmacokinetic and safety evaluation of various Oral doses of a novel 1:20 THC:CBD cannabis herbal extract in dogs. *Front Vet Sci*. (2020) 7:583404. doi: 10.3389/fvets.2020.583404

60. Polidoro D, Temmerman R, Devreese M, Charalambous M, van Ham L, Cornelis I, et al. Pharmacokinetics of cannabidiol following intranasal, intrarectal, and oral administration in healthy dogs. *Front. Vet. Sci.* (2022) 9:899940. doi: 10.3389/fvets.2022.899940

61. Hill AJ, Mercier MS, Hill TD, Glyn SE, Jones NA, Yamasaki Y, et al. Cannabidivarin is anticonvulsant in mouse and rat. *Br J Pharmacol*. (2012) 167:1629–42. doi: 10.1111/j.1476-5381.2012.02207.x

62. Borrelli F, Pagano E, Romano B, Panzera S, Maiello F, Coppola D, et al. Colon carcinogenesis is inhibited by the TRPM8 antagonist cannabigerol, a cannabis-derived non-psychotropic cannabinoid. *Carcinogenesis*. (2014) 35:2787–97. doi: 10.1093/carcin/bgu205

63. de Caro C, Cristiano C, Avagliano C, Bertamino A, Ostacolo C, Campiglia P, et al. Characterization of new TRPM8 modulators in pain perception. *Int J Mol Sci*. (2019) 20:20. doi: 10.3390/ijms20225544

64. Amstutz K, Schwark WS, Zakharov A, Gomez B, Lyubimov A, Ellis K, et al. Single dose and chronic oral administration of cannabigerol and cannabigerolic acid-rich hemp extract in fed and fasted dogs: physiological effect and pharmacokinetic evaluation. *J Vet Pharmacol Ther*. (2022) 45:245–54. doi: 10.1111/jvp.13048

65. Colasanti BK. A comparison of the ocular and central effects of delta 9-tetrahydrocannabinol and cannabigerol. *J Ocul Pharmacol*. (1990) 6:259–69. doi: 10.1089/jop.1990.6.259

66. Deiana S. "Potential medical uses of Cannabigerol: A brief Overview," In: *Preedy VR, editor. Handbook of Cannabis and Related Pathologies: Biology, Pharmacology, Diagnosis, and Treatment*. Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom: Elsevier Science (2017). 958–67.

67. Li L, Li X, Xu L, Sheng Y, Huang J, Zheng Q. Systematic evaluation of dose accumulation studies in clinical pharmacokinetics. *Curr Drug Metab*. (2013) 14:605–15. doi: 10.2174/13892002113149990002

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Therapeutic efficacy and pharmacokinetics of liposomal-cannabidiol injection: a pilot clinical study in dogs with naturally-occurring osteoarthritis

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Introduction: Osteoarthritis is a common disease in dogs resulting in chronic pain and decreased wellbeing. Common analgesics such as non-steroidal anti-inflammatories may fail to control pain and can produce major adverse effects. Study objectives were to evaluate pharmacokinetics, therapeutic efficacy, and safety of subcutaneous liposomal-cannabidiol (CBD) as an additional analgesic therapy in dogs suffering from naturally-occurring osteoarthritis.

Methods: Six such dogs were recruited following ethics approval and owner consent. Dogs were administered a single subcutaneous injection of 5 mg/kg liposomal-CBD. Plasma concentrations of CBD, blood work, activity monitoring collar data, wellbeing questionnaire (owners) and pain scoring (veterinarian) were performed at baseline and monitored up to six weeks following intervention. Data overtime were compared with baseline using linear-regression mixed-effects. *P*-value was set at 0.05.

Results: CBD plasma concentrations were observed for 6 weeks; median (range) peak plasma concentration (C_{max}) was 45.2 (17.8–72.5) ng/mL, time to C_{max} was 4 (2–14) days and half-life was 12.4 (7.7–42.6) days. Median (range) collar activity score was significantly increased on weeks 5–6; from 29 (17–34) to 34 (21–38). Scores of wellbeing and pain evaluations were significantly improved at 2–3 weeks; from 69 (52–78) to 53.5 (41–68), and from 7.5 (6–8) to 5.5 (5–7), respectively. The main adverse effect was minor local swelling for several days in 5/6 dogs.

Conclusion: Liposomal-CBD administered subcutaneously produced detectable CBD plasma concentrations for 6 weeks with minimal side effects and demonstrated reduced pain and increased wellbeing as part of multimodal pain management in dogs suffering from osteoarthritis. Further placebo-controlled studies are of interest.

KEYWORDS

analgesia, cannabidiol, CBD, dogs, liposomes, osteoarthritis, pharmacokinetics, prolonged release

1. Introduction

Osteoarthritis is one of the prevalent diseases in geriatric dogs, which usually results in chronic pain and decrease or loss of function (1–3). Conservative management of canine osteoarthritis uses long term non-steroidal anti-inflammatory drugs (NSAIDs) in order to reduce inflammation and control pain (4–7). However, NSAIDs may not be sufficient to control pain and their long-term use can be associated with gastrointestinal, hepatic, and renal adverse effects (7–9).

Cannabidiol (CBD) and tetrahydrocannabinol (THC) are the primary derivatives of the plant *Cannabis sativa*. While THC is highly psychoactive and may result in neurological signs in dogs (10, 11), CBD has no psychoactive activity and can be administered safely at high doses or for long periods (10, 12, 13). CBD was reported to alleviate chronic pain in people (14–16), and recently its effectiveness was reported in dogs with osteoarthritis (17–20). The recommended route of administration is orally with a frequency of twice daily (17, 19). In people, the bioavailability of CBD is considered to be as low as 6% (21). In dogs, bioavailability may be better, although, depending on the formulation and the dose used, plasma levels may be variable between studies and within a study between individual dogs (13, 17, 20, 22, 23). Another concern with oral oil-based CBD preparations is the palatability of the preparation, which may decrease dog compliance to the treatment (24).

Alternative, injectable route of CBD delivery using liposomes was reported recently (25). Liposomes are vesicles made of one or more bilayers of well-characterized phospholipids. They are attractive for pharmaceutical application because this delivery system is biocompatible, biodegradable, and non-toxic (26–28). Additionally, the US Food and Drug Administration (FDA) has approved many liposomal drug-products (28). Prolonged-release injectable liposomal-CBD formulation allows a more convenient administration route with better pet and owner compliance, and with the potential to increase CBD bioavailability (25).

The objectives of this pilot study were to evaluate the pharmacokinetics, therapeutic efficacy and safety of a single subcutaneous injection of liposomal-CBD using synthetic CBD in dogs with naturally-occurring osteoarthritis. Our hypotheses were that CBD will be detected for several weeks, there will be an improvement in dogs' activity, pain level and wellbeing without major adverse effects.

2. Methods

2.1. Animals

The study was approved by the Institutional Animal Care and Use Committee (IACUC; approval protocol MD-21-16,661-2), and a signed informed consent was obtained from all dog owners or legal guardians. Six dogs suffering from naturally-occurring osteoarthritis at least in one joint were recruited to this study. Following an orthopedic examination, osteoarthritis was confirmed radiographically, and complete blood count and biochemistry panel were performed before initiation of the study. Exclusion criteria included dogs that were younger than 2-or older than 15-years, orthopedic surgeon recommendation for any joint surgery, undergoing a surgical procedure 3 months before intervention, or suspected liver disease. For ethical reasons, all dogs continued receiving analgesics and joint supplements that were prescribed prior to recruitment.

2.2. Liposomal-CBD intervention

Liposomal-CBD formulation (CBD Liposome Platform Technology; LPT) was obtained from Innocan Pharma™ (Israel). According to the product certificate of analysis, the Liposomal-CBD was prepared under strict aseptic conditions. Prior to use samples were submitted to Hy-Labs (Rehovot, Israel), a certified and accredited laboratory by the Israeli Ministry of Health and FDA, to confirm the formulation was sterile and below the approved limit of endotoxins. The results of these tests met the requirements of extra-vascular administered drugs in people.

The liposomal-CBD formulation was composed of synthetic CBD (Purisys LLC., Athens, GA, United States; not considered a controlled substance) that was loaded at a concentration of 50 mg/mL into hydrogenated soy phosphatidylcholine (HSPC) liposomes (Lipoid GmbH, Ludwigshafen, Germany).

The injection was performed between the shoulders, after hair clipping and aseptic skin preparation. Liposomal-CBD was injected subcutaneously at a dose of 5 mg/kg (0.1 mL/kg) using a 21-gauge, 1-inch needle at the prepared skin area.

2.3. Monitoring

2.3.1. Pharmacokinetics

One mL blood was collected from a peripheral vein (cephalic or saphenous) for pharmacokinetic analysis at 2 and 6 h, 1, 2 and 4 days, and weekly 1–6 weeks following injection. Blood was collected into ethylenediamine tetra-acetic acid (EDTA) 1 mL tubes and centrifuged to separate the plasma within 5 min from collection. Plasma was immediately frozen at −20°C and then kept at −80°C until analysis. CBD quantification was performed using UHPLC-tandem mass spectrometry (LC–MS/MS) method, which was reported by the authors recently, and can be found in the Supplementary material at: https://www.frontiersin.org/articles/10.3389/fvets.2022.892306/full#supplementary-material (25). Pharmacokinetic parameters were calculated for 6 weeks following injection using a non-compartmental analysis with Phoenix WinNonlin (Certara™, NJ, United States, Version 6.3).

In 3/6 dogs an intravenous catheter was placed in the cephalic vein and left in place for 24–48 h to facilitate blood sampling.

2.3.2. Pain assessment

The Canine Brief Pain Inventory (CBPI) (29, 30) was used as an owner questionnaire assessment. Briefly, this questionnaire includes a pain (scale 0–40) and function (scale 0–60) assessments, summed to a total scale of 0–100, where 0 = normally functioning dog with no pain, and 100 = non-functioning dog with worse possible pain. In addition, an overall CBPI quality of life assessment is given using a descriptive scale: poor, fair, good, very good and excellent. A pain interactive visual analog scale (iVAS) was used for veterinary assessment with a scale of 0–10; 0 = no pain, 10 = worse possible pain.

Both assessments were completed at baseline before injection and then once weekly up to 6 weeks from injection.

2.3.3. Activity monitoring collar and vital signs

At least two weeks before intervention, an activity monitoring collar (PetPace, Burlington, MA, United States[1]) (31, 32) was placed on the dogs' neck. Data was collected from the collar for 2 weeks prior and 6 weeks following liposomal-CBD injection. For each dog the mean weekly score of four parameters (activity score, position score, calories expedite and sleep score) was obtained from the PetPace platform and analyzed for all dogs after completion of the study.

Physiologic parameters were monitored throughout the study period: heart rate (HR) using a stethoscope, respiratory frequency (f_R) by observing thoracic excursions, rectal temperature (RT) via digital thermometer, and mean arterial blood pressure using an oscillometric blood pressure monitor (CASMED 740; CAS Medical Systems Inc., Branford, CT, United States) with the cuff placed above the carpus over the radial artery while the dog was in sternal recumbency. The physiologic parameters were measured at baseline and then at 2 and 6 h, 1, 2 and 4 days, and weekly 1–6 weeks following injection.

2.3.4. Blood work

Blood samples (1–1.5 mL) were collected in EDTA tubes for complete blood count (CBC; ADVIA 2120i Hematology System, Siemens Healthineers, Erlangen, Germany; including clinical pathology assessment of blood smears) and in tubes containing a separator gel (CAT Serum Sep Clot Activator, Vacuette®, Greiner Bio-One, Kremsmünster, Austria; 2–2.5 mL) for biochemistry panel (cobas® 6,000, Roche Diagnostics Corporation, Indianapolis, IN, United States) at baseline and then at 1 and 4 weeks after intervention. In two of the dogs, additional blood work was performed 2 days following injection.

2.3.5. Adverse effects and follow-up

During the 6 weeks after injection, dogs were monitored closely for adverse effects; at the hospital during the first 6 h after injection, by the veterinarian at each time-point of blood sampling for PK, and by the owners at home throughout the 6 weeks. Following study termination, dog owners were contacted by phone once monthly for 6 more months, and then every 3–4 months. Additionally, owners were requested to inform the attending veterinarian of any change in health status of their dog.

2.4. Statistical analysis

Power analysis was not performed, as due to safety reasons the number of participants was limited to 6 dogs by the IACUC. Statistical analysis was performed using Stata/SE statistical software version 15.0 (StataCorp, College Station, TX, United States). Because sample size was small, descriptive statistics are expressed as median (range as minimum-maximum). Data analysis was performed with repeated measures mixed-effects with random intercept at the dog level. All values at time points following intervention were compared with baseline. Additionally, the association between CBD plasma concentrations and CBPI and iVAS scores were tested using mixed-effects linear regression. A p-value <0.05 was considered significant.

3. Results

3.1. Animals

Three spayed female and three male (1 neutered, 2 intact) dogs with a median age of 12 (9–14) years old and body weight of 34 (26–58) kg were recruited to the study and completed the 6 weeks monitoring period. Dogs' signalments, joints affected, osteoarthritic supplements and routine oral analgesics are presented in Table 1.

3.2. Pharmacokinetic data

CBD plasma concentrations were observed throughout the 6-weeks monitoring period, including at the 6-week time point (Figure 1; Table 2). The plasma profile obtained showed a gradual increase in CBD up to the maximal CBD plasma concentration (C_{max}), and then a decrease starting in most dogs (4/6) at one week following injection. In dog number 1 the increase and the decrease were very gradual, and in dog number 6 the decline started earlier, after 2-days from injection (Figure 1). Calculated pharmacokinetic data and CBD plasma concentrations at 3- and 6-weeks following injection are presented in Table 2.

3.3. Pain scores

Dogs had significantly improved CBPI pain scores compared with baseline at weeks 2–3 (p = 0.011 and 0.031, respectively), improved CBPI function scores at weeks 2 and 6 (p = 0.004 and 0.026, respectively), improved CBPI total scores at weeks 2–3 (p = 0.001 and 0.028, respectively) and borderline improvement at week 6 (p = 0.052), and improved CBPI quality of life at weeks 2–3 (p = 0.046 for both weeks; Table 3). iVAS pain scores were significantly improved at 1–3 weeks (p < 0.001) and at 4 weeks following injection (p = 0.034; Table 3). The improvement in pain scores was significantly associated with the pharmacokinetic profile obtained; total CBPI at weeks 1–6 (p < 0.001 to p = 0.039, coefficients −0.249 to −4.399) and iVAS at weeks 1 (p = 0.008, coefficient −0.018), 2 and 3 (p < 0.001, coefficients −0.09 and −0.326, respectively).

3.4. Activity monitoring collar and physiologic parameters

Collar activity scores were significantly increased on weeks 5–6 (p = 0.012 and 0.027, respectively). Position scores, calories expedite, and sleep scores did not change significantly from baseline recordings (Table 3).

1 https://petpace.com/smart-sensing-collar/

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TABLE 1 Data of 6 dogs suffering from osteoarthritis that were administered a single subcutaneous liposomal-cannabidiol (CBD) injection in addition to routine analgesic treatments.

Dog	Sex	Age (years)	Breed	Body weight (kg)	Affected joints	Supplements and analgesics	Other health conditions
1	Spayed female	14	Samoyed	26	Hip	Glucosamine, hyaluronic acid, chondroitin sulfate	
2	Neutered male	12	Mixed	58	Hip	Glucosamine, gabapentin	Kidney disease, suspected lumbar partial disc herniation
3	Spayed female	12	Mixed	28	Hip, stifles, and shoulders	Occasional previcox	
4	Spayed female	9	Mixed	36	Hip, stifles, and shoulders	Curcumin (turmeric), dipyrone	Kidney disease, lumbar pain
5	Male	14	Flat-coated retriever	36	Hip, stifles, and left shoulder	Glucosamine, occasional dipyrone	
6	Male	12	Malinois	32	Hip, stifles, tarsus, shoulders, elbows, carpus	Glucosamine, gabapentin, previcox	

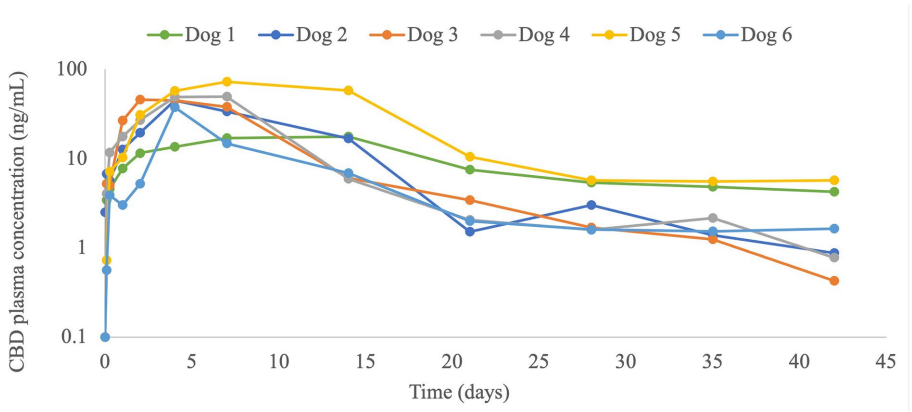

FIGURE 1
Plasma cannabidiol (CBD) concentrations (ng/mL) in 6 dogs with osteoarthritis before and up to 42 days (6 weeks) after a single subcutaneous liposomal-CBD injection at 5 mg/kg.

HR decreased significantly from baseline at 6 h ($p = 0.010$), 4 days ($p = 0.010$), 4 weeks ($p = 0.022$), and 6 weeks from injection ($p = 0.017$). f_R decreased significantly from baseline at 2 h ($p = 0.018$), 2 and 4 days ($p = 0.005$–0.008), and 5–6 weeks ($p = 0.003$–0.008). MAP decreased significantly from baseline at 4 days from injection ($p = 0.048$), and no difference was observed in RT throughout the study period (Table 4).

3.5. Blood work

Most median hematology and biochemistry values were within reference ranges at all measurement times, although, some parameters were changed significantly from baseline. White blood cells (WBCs), neutrophils and monocytes increased significantly from baseline at 2 days from injection ($p < 0.001$). These increases were mainly attributed to a dog that developed phlebitis around the intravenous catheter. At 1 week after injection WBCs increased in 3/6 dogs (in the reference range) and decreased in 1/6 dogs with no overall significant change. Eosinophils increased significantly at 4 weeks ($p = 0.009$). At 1 week, a significant decrease was observed in hematocrit ($p = 0.046$), packed cell volume (PCV; $p = 0.006$), mean corpuscular volume (MCV; $p = 0.017$) and reticulocytes ($p = 0.031$). Platelets decreased significantly at 2 days ($p = 0.046$) and increased significantly at 1 week ($p < 0.001$), and plateletcrit increased significantly at 1 week ($p = 0.007$; Table 5).

Clinical pathology assessment of blood smears revealed mature non-toxic neutrophils at baseline in all dogs. A mild number of neutrophils became bands with mild toxic appearance in 3 different dogs: at 2 days (1 dog that developed phlebitis associated with intravenous catheter positioning), at 1 week (1 dog) and at 4 weeks (1 dog). Mild number of reactive monocytes was observed at baseline in 5/6 dogs, which were absent at the 4-week assessment in 4 dogs and sustained in one of these dogs. Mild–moderate number of atypical granular lymphocytes was observed at baseline and throughout the monitoring period in 5/6 dogs. Although none of the dogs was anemic, occasional polychromasia was observed in 5/6 dogs at baseline and at the

TABLE 2 Pharmacokinetic data of plasma cannabidiol (CBD) from six dogs with osteoarthritis after a single subcutaneous 5 mg/kg liposomal-CBD injection.

Dog	C_{max} (ng/mL)	$C_{21\ days}$ (ng/mL)	$C_{42\ days}$ (ng/mL)	T_{max} (days)	Half-life (days)	AUC (ng·h/mL)	AUC/dose (ng·h/mL/mg/kg)
1	17.8	7.5	4.3	14	42.6	9,810	1962
2	44.8	1.5	0.9	4	10.5	11,877	2,375
3	45.7	3.4	0.4	2	7.7	11,630	2,326
4	49.4	2.1	0.8	7	12.1[#]	12,380	2,476
5	72.5	5.7	5.9	4	12.8[#]	19,275	3,855
6	37.7	1.6	2.0	2	14.7[#]	4,529	906
Median	**45.2**	**2.8**	**1.5**	**4**	**12.4**	**11,754**	**2,351**

[#]Lambda < 0.8. C_{max}, peak plasma concentration; $C_{21/42\ days}$, plasma concentration at 21/42 days from injection; T_{max}, time to maximum plasma concentration; AUC, area under the concentration–time curve. Blood samples were collected for 6 weeks following injection. The bold values are the median of the 6 dogs.

TABLE 3 Scoring of canine brief pain inventory (CBPI; pain scale 0–40, function scale 0–60, total scale 0–100; 0 = no pain/normal function, 100 = worse pain/no function, and overall quality of life: poor, fair, good, very good and excellent) by owners, interactive visual analog scale (iVAS; scale 0–10; 0 = no pain, 10 = worse pain) by an anesthesiologist, and activity monitoring collar (PetPace) scores from six dogs with osteoarthritis, before and six weeks after liposomal-cannabidiol (CBD) subcutaneous injection.

	Baseline	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
CBPI							
Pain	22 (17–32)	19.5 (17–28)	16.5 (14–28)*	18.5 (12–28)*	22.5 (19–28)	22.5 (18–33)	22.5 (15–31)
Function	44 (39–52)	41 (26–45)	36 (26–43)*	40 (26–54)	41 (30–49)	40 (20–55)	36 (18–49)*
Total	69 (62–78)	63 (43–68)	53.5 (41–68)*	57 (44–79)*	66 (50–72)	63.5 (40–82)	58 (33–80)
CBPI Quality of life	Fair (Fair-Good)	Good (Fair-Good)	Good (Fair-Very good)*	Good (Fair-Very good)*	Good (Fair-Good)	Fair-Good (Fair-Good)	Fair-Good (Fair-Good)
iVAS	7.5 (6–8)	6.5 (6–7)*	6.0 (5–7)*	5.5 (5–7)*	6.5 (6–8)*	7.0 (6–8)	7.5 (6–8)
PetPace							
Activity score	29 (17–34)	30 (17–38)	30 (20–37)	32 (21–36)	30 (20–43)	33 (21–42)*	34 (21–38)*
Position score	651 (552–820)	698 (527–937)	684 (495–766)	669 (559–791)	693 (533–872)	655 (587–837)	692 (539–903)
Calories expedite	1, 308 (1,009–1878)	1,311 (1,018–1735)	1,334 (1,041–1889)	1,327 (1,056-1,912)	1,303 (993–1,938)	1,318 (963–1,930)	1,309 (1,012-1,732)
Sleep score	83 (73–87)	83 (77–88)	83 (79–88)	82 (78–87)	81 (77–88)	83 (78–88)	84 (79–88)

Data is presented as median (range; minimum-maximum). *Significantly improved from baseline value ($p < 0.05$).

following assessments. The dog that did not show polychromasia had mild poikilocytosis at baseline, then mild spherocytosis and mild poikilocytosis at 1-week, which were not observed on the 4-week assessment.

Alkaline phosphatase (ALP) did not change significantly during the study, however, one dog (dog number 5) showed high ALP value at baseline, which was further increased at the 4-week measurement. Another dog (dog number 4) had ALP elevation only at the 2-day measurement, during an elevated HR event. Gamma-glutamyltransferase (GGT) decreased significantly from baseline at 1 and 4 weeks from injection ($p = 0.002$ and 0.015, respectively). Total bilirubin increased significantly at 2 days ($p < 0.001$). Albumin decreased significantly at 1 week ($p = 0.008$). Total protein decreased significantly at all time points ($p = 0.001$, $p < 0.01$ and $p = 0.004$, respectively). Creatinine decreased significantly from baseline at 1 and 4 weeks from injection ($p = 0.004$ and 0.044, respectively). When dog 2, who had a kidney disease, was excluded from the creatinine analysis, creatinine was still decreased significantly at 1 week ($p = 0.001$). Calcium and potassium decreased significantly at 2 days ($p < 0.001$ and $p = 0.010$, respectively). CO_2 decreased significantly at 2 days ($p < 0.001$) and increased significantly at 1 week ($p = 0.010$; Table 5).

3.6. Adverse effects and follow-up

Local response (minor, non-painful swelling at the injection site) was observed in 5/6 dogs. The swelling was resolved (i.e., absorbed completely) within 3–6 days following appearance without any treatment (Table 6). One dog had an increased HR to 140–160 beats per minute starting approximately 36 h after injection, which resolved without treatment a day later. An echocardiogram revealed sinus tachycardia. Another dog developed a fever, which was attributed to phlebitis around an intravenous catheter that was left for 24 h for blood sampling. The catheter was removed, oral antibiotics was initiated, and the fever was resolved within 12 h.

At the time of manuscript submission, one of the dogs died naturally more than a year following injection at the age of 15 years, and two dogs were euthanized 5- and 7-months following injection due to deterioration in their disease condition (Table 6).

TABLE 4 Physiologic parameters from six dogs with osteoarthritis, before and six weeks after liposomal-cannabidiol (CBD) subcutaneous injection.

Time	HR (bpm)	f_R (rpm)	RT (°C)	MAP (mmHg)
Baseline	114 (100–126)	24 (24–36)	38.4 (37.8–38.7)	110 (83–125)
2 h	102 (80–120)	20 (16–28)*	38.3 (37.6–38.7)	103 (89–116)
6 h	98 (64–116)*	24 (12–28)	38.4 (37.9–38.7)	109 (101–120)
1 day	116 (88–120)	22 (20–32)	38.3 (38.0–40.0)	102 (99–122)
2 days	104 (84–160)	20 (16–32)*	38.4 (38.0–39.6)	101 (95–120)
4 days	94 (84–112)*	20 (12–32)*	38.2 (37.8–38.7)	95 (89–111)*
1 week	100 (88–112)	24 (16–28)	38.0 (37.5–38.5)	104 (92–111)
2 weeks	100 (80–112)	26 (20–32)	38.1 (37.9–38.4)	103 (97–121)
3 weeks	102 (88–116)	24 (16–32)	38.3 (37.9–38.4)	104 (97–120)
4 weeks	94 (80–116)*	24 (16–32)	38.3 (37.9–38.5)	104 (95–123)
5 weeks	104 (84–112)	20 (20–24)*	38.0 (37.8–38.8)	100 (89–108)
6 weeks	94 (76–120)*	20 (16–24)*	38.3 (38.0–38.6)	104 (97–116)

Data is presented as median (range; minimum-maximum). HR, heart rate; f_R, respiratory frequency; RT, rectal temperature; MAP, mean arterial pressure; bpm, beats per minute; rpm, respirations per minute.
*Significantly different from baseline value ($p < 0.05$).

4. Discussion

4.1. Pharmacokinetics

Results from the present study suggest that a single subcutaneous liposomal-CBD administration provides long-term (i.e., several weeks) CBD plasma concentration and analgesia. Liposomal delivery systems provide a slow release of various encapsulated drugs (26, 28). Additionally, many liposomal-based formulations improve the therapeutic window of drugs and therefore reduce their toxicity (33). The use of liposomes as a delivery system for CBD in the present study, indeed provided slow drug release during the tested period, as was shown by the time it took to reach C_{max} (T_{max}; 2–14 days) and by the long half-life (7.7–42.6 days) (Table 2). Compared with various oral CBD-containing formulations administering a single 2 mg/kg dose in dogs, the median/mean C_{max}, T_{max}, and half-life were 102.3 ng/mL, 1.5 h, and 4.2 h, respectively ($n = 4$) (17); 213 ng/mL, 2.1 h, and 2.5 h, respectively ($n = 6$) (11); 301 ng/mL, 1.4 h, and 1.0 h, respectively, (22); 226 ng/mL, 2.5 h, and 3.8 h, respectively (34).

In people, bioavailability of CBD is very low (6%–10%) and depends on fasting conditions (21). In dogs, bioavailability is considered better than in people, and reported to be 13%–70% depending on the formulation used (23, 35, 36). First-pass liver metabolism is believed to be the primary reason for the low bioavailability of oral CBD (21, 37). Therefore, alternative routes of delivery, such as via mucosal absorption that would bypass the liver are of interest. A recent study investigated the pharmacokinetics of a single 1 mg/kg pure CBD in oil formulation via oral transmucosal (OTM) administration or orally (6 dogs per route). Mean C_{max} and T_{max} for OTM and oral routes were 200.3 ng/mL and 1.9 h, and 206.8 ng/mL and 2.2 h, respectively. Half-life was 2.6 h with both routes (37). Interestingly, there was no difference in pharmacokinetic parameters between administration routes, suggesting that absorption via oral mucosa was not optimal or that most of the drug was actually swallowed (37). CBD administration was also investigated via nasal mucosa (mean dose of 1.7 mg/kg) or intrarectally using suppositories (mean dose of 8.3 mg/kg) compared with oral route (mean dose of 8.3 mg/kg). Following rectal administration CBD levels were below the limit of quantification. Mean C_{max} and T_{max} for nasal and oral routes were 28 ng/mL and 0.5 h, and 217 ng/mL and 3.5 h, respectively. Terminal elimination half-life was 7.0 and 15.7 h, respectively (38). According to these studies, CBD administered via mucosal sites was inferior compared with oral administration in dogs, although more studies using different CBD formulations are required for conclusion. This is strengthened by a study in dogs with naturally occurring osteoarthritis reporting a significant improvement following OTM CBD compared with control dogs (19).

Administration of Sativex® (phytocannabinoid-based) sublingual spray was investigated in healthy young beagles, using an approximate dose of 0.5 mg/kg. Following a single dose, mean C_{max} and T_{max} of CBD were 10.5 ng/mL and 2 h, respectively (39). It should be noted that blood was sampled from the jugular vein in the Sativex® study, which may have resulted in a biased overestimation of CBD plasma concentrations, because the jugular sampling site was reported to affect concentration of drugs administered via the oral mucosal route (40).

Transdermal administration was also investigated in two studies; (i) one study administered CBD-infused transdermal cream applied to the pinnae, which was compared with two oral formulations (CBD-infused oil or microencapsulated oil beads). These formulations were tested at 5 mg/kg twice daily in young healthy beagles ($n = 10$ per treatment). Following a single dose, mean C_{max} and half-life reached 625.3 ng/mL and 3.3 h (infused oil), 346.3 ng/mL and 1.6 h (oil beads), and 74.3 ng/mL (transdermal cream), respectively. The half-life of the CBD-infused transdermal cream could not be determined due to lack of elimination phase (23). (ii) The second study administered a transdermal low-THC *Cannabis sativa* extract 4 mg/kg rubbed into the pinnae twice daily for two weeks in six healthy young beagles. Mean C_{max} was 12.8 and 10.6 ng/mL after 7- and 14-days of administration. The authors concluded that CBD absorption via the transdermal route was generally poor (41).

In the present study C_{max} was lower compared with CBD plasma/serum concentrations at steady-state following 2–6 weeks oral CBD administration in dogs; 60–125 ng/mL (34), 80–160 ng/mL (23),

TABLE 5 Complete blood count and biochemistry panel performed in six dogs with osteoarthritis, before and after a single liposomal-cannabidiol (CBD) subcutaneous injection at 5 mg/kg.

Parameter	Reference range	Baseline	2 days (n = 2)	1 week	4 weeks
Hematology					
White blood cells (10^3/μL)	5.2–13.9	8.2 (6.5–14.8)	14.1 (12.2–16.0)*	9.8 (7.5–11.7)	8.0 (6.6–10.6)
Neutrophils (10^3/μL)	3.9–8.0	5.1 (4.4–11.6)	10.8 (9.8–11.9)*	6.9 (4.8–7.1)	5.3 (3.6–7.1)
Monocytes (10^3/μL)	0.2–1.1	0.55 (0.36–0.65)	0.97 (0.65–1.29)*	0.61 (0.56–0.71)	0.51 (0.34–0.56)
Lymphocytes (10^3/μL)	1.3–4.1	2.0 (1.0–2.7)	2.0 (1.6–2.4)	1.9 (1.3–3.4)	1. 9 (1.2–2.4)
Eosinophils (10^3/μL)	0.0–0.6	0.37 (0.29–0.58)	0.13 (0.11–0.15)	0.45 (0.28–0.64)	0.46 (0.36–1.24)*
Basophils (10^3/μL)	0.0–0.1	0.01 (0.0–0.04)	0.02 (0.01–0.02)	0.01 (0.0–0.02)	0.01 (0.0–0.02)
Neutrophils (%)	42.5–77.3	65.6 (60.9–78.7)	77.2 (74.5–79.9)*	69.4 (61.0–71.1)	64.7 (54.1–71.3)
Monocytes (%)	3.3–10.3	5.8 (4.4–8.6)	6.7 (5.3–8.1)	6.7 (5.0–9.4)	5.8 (3.8–7.3)
Lymphocytes (%)	11.8–39.6	24.1 (12.6–27.6)	14.2 (13.3–15.1)	18.8 (16.8–29.2)	21.8 (16.2–29.3)
Eosinophils (%)	0.0–7.0	4.7 (0.3–6.4)	0.9 (0.9)	4.2 (2.8–6.6)	6.1 (4.7–11.6)*
Basophils (%)	0.0–1.3	0.1 (0.0–0.4)	0.1 (0.1)	0.1 (0.1–0.2)	0.1 (0.0–0.2)
Red blood cells (10^6/μL)	5.7–8.8	6.3 (4.6–6.7)	6.1 (5.5–6.6)	5.8 (4.6–6.5)	6.1 (5.8–6.8)
Hematocrit (%)	37.1–57.0	43.9 (35.1–51.3)	43.9 (38.9–48.8)	40.0 (34.8–45.8)*	45.0 (39.7–48.8)
Hemoglobin (g/dL)	12.9–18.4	15.0 (12.0–17.4)	14.8 (13.4–16.1)	13.6 (12.5–15.6)	15.1 (14.0–16.1)
Mean corpuscular volume (MCV; fL)	58.8–71.2	71.0 (67.8–76.3)	72.0 (70.1–73.8)	70.2 (67.7–75.0)*	70.4 (68.3–75.5)
Mean corpuscular hemoglobin (MCH; pg)	20.5–24.2	24.3 (23.4–26.0)	24.3 (24.2–24.4)	23.9 (23.0–26.9)	24.0 (23.2–25.8)
Mean corpuscular hemoglobin concentration (MCHC; g/dL)	31.0–36.2	34.0 (33.5–35.3)	33.8 (33.0–34.5)	34.3 (33.1–35.8)	33.9 (32.9–35.3)
Reticulocytes (10^9/L)	0.0–60.0	91.9 (14.0–235.7)	71.3 (44.8–97.7)	52.2 (33.6–129.3)*	69.1 (27.7–183.4)
Reticulocytes (%)	0.0–1.5	1.5 (0.3–3.5)	1.1 (0.8–1.5)	0.9 (0.6–2.0)*	1.2 (0.5–2.8)
Platelets (10^3/μL)	143–400	362 (242–495)	297 (253–340)*	437 (383–641)*	360 (312–449)
Plateletcrit (%)	0.1–0.4	0.43 (0.26–0.50)	0.32 (0.31–0.32)	0.48 (0.44–0.61)*	0.37 (0.32–0.43)
Mean platelet volume (MPV; fL)	7.0–11.0	10.7 (10.0–12.4)	11.0 (9.2–12.8)	10.8 (8.9–13.1)	10.1 (9.0–11.5)
Platelets distribution width (PDW; %)	40.6–65.2	54.8 (48.5–60.7)	51.9 (46.7–57.1)	59.0 (42.8–63.3)	50.1 (43.2–59.0)
Packed Cell Volume (PCV; %)		44 (35–54)	44 (38–49)	38 (35–44)*	42 (38–45)
Total solids (TS)		7.0 (6.0–9.0)	7.0 (6.5–7.4)	7.0 (6.2–8.2)	7.0 (6.8–8.4)
Biochemistry					
Creatine phosphokinase (CPK; IU/L)	51–399	152 (83–264)	80 (67–92)	120 (106–425)	122 (74–196)
Aspartate aminotransferase (AST; IU/L)	19–42	27 (24–30)	20 (19–21)	31 (18–40)	30 (20–45)
Alanine transaminase (ALT; IU/L)	19–67	65 (26–183)	79 (56–102)	51 (21–112)	95 (33–200)
Alkaline phosphatase (ALP; IU/L)	21–170	71 (29–874)	750 (737–762)	107 (34–701)	95 (29–1,005)
Gamma-glutamyl transferase (GGT; IU/L)	0–6	5 (3–9)	6 (5–6)	3 (3)*	3 (3–6)*
Amylase (U/L)	103–1,510	985 (673–1,994)	1,829 (1,114-2,543)	1,031 (630–1,612)	836 (607–1,709)
Triglyceride (mg/dL)	19–133	74 (45–410)	219 (96–342)	128 (52–246)	151 (82–280)
Cholesterol (mg/dL)	135–361	237 (165–371)	285 (227–343)	278 (161–358)	278 (169–409)
Total bilirubin (mg/dL)	0.0–0.2	0.15 (0.15)	0.19 (0.15–0.23)*	0.15 (0.15)	0.15 (0.15)
Glucose (mg/dL)	64–123	84 (76–96)	92 (89–94)	87 (66–96)	83 (71–92)
Albumin (g/dL)	3.0–4.4	3.8 (3.0–5.3)	3.5 (3.0–3.9)	3.2 (2.9–3.6)*	3.3 (2.9–4.7)
Total protein (g/dL)	5.4–7.6	7.1 (6.1–8.7)	6.2 (5.6–6.7)*	6.5 (6.0–8.0)*	6.7 (6.0–8.1)*
Urea (mg/dL)	10.7–53.5	31.1 (24.5–114.6)	26.7 (23.8–29.5)	26.3 (18.6–35.5)	30.5 (23.4–58.2)
Creatinine (mg/dL)	0.3–1.2	1.02 (0.79–1.78)	0.85 (0.62–1.08)	0.84 (0.72–0.99)*	0.92 (0.68–1.13)
Phosphate (mg/dL)	3.0–6.2	3.78 (2.23–4.81)	3.76 (3.52–3.99)	4.12 (2.85–4.32)	3.86 (3.16–4.42)

(Continued)

TABLE 5 (Continued)

Parameter	Reference range	Baseline	2 days (n = 2)	1 week	4 weeks
Calcium (mg/dL)	9.7–11.5	10.5 (9.7–11.5)	9.6 (8.8–10.4)*	10.3 (9.6–10.8)	10.5 (10.0–11.0)
Sodium (mmol/L)	140–154	147 (137–149)	147 (142–151)	148 (145–152)	148 (135–150)
Chloride (mmol/L)	104–118	106 (103–115)	104 (102–107)	108 (104–110)	106 (103–109)
Potassium (mmol/L)	3.6–5.3	5.45 (4.28–6.07)	4.56 (4.48–4.64)*	5.67 (4.52–6.05)	5.29 (4.62–5.98)
CO_2 (mmol/L)	16–26	19.7 (15.9–21.8)	18.2 (16.8–19.5)*	21.1 (19.3–23.7)*	20.6 (19.6–21.5)

*Significantly different from baseline value ($p < 0.05$). Data is presented as median (range; minimum-maximum).

5–860 (median 311) ng/mL (20), and 53–201 ng/mL (12). This difference in C_{max} could be the effect of the relatively lower dose used with the prolong-release liposomal formulation, which was based on the reported dose tested intravenously (36). In retrospect a higher dose could have been tested. On the other hand, in many studies of oral CBD in dogs, C_{max} among individuals was extremely variable, with some dogs reaching only 10th of CBD plasma concentrations of other dogs in the same study using the same formulation (20, 24, 35, 37). Reduced variability among dogs in the present study suggests a more uniform drug absorption across dogs. Subcutaneous injected CBD has the benefit of direct absorption and bypassing the high extraction ratio of CBD by the liver compared with the oral route (21). Furthermore, when evaluating prolong-release formulations, the area under the curve (AUC) is the most important assessment tool, as it presents the total drug exposure over time (28). When normalized to dose, the AUC following liposomal-CBD administration in the present study (2,351 ng·h/mL/mg/kg; Table 2) was higher in comparison to long-term/steady-state oral CBD administration; 241–480 ng·h/ml/mg/kg after 28 days, once a day 1–12 mg/kg (12), 346–588 ng·h/ml/mg/kg after cannabis herbal extract containing 1:20 THC:CBD at 2–10 mg/kg (11), or 328–423 ng·h/ml/mg/kg after 2 mg/kg twice daily for 2 weeks of three different forms of hemp extract (34). Therefore, it suggests that the exposure to CBD using the liposomal formulation is superior to the oral route.

4.2. Pain and analgesia

CBD is known to have anti-inflammatory and anti-nociceptive effects (42–44) and was described in the past few years as an efficacious analgesic in dogs suffering from osteoarthritis (17–20). The therapeutic efficacy reported in the present study is similar to previous studies with pain reduction and improved function in all dogs. The endocannabinoid system plays an important role in afferent and efferent nociceptive pathways (45). CBD is considered to exhibit its anti-inflammatory properties and analgesia via cannabinoid receptor 2 (CB2) as an inverse agonist and as an inhibitor of the reuptake of the endocannabinoid anandamide (15, 45, 46). Additionally, CBD was reported to interact with many other receptors and channels that are involved in nociception, such as activation of serotonin receptors (5-HT_{1A}), activation of transient receptor potential channels, vanilloid subfamily (TRPV1), inhibition of tumor necrosis factor-alpha (TNF-α), and inhibition of adenosine transporters (15, 45, 47). Furthermore, CB2 receptors expression is upregulated during inflammation in the affected tissue, as occurs in an osteoarthritic or rheumatoid joint. Therefore, treatment with cannabinoids activates CB2 receptors, and results in inhibition of cytokine production, decrease in leukocyte infiltration, reduction in bone destruction, and pain relief (45).

Unfortunately, the plasma CBD dose–response curve in dogs is still unknown. In the present study, a significant improvement in CBPI and iVAS pain scores was observed up to 3–4 weeks from injection, which corresponded to a median CBD concentration of 2.8 ng/mL. This may suggest that at this CBD plasma concentration there is still an analgesic effect, although, it is possible that the positive effect is also attributed to the overall high exposure observed.

4.3. Activity monitoring collar

Mobility in dogs can be affected by osteoarthritic pain, as previously reported (6, 32, 48). Therefore, the use of activity monitoring collars was chosen in order to provide an objective activity measurement. PetPace is a non-invasive monitoring collar that allows continuous monitoring of activity, position, certain vital signs, and sleep quality, and showed an excellent correlation with real-time variables (31, 49, 50). Recently, PetPace collar was suggested as a monitoring device to detect osteoarthritic pain, as it detected a significantly lower overall and high intensity activity levels in arthritic dogs when compared to healthy dogs (32). In the present study increased activity was observed 5–6 weeks following intervention, which was delayed from improvement in pain scoring evaluations, and CBD plasma levels. Factors other than pain can play a role in the pattern of dogs' daily activity, such as owner activities, car rides, or environmental conditions (rain/extreme heat). Therefore, activity data from the collar, including data from the present study, should be interpreted with caution.

4.4. Blood work

Although some of the blood work values changed significantly from baseline during the monitoring period, most changes were not clinically important, as values were kept within the reference range. WBCs increased in some of the dogs, but were not above the reference range, except the dog who had phlebitis. The increase in WBCs can be explained by a mild response of the immune system to injection of foreign materials (51), and it suits the local response observed at the injection site. The authors are not aware of published studies evaluating the effect of other liposomal formulations on WBC count administered subcutaneously in dogs. Epidurally administered liposomal-morphine in dogs did not show a systemic elevation of WBCs, but WBC count in the CSF was higher in the

TABLE 6 Adverse effects and follow-up of six dogs with osteoarthritis after a single liposomal-cannabidiol (CBD) subcutaneous injection at 5 mg/kg.

Dog	Local response	Adverse effects	Follow-up
1	None observed	None	Pancreatitis 8 weeks after injection (medications were given with butter). Resolved after 2-day hospitalization. Died in her sleep 1 year and 2 weeks after injection.
2	Yes, at 2 days	None	Euthanasia due to deterioration in lumbar neurologic condition 7 months after injection
3	Yes, at 4 days	None	Deterioration in osteoarthritis. At the time of manuscript submission, 1 year and 7 months following injection
4	Yes, at 4 days	Increased heart rate at 1–2 days after injection	Generally doing well. At the time of manuscript submission, 1.5 years following injection
5	Yes, at 4 days	Fever 1 day after injection (caused by phlebitis), resolved within 12 h of antibiotics administration	Generally doing well. At the time of manuscript submission,1.5 years following injection
6	Yes, at 1 week	None	Gastric ulcers 8 weeks after injection (high dose of Previcox for a long period). Euthanasia due to deterioration in life quality 5 months after injection

Local response (swelling at injection site) was resolved within 3–6 days without any treatment.

liposomal-morphine group (17 ± 18 cells/mm^3) versus the liposomal vehicle group (2 ± 1 cells/mm^3), with a value of <20 as the normal range (52). Hematocrit decreased a week post injection, but it was mild with no clinical importance. ALP was reported to significantly increase from baseline following long-term (weeks to months) administration of oral CBD in dogs, which was thought to result from induction of liver CYP isoenzymes (22, 24). However, a recent study reported that increased ALP correlated with significant elevation in bone-specific ALP, suggesting that the rise in total ALP can be partly attributed to osteoblastic activity (13). In the present study ALP increased in two dogs (33%);one of them had increased levels at baseline, and the other dog had an increase only at the 2-day measurement. Albumin level decreased during the present study, although in the reference range. A recent study investigating long-term CBD administration in dogs reported that albumin decreased gradually and reached significant difference at 6-months from initiation of the CBD administration. But the albumin values were still within the reference range (13). Albumin level may be decreased due to effects on the liver, but no other changes related to liver function were observed. Other effects of the liposomal-CBD, such as proteinuria or inflammation, may have resulted in decreased albumin and should be further investigated.

4.5. Adverse effects

The minimal local swelling at the injection site was not diagnosed further, because it was minor, did not require a medical intervention, and was self-limiting. A different liposomal formulation (Exparel, DepoFoam Bupivacaine; made of phospholipids, cholesterol, and triglycerides) was reported previously to produce local response at the injection site in dogs. That study used experimental dogs and described the formation of granulomatous inflammation following multiple injections, characterized by an increased number of multinucleated giant cells and vacuolated macrophages. The authors of the Exparel study considered the local response as a normal response to the liposomes and non-adverse (51).

4.6. CBD drug-products in veterinary medicine

In recent years CBD has gained popularity in the veterinary market (13). However, products' label can be misleading as many "CBD" products are actually extracts or enriched extracts from *Cannabis sativa*, and therefore they contain varying amounts of CBD in addition to many other chemically complex cannabis ingredients. A recent study reported that of 29 CBD products for dogs the total median CBD concentrations of their label claim was 93% (0%–154%) of claims (53). Valid CBD label-claims require rigorous analytical characterization and regulation (53). The FDA has published a guidance explaining that CBD products that are marketed without a prescription are not approved and may put users at risk (54, 55). Compared with cannabis-based products, synthetic CBD, which is FDA approved with a drug master file, provides a true THC and other cannabinoids-free product. The use of synthetic CBD as the active pharmaceutical ingredient of the liposomal-CBD formulation, can provide a reliable desired effect repeatedly.

4.7. Limitations

Limitations to this study include the small sample size, and the non-blinded study design, which could have introduced bias to the owner and veterinary evaluations. We calculated the bioavailability based on a study reporting intravenous CBD administration from 1988 (36), which may not be an accurate calculation, but no other study reporting intravenous CBD in dogs is available in the literature. Most of the dogs in this study were geriatric, which potentially can affect the absorption and elimination of the CBD, and younger animals may have different pharmacokinetic profile following liposomal-CBD. Although, this may also be a strength of this study, as some of the dogs had concurrent disease states and/or were receiving routine medications, and this is usually the population of dogs that can benefit from CBD treatment.

5. Conclusion

Liposomal-CBD administered subcutaneously had minor adverse effects, resulted in detectable CBD plasma concentrations for 6 weeks and showed high exposure in terms of AUC, which correlated with high bioavailability and decreased pain scores. This liposomal formulation can be used as an additional treatment as part of multimodal analgesia to increase wellbeing in dogs suffering from osteoarthritis. Further studies incorporating placebo-control, dose–response curve, and multiple injections (i.e., every several weeks) would provide more information as to the long-term efficacy and safety of this formulation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal studies were approved by the Ein-Kerem Animal Care and Use Committee, The Hebrew University of Jerusalem. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

YS-B and AC contributed to study conception, data acquisition and interpretation, and drafted the manuscript. NY, JM, DZ, AH, and DB contributed to the data acquisition. WA analyzed the data. EL and DB interpreted the results. YB contributed to study conception and interpreted the results. All authors contributed to the article and approved the submitted version.

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Conflict of interest

DZ, AH, and AC are supported by Innocan Pharma™. AC and YB have a patent pending on the liposomal-CBD formulation used in this study.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Riley LM, Satchell L, Stilwell LM, Lenton NS. Effect of massage therapy on pain and quality of life in dogs: a cross sectional study. *Vet Rec*. (2021) 189:e586. doi: 10.1002/vetr.586

2. Schachner ER, Lopez MJ. Diagnosis, prevention, and management of canine hip dysplasia: a review. *Vet Med (Auckl)*. (2015) 6:181–92. doi: 10.2147/VMRR.S53266

3. Alves JC, Santos A, Jorge P, Lavrador C, Carreira LM. Intra-articular injections with either triamcinolone Hexacetonide, stanozolol, Hylan G-F 20, or a platelet concentrate improve clinical signs in police working dogs with bilateral hip osteoarthritis. *Front Vet Sci*. (2020) 7:609889. doi: 10.3389/fvets.2020.609889

4. Vijarnsorn M, Kwananocha I, Kashemsant N, Jarudecha T, Lekcharoensuk C, Beale B, et al. The effectiveness of marine based fatty acid compound (PCSO-524) and firocoxib in the treatment of canine osteoarthritis. *BMC Vet Res*. (2019) 15:349. doi: 10.1186/s12917-019-2110-7

5. Reymond N, Speranza C, Gruet P, Seewald W, King JN. Robenacoxib vs. carprofen for the treatment of canine osteoarthritis; a randomized, noninferiority clinical trial. *J Vet Pharmacol Ther*. (2012) 35:175–83. doi: 10.1111/j.1365-2885.2011.01297.x

6. Muller C, Gines JA, Conzemius M, Meyers R, Lascelles BDX. Evaluation of the effect of signalment and owner-reported impairment level on accelerometer-measured changes in activity in osteoarthritic dogs receiving a non-steroidal anti-inflammatory. *Vet J*. (2018) 242:48–52. doi: 10.1016/j.tvjl.2018.10.005

7. Barbeau-Gregoire M, Otis C, Cournoyer A, Moreau M, Lussier B, Troncy E. A 2022 systematic review and meta-analysis of enriched therapeutic diets and nutraceuticals in canine and feline osteoarthritis. *Int J Mol Sci*. (2022) 23:10384. doi: 10.3390/ijms231810384

8. Belshaw Z, Asher L, Dean RS. The attitudes of owners and veterinary professionals in the United Kingdom to the risk of adverse events associated with using non-steroidal anti-inflammatory drugs (NSAIDs) to treat dogs with osteoarthritis. *Prev Vet Med*. (2016) 131:121–6. doi: 10.1016/j.prevetmed.2016.07.017

9. Innes JF, Clayton J, Lascelles BD. Review of the safety and efficacy of long-term NSAID use in the treatment of canine osteoarthritis. *Vet Rec*. (2010) 166:226–30. doi: 10.1136/vr.c97

10. Vaughn D, Kulpa J, Paulionis L. Preliminary investigation of the safety of escalating cannabinoid doses in healthy dogs. *Front Vet Sci*. (2020) 7:51. doi: 10.3389/fvets.2020.00051

11. Chicoine A, Illing K, Vuong S, Pinto KR, Alcorn J, Cosford K. Pharmacokinetic and safety evaluation of various Oral doses of a novel 1:20 THC:CBD cannabis herbal extract in dogs. *Front Vet Sci*. (2020) 7:583404. doi: 10.3389/fvets.2020.583404

12. Vaughn DM, Paulionis LJ, Kulpa JE. Randomized, placebo-controlled, 28-day safety and pharmacokinetics evaluation of repeated oral cannabidiol administration in healthy dogs. *Am J Vet Res*. (2021) 82:405–16. doi: 10.2460/ajvr.82.5.405

13. Bradley S, Young S, Bakke AM, Holcombe L, Waller D, Hunt A, et al. Long-term daily feeding of cannabidiol is well-tolerated by healthy dogs. *Front Vet Sci*. (2022) 9:977457. doi: 10.3389/fvets.2022.977457

14. Fitzcharles MA, Baerwald C, Ablin J, Hauser W. Efficacy, tolerability and safety of cannabinoids in chronic pain associated with rheumatic diseases (fibromyalgia syndrome, back pain, osteoarthritis, rheumatoid arthritis): a systematic review of randomized controlled trials. *Schmerz*. (2016) 30:47–61. doi: 10.1007/s00482-015-0084-3

15. Millar SA, Stone NL, Bellman ZD, Yates AS, England TJ, O'Sullivan SE. A systematic review of cannabidiol dosing in clinical populations. *Br J Clin Pharmacol*. (2019) 85:1888–900. doi: 10.1111/bcp.14038

16. O'Brien M, McDougall JJ. Cannabis and joints: scientific evidence for the alleviation of osteoarthritis pain by cannabinoids. *Curr Opin Pharmacol*. (2018) 40:104–9. doi: 10.1016/j.coph.2018.03.012

17. Gamble LJ, Boesch JM, Frye CW, Schwark WS, Mann S, Wolfe L, et al. Pharmacokinetics, safety, and clinical efficacy of cannabidiol treatment in osteoarthritic dogs. *Front Vet Sci*. (2018) 5:165. doi: 10.3389/fvets.2018.00165

18. Verrico CD, Wesson S, Konduri V, Hofferek CJ, Vazquez-Perez J, Blair E, et al. A randomized, double-blind, placebo-controlled study of daily cannabidiol for the treatment of canine osteoarthritis pain. *Pain*. (2020) 161:2191–202. doi: 10.1097/j.pain.0000000000001896

19. Brioschi FA, Di Cesare F, Gioeni D, Rabbogliatti V, Ferrari F, D'Urso ES, et al. Oral transmucosal cannabidiol oil formulation as part of a multimodal analgesic regimen: effects on pain relief and quality of life improvement in dogs affected by spontaneous osteoarthritis. *Animals (Basel)*. (2020) 10:1505. doi: 10.3390/ani10091505

20. Mejia S, Duerr FM, Griffenhagen G, McGrath S. Evaluation of the effect of cannabidiol on naturally occurring osteoarthritis-associated pain: a pilot study in dogs. *J Am Anim Hosp Assoc*. (2021) 57:81–90. doi: 10.5326/JAAHA-MS-7119

21. Perucca E, Bialer M. Critical aspects affecting cannabidiol Oral bioavailability and metabolic elimination, and related clinical implications. *CNS Drugs*. (2020) 34:795–800. doi: 10.1007/s40263-020-00741-5

22. Deabold KA, Schwark WS, Wolf L, Wakshlag JJ. Single-dose pharmacokinetics and preliminary safety assessment with use of CBD-rich hemp nutraceutical in healthy dogs and cats. *Animals (Basel)*. (2019) 9:832. doi: 10.3390/ani9100832

23. Bartner LR, McGrath S, Rao S, Hyatt LK, Wittenburg LA. Pharmacokinetics of cannabidiol administered by 3 delivery methods at 2 different dosages to healthy dogs. *Can J Vet Res*. (2018) 82:178–83.

24. McGrath S, Bartner LR, Rao S, Packer RA, Gustafson DL. Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. *J Am Vet Med Assoc*. (2019) 254:1301–8. doi: 10.2460/javma.254.11.1301

25. Shilo-Benjamini Y, Cern A, Zilbersheid D, Hod A, Lavy E, Barasch D, et al. A Case report of subcutaneously injected liposomal cannabidiol formulation used as a compassion therapy for pain management in a dog. *Front Vet Sci*. (2022) 9:892306. doi: 10.3389/fvets.2022.892306

26. Abu Lila AS, Ishida T. Liposomal delivery systems: design optimization and current applications. *Biol Pharm Bull*. (2017) 40:1–10. doi: 10.1248/bpb.b16-00624

27. Patil YP, Jadhav S. Novel methods for liposome preparation. *Chem Phys Lipids*. (2014) 177:8–18. doi: 10.1016/j.chemphyslip.2013.10.011

28. Rahnfeld L, Luciani P. Injectable lipid-based depot formulations: where do we stand? *Pharmaceutics*. (2020) 12:567. doi: 10.3390/pharmaceutics12060567

29. Brown DC, Boston RC, Coyne JC, Farrar JT. Ability of the canine brief pain inventory to detect response to treatment in dogs with osteoarthritis. *J Am Vet Med Assoc*. (2008) 233:1278–83. doi: 10.2460/javma.233.8.1278

30. Brown DC, Bell M, Rhodes L. Power of treatment success definitions when the canine brief pain inventory is used to evaluate carprofen treatment for the control of pain and inflammation in dogs with osteoarthritis. *Am J Vet Res*. (2013) 74:1467–73. doi: 10.2460/ajvr.74.12.1467

31. Belda B, Enomoto M, Case BC, Lascelles BDX. Initial evaluation of PetPace activity monitor. *Vet J*. (2018) 237:63–8. doi: 10.1016/j.tvjl.2018.05.011

32. Rowlison de Ortiz A, Belda B, Hash J, Enomoto M, Robertson J, Lascelles BDX. Initial exploration of the discriminatory ability of the PetPace collar to detect differences in activity and physiological variables between healthy and osteoarthritic dogs. *Front Pain Res (Lausanne)*. (2022) 3:949877. doi: 10.3389/fpain.2022.949877

33. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. *Pharmaceutics*. (2017) 9:12. doi: 10.3390/pharmaceutics9020012

34. Wakshlag JJ, Schwark WS, Deabold KA, Talsma BN, Cital S, Lyubimov A, et al. Pharmacokinetics of cannabidiol, Cannabidiolic acid, Delta9-tetrahydrocannabinol, Tetrahydrocannabinolic acid and related metabolites in canine serum after dosing with three Oral forms of hemp extract. *Front Vet Sci*. (2020) 7:505. doi: 10.3389/fvets.2020.00505

35. Corsato Alvarenga I, Panickar KS, Hess H, McGrath S. Scientific validation of cannabidiol for Management of dog and cat Diseases. *Annu Rev Anim Biosci*. (2023) 11:227–46. doi: 10.1146/annurev-animal-081122-070236

36. Samara E, Bialer M, Mechoulam R. Pharmacokinetics of cannabidiol in dogs. *Drug Metab Dispos*. (1988) 16:469–72.

37. Della Rocca G, Paoletti F, Conti MB, Galarini R, Chiaradia E, Sforna M, et al. Pharmacokinetics of cannabidiol following single oral and oral transmucosal administration in dogs. *Front Vet Sci*. (2023) 9:1104152. doi: 10.3389/fvets.2022.1104152

38. Polidoro D, Temmerman R, Devreese M, Charalambous M, Ham LV, Cornelis I, et al. Pharmacokinetics of cannabidiol following intranasal, intrarectal, and Oral Administration in Healthy Dogs. *Front Vet Sci*. (2022) 9:899940. doi: 10.3389/fvets.2022.899940

39. Fernandez-Trapero M, Perez-Diaz C, Espejo-Porras F, de Lago E, Fernandez-Ruiz J. Pharmacokinetics of Sativex® in dogs: towards a potential cannabinoid-based therapy for canine disorders. *Biomol Ther*. (2020) 10:249. doi: 10.3390/biom10020279

40. Hedges AR, Pypendop BH, Shilo Y, Stanley SD, Ilkiw JE. Impact of the blood sampling site on time-concentration drug profiles following intravenous or buccal drug administration. *J Vet Pharmacol Ther*. (2014) 37:145–50. doi: 10.1111/jvp.12075

41. Hannon MB, Deabold KA, Talsma BN, Lyubimov A, Iqbal A, Zakharov A, et al. Serum cannabidiol, tetrahydrocannabinol (THC), and their native acid derivatives after transdermal application of a low-THC *Cannabis sativa* extract in beagles. *J Vet Pharmacol Ther*. (2020) 43:508–11. doi: 10.1111/jvp.12896

42. Miranda-Cortes A, Mota-Rojas D, Crosignani-Outeda N, Casas-Alvarado A, Martinez-Burnes J, Olmos-Hernandez A, et al. The role of cannabinoids in pain modulation in companion animals. *Front Vet Sci*. (2023) 9:1050884. doi: 10.3389/fvets.2022.1050884

43. Fallahi S, Bobak L, Opalinski S. Hemp in animal diets-cannabidiol. *Animals (Basel)*. (2022) 12:2541. doi: 10.3390/ani12192541

44. Yu CHJ, Rupasinghe HPV. Cannabidiol-based natural health products for companion animals: recent advances in the management of anxiety, pain, and inflammation. *Res Vet Sci*. (2021) 140:38–46. doi: 10.1016/j.rvsc.2021.08.001

45. Mosley C, Gaynor J, Cital S, Brassard J. Cannabinoids for pain management In: S Cital, K Kramer, L Hughston and J Gaynor, editors. *Cannabis therapy in veterinary medicine, a complete guide*. Switzerland: Springer (2021). 117–42.

46. Dos Santos RG, Hallak JEC, Crippa JAS. Neuropharmacological effects of the Main Phytocannabinoids: a narrative review. *Adv Exp Med Biol*. (2021) 1264:29–45. doi: 10.1007/978-3-030-57369-0_3

47. Legare CA, Raup-Konsavage WM, Vrana KE. Therapeutic potential of cannabis, cannabidiol, and cannabinoid-based pharmaceuticals. *Pharmacology*. (2022) 107:131–49. doi: 10.1159/000521683

48. Brown DC, Boston RC, Farrar JT. Use of an activity monitor to detect response to treatment in dogs with osteoarthritis. *J Am Vet Med Assoc*. (2010) 237:66–70. doi: 10.2460/javma.237.1.66

49. Ortmeyer HK, Robey L, McDonald T. Combining Actigraph link and PetPace collar data to measure activity, proximity, and physiological responses in freely moving dogs in a natural environment. *Animals (Basel)*. (2018) 8:230. doi: 10.3390/ani8120230

50. Ortmeyer HK, Katzel LI. Effects of proximity between companion dogs and their caregivers on heart rate variability measures in older adults: a pilot study. *Int J Environ Res Public Health*. (2020) 17:2674. doi: 10.3390/ijerph17082674

51. Richard BM, Rickert DE, Newton PE, Ott LR, Haan D, Brubaker AN, et al. Safety evaluation of EXPAREL (DepoFoam bupivacaine) administered by repeated subcutaneous injection in rabbits and dogs: species comparison. *J Drug Deliv*. (2011) 2011:467429:1–14. doi: 10.1155/2011/467429

52. Yaksh TL, Provencher JC, Rathbun ML, Myers RR, Powell H, Richter P, et al. Safety assessment of encapsulated morphine delivered epidurally in a sustained-release multivesicular liposome preparation in dogs. *Drug Deliv*. (2000) 7:27–36. doi: 10.1080/107175400266768

53. Wakshlag JJ, Cital S, Eaton SJ, Prussin R, Hudalla C. Cannabinoid, terpene, and heavy metal analysis of 29 over-the-counter commercial veterinary hemp supplements. *Vet Med (Auckl)*. (2020) 11:45–55. doi: 10.2147/VMRR.S248712

54. Hahn SM. FDA advances work related to cannabidiol products with focus on protecting public health, providing market clarity (2020). Available at: https://www.fda.gov/news-events/press-announcements/fda-advances-work-related-cannabidiol-products-focus-protecting-public-health-providing-market.

55. Hahn SM. Better data for a better understanding of the use and safety profile of cannabidiol (CBD) PRO (2021). Available at: https://www.fda.gov/news-events/fda-voices/better-data-better-understanding-use-and-safety-profile-cannabidiol-cbd-products.

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Cannabidiol plasma determination and pharmacokinetics conducted at beginning, middle and end of long-term supplementation of a broad-spectrum hemp oil to healthy adult dogs

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Introduction: Veterinary hemp products containing cannabidiol (CBD) and negligible psychoactive (THC) have increased popularity since hemp (with <0.3% THC) was removed from schedule 1 substances under the Controlled Substances Act in 2018. This was accompanied by increased CBD research, mostly on the short-term safety and efficacy for inflammatory and neurological conditions. It is imperative to understand how CBD is metabolized or accumulated in the body long-term, thus the goal of the present work was to determine monthly plasma CBD concentrations, as well as changes in pharmacokinetic (PK) parameters in chronically dosed dogs.

Methods: The study was a masked, placebo-controlled, randomized design. Six adult beagles were assigned to placebo, 5 and 10 mg/kg/day CBD treatment groups. Dogs received oral oil treatment once daily for 36 weeks. Blood was collected once every 4 weeks pre- and postprandially for CBD plasma determination (at 0 and 2 h). Pharmacokinetics were conducted at 0, 18 and 36 weeks. Pharmacokinetics and monthly CBD plasma data of dogs who received CBD were analyzed as repeated measures over time using a mixed model, with significance at $\alpha = 0.05$.

Results: Average plasma CBD at 5 and 10 mg/kg were 97.3 ng/mL and 236.8 ng/mL pre-prandial, 341 ng/mL and 1,068 ng/mL postprandial, respectively. PK parameters suggested CBD accumulation over time, with significant increases in C_{max} and AUC at both the 18 and 36-week timepoints. C_{max} and AUC were dose proportional. Half-life demonstrated large inter-individual variations and increased ($p < 0.05$) at weeks 18 and 36 compared to baseline. Volume of distribution was not affected by time or treatment, while MRT increased, and clearance decreased over time ($p < 0.05$).

Conclusions and clinical importance: Chronic administration of CBD to healthy adult dogs led to a dose-proportional accumulation in the body for 36 weeks, which was confirmed by an increased half-life, total exposure, mean residence time and plasma peak. Our data also suggests that CBD plasma levels may have less daily variation if administered twice daily.

KEYWORDS

CBD, hemp, PK, canine, long-term, health

1. Introduction

Hemp (*Cannabis sativa*) originated from Central Asia and has a rich history of therapeutic use by humans that dates back thousands of years B.C. (1). Although hemp has known medicinal properties, regulations in the US prohibited its use with the Marihuana Tax Act of 1937, and it has only recently become available to the public with the Farm Bill of 2018. This regulatory change increased consumer and research interest in cannabidiol (CBD), which is among the most relevant phytocannabinoids (PC) in hemp. Specifically, CBD for canine patients in Veterinary Medicine is known for being well tolerated (2–5) and for having medicinal properties in alleviating symptoms of inflammatory (6–9) and neurological (10, 11) conditions. For instance, CBD was reported to reduce pruritus in canines with atopic dermatitis (8), to improve osteoarthritis symptoms (7), and to reduce seizure frequency in addition to antiepileptic drugs in dogs with drug-resistant idiopathic epilepsy (11, 12).

When any exogenous substance of interest is being introduced to the market, it is essential to understand its pharmacological properties in order to describe its functionality in the target species. Pharmacokinetics (PK) is an effective way to communicate functionality in terms of the spatial and temporal distribution of exogenous substances in a biological system (13). Exogenous substances introduced to biological systems undergo complex kinetic changes that comprise transportation, biochemical modification, and elimination (13). Although pharmacokinetic models may be calculated based on other previously published observed models, such as comparing dog to human and vice versa, gastrointestinal anatomy and physiology differences vastly alter absorption, distribution, metabolism, and elimination in each species (14), which impact the PK of any potentially studied exogenous substance. Therefore, PK studies on the target species are necessary. During a PK, the substance is introduced to the body and timed samples are collected for subsequent measurement of the target molecule. These timed measurements allow researchers to plot a PK curve, and both observe and calculate important parameters with mathematical models.

The majority of CBD PKs in dogs have been conducted with various doses, using different oral supplementation forms or carrier oils, to subjects naïve to the drug (3, 7, 15–18). Only one study, to our knowledge, conducted CBD PK in canines at the study baseline as well as after 28 days of daily CBD dosing, and they found indication of CBD accumulation (5).

Chronic health conditions like epilepsy (11), dermatitis (8) or osteoarthritis (7) require continuous CBD dosing, making it imperative to understand how PK is affected long-term. Therefore, the goal of the present study was to measure and calculate the PK on naïve dogs to CBD, as well as sequential PKs at 18 and 36 weeks of daily CBD administration. A secondary goal was to measure CBD monthly (every 4 weeks) at trough and peak to capture the fluctuation in CBD plasma levels of dogs dosed once daily.

2. Materials and methods

2.1. Animals and study design

Eighteen (nine neutered male, nine spayed female, all Beagle breed) adult healthy dogs, average age 2.3 years ±0.14 (range 2.1 to 2.6 years old), with body weight (BW) 9.5 kg ± 1.80 (range 7.1 to 12.8 kg) at study start were randomly assigned (n = 6) to one of three treatment groups; 5 mg/kg BW CBD,10 mg/kg BW CBD and 0 mg/kg BW CBD (vehicle only). Groups were balanced by sex and body weight, and dogs belonging to the same treatment were housed in pairs or trios when necessary. The study was approved by the Institutional Animal Care and Use Committee (IACUC) at Colorado State University (protocol number 2121).

All dogs were fed controlled amounts of adult dry dog food (Hill's Science Diet Adult Chicken & Barley Recipe; Hill's Pet Nutrition) once daily between 07:00 and 09:00, and dosed once daily with their respective treatment oils within 30 min of feeding. Fresh water was provided *ad libitum*. Dogs were weighed every 2 weeks and both their food offered and CBD doses were adjusted accordingly. All study personnel were masked except for one of the PIs. More detailed information about housing and enrichment has been previously described (19).

2.2. Treatments

Two broad spectrum industrial hemp extracts were formulated to deliver 5 and 10 mg/kg CBD once daily to study dogs in similar volumes. Oils contained 5.1 and 10.0% CBD in a medium-chain triglyceride (MCT) vehicle oil, respectively, where 95% of the cannabinoid profile was CBD. There was a negative control group that received the MCT oil without hemp. Cannabidiol concentrations were measured using high performance liquid chromatography (HPLC) with UV absorption and diode array detector (DAD) at an external laboratory [SC Labs, Denver CO, United States]. The CBD determination was conducted at the beginning, middle and end of the experiment, as previously described (19). There were non-detectable levels of delta-8 and delta-9 THC in both oils.

2.3. Plasma CBD determinations

Plasma samples were processed and quantified at the Flint Animal Cancer Center, Drug Discovery and Development Shared Resources Core facility at Colorado State University (Fort Collins, CO, United States). Plasma extracts were prepared by liquid–liquid extraction using D3-CBD (Cerilliant Corporation, Sigma Aldrich, Round Rock, TX, United States) as the internal standard (IS) at a final concentration of 200 ng/mL. A standard curve was created in acetonitrile ranging from 0.98 ng/mL to 2000 ng/mL by spiking 100 ul blank canine plasma with 10 µL of 10x Standard at each

concentration along with 10 uL of 10X D3-CBD as internal standard. One hundred microliters of sample plasma was spiked with 10 µL of 10X IS and 10 µl of acetonitrile then all samples were mixed with 500 µL ethyl acetate on a shaker for 10 min at room temperature. Samples were centrifuged for 10 min at 14,000x g to separate organic and aqueous phases, then 400 µL of organic phase from each extract was transferred to a fresh tube and evaporated to dryness in a SpeedVac on high heat for 30–40 min. The remaining pellet was reconstituted with 100 µL acetonitrile before transferring to vials with glass inserts. The CBD in processed plasma extracts was isolated by injecting 30 microliters using a LEAP autosampler onto a Waters Sunfire C18, 5 µm, 4.6 × 50 mm column, and quantified by mass spectrometry (Sciex 3,200 Q-TRAP triple quadrupole MS; Applied Biosystems, Inc., Foster City, CA, United States). The column oven was set to 30°C, flow rate set to 1,000 µL/min (LC-20 AD HPLC system, Shimadzu Corporation, Kyoto, Japan), and total run time was 7 min. The mobile phase was composed of acetonitrile with 0.1% formic acid and HPLC-grade water with 0.1% formic acid at the proportions 75:25 for 1.5 min, 99:1 for 3.5 min, and 75:25 for 2 min. Data acquisition was performed using Sciex Analyst software v1.7.1. Quantitation analysis of CBD was performed using a linear fit to calibration with a weighted least square (1/x2) regression using 12 standards.

As described in a recent CBD tolerability study (19), 0 h and 2 h post-prandial plasma collections occurred every 4 weeks for 36 weeks. Cannabidiol trough (0 h) and peak (2 h) plasma concentrations were measured. The 0 h represented time of fast and nearly 24 h after last dose, while the 2 h after feeding and dosing was an assumption that CBD peak concentrations would be captured based on previous research (7, 16, 18).

2.4. Pharmacokinetics

Pharmacokinetics were conducted at 3 instances during the study: at the beginning, when dogs were naïve to CBD (day 0), as well as at 18 and 36 weeks of chronic daily supplementation. During each PK, a catheter was first placed on one of the front limbs of each dog. Four mL blood were collected from the cephalic vein for timepoint 0 h, and 2 mL was transferred to a green-top tube (BD Vacutainer® sodium heparin 33 IU; BD Company, Franklin Lakes, NJ, United States) for CBD plasma determination (2 mL was stored as serum for metabolomics determination, not presented here). Dogs were fed in groups of 3 to stagger blood collections. Immediately after 10 min, food bowls were removed and dogs were dosed with their respective oils. The exact time of oil dosed was recorded, and 2 mL of blood was collected for CBD determinations at 2, 4, 8, 12, 24, and 48 h of the initial dosing time. Green-top tubes with blood were kept in ice for around 1 h until processed. Tubes were centrifuged (Avanti J-15R centrifuge; Beckman Coulter Company, Pasadena, CA, United States) at 4°C for 10 min at 2,000 G to separate plasma, which was immediately frozen at −80°C.

A non-compartmental modeling approach was used to measure PK parameters (13) and were calculated using a software (Phoenix WinNonlin™; Certara, Princeton, NJ, United States). These included time to reach maximum CBD peak (C_{max}), time to reach maximum CBD peak normalized by dose ($C_{max}D$), time at which CBD reached its peak (T_{max}), half-life or the time it took for plasma CBD concentration to be reduced to half of its peak ($T_{1/2}$), area under the curve from 0 to 48 h or total CBD exposure within this time frame ($AUC_{0\text{-}48h}$), area under the curve from 0 to 48 h normalized by dose ($AUCD_{0\text{-}48h}$), volume of distribution (Vz/F), clearance (Cl/F) and mean residence time (MRT). Because an intravascular arm was not included in this study, the bioavailability parameter "F" could not be calculated and is currently undetermined.

2.5. Statistical analysis

Data were analyzed as repeated measures over time using the generalized linear mixed model (GLIMMIX) procedure from statistical analysis software (SAS Institute v 9.4, Cary, NC). Specifically, changes in PK parameters were analyzed over 3 timepoints (0, 18, and 36 weeks), while changes in CBD plasma concentration (trough and peak) were analyzed every 4 weeks. Fixed effects were timepoint (time), treatment and their interaction. The subject was defined as dog nested within treatment, and covariance structure was defined as unstructured (UN) for plasma CBD and compound symmetry (CS) for PK parameters based on the Bayesian information criterion (BIC). When data did not meet model assumptions assessed by studentized residuals plot, natural logarithm transformation was performed, and data were back transformed to the original scale for reporting. Pairwise treatment comparisons were adjusted with Tukey–Kramer post-hoc test to protect against type I error. Significance was noted at an $\alpha = 0.05$.

3. Results

3.1. Pharmacokinetics

The 48 h PK parameters were compared both between treatments (5 and 10 mg/kg CBD) and across time (weeks 0, 18, and 36). The placebo group had non-detectable to negligible CBD levels throughout the entire study, as expected, and thus was not reported. A visual representation of the 3 PKs was plotted (Figure 1). There was clear evidence for a cumulative effect of CBD, as well as dose-proportional magnitude changes. Cannabidiol C_{max} was approximately twice as high in 10 versus 5 mg/kg dose treatment group when administered to naïve dogs, and nearly 3-fold greater in 10 vs. 5 mg/kg at weeks 18 and 36 [Table 1; P (Treatment) = 0.003 and P (Time) = 0.005]. When C_{max} was normalized by dose ($C_{max}D$), the treatment effect was lost but plasma CBD concentrations increased over each time of PK collection [P (Time) = 0.005]. Likewise, $AUC_{0\text{-}48h}$ at week 0 was approximately twice as high in dogs given 10 mg/kg vs. 5 mg/kg, and this difference increased over time [P (Treatment) = 0.001 and P (Time) < 0.0001]. The $AUC_{0\text{-}48h}$ normalized by dose ($AUC_{0\text{-}48h}D$), similar to $C_{max}D$, showed a clear increase in total CBD exposure at both weeks 18 and 36 [P (Time) < 0.0001]. The time to reach C_{max} was 2 h for most dogs, with a few exceptions where the CBD maximum observed level was closest to 8 or 12 h.

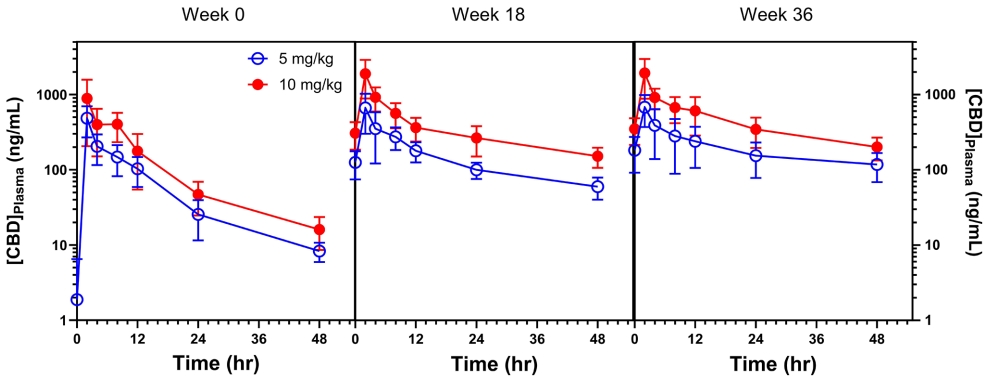

FIGURE 1
Pharmacokinetic curves (mean ± standard error) of dogs administered 5 and 10 mg/kg CBD at week 0 (baseline, naïve to CBD), and at weeks 18 and 36 of chronic daily CBD supplementation.

TABLE 1 Non-compartmental pharmacokinetics parameters means [95% CI] of beagles continuously dosed with 5 and 10 mg/kg CBD ($n = 6$) once daily for 36 weeks.

	Week 0		Week 18		Week 36		*P*	*P*
Treatment	5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg	(Treatment)	(Time)
C_{max}, ng/mL	441^{bx} [284, 686]	880^{ax} [566, 1,369]	585^{by} [376, 909]	$1{,}709^{ay}$ [1,099, 2,657]	616^{by} [396, 957]	$1{,}746^{ay}$ [1,123, 2,714]	0.003	0.005
$C_{max}D$, kg*ng/mL/mg	88.2^{x} [56.8, 137.2]	88.0^{x} [56.6, 136.9]	116.9^{y} [75.2, 181.8]	170.9^{y} [109.9, 265.7]	123.1^{z} [79.2, 191.4]	174.6^{z} [112.3, 271.4]	0.342	0.005
$AUC_{0\text{-}48h}$, h*ng/mL	$3{,}525^{bx}$ [2,650, 4,690]	$6{,}930^{ax}$ [5,209, 9,220]	$7{,}205^{by}$ [5,415, 9,586]	$18{,}033^{ay}$ [13,554, 23,992]	$9{,}346^{bz}$ [7,025, 12,434]	$22{,}138^{az}$ [16,640, 29,454]	0.001	<0.0001
$AUCD_{0\text{-}48h}$, hr.*kg*ng/ml/mg	705^{x} [530, 938]	693^{x} [521, 922]	$1{,}441^{y}$ [1,083, 1917]	$1{,}803^{y}$ [1,355, 2,399]	$1{,}869^{z}$ [1,405, 2,487]	$2{,}214^{z}$ [1,664, 2,945]	0.467	<0.0001
[1]T_{max}, h	3.67 ± 4.082 (2–12)	3 ± 2.45 (2–8)	2 ± 0.0 (2–2)	2 ± 0.0 (2–2)	2 ± 0.0 (2–2)	3 ± 2.45 (2–8)	–	–
[2]$T_{1/2}$, h	8.8^{y} [6.2, 12.5]	12.6^{y} [8.8, 17.9]	24.6^{z} [17.3, 35.0]	28.3^{z} [19.9, 40.3]	30.6^{z} [20.7, 45.3]	26.9^{z} [18.9, 38.2]	0.459	<0.0001
Vz/F, L/kg	18.0 [10.5, 30.9]	26.2 [15.2, 44.9]	24.6 [14.3, 42.2]	22.7 [13.2, 38.9]	35.1 [20.5, 60.3]	17.5 [10.2, 30.1]	0.597	0.856
CL, L/h/kg	1.418^{x} [1.066, 1.887]	1.444^{x} [1.085, 1.921]	0.694^{y} [0.522, 0.923]	0.555^{y} [0.417, 0.738]	0.535^{z} [0.402, 0.712]	0.452^{z} [0.340, 0.601]	0.468	<0.0001
$MRT_{0\text{-}48h}$	9.93^{x} [7.82, 12.04]	11.11^{x} [9.00, 13.22]	14.91^{y} [12.80, 17.02]	14.63^{y} [12.52, 16.74]	17.82^{z} [15.71, 19.93]	15.50^{z} [13.39, 17.61]	0.697	<0.0001

Pharmacokinetics was conducted at weeks 0, 18, and 36, and effects of treatment and time are shown below.
[a,b]Different superscripts denounce significance between treatments within each parameter in a row.
[x–z]Different superscripts denounce significance between timepoints within each parameter in a row.
[1]Mean ± Standard deviation (range) was reported for T_{max} due to non-parametric data.
[2]One outlier in the 5 mg/kg CBD treatment at week 36 was removed from the table.

Half-life ($T_{1/2}$) was similar between treatments [*P* (Treatment) = 0.459] and increased with chronic dosing from week 0 to 18, plateauing from week 18 to 36 [*P* (Time) < 0.0001]. One dog in group 5 mg/kg had a calculated $T_{1/2}$ of 316 h at the third PK (week 36), so it was considered an outlier and removed from statistical analysis.

Volume of distribution (Vz/F) was similar between treatments and did not change over time, ranging from 17.5 to 35.1 L/kg (Table 1). Clearance (CL) decreased over time [*P* (Time) < 0.0001], whereas MRT increased at both weeks 18 and 36 [*P* (Time) < 0.0001].

3.2. Monthly plasma CBD

Plasma CBD was determined at its hypothesized trough level (at fast, nearly 24 h after its last dose, Figure 2A), and close to its peak at 2 h after feeding and dosing, Once every 4 weeks for 36 weeks (Figure 2B). The

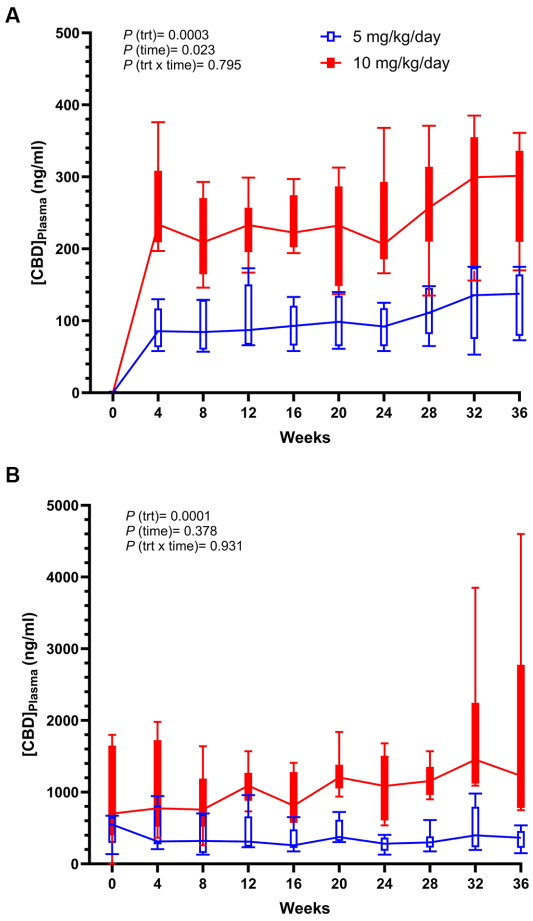

FIGURE 2
Box plots of trough (0 h) **(A)** and peak (2 h) **(B)** plasma CBD measured over time of dogs supplemented daily with 10 mg/kg CBD (solid red) and 5 mg/kg CBD (unfilled blue). *p*-values reported include treatment (trt), time, and treatment by time (trt x time).

concentration of CBD present in plasma averaged from 104 to 390 ng/mL in dogs supplemented with 5 mg/kg CBD, and from 246 to 1,216 ng/mL in dogs supplemented with 10 mg/kg CBD. There were markedly treatment differences at both trough ($p = 0.0003$) and peak ($p = 0.0001$) collections, and only CBD trough levels had a significant time effect (Figure 2A), although pairwise comparisons between months did not show a difference after Tukey adjustment. The monthly variation in plasma CBD at 2 h collection among dogs receiving the same treatment was greater than that at fast, and dogs receiving 10 mg/kg/d CBD had the greatest variation at weeks 32 and 36 (Figure 2B).

4. Discussion

The primary goal of the present work was to quantify PK changes over time in orally supplemented dogs with CBD at 5 and 10 mg/kg using an MCT oil vehicle. There was a placebo group not presented in this work that served as negative control for a previous CBD tolerability study (19). The target dose 5 mg/kg was chosen based on what has been previously studied in dogs with idiopathic epilepsy (11), osteoarthritis (7, 9), and anxiety in humans (20). The 10 mg/kg dose was chosen as in previous studies also showed tolerability and safety (7, 15), with differences only found with an increased C_{max} compared with lower CBD dosage (7). There have been some single-dose PK studies in dogs (3, 7, 15–18) that were conducted over 24 h using different CBD oral formulations and doses, whereas the present work performed multiple 48 h-PKs during long-term administration. To the authors' knowledge, there are only two studies to date that investigated the effects of repeated CBD doses on PK parameters of dogs (5, 21) which had some common findings to the present work that will be discussed in more detail below.

When administered to naïve dogs, broad spectrum hemp extracts containing mainly CBD present differences in PK parameters across studies that can be attributed to factors like vehicle choice (15, 18), fed versus fasted state of dogs (17, 22, 23) and PK length. For instance, when comparing two oils and a soft chew with similar concentrations of CBD, (18) found that the solid form had a delayed and higher absorption peak relative to the oils. Similarly, three different CBD delivery formats (oil, capsule and dermal cream) had a much greater impact on PK, affecting most parameters (15). Since each hemp formula and format are metabolized differently, direct comparisons among studies must be made with caution. When supplementing the same doses as the current study, but using a herbal extract containing 1:20 THC:CBD (16), CBD C_{max} were reported to be twice as high.

There have been a few studies that reported a CBD dose-dependent C_{max} and AUC (7, 16), similar to what was observed in the present study. This dose effect does not seem to be linear, as CBD administered at 20 mg/kg in dogs (17) and over 3,000 mg in humans (24) did not reflect proportional increases in C_{max} and AUC relative to smaller doses. Dogs in the present work seemed to fall within the linear window of a dose magnitude effect on C_{max} and AUC at 5 and 10 mg/kg/d CBD. When normalizing both C_{max} and $AUC_{0\text{-}48h}$ by dose, our work clearly showed a cumulative effect of CBD over time, which was also reported in dogs (5) and rats (25) after 28 days of daily CBD supplementation. The latter also found significant PK differences between males and females (25), which were not evidenced in the present study because dogs in each treatment were balanced by sex, age and weight. However, we were able to compare sex differences herein and found that Cl/F was higher in females ($p = 0.003$), and Cmax_D had a tendency ($p = 0.087$) to be higher in males.

The novelty about the present work is that values of $AUCD_{0\text{-}48h}$, $AUC_{0\text{-}48h}$, and $C_{max}D$ increased from week 0 to 18, as well as from week 18 to 36, indicating continuous accumulation over a long period of time. These results could be expected because cannabinoids are highly lipophilic (25, 26), and CBD was observed to accumulate 10–100 fold greater in adipose tissue than in hepatic or muscular tissues of rats (25). Thus, cannabidiol accumulation in adipose tissue would also be expected to occur in dogs. Long-term increases in C_{max} and $AUC_{0\text{-}48h}$ could be a consequence of CBD being mobilized from adipose tissue, but this was not measured. Trough and peak CBD plasma levels did not increase over time to corroborate PK findings, what emphasizes the importance in conducting the 48-h PKs. Like CBD measured at trough and peak timepoints, the average plasma CBD measured at the same timepoints during the 3 PKs in Figure 1 were at similar levels; however, the slopes between weeks 0 and 18 had a drastic change, and the negative slope had a further decrease at week 36 PK. These changes in slopes led to the increases in $AUCD_{0\text{-}48h}$ and $AUC_{0\text{-}48h}$.

Cannabidiol's T_{max}, or time to reach its plasma peak, has been relatively consistent among studies (3, 5, 7, 16, 18) at 1 to 4 h, and is not

influenced by dose or chronic administration according to both the present work and (18). Conversely, half-life has been reported to widely vary among dogs, and to range from nearly 1 to 24 h in single-dose PK studies (5, 7, 15, 16, 18). Elimination $T_{1/2}$ is a dependent variable directly related to volume of distribution and inversely related to clearance, which are both independent variables (27). Although $T_{1/2}$ does not have much value in predicting drug elimination with a single dose, it is valuable in predicting the rate of drug accumulation and elimination after consecutive doses (27). In the present study, half-life almost tripled after 18 weeks, and remained similar at week 36, strengthening the argument that CBD accumulates in dogs over time. This also corroborates with previous findings in regard to a half-life increase after 28d of CBD administration twice daily at various doses (5).

Volume of distribution via extravascular (Vz/F) is calculated as the collective amount of a compound present in the body that was absorbed over the PK. It can be defined as the volume of plasma that would be necessary to account for the total amount of drug in the patient's body, if that exogenous substance were present throughout the body at the same concentration as found in the plasma. A high Vz/F indicates that the substance or drug is extensively distributed in other tissues rather than present in the blood (27). Volume of distribution of CBD found in dogs in the present work was comparable to what has been reported in humans (24) and horses (28). In contrast, volume of CBD distribution in cats was reported to be higher (29); although there is limited research in cats, this may indicate that cats and dog Vz/F cannot be compared. Clearance (CL) refers to the hypothetical volume of plasma from which a drug is completely removed per hour (27), and in this study, it decreased over 36 weeks indicating a lower rate of CBD elimination with chronic administration. The CL rate was also similar in horses (28) at the week 0 timepoint in this study. Cannabidiol accumulation and rate of elimination may be attributed to its lipophilic nature, as well as to anatomical differences of mammals. For instance, the adipose distribution of fascia in both dogs and humans have a superficial adipose tissue, whereas the horse fascia lacks this adipose layer (30). Thus, if CBD was administered to horses long-term, we could expect consecutive CL measurements to differ from the dog due to a greater accumulation in the fascia superficial adipose tissue of canines. This theory would need scientific evidence to be validated. Finally, MRT refers to the average time CBD spends in the body before being eliminated (13), and in this study MRT also reflected CBD accumulation over time. Although both CL and MRT indicated CBD storage mostly in adipose tissue, Vz/F was unaffected by time. This lack of significance could have happened because of the high intraspecies variation, as well as due to Vz/F calculation that does not account for the ratio of CBD distribution among body tissues.

A secondary goal of the present study was to determine plasma CBD concentrations over 36 weeks. Plasma CBD presented monthly variations when measured both at trough and peak (pre-prandially and 2 h pos-feeding and dosing). After the first month (week 4) of chronic administration, CBD had already reached high levels. It might be possible that plasma CBD had a weekly incline during the first month, similar to what was (2) reported, but this was not captured here. High intragroup variation was found in the current study and also corroborates previous plasma CBD research (2). In their work CBD peak levels were not measured, so they could only assume what it was before these were measured in the current study. After nearly 24 h of dosing, plasma CBD levels dropped 3.8–4.9 times that of its supposed peak. It has been suggested that CBD plasma levels correlates with a reduction in seizure activity in dogs (11), so it might be necessary to administer CBD twice daily to maintain consistent therapeutic CBD plasma levels. Twice daily chronic dosing might also lower the impact on liver enzymes such as ALP, which has been vastly reported to increase in dogs taking CBD (2, 4, 5, 7, 17, 31) but this still needs scientific evidence. Dosing recommendations including frequency for chronic use should be further investigated in regard to how it may influence clinical outcomes of dogs.

Some study strengths included sample size, PK duration, and repeated PKs over a long-time interval. Although sample size may be deemed a small representation ($n = 6$), the repeated measures for each treatment at 3 timepoints allowed it to be sufficient to detect both treatment and time differences, with a power of 93% for treatment and 86% for time based on $AUC_{0\text{-}48h}$, determined by the GLMPOWER procedure from SAS (v 9.4).

A study limitation was that intravenous CBD AUC was not determined, and that would be necessary to calculate absolute bioavailability. A single-PK study with 8 horses dosed at 10 mg/kg CBD found oral bioavailability to be low (14%) for CBD formulated with sesame oil as the vehicle (28). Likewise, a study in humans found that single doses of 3 forms of oral CBD had <7% bioavailability, and it was lower in fasted than fed states (24). Doran et al. (17) reported that both C_{max} and AUC were greater in fed vs. fasted dogs, which indicates that dosing dogs at a fed state in the current study likely contributed to a higher CBD absorption and bioavailability. Although bioavailability of any oral CBD suspension has not yet been determined for canines to the authors' knowledge, it would be expected to be low if we extrapolate what has been found in other monogastric animals (24, 28). Future studies should focus on determining CBD absolute bioavailability, as well as understand the effect of CBD that bypasses small intestine digestion on the colonic microbiome of dogs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Institutional Animal Care and Use Committee (IACUC) at Colorado State University (protocol number 2121). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

IC: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft. DG: Data curation, Methodology, Software, Validation, Visualization, Writing – review & editing. KB: Data curation, Methodology, Validation, Writing – review & editing. KW: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. SM: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

KW works at Hill's Pet Nutrition and SM consults for a CBD company.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Rehman M, Fahad S, du G, Cheng X, Yang Y, Tang K, et al. Evaluation of hemp (*Cannabis sativa* L.) as an industrial crop: a review. *Environ Sci Pollut Res*. (2021) 28:52832–43. doi: 10.1007/s11356-021-16264-5

2. Bradley S, Young S, Bakke AM, Holcombe L, Waller D, Hunt A, et al. Long-term daily feeding of cannabidiol is well-tolerated by healthy dogs. *Front Vet Sci*. (2022) 9:7457. doi: 10.3389/fvets.2022.977457

3. Deabold KA, Schwark WS, Wolf L, Wakshlag JJ. Safety assessment with use of CBD-rich hemp nutraceutical in healthy dogs and cats. *Animals*. (2019) 9:1–13. doi: 10.3390/ani9100832

4. McGrath S, et al. A report of adverse effects associated with the administration of cannabidiol in healthy dogs. *J Am Hol Vet Med Assoc*. (2018) 52:34–8.

5. Vaughn DM, Paulionis LJ, Kulpa JE. Randomized, placebo-controlled, 28-day safety and pharmacokinetics evaluation of repeated oral cannabidiol administration in healthy dogs. *Am J Vet Res*. (2021) 82:405–16. doi: 10.2460/ajvr.82.5.405

6. Brioschi FA, di Cesare F, Gioeni D, Rabbogliatti V, Ferrari F, DUrso ES, et al. Oral transmucosal cannabidiol oil formulation as part of a multimodal analgesic regimen: effects on pain relief and quality of life improvement in dogs affected by spontaneous osteoarthritis. *Animals*. (2020) 10:1–14. doi: 10.3390/ani10091505

7. Gamble L-J, Boesch JM, Frye CW, Schwark WS, Mann S, Wolfe L, et al. Pharmacokinetics, safety, and clinical efficacy of Cannabidiol treatment in osteoarthritic dogs. *Front Vet Sci*. (2018) 5:1–9. doi: 10.3389/fvets.2018.00165

8. Loewinger M, Wakshlag JJ, Bowden D, Peters-Kennedy J, Rosenberg A. The effect of a mixed cannabidiol and cannabidiolic acid based oil on client-owned dogs with atopic dermatitis. *Vet Dermatol*. (2022) 33:329–e77. doi: 10.1111/vde.13077

9. Mejia S, Duerr FM, Griffenhagen G, McGrath S. Evaluation of the effect of Cannabidiol on naturally occurring osteoarthritis-associated pain: a pilot study in dogs. *J Am Anim Hosp Assoc*. (2021) 57:81–90. doi: 10.5326/JAAHA-MS-7119

10. Hunt ABG, Flint HE, Logan DW, King T. A single dose of cannabidiol (CBD) positively influences measures of stress in dogs during separation and car travel. *Front Vet Sci*. (2023) 10:13–5. doi: 10.3389/fvets.2023.1112604

11. McGrath S, Bartner LR, Rao S, Packer RA, Gustafson DL. Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. *J Am Hol Vet Med Assoc*. (2019) 254:1301–8. doi: 10.2460/javma.254.11.1301

12. Rozental A, et al. The efficacy and safety of cannabidiol as adjunct therapy for drug-resistant idiopathic epilepsy in 51 dogs: a double-blinded crossover study. *J Vet Intern Med*. (2023).

13. Foster DM, Vicine P. Chapter 8 - Non-compartmental and compartmental approaches to pharmacokinetics data analysis. In: Atkinson AJ, Huang S-M, Lertora JJL, Markey SP, editors. *Principles of clinical pharmacology*. *3rd* ed. Academic Press (2012). p. 97–116.

14. Martinez MN, Mochel JP, Neuhoff S, Pade D. Comparison of canine and human physiological factors: understanding interspecies differences that impact drug pharmacokinetics. *AAPS J*. (2021) 23:59. doi: 10.1208/s12248-021-00590-0

15. Bartner LR, McGrath S, Rao S, Hyatt LK, Wittenburg LA. Pharmacokinetics of cannabidiol administered by 3 delivery methods at 2 different dosages to healthy dogs. *Can J Vet Res*. (2018) 82:178–83.

16. Chicoine A, Illing K, Vuong S, Pinto KR, Alcorn J, Cosford K. Pharmacokinetic and safety evaluation of various Oral doses of a novel 1:20 THC:CBD Cannabis herbal extract in dogs. *Front Vet Sci*. (2020) 7:1–12. doi: 10.3389/fvets.2020.583404

17. Doran CE, McGrath S, Bartner LR, Thomas B, Cribb AE, Gustafson DL. Drug-drug interaction between cannabidiol and phenobarbital in healthy dogs. *Am J Vet Res*. (2022) 83:86–94.

18. Wakshlag JJ, Schwark WS, Deabold KA, Talsma BN, Cital S, Lyubimov A, et al. Pharmacokinetics of Cannabidiol, Cannabidiolic acid, Δ9-tetrahydrocannabinol, Tetrahydrocannabinolic acid and related metabolites in canine serum after dosing with three Oral forms of hemp extract. *Front Vet Sci*. (2020) 7:1–12. doi: 10.3389/fvets.2020.00505

19. Corsato Alvarenga I, Wilson K, McGrath S. Tolerability of long-term CBD supplementation to healthy adult dogs. *J Vet Intern Med*. (2023)

20. Bahji A, Meyyappan AC, Hawken ER. Efficacy and acceptability of cannabinoids for anxiety disorders in adults: a systematic review & meta-analysis. *J Psychiat Res*. (2020) 129:257–64. doi: 10.1016/j.jpsychires.2020.07.030

21. Fernández-Trapero M, Pérez-Díaz C, Espejo-Porras F, de Lago E, Fernández-Ruiz J. Pharmacokinetics of sativex® in dogs: towards a potential cannabinoid-based therapy for canine disorders. *Biomol Ther*. (2020) 10:1–8. doi: 10.3390/biom10020279

22. Amstutz K, Schwark WS, Zakharov A, Gomez B, Lyubimov A, Ellis K, et al. Single dose and chronic oral administration of cannabigerol and cannabigerolic acid-rich hemp extract in fed and fasted dogs: physiological effect and pharmacokinetic evaluation. *J Vet Pharmacol Ther*. (2022) 45:245–54. doi: 10.1111/jvp.13048

23. Łebkowska-Wieruszewska B, Stefanelli F, Chericoni S, Owen H, Poapolathep A, Lisowski A, et al. Pharmacokinetics of Bedrocan® a cannabis oil extract, in fasting and fed dogs: an explorative study. *Res Vet Sci*. (2019) 123:26–8. doi: 10.1016/j.rvsc.2018.12.003

24. Lim SY, Sharan S, Woo S. Model-based analysis of Cannabidiol dose-exposure relationship and bioavailability. *Pharmacotherapy*. (2020) 40:291–300. doi: 10.1002/phar.2377

25. Child RB, Tallon MJ. Cannabidiol (CBD) dosing: plasma pharmacokinetics and effects on accumulation in skeletal muscle, liver and adipose tissue. *Nutrients*. (2022) 14:2101. doi: 10.3390/nu14102101

26. Jewell A, Brookes A, Feng W, Ashford M, Gellert P, Butler J, et al. Distribution of a highly lipophilic drug cannabidiol into different lymph nodes following oral administration in lipidic vehicle. *Eur J Pharm Biopharm*. (2022) 174:29–34. doi: 10.1016/j.ejpb.2022.03.014

27. Greenblatt DJ. Elimination half-life value and limitations. *Ann Rev Med*. (1985) 36:421–7. doi: 10.1146/annurev.me.36.020185.002225

28. Sánchez de Medina A, et al. Pharmacokinetics and oral bioavailability of cannabidiol in horses after intravenous and oral administration with oil and micellar formulations. *Equine Vet J*. (2023):1–10. doi: 10.1111/evj.13923

29. Rozental AJ, Gustafson DL, Kusick BR, Bartner LR, Castro SC, McGrath S. Pharmacokinetics of escalating single-dose administration of cannabidiol to cats. *J Vet Pharmacol Ther*. (2023) 46:25–33. doi: 10.1111/jvp.13100

30. Ahmed W, Kulikowska M, Ahlmann T, Berg LC, Harrison AP, Elbrønd VS. A comparative multi-site and whole-body assessment of fascia in the horse and dog: a detailed histological investigation. *J Anat*. (2019) 235:1065–77. doi: 10.1111/joa.13064

31. Vaughn D, Kulpa J, Paulionis L. Preliminary investigation of the safety of escalating cannabinoid doses in healthy dogs. *Front Vet Sci*. (2020) 7:1–13. doi: 10.3389/fvets.2020.00051

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Dermatological evaluation in dogs with atopic dermatitis treated with full-spectrum high cannabidiol oil: a pre study part 1

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Introduction: Dermatological consultations represent a great part of the small animal medical clinic routine. Canine atopic dermatitis (CAD) is a common skin disease that affects a significant amount of dogs, making it a relevant consideration in clinical practice. The role of the endocannabinoid system on skin homeostasis has been described and its deregulation contributes to dermatopathies. Its function in specialized skin cells reveals an expressive therapeutic potential. Due to the difficulties and the growing scientific evidence of the therapeutic benefits of cannabis on animals, this work aimed to evaluate the anti-inflammatory effects of cannabis-derived oil in the treatment of CAD.

Methods: Fourteen canines diagnosed with CAD were divided into two groups: T: full spectrum high cannabidiol (CBD) cannabis oil, 2,5 mg/kg; and C: control group (treated with olive oil alone). The effectiveness was evaluated based on the degree of pruritus, dermatological evaluation (CADESI-4) and histopathological evaluation of the skin including mast cell count.

Results: Despite the theoretical basis, there were no significant results obtained between the compared treatments.

Discussion: Thus, it can be concluded that although full spectrum high cannabinoids therapy presents a promising approach to immunological diseases, further research is required in order to establish the actual effective cannabinoid ratio within the myriad possible combinations and for multi-target therapy of CAD.

KEYWORDS

canine atopic dermatitis, cannabidiol, cannabis, histopathology, mast cell count, veterinary dermatology

Introduction

Canine atopic dermatitis (DAC) is a disorder resulting in chronic inflammation and pruritus (1, 2). DAC is multifactorial and its pathogenicity is not well elucidated. It is known to be genetic predisposition with immunological alterations leading to defective cutaneous barriers and hyperinflation of the skin (3). The syndrome begins with sensitivity to environmental allergens, mainly house dust mites, which penetrate the skin and stimulate the recruitment of inflammatory cells and IgE-mediated mast cell degranulation (2, 4). The management of this disease requires a multimodal therapy aimed at improving the skin barrier, immunomodulation, and prevention of allergies (5).

According to Tóth et al. (6), the endocannabinoid system plays a key role in skin homeostasis, barrier formation, and regeneration. Disruptions to this system can lead to diseases and disorders, such as atopic dermatitis. The endocannabinoid system is present in immune cells and skin-specific cells such as keratinocytes, fibroblasts, melanocytes, and sebocytes, making them all potential therapeutic targets (7). Current literature suggests that the effects of cannabinoids and cannabinoid-related receptors on specialized skin cells modulate inflammation and offer a novel approach to treating atopic dermatitis by regulating different mechanisms of the disease (8).

In light of the strong relationship between the endocannabinoid system and skin homeostasis, the objective was to evaluate the effectiveness of full-spectrum cannabis oil rich in CBD in dogs with atopic dermatitis through dermatological assessment, using CADESI-4 and pruritus degree, as well as histopathological analysis with mast cell counting in three regions affected by atopic dermatitis.

Materials and methods

Ethics committee

This study was approved by the Ethics Committee for Animal Use and Experimentation of the Federal University of Santa Maria (CEUA/UFSM) (number 8656301121 - ID 003662) and was conducted in accordance with the ethical principles of the National Council for Animal Experimentation Control (CONCEA).

Animal selection

Canine subjects with a diagnosis of atopic dermatitis (AD), with prior exclusion of food allergy and flea bite dermatitis, were selected according to the criteria of Favrot.

The inclusion criteria were: AD diagnosis; Absence of concurrent diseases; No systemic treatment within the past 30 days; The exclusion criteria were: Lack of regular flea control; Presence of dental calculus; Moderate-to-severe gingivitis; Any other concurrent disease. No maximum or minimum CADESI values were stipulated for selection or exclusion. Additionally, there was no standardization in the use of shampoo for topical treatment or type of diet used, in order to mimic clinical routine.

The fourteen canines (Table 1) were divided into two groups: T: the treatment group, treated with cannabis oil, and C: the control group, treated with olive oil (diluent of the product used in the treatment group with maximum acidity of 0,4%). The cannabis oil used contained a full spectrum with a higher concentration of cannabidiol (CBD) at 1,500 mg, in a ratio of 21:1 for CBD:THC (AMA+ME®). The treatment involved administering 2.5 mg/kg, twice a day for 60 days. Assessments were conducted before (T0) and after (T60) the treatment. The frequency of baths, shampoos, and diets already in use, whether therapeutic or not, was maintained. It was also recommended to withhold any type of treat from the animal.

Dermatological evaluation

The animal was assessed by a dermatologist using the CADESI-4 score, and according to the owner, the degree of pruritus was evaluated using the scale adapted. Both the dermatologist and the owner were blinded to the treatment.

Histopathology and mast cells count

For skin biopsy, intravenous sedation was performed using 3 μg/kg fentanyl and 4 μg/kg of dexmedetomidine (Dexdomitor®, Zoetis). The biopsy was taken from the interdigital, axillary, and inguinal regions with the aid of a scalpel or punch. The tissue samples were preserved in 10% formalin for subsequent histopathological evaluation and mast cell counting using special toluidine blue staining under a 400× objective. Suturing was done using 3-0 Sultan nylon sutures.

The histopathological evaluation criteria included (a) Orthokeratotic hyperkeratosis, (b) acanthosis, and (c) perivascular and periadnexal lymphoplasmacytic dermal inflammation. These criteria were subsequently classified into four grades (absent, mild, moderate, and severe) for the interdigital, axillary, and inguinal regions.

Statistics analysis

Descriptive statistics were performed for qualitative variables, including calculating frequencies (both absolute and relative), and for quantitative variables, measures of central tendency and variability were calculated. The Shapiro-Wilk test was conducted to test the normality of the data. For comparisons between two groups, the Student's *t*-test (independent groups) or paired *t*-test (dependent groups) were used when the data did not follow a normal distribution. The Mann-Whitney *U* test (independent groups) or Wilcoxon signed-rank test (dependent groups) were applied in case of non-normality. The Chi-Square test was used for the association between qualitative variables. Statistical Package for Social Sciences (SPSS) version 17.0 software was used for the analyses, and the significance level was set at 5%.

Results

Dermatological evaluation

In the CADESI-4 evaluation (Table 2), there was a difference in pre- and post-treatment for group C ($p = 0{,}042$), but there was no difference within group T ($p = 0{,}398$) or between the treatments performed ($p = 0.654$). The limit values obtained from this evaluation are shown in Figure 1.

Regarding the degree of pruritus (Table 2), assessed according to the tutor's evaluation, there was no difference the tested treatments ($p = 0.396$), as well as in the pre- and post-treatment within group T ($p = 0.186$), but there was a significant decrease in group C ($p = 0.039$) (Figure 1).

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TABLE 1 Animal demographics.

Group	Sex	Age	Race	Age of disease	Previous treatment
Control					
Animal 1	Male	12 years	Shih-tzu	6 months	Corticosteroid
Animal 2	Female	1 year	Lhasa apso	9 months	Corticosteroid
Animal 3	Female	5 years	Shih-tzu	1 year	Oclacitinib
Animal 4	Female	1 year	Dachshund	10 months	Corticosteroid, oclacitinib
Animal 5	Female	11 years	Shih-tzu	1 year	Corticosteroid, oclacitinib, caninized monoclonal antibody
Animal 6	Male	7 years	Shih-tzu	3 years	Corticosteroid
Animal 7	Male	6 years	Dachshund	3 years	Corticosteroid
CBD					
Animal 1	Female	8 years	Shih-tzu	6 months	Corticosteroid
Animal 2	Female	10 years	Shih-tzu	3 years	Corticosteroid, oclacitinib, caninized monoclonal antibody
Animal 3	Male	6 years	Golden Retriever	1 year	Corticosteroid
Animal 4	Female	7 years	Shih-tzu	3 years	Corticosteroid
Animal 5	Female	8 years	Lhasa apso	2 years	Corticosteroid, oclacitinib
Animal 6	Male	9 years	Shih-tzu	1 year	Corticosteroid
Animal 7	Male	11 years	Shih-tzu	3 years	Corticosteroid, oclacitinib

TABLE 2 Pre- and post-treatment CADESI-04, itching degree (PVAS), and mast cell evaluation in dogs with atopic dermatitis.

Group	Animal	CADESI-04		Itching degree		Mast cells					
						Armpit		Interdigit		Groin	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Control	1	67	44	7	2	62	104	334	172	186	146
	2	14	18	9	8	84	102	296	134	76	46
	3	36	26	5	7	88	94	196	293	105	120
	4	13	7	5	6	138	45	60	143	34	43
	5	82	114	10	10	96	78	226	136	122	165
	6	24	12	8.5	2	66	90	6	168	80	67
	7	9	7	7	4	96	52	110	124	50	82
CBD	1	32	19	3	0	110	156	202	350	102	360
	2	18	7	9	4.5	102	176	360	320	146	90
	3	7	11	8	9	440	101	248	114	34	51
	4	57	49	4	5	228	232	404	260	167	230
	5	23	12	6	0	115	152	304	98	112	178
	6	65	55	4	0	155	148	404	203	194	161
	7	67	66	10	2	103	84	242	208	205	110

Pre, pre-treatment; Post, post-treatment.

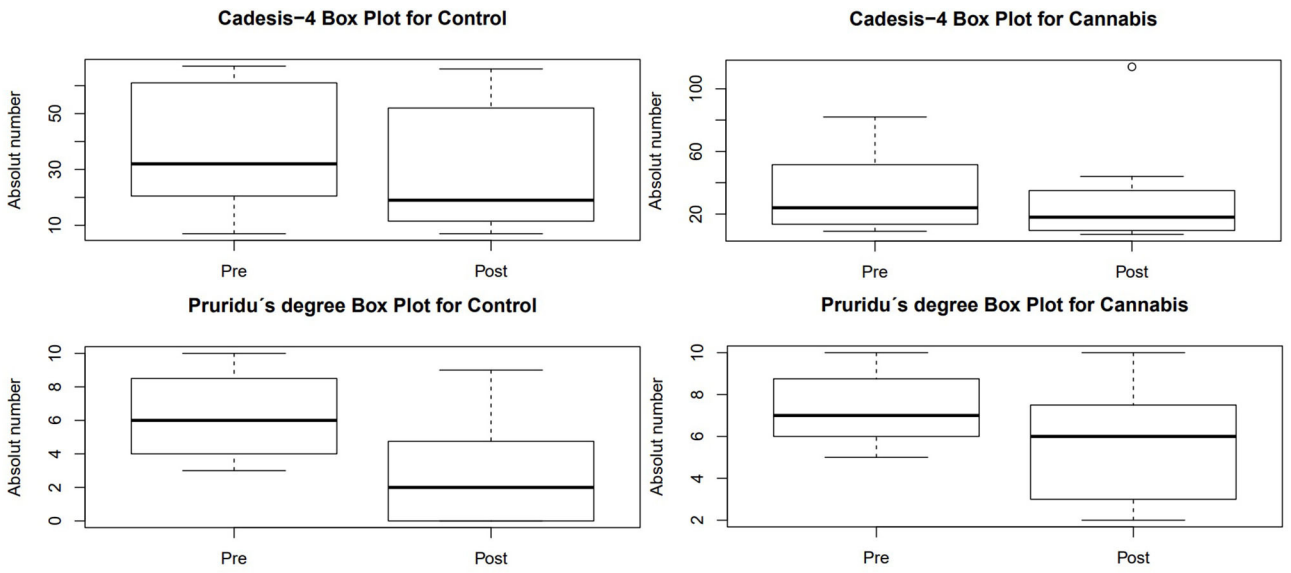

FIGURE 1
Box plot obtained in the evaluations of CADESI-4 and degree of pruritus in the pre and pros-treatment groups.

Histopathology and mast cells count

In the axillary region (Table 3), group T showed no changes in five animals (71.4%) in the evaluation of orthokeratotic hyperkeratosis, but it revealed a worsening of the lesion grade in two animals, with one previously classified as absent but now showing moderate lesions. As for the presence of acanthosis, five animals (71.4%) also showed no alteration, while two presented a worsening of the clinical condition. Perivascular inflammation remained in three animals (42.9%), and two showed an improvement in the evaluation grade, while two showed worsening. On the other hand, group C had four dogs (57.1%) with the same grade of Orthokeratotic hyperkeratosis, two with worsening, and one with a significant improvement, progressing from a moderate grade to absent. Concerning acanthosis, four patients (57.1%) maintained the same grade, while two (28.6%) showed improvement, and the only one that worsened progressed from absent to moderate. Perivascular inflammation remained in only one animal, while three (42.9%) showed worsening, and two (28.6%) showed improvement, with one representative experiencing a decrease in the grade from severe to mild.

In the interdigital region (Table 3), group T showed a 57.1% improvement in Orthokeratotic hyperkeratosis lesions, with one remaining at the same level and two experiencing worsening, including one from absent to severe. As for the presence of acanthosis in the same group, 71.4% remained at the same grade, while two showed worsening. Perivascular inflammation had the same proportion as acanthosis, but without a significant change in the grade of the lesion. In group C, there was an equal proportion (42.9%) of animals with worsening and improvement in the grade of Orthokeratotic hyperkeratosis, except for one animal that progressed from severe to absent, and only one maintained the same grade. Regarding acanthosis in this group, three (42.9%) maintained the same grade of the lesion, and the same proportion (28.6%) showed worsening and improvement, progressing from mild to severe and moderate to absent, respectively. Perivascular inflammation in the control group revealed 71.4% maintaining the grade and two with and improvement in the grades previously presented.

In the inguinal region (Table 3), Orthokeratotic hyperkeratosis in group T remained in 57.1% of the animals, with a worsening of grade in only one and improvement in two patients, with one progressing from moderate to absent. As for the evaluation of acanthosis, maintenance was observed in 42.9% of the animals, while 57.1% showed worsening. Concerning perivascular inflammation, three animals maintained the same grade, and two (28.6%) showed improvement and worsening. Group C presented 57.1% maintenance in Orthokeratotic hyperkeratosis lesions, two showed improvement, and two worsened in grade. As for the evaluation of acanthosis, three (42.9%) showed worsening, two remained at the same grade, and two improved, with one progressing from severe to mild. Regarding perivascular inflammation, 42.9% showed improvement, and 28.6% showed worsening and maintenance of the grade.

The mast cell count (Table 2) did not show statistical difference between pre- and post-treatment within and between the groups (Figure 2).

Discussion

Dermatological evaluation

The onset of clinical signs of canine atopic dermatitis (CAD) is characterized by alesional pruritus, represented by excessive scratching, biting, or licking. The primary lesions are erythema and papules, which may lead to secondary lesions due to self-trauma, resulting in chronic inflammation and secondary infections, represented by excoriations, alopecia, lichenification,

TABLE 3 Histopathological findings in dogs with atopic dermatitis treated with high-CBD oil.

Group	Animal	Orthokeratotic hyperkeratosis						Acanthosis						Perivascular and periadnexal dermal lymphoplasmacytic inflammation					
		Armpit		Groin		Interdigit		Armpit		Groin		Interdigit		Armpit		Groin		Interdigit	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Control	1	G	G	G	G	Mo	G	Mo	Mo	Mo	Mo	Mo	Mo	G	Mo	Mo	Mo	Mo	Mo
	2	A	Mo	G	G	G	Mo	A	G	A	G	G	G	A	G	A	G	Mo	Mo
	3	A	A	Mo	A	Mo	G	A	G	G	Mo	Mo	Ma	G	G	G	G	Mo	Ma
	4	Mo	Mo	Mo	Mo	Ma	Mo	A	A	A	A	A	G	G	G	G	G	G	Mo
	5	G	G	G	G	Mo	G	G	G	Mo	Mo	Mo	Mo	Mo	G	Mo	G	Mo	Mo
	6	G	Mo	G	G	G	G	A	A	A	G	Mo	Mo	G	G	Mo	G	Mo	Mo
	7	Mo	Mo	G	Mo	A	Ma	A	A	A	G	A	A	G	A	A	G	G	G
CBD	1	G	Mo	G	G	Ma	A	A	Mo	G	Mo	G	G	G	Mo	Ma	Mo	Mo	Mo
	2	G	G	G	A	Mo	Ma	A	A	A	A	G	G	G	G	G	G	Mo	Mo
	3	Mo	A	G	G	G	A	G	A	A	A	Mo	A	Ma	G	G	G	G	G
	4	G	G	G	G	Mo	G	Ma	Ma	Mo	Ma	Mo	Mo	Ma	Mo	Mo	Ma	Ma	Mo
	5	Mo	Mo	Mo	Mo	G	Mo	A	A	A	G	Mo	G	G	Mo	G	Mo	Mo	G
	6	G	G	G	Mo	G	Mo	Ma	Ma	Ma	G	G	Ma	Ma	Mo	Ma	Mo	Mo	Mo
	7	Mo	Ma	Mo	G	G	G	Ma	Mo	Mo	G	G	Mo	Mo	Ma	Mo	G	Mo	Mo

Pre, pre-treatment; Post, post-treatment; A, Absent; G, Gentle; Mo, Moderate; Ma, Marked.

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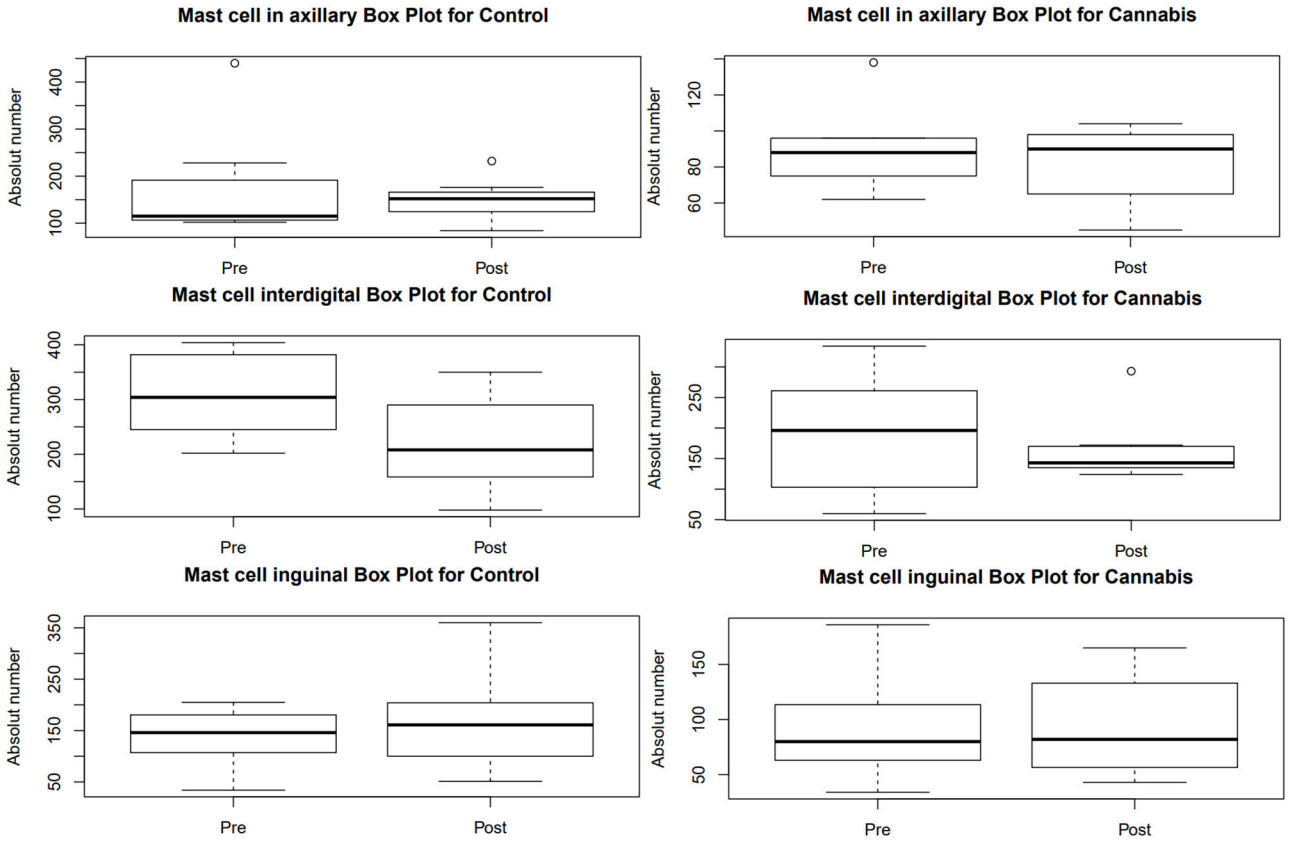

FIGURE 2
Box plot obtained in the mast cell count performed on biopsies with special toluidine blue staining at 400× magnification in dogs with AD. Pre, pre-treatment; Post, post-treatment.

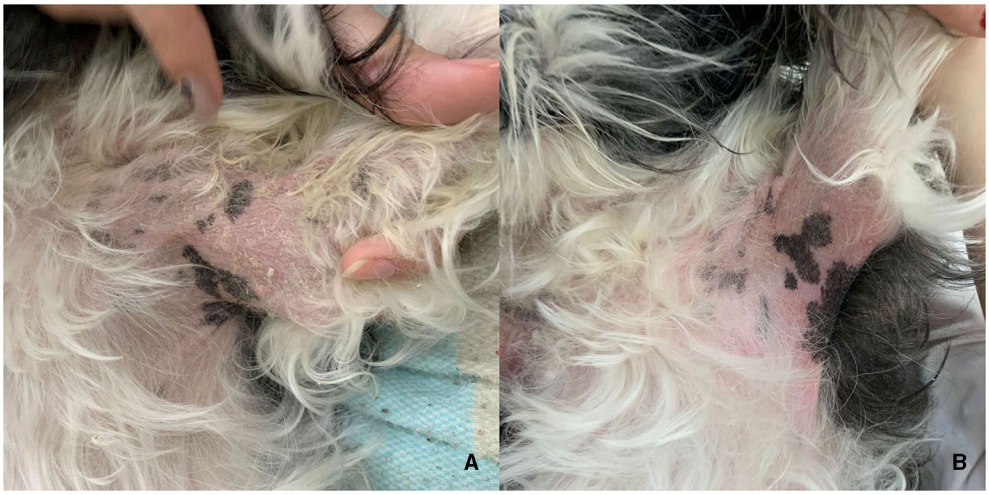

FIGURE 3
Axilla of a dog from the group treated with CBD-rich cannabis oil, before **(A)** and after **(B)** 60 days of treatment. Notice the reduction of erythema and absence of scaling in **(B)**.

hyperpigmentation, crusting, and seborrhea (1). Luz-Veiga et al. (9) suggest that phytocannabinoids CBD and CBG have promising antimicrobial effects when topically Applied, without altering the skin's microbiota, which could become an ally in the treatment of dermatopathies. Campora et al. (10) detected increased immunoreactivity of CB1 and CB2 receptors in various cell types in

the epidermis and dermis of dogs diagnosed with atopic dermatitis, including mast cells, fibroblast, and endothelial cells.

In a treatment with 2 mg/kg of CBD and CBDA for 4 weeks conducted by Loewinger et al. (11) in atopic dogs and evaluated using CADESI-4 and pruritus scores, no statistical improvement was observed. This study corroborates with the current study, which also did not find significance in these analyses, even when extended to 6 weeks of treatment. It is worth nothing that the treatment duration may be considered short to observe significant improvement in the skin. Additionally, we emphasize that there were no specific maximum and minimum limits for CADESI-4 evaluation for inclusion and/or exclusion in this study, as stipulated by Loewinger et al. (11). However, Mogi et al. (12) revealed clinical improvement in both CADESI-4 and pruritus scores in atopic dogs treated with THC-free CBD oil twice daily for 8weeks. However, this study did not include a control group for comparison of the obtained data. Despite the lack of significance in laboratory analyses, Figure 3 shows clinical improvement presented by a dog in the cannabis-treated group, which none of the dogs in the control group showed.

A study conducted by de Santiago et al. (13) revealed that a diet with high concentrations of antioxidants, polyphenols, docosahexaenoic omega-3 fatty acids, and eicosapentaenoic acid improves skin health, reduces inflammation, and enhances clinical signs of CAD, as assessed by CADESI-4 and pruritus scores. The lack of significance between the treatment groups and the significance observed between the pre- and post-treatment within the control group can be attributed, primarily, to the non-inert diluent, olive oil, used in the control group. More than 50 different phenols have been identified in olive oil wastewater, and they have been associated with antioxidant, anti-inflammatory, and antimicrobial properties (14, 15). The current literature diverges on the efficacy of olive oil in atopic dermatitis, with some authors justifying positive results when administered concomitantly with other substances (16). There are no references found on the effects of oral administration of olive oil for dermatopathies, especially for canine atopic dermatitis. Additionally, it is essential to consider the various pruritogenic mechanisms involved, including hyperinnervation of CAD lesions, pro-inflammatory molecules, keratinocytes, monocytes, cutaneous nerve fibers, and the involvement of the central and peripheral nervous systems (17, 18). Although the complete understanding of pruritus is still lacking, it had been shown that TRPV1 activation minimizes itching induced by dust mite allergens in mice with atopic dermatitis (19), the main allergen in dogs of this region reported by Pereira et al. (4).

The results obtained according to the responses of the tutors regarding the pruritus grade disagreed with the dermatologist's evaluation. There are a few studies on the placebo effect in dogs, but there are some theories that do not involve a real placebo effect. (A) Placebo effect of the caregiver or by proxy: this means that due to the investment made, the caregiver believes that they will get a return. As a result, the tutor is highly susceptible to this type of control and may perceive improvements even when they do not exist. A study conducted by Conzemius and Evans (20) reported a prevalence of 39.7% and 44.8% of proxy control effects in dogs with lameness as evaluated by tutors and veterinarians, respectively. (B) Regression to the mean: this refers to a real improvement in the animal, but it occurs as a natural course of regression. This is associated with chronic diseases that fluctuate in severity naturally. A study conducted by Muñana et al. (21) concluded that 79% of dogs that received control for epilepsy showed improvement in seizure frequency. Both types of effects emphasize the importance of having a control group in scientific research in veterinary medicine to ensure that the evidence obtained is not poor and to accurately evaluate the success or failure of the intervention.

Given the chronicity, difficulty in controlling clinical signs, and the laborious maintenance of canine atopic dermatitis, the placebo effect concerning the tutor's evaluation can be a reasonable justification, even if not well-supported by the literature. There are reports of improvement according to some tutors, but it is not observed in all cases, which can be related to the placebo effect described above. In other words, there may have been a clinical improvement, but without statistical significance. Recalling the discussion in the "dermatological evaluation" section, it was possible to justify an improvement in the control group because it was not treated with an inert substance, thereby approximating the possible differences between the cannabis-treated group and the control group.

It is believed that the main limitation regarding the pruritus scale was the lack of prior information about the pruritus grade. As a result, tutors would confirm a certain level of pruritus that was often higher than the confirmed previous grade, subsequently reporting that the pruritus had indeed decreased. This discrepancy in reporting could be attributed to the absence of initial information and may have influenced the perceived improvement in pruritus.

Histopathology and mast cells count

The presence of mast cells is directly proportional to the severity of the clinical condition, pruritus pathogenesis, and disease progression (22). The results obtained from the tutor's assessment of pruritus grade are consistent with the results regarding the number of mast cells. Despite the mention of the possibility of the placebo effect in tutors, the number of mast cells did not show statistical difference in dogs with atopic dermatitis treated with cannabis. Nam et al. (23) reported a reduction in activation and degranulation of mast cells, as well as decreased recruitment of these cells and local inflammation in atopic dermatitis through CB1 receptors. The antipruritic action is also attributed to mast cells, primarily related to the endocannabinoid PEA (24). Additionally, Mogi et al. (12) emphasized the importance of adding phytocannabinoid supplementation early in the disease course, as using it as a single agent in refractory or severe cases does not show significant clinical improvement. The same authors also advocate combining phytocannabinoids with conventional drugs to potentially reduce dosages, financial costs, and enhance the overall efficacy of the treatment plan. It is essential to highlight that the anti-inflammatory mechanisms are not yet fully understood (25).

The histopathological lesions of CAD reveal an inflammatory pattern characterized by chronicity, perivascular dermatitis, hyperplasia, and spongiosis (26). Scott (27) reported the presence of epidermal hyperplasia, orthokeratotic or parakeratotic hyperkeratosis, hypergranulosis, spongiosis, melanosis, and leukocytic exocytosis. This same author also mentioned dermal changes such as congestion, vasodilation, and angiocentric inflammation with predominantly mononuclear and neutrophilic infiltrated. Campora et al. (10) revealed hyperplastic epidermis and focal hyperkeratosis in five dogs with CAD. In a detailed study, Chiocchetti et al. (8) described the presence of moderate to severe hyperkeratosis and acanthosis, with focal to diffuse distribution in eight dogs with CAD. They also reported superficial and interstitial perivascular inflammatory infiltrates, consisting of cells such as lymphocytes, histiocytes, mast cells, plasma cells, and some eosinophils. In four of these animals, predominantly neutrophils were present. The latter authors did not disclose the biopsy site or whether any treatment was used. Chiocchetti et al. (8) aimed to investigate the expression of CB2, GPR55, TRPV1, and TRPA1 receptors in skin cells of dogs with atopic dermatitis. The authors concluded that these receptors are highly expressed in infiltrative inflammation in dogs with atopic dermatitis and that cannabis has a considerable theoretical basis as a potential therapeutic option for this disease, alleviating pruritus and inflammation. The findings of the present study, which provide a more detailed description of the histopathological changes in the most affected regions of dogs with atopy, contradict the literature that supports the therapeutic potential. In other words, no improvement in the evaluation criteria specified in this study was observed.

The absence of significant findings in the different evaluations conducted in this study can be mainly justified by the fact that the animals had a dermatopathy, and these changes are primarily related to the dose used. In the present study, the body condition of each patient was not taken into consideration, whereas in the literature, there is evidence of phytocannabinoid deposition in adipose tissue due to their liposolubility. Studies that accounted for this factor revealed an increase of 20% in the dose for obese animals to compensate for this characteristic (28).

Limitations of this study include: small number and uniformized animals; short-term therapy; and possible influence of olive oil. However, despite the absence of clinical improvement in this study, cannabinoids are a promising option to ameliorate the pathophysiology of this disease.

Conclusion

Despite the absence of significance in the dermatological evaluations for the canine atopic dermatitis, it is worth noting the individuality of each animal concerning the dosage used, as a very positive result was obtained in the cannabis group. This study reveals that the full-spectrum cannabis oil rich in CBD at a dosage of 2.5 mg/kg does not show therapeutic advantage when compared to olive oil. This is mainly due to the complexity of controlling this disease, which demands a multimodal therapy. Further clinical research involving this topic is recommended to either confirm or definitively rule out potential therapeutic means to aid in controlling this dermatopathy that greatly affects the quality of life.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal studies were approved by Ethics Committee for Animal Use and Experimentation of the Federal University of Santa Maria (CEUA/UFSM) (protocol number 8656301121, identification number 003662). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

CM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review & editing. ALS: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Writing—original draft, Writing—review & editing. ÂIS: Data curation, Formal analysis, Writing—review & editing. APS: Writing—review & editing, Formal analysis. MM: Investigation, Methodology, Writing—review & editing. AVS: Writing—review & editing, Methodology, Supervision. EA: Conceptualization, Resources, Writing—review & editing, Funding acquisition. ST: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing—review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Hensel P, Santoro D, Favrot C, Hill P, Griffin C. Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification. *BMC Vet Res.* (2015) 11:196. doi: 10.1186/s12917-015-0515-5

2. Gugliandolo E, Palma E, Cordaro M, D'Amico R, Peritore AF, Licata P, et al. Canine atopic dermatitis: role of luteolin as new natural treatment. *Vet Med Sci.* (2020) 6:926–32. doi: 10.1002/vms3.325

3. Gedon NKY, Mueller RS. Atopic dermatitis in cats and dogs: a difficult disease for animals and owners. *Clin Transl Allergy*. (2018) 8:41. doi: 10.1186/s13601-018-0228-5

4. Pereira DT, Cunha VES, Schmidt C, Magnus T, Krause A. Sensitization study of dogs with atopic dermatitis in the central region of Rio Grande do Sul. *Arq Bras Med Veterinária e Zootec.* (2015) 67:1533–8. doi: 10.1590/1678-4162-8224

5. Olivry T, DeBoer DJ, Favrot C, Jackson HA, Mueller RS, Nuttall T, et al. Treatment of canine atopic dermatitis: 2010 clinical practice guidelines from the International Task Force on Canine Atopic Dermatitis. *Vet Dermatol.* (2010) 21:233–48. doi: 10.1111/j.1365-3164.2010.00889.x

6. Tóth KF, Ádám D, Bíró T, Oláh A. Cannabinoid signaling in the skin: Therapeutic potential of the "c(ut)annabinoid" system. *Molecules.* (2019) 24:1–56. doi: 10.3390/molecules24050918

7. Martinelli G, Magnavacca A, Fumagalli M, Dell'Agli M, Piazza S, Sangiovanni E. Cannabis sativa and skin health: dissecting the role of Phytocannabinoids. *Planta Med.* (2022) 88:492–506. doi: 10.1055/a-1420-5780

8. Chiocchetti R, Salamanca G, De Silva M, Gobbo F, Aspidi F, Cunha RZ, et al. Cannabinoid receptors in the inflammatory cells of canine atopic dermatitis. *Front Vet Sci.* (2022) 15:9. doi: 10.3389/fvets.2022.987132

9. Luz-Veiga M, Amorim M, Pinto-Ribeiro I, Oliveira ALS, Silva S, Pimentel LL, et al. Cannabidiol and cannabigerol exert antimicrobial activity without compromising skin microbiota. *Int J Mol Sci.* (2023) 24:2389. doi: 10.3390/ijms24032389

10. Campora L, Miragliotta V, Ricci E, Cristino L, Di Marzo V, Albanese F, et al. Cannabinoid receptor type 1 and 2 expression in the skin of healthy dogs and dogs with atopic dermatitis. *Am J Vet Res.* (2012) 73:988–95. doi: 10.2460/ajvr.73.7.988

11. Loewinger M, Wakshlag JJ, Bowden D, Peters-Kennedy J, Rosenberg A. The effect of a mixed cannabidiol and cannabidiolic acid based oil on client-owned dogs with atopic dermatitis. *Vet Dermatol.* (2022) 33:329. doi: 10.1111/vde.13077

12. Mogi C, Yoshida M, Kawano K, Fukuyama T, Arai T. Effects of cannabidiol without delta-9-tetrahydrocannabinol on canine atopic dermatitis: a retrospective assessment of 8 cases. *Can Vet J.* (2022) 63:423–6.

13. de Santiago MS, Arribas JLG, Llamas YM, Becvarova I, Meyer H. Randomized, double-blind, placebo-controlled clinical trial measuring the effect of a dietetic food on dermatologic scoring and pruritus in dogs with atopic dermatitis. *BMC Vet Res.* (2021) 17:354. doi: 10.1186/s12917-021-03063-w

14. Zbakh H, El Abbassi A. Potential use of olive mill wastewater in the preparation of functional beverages: a review. *J Funct Foods.* (2012) 4:53–65. doi: 10.1016/j.jff.2012.01.002

15. Carrara M, Kelly MT, Roso F, Larroque M, Margout D. Potential of Olive oil mill wastewater as a source of polyphenols for the treatment of skin disorders: a review. *J Agric Food Chem.* (2021) 69:7268–84. doi: 10.1021/acs.jafc.1c00296

16. Kiechl-Kohlendorfer U, Berger C, Inzinger R. The effect of daily treatment with an olive oil/lanolin emollient on skin integrity in preterm infants: a randomized controlled trial. *Pediatr Dermatol.* (2008) 25:174–8. doi: 10.1111/j.1525-1470.2008.00627.x

17. Laprais A, Dunston SM, Torres SMF, Favrot C, Olivry T. Evaluation of intraepidermal nerve fibres in the skin of normal and atopic dogs. *Vet Dermatol.* (2017) 28:355–e80. doi: 10.1111/vde.12420

18. Mollanazar NK, Smith PK, Yosipovitch G. Mediators of chronic pruritus in atopic dermatitis: getting the itch out? *Clin Rev Allergy Immunol.* (2016) 51:263–92. doi: 10.1007/s12016-015-8488-5

19. Yun J-W, Seo JA, Jang W-H, Koh HJ, Bae I-H, Park Y-H, et al. Antipruritic effects of TRPV1 antagonist in murine atopic dermatitis and itching models. *J Invest Dermatol.* (2011) 131:1576–9. doi: 10.1038/jid.2011.87

20. Conzemius MG, Evans RB. Caregiver placebo effect for dogs with lameness from osteoarthritis. *J Am Vet Med Assoc.* (2012) 241:1314–9. doi: 10.2460/javma.241.10.1314

21. Muñana KR, Zhang D, Patterson EE. Placebo effect in canine epilepsy trials. *J Vet Intern Med.* (2010) 24:166–70. doi: 10.1111/j.1939-1676.2009.0407.x

22. Forsythe P, Paterson S. Ciclosporin 10 years on: indications and efficacy. *Vet Rec.* (2014) 174:13–21. doi: 10.1136/vr.102484

23. Nam G, Jeong SK, Park BM, Lee SH, Kim HJ, Hong S-P, et al. Selective cannabinoid receptor-1 agonists regulate mast cell activation in an oxazolone-induced atopic dermatitis model. *Ann Dermatol.* (2016) 28:22. doi: 10.5021/ad.2016.28.1.22

24. Miragliotta V, Noli C. Dermatology: endocannabinoids and related n-acylethanolamines in the skin. In: *Cannabis Therapy in Veterinary Medicine.* Cham: Springer International Publishing. (2021). p. 207–30. doi: 10.1007/978-3-030-68317-7_14

25. Anil SM, Peeri H, Koltai H. Medical cannabis activity against inflammation: active compounds and modes of action. *Front Pharmacol.* (2022) 13:l doi: 10.3389/fphar.2022.908198

26. Olivry T, Hill PB. The ACVD task force on canine atopic dermatitis (XVIII): histopathology of skin lesions. *Vet Immunol Immunopathol.* (2001) 81:305–9. doi: 10.1016/S0165-2427(01)00305-1

27. Scott DW. Observations on canine atopy. *J Am Anim Hosp Assoc.* (1981) 15:91–100. doi: 10.1017/S0026318400010932

28. Corsetti S, Borruso S, Malandrucco L, Spallucci V, Maragliano L, Perino R, et al. *Cannabis sativa* L. may reduce aggressive behaviour towards humans in shelter dogs. *Sci Rep.* (2021) 2:1–10. doi: 10.1038/s41598-021-82439-2

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Pharmacokinetics of cannabidiol-/cannabidiolic acid-rich hemp oil in juvenile cynomolgus macaques (*Macaca fascicularis*)

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Introduction: Cannabinoids are increasingly popular in human and veterinary medicine and have been studied as an alternative treatment for a wide range of disorders. The goal of this study was to perform a pharmacokinetic analysis of oral cannabidiol (CBD)-/cannabidiolic acid (CBDA)-rich hemp oil (CBD/ArHO) in juvenile cynomolgus macaques (*Macaca fascicularis*).

Methods: After a 2 mg/kg CBD/ArHO pilot study, 4 and 8 mg/kg direct-to-mouth CBD/ArHO were administered ($n = 4$ per dose) once daily for 14 days and blood was collected at 0-, 0.5-, 1-, 2-, 4-, 8-, 12-, and 24-h, and on Days 7 and 14, to quantify serum cannabinoid concentrations by high-performance liquid chromatography–tandem mass spectrometry. Serum biochemistries and complete blood counts were performed on Days 0, 1, and 14.

Results: The maximum mean serum concentration (C_{max}) of CBDA was 28.6–36.2 times that of CBD at 4 and 8 mg/kg. At 8 mg/kg, the C_{max} of CBD was 1.4 times higher ($p = 0.0721$), and CBDA was significantly 1.8 times higher ($p = 0.0361$), than at 4 mg/kg. The maximum mean serum concentration of Δ^9-tetrahydrocannabinol (THC) was 4.80 ng/mL at 8 mg/kg. Changes in serum biochemistries and complete blood counts over time were not clinically significant.

Discussion: Given the low serum CBD concentrations, the doses and frequency used in this study may be insufficient for a therapeutic effect of CBD in particular; therefore, clinical studies are needed to determine the therapeutic dose of CBD and CBDA for macaques, which may differ based on the disorder targeted.

KEYWORDS

pharmacokinetics, monkey, nonhuman primate, cannabidiol, cannabidiolic acid, cannabinoids, hemp (*Cannabis sativa* L.), noncompartmental analysis

1 Introduction

The hemp plant (*Cannabis sativa L.*) is a source of a variety of cannabinoids, with the most notable abundant bioactive components being cannabidiol (CBD), Δ^9-tetrahydrocannabiol (THC), and their acids (1). Temperature, humidity, precipitation, and genetic variety affect the phytocannabinoid profile of hemp plants (2), wherein cannabigerolic acid (CBGA) is converted to cannabidiolic acid (CBDA) by CBDA synthase or to Δ^9-tetrahydrocannabinolic acid (THCA)

by THCA synthase (3, 4). Then, light, heat, or oxygenation cause decarboxylation of these acids to cannabigerol (CBG), CBD, or THC during storage and processing (5, 6). Although CBD can be converted to THC under artificial gastric conditions *in vitro* (7), studies in humans and other species have failed to demonstrate this conversion when CBD is administered orally (8–10). The hemp plant also produces other phytochemicals, such as terpenes and hydrocarbons, that may potentiate cannabinoid activity—known as the *entourage effect* (11).

Due to its psychoactive properties, THC is currently a schedule I controlled drug in the United States except for a few synthetic prescription-only forms (12, 13); however, there is a lack of federal regulation regarding the sale and use of CBD-containing products. The Agricultural Improvement Act of 2018 resulted in federal legalization of cannabinoid products containing less than 0.3% THC (14). In addition, other commercially available products widely vary in CBD concentration, recommended dose, and routes of administration. Independent analyzes of numerous products have also demonstrated inconsistencies between the CBD concentrations provided on the label and their actual contents (15, 16).

While the only current FDA-approved use for CBD is as an anticonvulsant to treat seizures associated with Lennox-Gastaut syndrome, Dravet syndrome, or tuberous sclerosis complex (17), recent studies have indicated additional therapeutic benefits of CBD including anti-inflammatory (18), antioxidant (19, 20), analgesic (21), antiemetic (22), anxiolytic (23), anticarcinogenic (24, 25), antimicrobial (26, 27), and immunomodulatory (21, 28) properties. More specifically, research in various species has evaluated its efficacy in treating diseases such as diabetes mellitus (29–31), osteoarthritis (21, 28, 32), and neurologic diseases (e.g., Alzheimer's, Parkinson's) (33–37). In addition, CBD has been proposed to treat symptoms associated with stress (38), gastrointestinal disturbances (39, 40), orofacial pain (41, 42), hypertension (43–45), and neoplastic processes (46). Prior to cisplatin chemotherapy in the house musk shrew (*Suncus murinus*; $n = 20$), intraperitoneal CBD demonstrated a biphasic response—low doses (5 mg/kg) reduced emesis and higher doses (40 mg/kg) potentiated it (22); similarly, prior to lithium chloride administration ($n = 36$), intraperitoneal CBDA significantly reduced the incidence of emesis at low doses (0.1–0.5 mg/kg) but increased dosing (5 mg/kg) did not significantly reduce emesis compared to the control (47). Far fewer studies have been conducted regarding the other therapeutic benefits of CBDA but include anti-inflammatory (48), analgesic (49, 50), anticonvulsant (51), and anxiolytic (52–54) properties. Compared to CBD, CBDA was a more potent antihyperalgesic (49) but was less effective in reducing cancer cell proliferation *in vitro* (24). Unheated hemp extract contained higher concentrations of CBDA than CBD but resulted in a higher maximum serum CBD concentration compared to heated hemp extract (1).

Overall, CBD is reportedly safe with minimal side effects or signs of toxicity. Most negative effects have been anecdotal, inconsistent among studies, or occurred with high doses or extended use. Rarely reported potential side effects include sedation, mild diarrhea, inappetence, agitation, hypersensitivity, poor sleep quality, ataxia, pyrexia, infection, dry mouth, head-shaking, and excessive licking (55–59). Mildly elevated serum liver enzymes, including alanine transaminase, alkaline phosphatase (ALP), and aspartate aminotransferase (AST), were reported at typical daily CBD doses (0.5–2.8 mg/kg) in some human, canine, and feline subjects (28, 60, 61); although the increases were statistically significant, they were not of clinical concern. Conversely, higher CBD doses (≥ 20 mg/kg) in humans may lead to drug-induced liver injury (62, 63). There is also evidence that CBD may interfere with some drug metabolism by inhibition of cytochrome P450 enzymes (64, 65). Therefore, caution should be used prior to concurrent administration with other drugs, especially those with hepatic metabolism or affected by cytochrome P450 inhibition.

There are no current studies published about the use of CBD or CBDA as a potential therapeutic agent in nonhuman primates (NHP); however, oral high-dose CBD (30–300 mg/kg) for 90 days ($n = 16$) did not affect rhesus macaque growth rates but increased liver and kidney weights, decreased testicular weight and inhibited spermatogenesis, dose-dependently decreased red blood cell counts, and occasionally resulted in transient diarrhea (66). Based on evidence of the therapeutic benefits in humans and other species (32, 38, 40), low-dose CBD could be a candidate as an adjunctive therapy for some common NHP medical conditions, including osteoarthritis (67), environmental stress (68), and inflammatory diarrheal disease (69). As a first step, a pharmacokinetic (PK) analysis of CBD and CBDA would inform whether doses recently reported in other species would be appropriate in macaques and provide a baseline for future studies evaluating its PK or therapeutic potential.

Based on the therapeutic doses of CBD and CBDA reported in other species (28, 55, 61, 70, 71), we selected a commercially available oral veterinary CBD-/CBDA-rich hemp oil (CBD/ArHO; approximately 1:1 CBD:CBDA) with a guaranteed content analysis (Supplementary Table S1) that was previously evaluated in domestic dogs (70). We also performed a pilot study at 2 mg/kg CBD/ArHO in cynomolgus macaques and determined that serum CBD concentrations were lower than in some species, but serum CBDA concentrations were 3.8 times higher than CBD (72). The goal of this study was to perform a PK analysis of CBD/ArHO in juvenile cynomolgus macaques (*Macaca fascicularis*). Our objectives were to (1) perform a 24-h, single-dose PK study at 4 or 8 mg/kg CBD/ArHO; (2) determine any physical, biochemical, or hematological effects over time; (3) determine if any cannabinoids accumulate in the serum after 14 days of daily dosing, and (4) compare the results to reported findings in other species. We hypothesized that 8 mg/kg CBD/ArHO would provide higher serum concentrations of CBD and CBDA than 4 mg/kg, with negligible amounts of serum THC, and CBDA being significantly higher than CBD. We also hypothesized that there would be minimal negative physical effects, and no clinically significant serum biochemical or hematological changes.

2 Materials and methods

2.1 Humane animal care and use

All procedures were approved by The Mannheimer Foundation IACUC, an AAALAC-accredited facility, and adhered to all approved standard operating procedures. Animals were maintained according to Animal Welfare Act (73, 74) and Regulations (75), and the *Guide for the Care and Use of Laboratory Animals* (76).

2.2 Animals

Male [$n = 4$; weight (mean ± 1 SD), 3.70 ± 0.67 kg] and female ($n = 4$; weight, 3.63 ± 0.32 kg) juvenile cynomolgus macaques (aged

3.29 ± 0.13 years) were selected. Inclusion criteria were subjects born at The Mannheimer Foundation, open to positive human interactions (determined by colony manager), and naïve to experimental and medical interventions outside of the routine preventative medicine program. This program included semiannual physical examination, tuberculin skin testing (10 µL intradermally every 6 months; Tuberculin OT, Colorado Serum Company, Denver, CO), deworming with ivermectin [0.4 mg/kg intramuscularly (IM) every 6 months; Vetrimec 1%, MWI Animal Health, Boise, ID], and routine vaccination against *Clostridium tetani* (0.5 mL IM every 5 years; tetanus toxoid, Fort Dodge Animal Health, Fort Dodge, IA), *Measles morbillivirus* [1 mL subcutaneously (SC) every 6 months; Vanguard DM, Zoetis, Parsippany, NJ], and *Rabies lyssavirus* (1 mL SC every 3 years; Rabvac 3, Elanco US, Fort Dodge, IA). Animals were serologically negative for *Macacine herpesvirus 1*, *Simian retrovirus 1*, *Simian T-lymphotrophic virus 1*, and *Simian immunodeficiency virus*, and identified by a SC passive integrated transponder (PetLink™ Slim, Datamars, Inc., Woburn, MA) from birth and a chest tattoo of their unique identification number after 6 months old.

Subjects were individually caught in nets, anesthetized with ketamine hydrochloride (10–15 mg/kg IM), boxed (Prima-Carrier, Primate Products, Immokalee, FL), and transferred from outdoor, same-sex and -age social housing units. Animals were weighed, physically examined, collared (medium, aluminum; Primate Products, Immokalee, FL), and same-sex pair-housed in 2 stainless-steel squeeze-back cages with an open pass-through door (floor area, 0.4 m^2 each; height, 76.2 cm) in indoor, climate-controlled rooms [70.3–80.2°F (21.3–26.8°C); relative humidity, 40–95%] on a 12:12-h light:dark cycle (07:00–19:00); cages were sanitized daily and disinfected at least every 15 days. Animals were fed a standard commercial primate diet (5049, Lab Diet, St Louis, MO) twice daily, and watered free-choice through an automated watering system. Each animal was environmentally enriched with a mirror, plastic ball, foraging board, and daily forage, including seeds, fruit, popcorn, multigrain fruit cereal (Fruit Spins, Great Value, Bentonville, Arkansas), and FiberBites (ClearH2O, Westbrook, ME). Animals were observed at least once daily to assess and document mentation, feed consumption, hydration status, stool quality, and any behavioral or health concerns.

Subjects were individually acclimated to human interactions and trained by positive reinforcement with handfed forage at least once daily after temporary separation by closing the mesh pass-through door. Soft classical music was played during handling. After the animals readily took forage directly from the trainers' hand, they were acclimated to the pole (Primate Products, Immokalee, FL). Once the animal ignored the pole when held by the trainers, the pole was latched transversely through the cage mesh until it was accepted as a neutral object. Then, the pole was repeatedly held as close to the collar as the animal would allow until it could be latched to the collar. Once latching and unlatching of the pole to the collar was repeatedly successful, the animals were trained to allow two trainers to each latch their pole on opposite sides of the collar, climb from their cage to the floor, sit on the primate restraint chair (Primate Products, Immokalee, FL) in the same animal room, and allow their collar to be secured into the chair for 10–15 min intervals. After study completion, macaques were anesthetized again with ketamine hydrochloride for collar removal, weighed, boxed, and returned to their outdoor enclosures.

2.3 Study design

Eight subjects were selected to receive 4 (2 male, 2 female) or 8 (2 male, 2 female) mg/kg of CBD/ArHO (70 mg/mL). Then, a randomized block design was applied. Animals were grouped by sex before being randomly assigned to dose groups using a random sequence generator;[1] the order of subject dosing was also randomized. The CBD/ArHO was administered orally with a 12-gauge curved gavage needle and 1-mL syringe after chairing. Subsequently, each animal was accessed by employing the squeeze-back mechanism on cage to allow for daily dosing for the study duration unless the animal was chaired for blood collection. All doses of CBD/ArHO (ElleVet Sciences, South Portland, ME) were administered between 07:00 and 09:00 on a given day prior to feeding. After a maximum chairing time of 4 h, animals were returned to their individual enclosures until the next timepoint for sample collection. Feed was provided immediately after the first 4 h of chairing. CBD/ArHO was administered for 14 days to determine any acute or subacute physiologic parameters over time, or if there was a cumulative effect of repeated administration. A procedural timeline is provided in Figure 1.

Immediately following CBD/ArHO administration, blood was collected at Days 0 (0 h), 1 (24 h), and 14 (336 h) by femoral venipuncture using a 22-gauge vacuum phlebotomy system (Vacutainer, BD, Franklin Lakes, NJ), 4-mL serum-separator tube (8881302072, Medtronic, Minneapolis, MN), and 3-mL K2-EDTA tube (367856, Vacutainer, BD, Franklin Lakes, NJ). The serum tubes were held at 4°C for at least 30 min before centrifugation at 1,300 × *g* for 15 min at 4°C. The serum was divided into two 2-mL screw-top microtubes (72.694.406, Sarstedt, Nümbrecht, HR); one was stored at −80°C for PK analysis, while the other was refrigerated at 4°C with the K2-EDTA tube for serum biochemistry and complete blood count, respectively. Additional blood was collected at 0.5, 1, 2, 4, 8, and 12 h, and Day 7 (168 h) from alternating saphenous veins with a 25-gauge needle and 1-mL luer-lock syringe (ML12558, Air-Tite Products, Virginia Beach, VA); a cephalic or femoral vein was used if the saphenous collection failed. The blood was transferred to a 0.5-mL serum separator tube (365967, Becton Dickinson, Franklin Lakes, NJ) and held at 4°C for at least 30 min before centrifugation at 1,300 × *g* for 15 min at 4°C. Serum samples were transferred into sterile 2-mL screw-top microtubes and stored at −80°C until all samples were submitted for PK analysis.

2.4 Sample analysis

All samples were wrapped in an absorbable pad, placed in a 1-gallon Ziploc Freezer bag, and packaged in an insulating extruded polystyrene box within a cardboard box for overnight shipment according to the requirements of the International Air Transport Association Dangerous Goods Regulations for Category B biologic substances (UN 3373). Refrigerated serum and K2-EDTA samples were shipped with cold packs to VRL Laboratories (San Antonio, TX) for biochemistry and complete blood count (5506, Chem Profile II

1 http://www.random.org/sequences

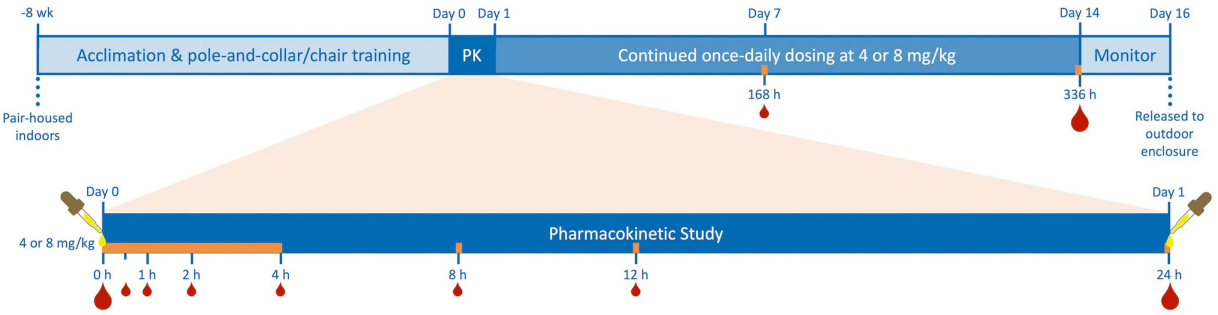

FIGURE 1
Timeline of juvenile cynomolgus macaque (4 males, 4 females) pair-housing, indoor acclimation, and pole-and-collar/chair training, 24-h pharmacokinetic study (PK) after single-dose oral administration of cannabidiol-/cannabidiolic acid-rich hemp oil (Day 0) at 4 or 8 mg/kg (2 males and 2 females per dose), continued once-daily dosing for an additional 13 days, and a two-day period of monitoring prior to release to their outdoor enclosures. Subjects were dosed daily at the same time as on their individual Day 0 and blood was always drawn immediately following the dose on Days 1, 7, and 14 as on Day 0. Blood was collected at 0-, 0.5-, 1-, 2-, 4-, 8-, 12-, 24-, 168-, and 336-h to quantify serum cannabinoid concentrations by high-performance liquid chromatography–tandem mass spectrometry. Additional blood was drawn for serum biochemistries and complete blood counts on Days 0, 1, and 14 (indicated by the larger blood drops). The orange bar represents the times of chair restraint for blood collection during the study, never exceeding 4 h.

and CBC) within 48 h. Serum samples for cannabinoid analysis were shipped in dry ice (UN 1845) to the University of Illinois at Chicago Toxicology Research Laboratory.

Serum cannabinoid analyzes were performed using an exploratory (fit-for-purpose) method for fast measurement of 11 cannabinoids and their metabolites. The reference standards for CBD and CBDA were obtained from Restek Corporation (Bellefonte, PA); all other reference and internal standards were obtained from Cerilliant Corporation (Round Rock, TX). Cannabinoid serum concentration for CBD, CBDA, THC, THCA, cannabinol (CBN), cannabichromene, CBG, and CBGA and their metabolites 7-Nor-7-carboxycannabidiol (7-COOH-CBD), 11-nor-9-Carboxy-Δ9-tetrahydrocannabinol (COOH-THC), and 11-nor-9-Carboxy-Δ9-tetrahydrocannabinol-Glucuronide (COOH-THC-Glu) was determined using high-performance liquid chromatography–tandem mass spectrometry (Nexera X2 and LCMS 8050, Shimadzu Corp., Kyoto, Japan).

Each serum sample (40 µL) was mixed with internal standards (20 µL, 100 ng/mL each) in 1:1 water:methanol in a 96 well plate. Then, proteins were precipitated, and compounds were extracted by adding ice-cold acetonitrile (80 µL), vortexing for 1–2 min, and centrifuging at 4,000 rpm for 10 min at 4°C. Supernatants (70 µL) were diluted with deionized water (70 µL) in a another 96 well plate and centrifuged again. 10 µL of the processed samples were injected into a column (100 Å, 3 µm, 2.1 × 50 mm, Atlantis T3 column, Waters, Milford, MA) coupled to liquid chromatography–tandem mass spectrometry. A guard cartridge (100 Å, 3 µm, 2.1 × 5 mm, Atlantis T3 VanGuard, Waters, Milford, MA) was also used for columnar protection. The column was equilibrated with mobile phase A (0.1% formic acid in water) and mobile phase B (acetonitrile) at 50% B. The compounds were eluted by a linear gradient from 50% B to 95% B over 6 min, and then held at 95% B for 1 min. Subsequently, the column was reequilibrated at initial composition for 1 min at a flow rate of 0.3 mL/min. The autosampler and column temperature were set a 4°C and 30°C, respectively. The cannabinoids and their metabolites were detected in electrospray ionization positive or negative mode. Interface voltage was 4 kV or −3.5 kV. Interface, desolvation line, and heat block temperatures were 300, 250, and 400°C, respectively. Nebulizing, heating, and drying gas flow were 2.7, 5, and 5 L/min, respectively. Serum cannabinoids concentrations were calculated by LabSolutions software (Shimadzu Corp., Kyoto, JP) using a quadratic calibration curve with 1/concentration^2 weighing based on relative response (peak area of cannabinoids/peak area of internal standards). The reference standards, their multiple reaction monitoring, polarity, and retention time, internal standards and their multiple reaction monitoring and polarity, and calibration curve range are summarized in Table 1.

2.5 Pharmacokinetic analysis

The PK analyzes were performed using Phoenix WinNonlin™ v8.3 (Certara, Princeton, NJ) by employing a plasma model (200–202) with extravascular dosing and the best fit method, which calculated the coefficient of determination (R^2), maximum serum concentration (C_{max}; ng/mL), time to maximal serum concentration (T_{max}; h), and half-life of the terminal phase ($t_{1/2-\lambda z}$; h); a linear trapezoidal linear interpolation was used to calculate the area under the curve until the last measurement (AUC_{last}; h·ng/mL), area under the moment curve until the last measurement ($AUMC_{last}$; h^2·ng/mL) and mean residence time until the last measurement (MRT_{last}; h). All values below the quantification level (BQL) before C_{max} were set to zero. The first value BQL after C_{max} was calculated as half of the lower limit of quantification (Table 1) for each cannabinoid; the subsequent values BQL were set to zero. Based on a limited sample size, lack of statistical difference by sex, and relatively low serum cannabinoid concentrations resulting in too few data points to calculate all non-compartmental analysis parameters by individual, the mean data were used for group analysis.

2.6 Statistical analysis

Mixed-effects models with the Geisser–Greenhouse correction were performed using Prism v10.0.0 for macOS X (GraphPad, Boston, MA) to determine statistical significance ($p \leq 0.05$). Random effects

TABLE 1 High-performance liquid chromatography–tandem mass spectrometry reference standards (RS) for cannabidiol (CBD), cannabidiolic acid (CBDA), (–)-Δ^9-tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabinolic acid A (THCA), cannabigerol (CBG), cannabigerolic acid (CBGA), 7-Nor-7-carboxycannabidiol, (7-COOH-CBD), (+)-11-nor-9-Carboxy-Δ^9-THC (COOH-THC), (+)-11-nor-9-Carboxy-Δ^9-THC-Glucuronide (COOH-THC-Glu), cannabichromene (CBC), and cannabinol (CBN) with multiple reaction monitoring (MRM) and polarity, and retention time, as well as internal standards with MRM and polarity, and the overall calibration curve range (lower and upper limits of quantification) are presented.

Reference standard				Internal standard		Calibration curve range (ng/mL)
Cannabinoid	Catalog number	MRM (Polarity)	Retention time	Name	MRM (Polarity)	
CBD	34,011	315 > 193 (+)	4.55	CBD-d3	318 > 196 (+)	2.5–1,000
CBDA	34,099	359 > 219 (+)	4.20	CBD-d3	318 > 196 (+)	1–2,500
THC	T-005	315 > 193 (+)	5.60	THC-d3	318 > 196 (+)	1–1,000
THCA	T-093	357 > 245 (–)	6.10	THCA-d3	357 > 248 (–)	1–1,000
CBG	C-141	317 > 193 (+)	4.45	CBD-d3	318 > 196 (+)	1–1,000
CBGA	C-142	361 > 219 (+)	4.35	CBD-d3	318 > 196 (+)	1–1,000
7-COOH-CBD	B140796	343 > 299 (–)	2.25	7-COOH-CBD-d3	346 > 302 (–)	1–1,000
COOH-THC	T-006	345 > 299 (+)	3.55	COOH-THC-d9	354 > 308 (+)	1–250
COOH-THC-Glu	T-038	519 > 345 (–) 521 > 345 (+)	2.00–2.10 (RS) 2.15–2.25 (Animals)	COOH-THC-Glu-d3	522 > 346 (–) 524 > 348 (+)	1–250
CBC	C143	315 > 193 (+)	5.95	THC-d3	318 > 196 (+)	2.5–1,000
CBN	C-046	311 > 223 (+)	5.20	CBD-d3	318 > 196 (+)	1–1,000

zero or less were removed to simplify the model. The independent variables included CBD/ArHO dose (mg/mL), sex (male, female), and time (h). Dependent variables included serum cannabinoid concentrations (ng/mL), serum biochemical concentrations, and complete blood count parameters, which were square-root transformed to improve normality. Residual plots were used to confirm model correctness and Quantile-Quantile plots of predicted versus actual residuals were used to confirm distribution normality.

3 Results

3.1 Pharmacokinetic study

All animals were bright, alert, and responsive throughout the study. During daily observations, they consistently maintained normal hydration, appetites, and stool quality. Most animals were reluctant to take the CBD/ArHO, many of whom turned their heads away from the gavage needle and syringe, and actively pushed them away during administration. Immediately following administration of the CBD/ArHO, some animals had mild hypersalivation, which resolved prior to the next blood collection timepoint.

3.1.1 Serum cannabinoids

Each cannabinoid significantly differed over time ($p \leq 0.0017$). There were no significant differences between males and females for any of the cannabinoids. CBDA was the only cannabinoid that significantly differed by dose over time ($p = 0.0361$); the 8 mg/kg dose was significantly higher than the 4 mg/kg dose. The PK curves for CBD, CBDA, THC, THCA, and 7-COOH-CBD are displayed in Figure 2. The PK results for all detectable cannabinoids are summarized in Table 2 and the serum cannabinoid concentrations on Days 1, 7, and 14 are summarized in Table 3.

For CBD, the model's goodness-of-fit was $R^2 \geq 0.91$ at both doses. The C_{max} was 1.4 times higher at 8 mg/kg than at 4 mg/kg. The T_{max} occurred at 1 h at 4 mg/kg, and 2 h at 8 mg/kg. At 8 mg/kg, the AUC_{last} was 2.1 times, and the $AUMC_{last}$ was 2.2 times, higher than at 4 mg/kg. At 4 mg/kg, the $t_{1/2-\lambda z}$ was 0.24 h less, and the MRT was 0.18 h less, than at 8 mg/kg; the $t_{1/2-\lambda z}$ was 0.26 h less than the MRT at 4 mg/kg and 0.20 h less than the MRT at 8 mg/kg. At 4 mg/kg, the C_{max} for one animal was at least 5.0 times higher than any other individual C_{max}, and 8.0 times higher than any other animal at the 1-h timepoint. At 8 mg/kg, the C_{max} for one animal was at least 1.9 times lower, and its T_{max} was at least 6 h later, than any other individual. By the 24-h timepoint, all serum CBD concentrations were BQL and remained BQL at 4 mg/kg. On Day 7 at 8 mg/kg, all serum CBD concentrations were 2.73–4.23 ng/mL; however, on Day 14 at 8 mg/kg, the serum CBD concentration was BQL for all but one animal (less than 6.85 ng/mL).

For CBDA, the model's goodness-of-fit was $R^2 \geq 0.94$ at both doses. The C_{max} was 1.8 times higher at 8 mg/kg than at 4 mg/kg. The T_{max} occurred at 0.5 h at 4 mg/kg, and 1 h at 8 mg/kg. At 8 mg/kg, the AUC_{last} was 3.3 times, and the $AUMC_{last}$ was 3.9 times, higher than at 4 mg/kg. At 4 mg/kg, the $t_{1/2-\lambda z}$ was 0.26 h greater, and the MRT was 0.69 h less, than at 8 mg/kg; the $t_{1/2-\lambda z}$ was 2.91 h greater than MRT at 4 mg/kg and 1.96 h greater than MRT at 8 mg/kg. At 4 mg/kg, the C_{max} for one animal was at least 2.5 times higher than any other individual C_{max}, while another was at least 3.8 times lower than any other individual C_{max}. At 8 mg/kg, the individual C_{max} values ranged from 223.54 ng/mL at 1 h to 1391.74 ng/mL at 0.5 h. By the 24-h timepoint, all serum CBDA concentrations ranged from 1.92–4.73 ng/mL at 4 mg/kg and 5.47–23.34 ng/mL at 8 mg/kg. On Day 7, the serum CBDA concentration for only one individual (at 4 mg/kg) was BQL; similarly, on Day 14, the serum CBDA concentration for only one individual (at 8 mg/kg) was BQL.

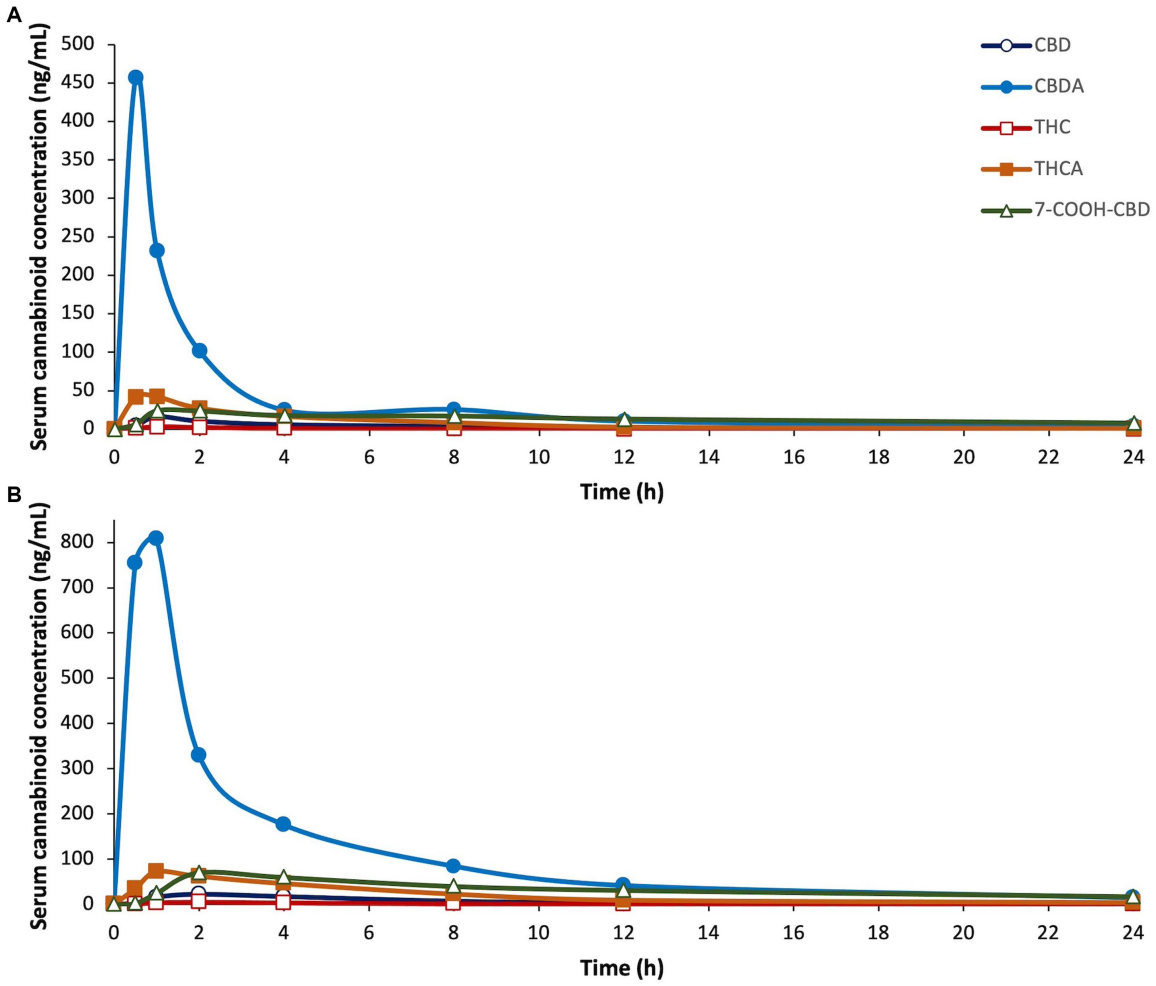

FIGURE 2
Serum concentrations of cannabidiol (CBD), cannabidiolic acid (CBDA), Δ^9-tetrahydrocannabinol (THC), tetrahydrocannabinolic acid (THCA), and 7-carboxy cannabidiol, (7-COOH-CBD), after a single dose of oral administration of cannabidiol-/cannabidiolic acid-rich hemp oil at **(A)** 4 or **(B)** 8 mg/kg to juvenile cynomolgus macaques ($n = 4$ per dose) over 24 h. Note differences in the X-axes due to the sizeable increases in serum cannabinoid concentrations between doses. Each cannabinoid significantly differed over time ($p \leq 0.0017$).

For THC, the model's goodness-of-fit was $R^2 = 0.9989$ at 4 mg/kg and $R^2 = 0.8395$ at 8 mg/kg. The C_{max} was 1.2 times higher at 8 mg/kg than at 4 mg/kg. The T_{max} occurred at 1 h at 4 mg/kg, and 2 h at 8 mg/kg. At 8 mg/kg, the AUC_{last} was 2.1 times, and the $AUMC_{last}$ was 4.0 times, higher than at 4 mg/kg. At 4 mg/kg, the $t_{1/2-\lambda z}$ was 2.57 h less, and the MRT was 2.35 h less, than at 8 mg/kg; the $t_{1/2-\lambda z}$ was 0.70 h less than the MRT at 4 mg/kg and 0.48 h less than the MRT at 8 mg/kg. The C_{max} for one animal that received 4 mg/kg was BQL at all timepoints, while another that received 8 mg/kg was the only animal with detectable serum THC concentrations by the 8-h timepoint (less than 2.99 ng/mL). All serum THC concentrations were BQL for all animals at the 24-h, 7-day, and 14-day timepoints.

For THCA, the model's goodness-of-fit was $R^2 \geq 0.94$ at both doses. The C_{max} was 1.7 times higher at 8 mg/kg than at 4 mg/kg. The T_{max} occurred at 1 h at both doses. At 8 mg/kg, the AUC_{last} was 2.4 times, and the $AUMC_{last}$ was 2.9 times, higher than at 4 mg/kg. At 4 mg/kg, the $t_{1/2-\lambda z}$ was 0.76 h less, and the MRT was 1.11 h less, than at 8 mg/kg; the $t_{1/2-\lambda z}$ was 0.30 h less than the MRT at 4 mg/kg and 0.65 h less than the MRT at 8 mg/kg. At 4 mg/kg, the C_{max} for one animal was at least 2.5 times higher than any other individual C_{max} and 5.8 times higher than any other animal at the 0.5-h timepoint; by the 2-h timepoint, the serum THCA concentration of that animal was less than the mean. The C_{max} for another animal at 4 mg/kg was 5.3 times lower than any other C_{max}. On Day 7, the serum THCA concentration was BQL for all but one animal (less than 1.50 ng/mL) at 4 mg/kg and none of the animals at 8 mg/kg; however, on Day 14, all but one individual at 4 mg/kg, and only one animal 8 mg/kg, had serum THCA concentrations less than 1.00 ng/mL.

For 7-COOH-CBD, the model's goodness-of-fit was $R^2 \geq 0.99$ at both doses. The C_{max} was 2.9 times higher at 8 mg/kg than at 4 mg/kg. The T_{max} occurred at 1 h at 4 mg/kg and 2 h at 8 mg/kg. At 8 mg/kg, the AUC_{last} was 2.4 times, and the $AUMC_{last}$ was 2.3 times, higher than at 4 mg/kg. At 4 mg/kg, the $t_{1/2-\lambda z}$ was 1.77 h greater, and the MRT was 0.44 h greater, than at 8 mg/kg; the $t_{1/2-\lambda z}$ was 5.25 h greater than the MRT at 4 mg/kg and 3.92 h

TABLE 2 Pharmacokinetic summary of cannabidiol (CBD), cannabidiolic acid (CBDA), Δ^9-tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabinolic acid (THCA), cannabigerolic acid (CBGA), 7-Nor-7-carboxycannabidiol (7-COOH-CBD), 11-nor-9-Carboxy-Δ^9-tetrahydrocannabinol (COOH-THC), and 11-nor-9-Carboxy-Δ^9-tetrahydrocannabinol-Glucuronide (COOH-THC-Glu) after a single dose of oral administration of cannabidiol-/cannabidiolic acid-rich hemp oil at 4 or 8 mg/kg to juvenile cynomolgus macaques ($n = 4$ per dose) over 24 h.

Cannabinoid	R^2	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2-\lambda z}$ (h)	AUC_{last} (h·ng/mL)	$AUMC_{last}$ (h^2·ng/mL)	MRT_{last} (h)
4 mg/kg							
CBD	0.9124	15.98 ± 11.07	1	5.57	75.12	437.71	5.83
CBDA	0.9417	456.75 ± 187.48	0.5	6.80	838.28	3264.70	3.89
THC	0.9989	3.94 ± 2.42	1	1.94	12.50	33.03	2.64
THCA	0.9464	41.90 ± 12.77	1	4.60	204.62	1002.26	4.90
CBGA	0.8446	12.14 ± 6.92	0.5	2.20	18.82	40.81	2.17
7-COOH-CBD	0.9909	24.19 ± 15.30	1	15.11	335.16	3304.96	9.86
COOH-THC	0.9727	3.74 ± 2.08	2	11.30	27.33	218.66	8.00
COOH-THC-Glu	0.5460	9.18 ± 4.95	2	12.76	19.15	663.43	8.66
8 mg/kg							
CBD	0.9735	22.31 ± 5.90	2	5.81	157.05	943.76	6.01
CBDA	0.9736	807.33 ± 281.65	1	6.54	2759.26	12634.97	4.58
THC	0.8495	4.80 ± 1.12	2	4.51	26.36	131.61	4.99
THCA	0.9427	71.90 ± 25.65	1	5.36	485.25	2916.39	6.01
CBGA	0.9543	18.23 ± 6.61	1	8.66	63.37	378.14	5.97
7-COOH-CBD	0.9979	70.31 ± 31.86	2	13.34	817.24	7698.93	9.42
COOH-THC	0.5539	8.02 ± 3.54	2	9.69	56.14	425.79	7.58
COOH-THC-Glu	0.8082	19.98 ± 9.03	4	20.34	21.16	1495.14	8.83

The goodness-of-fit (R^2), mean maximum serum concentration (C_{max}; mean ± SEM), time to maximum serum concentration (T_{max}), half-life of the terminal phase ($t_{1/2-\lambda z}$), area under the curve until the last measurement (AUC_{last}), area under the moment curve until the last measurement ($AUMC_{last}$), and mean residence time (MRT_{last}) are presented.

TABLE 3 Mean serum cannabinoid concentration (mean ± SEM) of cannabidiol (CBD), cannabidiolic acid (CBDA), Δ^9-tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabinolic acid (THCA), cannabigerolic acid (CBGA), 7-Nor-7-carboxycannabidiol (7-COOH-CBD), 11-nor-9-Carboxy-Δ^9-tetrahydrocannabinol (COOH-THC), and 11-nor-9-Carboxy-Δ^9-tetrahydrocannabinol-Glucuronide (COOH-THC-Glu) after once daily oral administration of cannabidiol-/cannabidiolic acid-rich hemp oil at 4 or 8 mg/kg to juvenile cynomolgus macaques ($n = 4$ per dose) on Days 1, 7, and 14 at 24, 168, and 336 h, respectively.

Cannabinoid	Serum Concentration (ng/mL)					
	Day 1		Day 7		Day 14	
	4 mg/kg	8 mg/kg	4 mg/kg	8 mg/kg	4 mg/kg	8 mg/kg
CBD	BQL	BQL	BQL	3.40 ± 0.34	BQL	1.71 ± 1.71
CBDA	3.74 ± 0.63	14.25 ± 3.68	3.19 ± 1.63	18.15 ± 7.15	2.56 ± 0.73	8.50 ± 3.59
THC	BQL	BQL	BQL	BQL	BQL	BQL
THCA	0.98 ± 0.18	3.73 ± 0.89	0.37 ± 0.37	2.86 ± 0.57	0.64 ± 0.39	1.55 ± 0.58
CBGA	BQL	0.66 ± 0.26	BQL	0.28 ± 0.28	BQL	BQL
7-COOH-CBD	8.10 ± 2.76	17.20 ± 5.58	13.76 ± 7.40	21.81 ± 7.82	11.48 ± 3.63	12.02 ± 2.84
COOH-THC	0.46 ± 0.31	1.11 ± 0.68	0.84 ± 0.84	0.91 ± 0.56	0.40 ± 0.40	BQL
COOH-THC-Glu	1.91 ± 0.51	3.76 ± 1.14	1.90 ± 1.09	3.99 ± 1.90	1.17 ± 0.52	1.89 ± 1.11

greater than the MRT at 8 mg/kg. At 4 mg/kg, the C_{max} for one animal was at least 2.3 times higher than any other individual C_{max} and 4.6 times higher than any other animal at the 1-h timepoint. The C_{max} and T_{max} results at 8 mg/kg ranged widely by individual: 151.02 ng/mL at 2 h, 103.05 ng/mL at 4 h, 31.99 ng/mL at 2 h, and 16.70 ng/mL at 8 h. Once the serum 7-COOH-CBD concentration was above the BQL, it did not fall below BQL in any subsequent measurements.

3.1.2 Serum biochemistry and complete blood count

A total of 22 biochemistry analytes were evaluated on Days 0, 1, and 14 and summarized in Supplementary Table S2. Of the liver parameters, alanine transaminase and total bilirubin did not significantly differ by time or sex. ALP ($p = 0.0218$) and gamma-glutamyl transferase (GGT; $p = 0.0087$) significantly differed by sex. The mean ALP was consistently higher in males than females. One

male at 4 mg/kg (898 U/L) and another at 8 mg/kg (954 U/L) were higher than the recommended ALP reference range at Day 0, and the other male at 8 mg/kg (1,392 U/L) was higher than the recommended ALP reference range on Day 1. ALP was within the recommended reference range for all individuals on Day 14. GGT was consistently higher in all males than females, but all values were still within the recommended reference range. AST significantly differed over time ($p = 0.0094$). The mean AST was 1.9–2.5 times higher at Day 1 compared to Day 0 and Day 14; one individual at 4 mg/kg and two individuals at 8 mg/kg were higher than the recommended reference range at Day 1. AST was within the recommended reference range for all individuals by Day 14. All liver parameters are summarized in Table 4.

The complete blood count parameters are summarized in Supplementary Table S3. Three Day 14 samples (2 females at 4 mg/kg, 1 female at 8 mg/kg) submitted for hematologic analysis were reported as frozen by the laboratory, invalidating the results, and excluding them from analysis. No parameters significantly differed by dose.

4 Discussion

Few studies have been published regarding the PK or use of CBD in NHP. The PK of intravenous (1.4 mg/kg; $n = 2$) and oral (114 mg/kg; $n = 1$) CBD administration in rhesus macaques (*Macaca mulatta*) were reported while evaluating if electron-capture gas chromatography could be used to analyze serum CBD concentrations (77). The PK of a combination product, CBD (3 mg/kg) and THC (1 mg/kg), administered IM daily for 4 months were determined in common squirrel monkeys (*Saimiri sciureus*; $n = 4$) (78). High-dose CBD (150–300 mg/kg) administered intravenously to rhesus macaques ($n = 12$) resulted in a median lethal dose of 212 mg/kg, and side effects included tremors, emesis, and abnormal respiratory rate (66). Finally, multiple NHP studies have demonstrated that CBD can attenuate the behavioral and neurological (cognition, memory, task performance) effects of THC (78–82).

In addition to NHP, PK studies of CBD or CBDA have been conducted in dogs (28, 55, 70, 71), cats (55, 61, 83), humans (84–86), horses (87, 88), cows (89–91), rabbits (92), guinea pigs (93), rats (94), mice (51), and parrots (95). The CBD doses examined have been highly variable, ranging from 0.5–300 mg/kg in humans (96). Except for Epidiolex, an oral CBD oil, CBD and CBDA products are not FDA approved or regulated, which can affect quality and cannabinoid concentrations. Other products vary in form and administration route, including oral chews (70), oral pastes (61), oral soft gels (71), transmucosal sprays (21), transdermal gels (97), inhalational powders (98), and SC (94), IM (80), intraperitoneal (49), or intravenous (77) injections, which affect serum cannabinoid concentrations and bioavailability.

Based on the CBD:CBDA ratio of the CBD/ArHO used in this study, administration at a 4 mg/kg dose equated to approximately 2 mg/kg of each CBD and CBDA; these doses must be considered when comparing our findings with other studies, especially those that utilized pure CBD isolates. There is conflicting evidence of the pharmacokinetic interactions of CBD and CBDA when administered in a multi-cannabinoid product compared to a CBD isolate (1, 99).

4.1 Pharmacokinetic study

Based on our pilot study (72), low-dose human recommendations (84–86, 100), and other studies (61, 70), increased doses of 4 and 8 mg/kg/day were selected for our PK study. Throughout this study, the animals actively avoided direct-to-mouth CBD/ArHO administration and mildly hypersalivated post-administration, a reported sign of unpalatability in macaques (101). Similarly, cats were reported with signs associated with unpalatability (i.e., lip-licking, head-shaking, and drooling) post-CBD/ArHO administration (55). In addition, use of the squeeze-back cage mechanism was necessary for dosing and likely resulted in increased environmental stress compared to more passive drug delivery systems; however, no additional adverse effects were observed, supporting the relative tolerance of CBD/ArHO.

Due to the number of animals available of similar age and weight meeting inclusion criteria, sample size in this study was low; this, the need to group individuals for the PK analysis due to the frequency of cannabinoid BQL values, and drastic differences in individual C_{max} or T_{max} skewed the overall means, limited statistical power, and prevented identification and exclusion of outliers. High inter-subject variability in cannabinoid concentration has been reported previously in humans (86).

4.1.1 Serum cannabinoids

While all serum cannabinoid concentrations were higher at 8 mg/kg than 4 mg/kg, serum CBDA concentration over time was the only statistically significant difference detected between doses ($p = 0.0361$). At 8 mg/kg, the C_{max} of CBD, CBDA, THC, THCA, and CBGA were less than twice those at 4 mg/kg, while the C_{max} for 7-COOH-CBD,

TABLE 4 Serum liver biochemistry analytes (mean ± SEM) after once daily oral administration of cannabidiol-/cannabidiolic acid-rich hemp oil at 4 or 8 mg/kg to juvenile cynomolgus macaques ($n = 4$ per dose) on Days 0, 1, and 14.

Parameter	Reference range	Day 0		Day 1		Day 14	
		4 mg/kg	8 mg/kg	4 mg/kg	8 mg/kg	4 mg/kg	8 mg/kg
Alkaline phosphatase†	46–875 U/L	686 ± 129	668 ± 146	594 ± 102	758 ± 241	525 ± 99	544 ± 129
Alanine transaminase	0–120 U/L	47 ± 9	66 ± 23	64 ± 15	74 ± 14	49 ± 6	64 ± 24
Aspartate transaminase**	16–88 U/L	33 ± 2	38 ± 3	80 ± 23	71 ± 16	33 ± 3	33 ± 3
Gamma-glutamyltransferase‡	21–184 U/L	97 ± 16	104 ± 23	88 ± 14	102 ± 21	91 ± 13	96 ± 20
Total bilirubin	0.00–2.00 mg/dL	0.09 ± 0.02	0.11 ± 0.03	0.11 ± 0.03	0.17 ± 0.07	0.10 ± 0.02	0.12 ± 0.03

Significantly changed over time **($p \leq 0.01$); Significantly differed by sex †($p \leq 0.05$), ‡($p \leq 0.01$). Results were rounded to match the reference range.

COOH-THC, and COOH-THC-Glu were more than twice those at 4 mg/kg. While a linear relationship between dose and AUC of cannabinoids has been reported (86), other studies have reported a less than dose-proportional increase in C_{max} and AUC as dose increased, suggesting the potential of dose-based difference in bioavailability or metabolism (102). Interestingly, at twice the dose, our results found that the C_{max} for CBD and CBDA less than doubled, while the AUC was 2.1–3.3 times higher.

One PK analysis in dogs at 8 mg/kg had a C_{max} for CBD of 591 ng/mL (28), 26.5 times higher than in our subjects, indicating a higher absorption and bioavailability, or differences in metabolism, distribution, or elimination, compared to cynomolgus macaques. Cats have a lower serum concentration of cannabinoids compared to dogs but varied by study. The C_{max} of an orally administered pure CBD isolate in oil was 17.8 ng/mL at 2.5 mg/kg, 61.1 ng/mL at 5 mg/kg, and 132.6 ng/mL at 10 mg/kg (83). In another study administering CBD/ArHO at 2 mg/kg, the C_{max} of CBD was 43 ng/mL in cats, 6 times lower than dogs in the same study (55); however, this C_{max} was twice that of the C_{max} in our subjects at 8 mg/kg. In a third study administering an oral paste at approximately 2.5 mg/kg (1.37 mg/kg CBD + 1.13 mg/kg CBDA), the C_{max} of CBD was 6.6 times higher than cats receiving 2 mg/kg CBD/ArHO (55, 61). In humans, pure CBD at 1.25 mg/kg resulted in a serum CBD concentration of 37.6 ng/mL at the 2.5-h timepoint, closer to our subjects, albeit 1.7 times higher than our 8 mg/kg CBD/ArHO (86). At a 200 mg/subject dose, the C_{max} was 153 ng/mL in healthy patients (85), similar to dogs dosed with CBD/ArHO at 2 mg/kg (28, 70).

The C_{max} of CBDA in cats at 2.5 mg/kg (1.37 mg/kg CBD + 1.13 mg/kg CBDA) of oral paste was higher (1011.3 ng/mL) than for our subjects (807.33 ng/mL) at 8 mg/kg CBD/ArHO, although the AUC_{last} in our subjects was minimally higher (2759.26 ng/mL) than in cats (2638.7 ng/mL) (61). This suggests that a 4 times higher dose would be required to reach the same CBDA absorption. The C_{max} for CBDA was at more than 28.6 times that of CBD, indicating a higher oral absorption and bioavailability of CBDA, consistent with cannabis extract administration in humans (103); however, few studies have determined the therapeutic dose for CBDA. In dogs, the C_{max} for CBDA was 2–6 times higher than CBD when administered at 2 mg/kg (70, 71).

In our study, the T_{max} was 1–2 h for CBD, and 0.5–1 h for CBDA while the $t_{1/2-\lambda z}$ was 5.57–5.81 h for CBD and 6.54–6.80 h CBDA. While no animal fell BQL for CBDA by the 24-h timepoint, for CBD, two individuals at 4 mg/kg were BQL at the 8-h timepoint, and one animal at 8 mg/kg was BQL at the 12-h timepoint; thus, dosing every 6–12 h would provide better coverage. Twice daily dosing was most reported in dogs (28), cats (55), and humans (102), was therapeutically efficacious in dogs and humans, and may be a more appropriate dosing regimen for NHP.

With the exception of THCA at 4 mg/kg, the T_{max} of the cannabinoid acids (CBDA, THCA, and CBGA) were earlier than the other cannabinoids detected, indicating rapid serum absorption as also reported in mice (51); however, in our study, the $t_{1/2-\lambda z}$ of cannabinoid acids was longer than the corresponding neutral cannabinoid. In this study, the serum CBDA concentration was 10.9–11.2 times higher than the second highest cannabinoid, THCA, and 28.6–36.2 times higher than CBD, despite approximately equal concentrations of CBD and CBDA in the CBD/ArHO. Like CBDA, the THCA C_{max} was higher than THC, consistent with a similar study in dogs and indicating better absorption (70). As in other studies using CBD/ArHO (70, 71), serum THC concentrations were considerably lower than other metabolites, reaching less than 4.81 ng/mL even at 8 mg/kg; given the low THC concentration (0.15 weight percent) in the CBD/ArHO, this was expected.

In humans, 7-COOH-CBD is the major circulating cannabinoid (85, 86, 102). At 1.25 mg/kg of a pure CBD isolate, the serum 7-COOH-CBD (157 ng/mL) concentration was 4.2 times higher than circulating CBD (37.6 ng/mL) (86). Conversely, at 2 mg/kg CBD/ArHO, the 7-COOH-CBD C_{max} (13 ng/mL) in dogs was 9.5 times lower than that of CBD (124 ng/mL) (70), and, at 2.5 mg/kg (1.37 mg/kg CBD + 1.13 mg/kg CBDA), the 7-COOH-CBD C_{max} (41.4 ng/mL) in cats was 6.8 times lower than CBD (282 ng/mL) (61). More similarly to humans but at much lower serum concentrations, the 7-COOH-CBD C_{max} (24.19 ng/mL) in our study was 1.5 times the CBD C_{max} (15.98 ng/mL) at 4 mg/kg and 3.2 times (70.31 ng/mL) the CBD C_{max} (22.31 ng/mL CBD) at 8 mg/kg. On Days 1, 7, and 14, 7-COOH-CBD was higher than serum CBD and CBDA concentrations, potentially due to conversion to or reduced elimination of 7-COOH-CBD. Despite its persistent serum concentration, 7-COOH-CBD was not responsible for the anticonvulsant effects of CBD in animals (104).

4.1.2 Serum biochemistry and complete blood count

Of the liver parameters, males had significantly higher ALP and GGT levels compared to females. Male cynomolgus macaques (after 36 months of age) and humans have also been reported to have higher ALP and GGT than females; ALP elevations also occur in young animals due to bone growth (105–107). AST was the only analyte that significantly differed over time ($p = 0.0094$). While AST is a biomarker of hepatocellular injury, elevations may also be due to normal variation, hemolysis, exercise, or muscle injury or disease. Concurrently elevated CK, as seen in 2 of the 3 animals at Day 0, often occurs in myopathies or issues with phlebotomy technique. Without elevations in alanine transaminase, high AST is less likely related to hepatopathy (108). After 2-week administration of CBD/ArHO, our results indicated that no clinically significant biochemical changes occurred over time.

Three complete blood count samples collected on Day 14 (all female, two at 4 mg/kg, one at 8 mg/kg) froze between shipment and analysis. This resulted in artificially low hematologic parameters, especially for the white blood cells. Similarly, whole-blood storage at −70°C for 15–30 days prior to a complete blood cell count significantly lowered all mean parameters other than hemoglobin and platelet count compared to fresh whole blood (109). After 2-week administration of CBD/ArHO, our overall results indicated that no clinically significant hematological changes occurred over time.

4.2 Other considerations

As terpenes are likely responsible for the smell and flavor of cannabinoid products, contribute to their bitter and unpleasant taste, and reduce patient compliance, the removal of terpenes from the formulation could improve palatability; however, given that terpenes may increase cannabinoid efficacy due to the entourage effect (11), this could reduce dose potency. Due to these concerns and to reduce stress associated with direct-to-mouth administration,

we attempted CBD/ArHO administration in a 10-mL gelatin-based gummy vehicle; however, we encountered additional compliance issues including wide-ranging consumption times or refusal to consume the gummy at all. Alternatively, combining the CBD/ArHO with other, more palatable or aromatic substances such as peppermint oil may mask its bitter taste and improve palatability (110).

The pharmacokinetics of cannabinoids vary based on diet and feeding schedule. Feeding a high-fat diet increased serum CBD concentrations in dogs (70) and humans (102, 111). Conversely, feeding rabbits immediately after CBD/ArHO administration resulted in decreased serum CBD and CBDA concentrations (92). Unlike dogs and humans, rabbits are hindgut fermenters and consume a high-fiber diet, which may absorb the oil. Based on the results in non-hindgut fermenters, feeding our cynomolgus macaques could improve the C_{max} of cannabinoids, as in dogs and humans. On collection days, feed was provided after the 4-h collection; however, feed consumption varied among individuals over the course of each day, so some animals may have had more feed in their gastrointestinal tract than others. Providing a high-fat meal prior to dosing could decrease inter-individual variability and increase absorption.

Although cannabinoid serum accumulation did not appear to occur in our study, the once daily dosing may have been insufficient for this effect. Given that CBD was BQL by the 24-h timepoint, increased CBD/ArHO dosing frequency and duration would be necessary to demonstrate any cumulative effects on serum cannabinoid concentration. Human studies suggest a minimal to moderate CBD accumulation over time (102, 112), but metabolites such as 7-COOH-CBD have greater accumulation (86).

Therapeutic doses and serum concentrations of cannabinoids have not been determined in most species, including macaques. For CBD, the recommended therapeutic doses and serum concentrations vary in reports in other species depending on its intended use. In humans, plasma CBD concentrations of 100 ng/mL were effective in reducing seizures and doses up to 50 mg/kg had a linear increase in plasma concentration and efficacy (100). In dogs, 2 mg/kg of CBD resulted in a median C_{max} of 102 ng/mL and twice daily dosing effectively reduced osteoarthritis-related discomfort (28). In our subjects at 8 mg/kg, the C_{max} of CBD only reached 22 ng/mL, 4.5 times less, suggesting that it would be a subtherapeutic dose if efficacy is similar to dogs and humans.

A biphasic or inverted U-shaped curve effect of CBD and other cannabinoids has been reported in multiple species. While anxiolytic or anti-emetic at certain doses, at some point increasing or decreasing the dose had no effect or even exacerbated the condition (22, 113). Doses of 0.1 and 0.5 mg/kg CBDA were effective in reducing vomiting in house shrews; however, vomiting was comparable to the control at 5 mg/kg (47). Similarly, 0.01 mg/kg of oral CBDA sufficiently reduced hyperalgesia in rats but 1 mg/kg did not (49). Little additional information is currently available regarding this effect; therefore, developing the therapeutic dose of CBD and CBDA in macaques should account for these potential issues.

As they are lipid soluble, cannabinoid concentrations should also be quantified in excreta, adipose, and target tissues (114). Cannabinoids concentrations in target tissues, such as the brain (51), joints (93), or gastrointestinal tract could affect efficacy. Histopathology could help evaluate potential beneficial or deleterious effects to healthy or disease-affected tissues after CBD/ArHO administration and determine therapeutic doses (115).

Other studies have shown anatomical and physiological side effects (66), and biochemical changes (28), after 30 days of dosing. Although our study did not detect any clinically significant changes in liver parameters, changes could be seen after long-term administration; therefore, it should be used cautiously in animals with hepatopathies, prone to liver failure, or receiving other treatments that involve cytochrome P450 mechanisms. Additionally, its use in NHP breeding colonies at lower doses should be evaluated, as high doses were shown to reduce spermatogenesis (66).

4.3 Conclusion

Due to poor regulation of commercially available products, which are frequently mislabeled and often widely variable in cannabinoid composition, products which provide a guaranteed analysis by an independent laboratory are most reliable. Clinical studies are needed to determine the therapeutic dose of CBD and CBDA for macaques, which may differ based on the disorder targeted. Additionally, CBD/ArHO should be evaluated as an adjunctive therapy in non-human primates. Given the low serum CBD concentrations, the doses and frequency used in this study may be insufficient for a therapeutic effect; however, if CBDA has similar therapeutic benefit to CBD, then CBD/ArHO has promise. During a clinical pilot study, we observed that accessing animals for multiple daily dosing may have increased environmental stress, which may limit the usefulness of CBD/ArHO unless an alternative, palatable vehicle is developed. In addition, once daily dosing would be more convenient and increase compliance of use as some facilities do not have 24-h personnel able to medicate the animals every 6–12 h; however, our PK data suggested that once daily dosing was insufficient in maintaining serum CBD concentrations. Given the considerable inter-subject variability and differences of our results compared to other species, CBD/ArHO should be evaluated for reproducibility in other cynomolgus macaques and other NHP to determine if results are similar to our findings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by the Mannheimer Foundation's Institutional Animal Care and Use Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

TJ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing, Funding

acquisition, Resources. JW: Conceptualization, Funding acquisition, Resources, Writing – review & editing. AL: Data curation, Formal analysis, Methodology, Validation, Writing – original draft. AZ: Data curation, Formal analysis, Methodology, Validation, Writing – review & editing. WB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

JW is a paid consultant of ElleVet Sciences. Although the CBD/ArHO and serum cannabinoid analysis and publication costs were financially supported by ElleVet, the study was conducted by the staff at The Mannheimer Foundation (TJ and WB) and independently analyzed by the Toxicology Research Laboratory of the University of Illinois at Chicago (AL and AZ).

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1286158/full#supplementary-material

References

1. Eichler M, Spinedi L, Unfer-Grauwiler S, Bodmer M, Surber C, Luedi M, et al. Heat exposure of *Cannabis sativa* extracts affects the pharmacokinetic and metabolic profile in healthy male subjects. *Planta Med*. (2012) 78:686–91. doi: 10.1055/s-0031-1298334

2. Sikora V, Berenji J, Latković D. Influence of agroclimatic conditions on content of main cannabinoids in industrial hemp (*Cannabis sativa* L.). *Genetika-Belgrade*. (2011) 43:449–56. doi: 10.2298/GENSR1103449S

3. Taura F, Morimoto S, Shoyama Y. Purification and characterization of cannabidiolic-acid synthase from *Cannabis sativa* L.: biochemical analysis of a novel enzyme that catalyzes the oxidocyclization of cannabigerolic acid to cannabidiolic acid. *J Biol Chem*. (1996) 271:17411–6. doi: 10.1074/jbc.271.29.17411

4. Taura F, Sirikantaramas S, Shoyama Y, Yoshikai K, Shoyama Y, Morimoto S. Cannabidiolic-acid synthase, the chemotype-determining enzyme in the fiber-type *Cannabis sativa*. *FEBS Lett*. (2007) 16:2929–34. doi: 10.1016/j.febslet.2007.05.043

5. Marks MD, Tian L, Wenger JP, Omburo SN, Soto-Fuentes W, He J, et al. Identification of candidate genes affecting Δ^9-tetrahydrocannabinol biosynthesis in *Cannabis sativa*. *J Exp Bot*. (2009) 60:3715–26. doi: 10.1093/jxb/erp210

6. Russo EB. Cannabidiol claims and misconceptions. *Trends Pharmacol Sci*. (2017) 38:198–201. doi: 10.1016/j.tips.2016.12.004

7. Merrick J, Lane B, Sebree T, Yaksh T, O'Neill C, Banks SL. Identification of psychoactive degradants of cannabidiol in simulated gastric and physiological fluid. *Cannabis Cannabinoid Res*. (2016) 1:102–12. doi: 10.1089/can.2015.0004

8. Crippa JAS, Zuardi AW, Hallak JEC, Miyazawa B, Bernardo SA, Donaduzzi CM, et al. Oral cannabidiol does not convert to Δ^8-THC or Δ^9-THC in humans: a pharmacokinetic study in healthy subjects. *Cannabis Cannabinoid Res*. (2020) 5:89–98. doi: 10.1089/can.2019.0024

9. Wray L, Stott C, Jones N, Wright S. Cannabidiol does not convert to Δ^9-tetrahydrocannabinol in an in vivo animal model. *Cannabis Cannabinoid Res*. (2017) 2:282–7. doi: 10.1089/can.2017.0032

10. Palazzoli F, Citti C, Licata M, Vilella A, Manca L, Zoli M, et al. Development of a simple and sensitive liquid chromatography triple quadrupole mass spectrometry (LC–MS/MS) method for the determination of cannabidiol (CBD), Δ^9-tetrahydrocannabinol (THC) and its metabolites in rat whole blood after oral administration of a single high dose of CBD. *J Pharm Biomed Anal*. (2018) 150:25–32. doi: 10.1016/j.jpba.2017.11.054

11. Nahler G, Jones TM, Russo EB. Cannabidiol and contributions of major hemp phytocompounds to the "entourage effect"; possible mechanisms. *Altern Complement Integr Med*. (2019) 5:1–16. doi: 10.24966/ACIM-7562/100066

12. CFR. *Schedules of controlled substance–schedule I. 21 CFR § 1308.11*. (2022). Available at:https://www.govinfo.gov/app/details/CFR-2022-title21-vol9/CFR-2022-title21-vol9-sec1308-11 (Accessed August 21, 2023).

13. USC. *Schedule of controlled substances. 21 USC § 812*. (2021). Available at:https://www.govinfo.gov/content/pkg/USCODE-2021-title21/pdf/USCODE-2021-title21-chap13-subchapI-partB-sec812.pdf (Accessed August 21, 2023).

14. PubL. *Agriculture improvement act of 2018. PubL 115–334*. (2018). Available at:https://www.govinfo.gov/content/pkg/PLAW-115publ334/pdf/PLAW-115publ334.pdf (Accessed August 21, 2023).

15. Wakshlag JJ, Cital S, Eaton SJ, Prussin R, Hudalla C. Cannabinoid, terpene, and heavy metal analysis of 29 over-the-counter commercial veterinary hemp supplements. *Vet Med (Auckl)*. (2020) 11:45–55. doi: 10.2147/VMRR.S248712

16. Bonn-Miller MO, Loflin MJE, Thomas BF, Marcu JP, Hyke T, Vandrey R. Labeling accuracy of cannabidiol extracts sold online. *JAMA*. (2017) 318:1708–9. doi: 10.1001/jama.2017.11909

17. Mazurkiewicz-Bełdzińska M, Zawadzka M. Use of cannabidiol in the treatment of epilepsy. *Neurol Neurochir Pol*. (2022) 56:14–20. doi: 10.5603/PJNNS.a2022.0020

18. Li K, Yan FJ, Yu LY, Yuece B, Hong LX, Ying YL, et al. Anti-inflammatory role of cannabidiol and O-1602 in cerulein-induced acute pancreatitis in mice. *Pancreas*. (2013) 42:123–9. doi: 10.1097/MPA.0b013e318259f6f0

19. Jastrząb A, Jarocka-Karpowicz I, Markowska A, Wroński A, Gęgotek A, Skrzydlewska E. Antioxidant and anti-inflammatory effect of cannabidiol contributes to the decreased lipid peroxidation of keratinocytes of rat skin exposed to UV radiation. *Oxidative Med Cell Longev*. (2021) 2021:e6647222:1–13. doi: 10.1155/2021/6647222

20. Pan H, Mukhopadhyay P, Rajesh M, Patel V, Mukhopadhyay B, Gao B, et al. Cannabidiol attenuates cisplatin-induced nephrotoxicity by decreasing oxidative/nitrosative stress, inflammation, and cell death. *J Pharmacol Exp Ther*. (2009) 328:708–14. doi: 10.1124/jpet.108.147181

21. Brioschi FA, Di Cesare F, Gioeni D, Rabbogliatti V, Ferrari F, D'Urso ES, et al. Oral transmucosal cannabidiol oil formulation as part of a multimodal analgesic regimen: effects on pain relief and quality of life improvement in dogs affected by spontaneous osteoarthritis. *Animals*. (2020) 10:1505. doi: 10.3390/ani10091505

22. Kwiatkowska M, Parker LA, Burton P, Mechoulam R. A comparative analysis of the potential of cannabinoids and ondansetron to suppress cisplatin-induced emesis in the *Suncus murinus* (house musk shrew). *Psychopharmacology*. (2004) 174:254–9. doi: 10.1007/s00213-003-1739-9

23. Guimarães FS, de Aguiar JC, Mechoulam R, Breuer A. Anxiolytic effect of cannabidiol derivatives in the elevated plus-maze. *Gen Pharmacol Vasc Syst*. (1994) 25:161–4. doi: 10.1016/0306-3623(94)90027-2

24. Henry JG, Shoemaker G, Prieto JM, Hannon MB, Wakshlag JJ. The effect of cannabidiol on canine neoplastic cell proliferation and mitogen-activated protein kinase activation during autophagy and apoptosis. *Vet Comp Oncol*. (2021) 19:253–65. doi: 10.1111/vco.12669

25. Ligresti A, Moriello A, Starowicz K, Matias I, Pisanti S, De Petrocellis L, et al. Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *J Pharmacol Exp Ther*. (2006) 318:1375–87. doi: 10.1124/jpet.106.105247

26. Luz-Veiga M, Amorim M, Pinto-Ribeiro I, Oliveira ALS, Silva S, Pimentel LL, et al. Cannabidiol and cannabigerol exert antimicrobial activity without compromising skin microbiota. *Int J Mol Sci*. (2023) 24:2389. doi: 10.3390/ijms24032389

27. Abichabki N, Zacharias LV, Moreira NC, Bellissimo-Rodrigues F, Moreira FL, Benzi JRL, et al. Potential cannabidiol (CBD) repurposing as antibacterial and promising therapy of CBD plus polymyxin B (PB) against PB-resistant gram-negative bacilli. *Sci Rep*. (2022) 12:6454. doi: 10.1038/s41598-022-10393-8

28. Gamble LJ, Boesch JM, Frye CW, Schwark WS, Mann S, Wolfe L, et al. Pharmacokinetics, safety, and clinical efficacy of cannabidiol treatment in osteoarthritic dogs. *Front Vet Sci*. (2018) 5:165. doi: 10.3389/fvets.2018.00165

29. Weiss L, Zeira M, Reich S, Har-Noy M, Mechoulam R, Slavin S, et al. Cannabidiol lowers incidence of diabetes in non-obese diabetic mice. *Autoimmunity*. (2006) 39:143–51. doi: 10.1080/08916930500356674

30. Weiss L, Zeira M, Reich S, Slavin S, Raz I, Mechoulam R, et al. Cannabidiol arrests onset of autoimmune diabetes in NOD mice. *Neuropharmacology*. (2008) 54:244–9. doi: 10.1016/j.neuropharm.2007.06.029

31. El-Remessy AB, Al-Shabrawey M, Khalifa Y, Tsai NT, Caldwell RB, Liou GI. Neuroprotective and blood-retinal barrier-preserving effects of cannabidiol in experimental diabetes. *Am J Pathol*. (2006) 168:235–44. doi: 10.2353/ajpath.2006.050500

32. Halpert M, Verrico C, Wesson S, Konduri V, Hofferek C, Vazquez-Perez J, et al. A randomized, double-blind, placebo-controlled study of daily cannabidiol for the treatment of canine osteoarthritis. *J Immunol*. (2020) 204:67.11. doi: 10.4049/jimmunol.204.Supp.67.11

33. Cheng D, Spiro AS, Jenner AM, Garner B, Karl T. Long-term cannabidiol treatment prevents the development of social recognition memory deficits in Alzheimer's disease transgenic mice. *J Alzheimers Dis*. (2014) 42:1383–96. doi: 10.3233/JAD-140921

34. Khodadadi H, Salles ÉL, Jarrahi A, Costigliola V, Khan MB, Yu JC, et al. Cannabidiol ameliorates cognitive function via regulation of IL-33 and TREM2 upregulation in a murine model of Alzheimer's disease. *J Alzheimers Dis*. (2021) 80:973–7. doi: 10.3233/JAD-210026

35. Crivelaro Do Nascimento G, Ferrari DP, Guimaraes FS, Del Bel EA, Bortolanza M, Ferreira NC. Cannabidiol increases the nociceptive threshold in a preclinical model of Parkinson's disease. *Neuropharmacology*. (2020) 163:107808. doi: 10.1016/j.neuropharm.2019.107808

36. Zuardi AW. Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. *Braz J Psychiatry*. (2008) 30:271–80. doi: 10.1590/S1516-44462008000300015

37. Chagas MHN, Zuardi AW, Tumas V, Pena-Pereira MA, Sobreira ET, Bergamaschi MM, et al. Effects of cannabidiol in the treatment of patients with Parkinson's disease: an exploratory double-blind trial. *J Psychopharmacol (Oxf)*. (2014) 28:1088–98. doi: 10.1177/0269881114550355

38. Hunt ABG, Flint HE, Logan DW, King T. A single dose of cannabidiol (CBD) positively influences measures of stress in dogs during separation and car travel. *Front Vet Sci*. (2023) 10:1112604. doi: 10.3389/fvets.2023.1112604

39. Scicluna RL, Wilson BB, Thelaus SH, Arnold JC, McGregor IS, Bowen MT. Cannabidiol reduced the severity of gastrointestinal symptoms of opioid withdrawal in male and female mice. *Cannabis Cannabinoid Res*. (2022) 2022:36. doi: 10.1089/can.2022.0036

40. Schicho R, Storr M. Topical and systemic cannabidiol improves trinitrobenzene sulfonic acid colitis in mice. *Pharmacology*. (2012) 89:149–55. doi: 10.1159/000336871

41. Vivanco-Estela AN, Dos-Santos-Pereira M, Guimaraes FS, Del-Bel E, Nascimento GC. Cannabidiol has therapeutic potential for myofascial pain in female and male parkinsonian rats. *Neuropharmacology*. (2021) 196:108700. doi: 10.1016/j.neuropharm.2021.108700

42. Nitecka-Buchta A, Nowak-Wachol A, Wachol K, Walczyńska-Dragon K, Olczyk P, Batoryna O, et al. Myorelaxant effect of transdermal cannabidiol application in patients with TMD: a randomized, double-blind trial. *J Clin Med*. (2019) 8:1886. doi: 10.3390/jcm8111886

43. Krzyżewska A, Baranowska-Kuczko M, Kasacka I, Kozłowska H. Cannabidiol inhibits lung proliferation in monocrotaline-induced pulmonary hypertension in rats. *Biomed Pharmacother*. (2023) 159:114234. doi: 10.1016/j.biopha.2023.114234

44. Kumric M, Dujic G, Vrdoljak J, Svagusa K, Kurir TT, Supe-Domic D, et al. CBD supplementation reduces arterial blood pressure via modulation of the sympatho-chromaffin system: a substudy from the HYPER-H21-4 trial. *Biomed Pharmacother*. (2023) 160:114387. doi: 10.1016/j.biopha.2023.114387

45. Batinic A, Sutlović D, Kuret S, Matana A, Kumric M, Bozic J, et al. Trial of a novel oral cannabinoid formulation in patients with hypertension: a double-blind, placebo-controlled pharmacogenetic study. *Pharmaceuticals*. (2023) 16:645. doi: 10.3390/ph16050645

46. Rossignol J, Hatton S, Ridley A, Hermine O, Greco C. The effectiveness and safety of pharmaceutical-grade cannabidiol in the treatment of mastocytosis-associated pain: a pilot study. *Biomedicine*. (2023) 11:520. doi: 10.3390/biomedicines11020520

47. Bolognini D, Rock E, Cluny N, Cascio M, Limebeer C, Duncan M, et al. Cannabidiolic acid prevents vomiting in Suncus murinus and nausea-induced behaviour in rats by enhancing 5-HT1A receptor activation. *Br J Pharmacol*. (2013) 168:1456–70. doi: 10.1111/bph.12043

48. Takeda S, Misawa K, Yamamoto I, Watanabe K. Cannabidiolic acid as a selective cyclooxygenase-2 inhibitory component in cannabis. *Drug Metab Dispos*. (2008) 36:1917–21. doi: 10.1124/dmd.108.020909

49. Rock EM, Limebeer CL, Parker LA. Effect of cannabidiolic acid and Δ^9-tetrahydrocannabinol on carrageenan-induced hyperalgesia and edema in a rodent model of inflammatory pain. *Psychopharmacology*. (2018) 235:3259–71. doi: 10.1007/s00213-018-5034-1

50. Vigli D, Cosentino L, Pellas M, De Filippis B. Chronic treatment with cannabidiolic acid (CBDA) reduces thermal pain sensitivity in male mice and rescues the hyperalgesia in a mouse model of Rett syndrome. *Neuroscience*. (2020) 453:113–23. doi: 10.1016/j.neuroscience.2020.09.041

51. Anderson LL, Low IK, Banister SD, McGregor IS, Arnold JC. Pharmacokinetics of phytocannabinoid acids and anticonvulsant effect of cannabidiolic acid in a mouse model of Dravet syndrome. *J Nat Prod*. (2019) 82:3047–55. doi: 10.1021/acs.jnatprod.9b00600

52. Calapai F, Cardia L, Calapai G, Di Mauro D, Trimarchi F, Ammendolia I, et al. Effects of cannabidiol on locomotor activity. *Life*. (2022) 12:652. doi: 10.3390/life12050652

53. Kleinhenz MD, Weeder M, Montgomery S, Martin M, Curtis A, Magnin G, et al. Short term feeding of industrial hemp with a high cannabidiolic acid (CBDA) content increases lying behavior and reduces biomarkers of stress and inflammation in Holstein steers. *Sci Rep*. (2022) 12:3683. doi: 10.1038/s41598-022-07795-z

54. Assareh N, Gururajan A, Zhou C, Luo JL, Kevin RC, Arnold JC. Cannabidiol disrupts conditioned fear expression and cannabidiolic acid reduces trauma-induced anxiety-related behaviour in mice. *Behav Pharmacol*. (2020) 31:591–6. doi: 10.1097/FBP.0000000000000565

55. Deabold KA, Schwark WS, Wolf L, Wakshlag JJ. Single-dose pharmacokinetics and preliminary safety assessment with use of CBD-rich hemp nutraceutical in healthy dogs and cats. *Animals*. (2019) 9:832. doi: 10.3390/ani9100832

56. Devinsky O, Marsh E, Friedman D, Thiele E, Laux L, Sullivan J, et al. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. *Lancet Neurol*. (2016) 15:270–8. doi: 10.1016/S1474-4422(15)00379-8

57. Contin M, Mohamed S, Santucci M, Lodi MAM, Russo E, Mecarelli O, et al. Cannabidiol in pharmacoresistant epilepsy: clinical pharmacokinetic data from an expanded access program. *Front Pharmacol*. (2021) 12:179. doi: 10.3389/fphar.2021.637801

58. McGrath S, Bartner LR, Rao S, Packer RA, Gustafson DL. Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. *J Am Vet Med Assoc*. (2019) 254:1301–8. doi: 10.2460/javma.254.11.1301

59. McGrath S, Bartner LR, Rao S, Kogan LR, Hellyer PW. A report of adverse effects associated with the administration of cannabidiol in healthy dogs. *Am Holistic Vet Med Assoc*. (2018) 52:34–8.

60. Kaufmann R, Aqua K, Lombardo J, Lee M. Observed impact of long-term consumption of oral cannabidiol on liver function in healthy adults. *Cannabis Cannabinoid Res*. (2021) 8:148–54. doi: 10.1089/can.2021.0114

61. Wang T, Zakharov A, Gomez B, Lyubimov A, Trottier NL, Schwark WS, et al. Serum cannabinoid 24 h and 1 week steady state pharmacokinetic assessment in cats using a CBD/CBDA rich hemp paste. *Front Vet Sci*. (2022) 9:895368. doi: 10.3389/fvets.2022.895368

62. Devinsky O, Cross JH, Laux L, Marsh E, Miller I, Nabbout R, et al. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. *N Engl J Med*. (2017) 376:2011–20. doi: 10.1056/NEJMoa1611618

63. Watkins PB, Church RJ, Li J, Knappertz V. Cannabidiol and abnormal liver chemistries in healthy adults: results of a phase I clinical trial. *Clin Pharmacol Ther*. (2021) 109:1224–31. doi: 10.1002/cpt.2071

64. Doohan PT, Oldfield LD, Arnold JC, Anderson LL. Cannabinoid interactions with cytochrome P450 drug metabolism: a full-spectrum characterization. *AAPS J*. (2021) 23:91. doi: 10.1208/s12248-021-00616-7

65. Nasrin S, Watson CJW, Perez-Paramo YX, Lazarus P. Cannabinoid metabolites as inhibitors of major hepatic CYP450 enzymes, with implications for cannabis-drug interactions. *Drug Metab Dispos Biol Fate Chem*. (2021) 49:1070–80. doi: 10.1124/dmd.121.000442

66. Rosenkrantz H, Fleischman RW, Grant RJ. Toxicity of short-term administration of cannabinoids to rhesus monkeys. *Toxicol Appl Pharmacol*. (1981) 58:118–31. doi: 10.1016/0041-008X(81)90122-8

67. Carlson CS, Loeser RF, Jayo MJ, Weaver DS, Adams MR, Jerome CP. Osteoarthritis in cynomolgus macaques: a primate model of naturally occurring disease. *J Orthop Res*. (1994) 12:331–9. doi: 10.1002/jor.1100120305

68. Gottlieb DH, Capitanio JP, McCowan B. Risk factors for stereotypic behavior and self-biting in rhesus macaques (*Macaca mulatta*): Animal's history, current environment, and personality. *Am J Primatol*. (2013) 75:995–1008. doi: 10.1002/ajp.22161

69. Hird DW, Anderson JH, Bielitzki JT. Diarrhea in nonhuman primates: a survey of primate colonies for incidence rates and clinical opinion. *Lab Anim Sci*. (1984) 34:465–70.

70. Wakshlag JJ, Schwark WS, Deabold KA, Talsma BN, Cital S, Lyubimov A, et al. Pharmacokinetics of cannabidiol, cannabidiolic acid, Δ^9-tetrahydrocannabinol, tetrahydrocannabinolic acid and related metabolites in canine serum after dosing with three oral forms of hemp extract. *Front Vet Sci*. (2020) 7:7. doi: 10.3389/fvets.2020.00505

71. Tittle DJ, Wakshlag JJ, Schwark WS, Lyubimov A, Zakharov A, Gomez B. Twenty-four hour and one-week steady state pharmacokinetics of cannabinoids in two formulations of cannabidiol and cannabidiolic acid rich hemp in dogs. *Med Res Arch*. (2022) 10:2907. doi: 10.18103/mra.v10i7.2907

72. Johns TN, Cameron AI, Burnside WM. Cannabidiol and cannabidiolic acid-rich hemp oil for the treatment of diarrheal disease in cynomolgus macaques (*Macaca fascicularis*). Abstracts of scientific presentations: 2020 AALAS virtual national meeting. *J Am Assoc Lab Anim Sci*. (2020) 59:659.

73. PubL. *Animal welfare act as amended. PubL 89–544*. (2018). Available at:https://www.govinfo.gov/content/pkg/COMPS-10262/pdf/COMPS-10262.pdf (Accessed August 21, 2023).

74. USC. *Transportation, Sale, and handling of certain animals. 7 USC § 2131–2160*. (2022). Available at:https://www.govinfo.gov/content/pkg/USCODE-2021-title7/pdf/USCODE-2021-title7-chap54.pdf (Accessed August 21, 2023).

75. CFR. *Animal welfare regulations. 9 CFR § 1–12*. (2022). https://www.govinfo.gov/content/pkg/CFR-2022-title9-vol1/pdf/CFR-2022-title9-vol1-chapI-subchapA.pdf (Accessed August 21, 2023).

76. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. *Guide for the care and use of laboratory animals. 8th* ed. Washington, DC: National Academies Press (2011). 220 p.

77. Jones AB, Elsohly MA, Bedford JA, Turner CE. Determination of cannabidiol in plasma by electron-capture gas chromatography. *J Chromatogr B Biomed Sci App*. (1981) 226:99–105. doi: 10.1016/S0378-4347(00)84210-3

78. Withey SL, Eisold JE, Chelini G, Kangas BD, Bergman J, Madras BK. Behavioral and molecular effects of THC and CBD in adolescent nonhuman primates. *FASEB J*. (2020) 34:1. doi: 10.1096/fasebj.2020.34.s1.09462

79. Brady KT, Balster RL. The effects of Δ^9-tetrahydrocannabinol alone and in combination with cannabidiol on fixed-interval performance in rhesus monkeys. *Psychopharmacology*. (1980) 72:21–6. doi: 10.1007/BF00433803

80. Withey SL, Kangas BD, Charles S, Gumbert AB, Eisold JE, George S, et al. Effects of daily Δ^9-tetrahydrocannabinol (THC) alone or combined with cannabidiol (CBD) on cognition-based behavior and activity in adolescent nonhuman primates. *Drug Alcohol Depend*. (2021) 221:108629. doi: 10.1016/j.drugalcdep.2021.108629

81. Wright MJ Jr, Vandewater SA, Taffe MA. Cannabidiol attenuates deficits of visuospatial associative memory induced by Δ^9tetrahydrocannabinol. *Br J Pharmacol*. (2013) 170:1365–73. doi: 10.1111/bph.12199

82. Jacobs DS, Kohut SJ, Jiang S, Nikas SP, Makriyannis A, Bergman J. Acute and chronic effects of cannabidiol on Δ^9-tetrahydrocannabinol (Δ^9-THC)-induced disruption in stop signal task performance. *Exp Clin Psychopharmacol*. (2016) 24:320–30. doi: 10.1037/pha0000081

83. Rozental AJ, Gustafson DL, Kusick BR, Bartner LR, Castro SC, McGrath S. Pharmacokinetics of escalating single-dose administration of cannabidiol to cats. *J Vet Pharmacol Ther*. (2023) 46:25–33. doi: 10.1111/jvp.13100

84. Taylor L, Crockett J, Tayo B, Morrison G. A phase I, open-label, parallel-group, single-dose trial of the pharmacokinetics and safety of cannabidiol (CBD) in subjects with mild to severe hepatic impairment. *J Clin Pharmacol*. (2019) 59:1110–9. doi: 10.1002/jcph.1412

85. Tayo B, Taylor L, Sahebkar F, Morrison G. A phase I, open-label, parallel-group, single-dose trial of the pharmacokinetics, safety, and tolerability of cannabidiol in subjects with mild to severe renal impairment. *Clin Pharmacokinet*. (2020) 59:747–55. doi: 10.1007/s40262-019-00841-6

86. Devinsky O, Patel AD, Thiele EA, Wong MH, Appleton R, Harden CL, et al. Randomized, dose-ranging safety trial of cannabidiol in Dravet syndrome. *Neurology*. (2018) 90:1204–11. doi: 10.1212/WNL.0000000000005254

87. Turner SE, Knych HK, Adams AA. Pharmacokinetics of cannabidiol in a randomized crossover trial in senior horses. *Am J Vet Res*. (2022) 83:28. doi: 10.2460/ajvr.22.02.0028

88. Declan R, McKemie DS, Kass PH, Puschner B, Knych HK. Pharmacokinetics and effects on arachidonic acid metabolism of low doses of cannabidiol following oral administration to horses. *Drug Test Anal*. (2021) 13:1305–17. doi: 10.1002/dta.3028

89. Meyer K, Hayman K, Baumgartner J, Gorden PJ. Plasma pharmacokinetics of cannabidiol following oral administration of cannabidiol oil to dairy calves. *Front Vet Sci*. (2022) 9:789495. doi: 10.3389/fvets.2022.789495

90. Kleinhenz MD, Magnin G, Lin Z, Griffin J, Kleinhenz KE, Montgomery S, et al. Plasma concentrations of eleven cannabinoids in cattle following oral administration of industrial hemp (*Cannabis sativa*). *Sci Rep*. (2020) 10:12753. doi: 10.1038/s41598-020-69768-4

91. Kleinhenz MD, Magnin G, Lin J, Griffin J, Kleinhenz KE, Curtis A, et al. Pharmacokinetics of cannabidiolic acid in cattle following oral dosing of industrial hemp (*Cannabis sativa*). *Am Assoc Bov Pract Conf Proc*. (2020) 53:391. doi: 10.21423/aabppro20208136

92. Rooney TA, Carpenter JW, KuKanich B, Gardhouse SM, Magnin GC, Tully TN. Feeding decreases the oral bioavailability of cannabidiol and cannabidiolic acid in hemp oil in New Zealand white rabbits (*Oryctolagus cuniculus*). *Am J Vet Res*. (2022) 83:6. doi: 10.2460/ajvr.22.01.0006

93. Spittler AP, Helbling JE, McGrath S, Gustafson DL, Santangelo KS, Sadar MJ. Plasma and joint tissue pharmacokinetics of two doses of oral cannabidiol oil in guinea pigs (*Cavia porcellus*). *J Vet Pharmacol Ther*. (2021) 44:967–74. doi: 10.1111/jvp.13026

94. Hložek T, Uttl L, Kadeřábek L, Balíková M, Lhotková E, Horsley RR, et al. Pharmacokinetic and behavioural profile of THC, CBD, and THC+CBD combination after pulmonary, oral, and subcutaneous administration in rats and confirmation of conversion in vivo of CBD to THC. *Eur Neuropsychopharmacol*. (2017) 27:1223–37. doi: 10.1016/j.euroneuro.2017.10.037

95. Carpenter JW, Tulley TN, Rockwell K, KuKanich B. Pharmacokinetics of cannabidiol in the Hispaniolan Amazon parrot (*Amazona ventralis*). *J Avian Med Surg*. (2022) 36:121–7. doi: 10.1647/20-00076

96. Leo A, Russo E, Elia M. Cannabidiol and epilepsy: rationale and therapeutic potential. *Pharmacol Res*. (2016) 107:85–92. doi: 10.1016/j.phrs.2016.03.005

97. Hammell DC, Zhang LP, Ma F, Abshire SM, McIlwrath SL, Stinchcomb AL, et al. Transdermal cannabidiol reduces inflammation and pain-related behaviours in a rat model of arthritis. *Eur J Pain*. (2016) 20:936–48. doi: 10.1002/ejp.818

98. Devinsky O, Kraft K, Rusch L, Fein M, Leone-Bay A. Improved bioavailability with dry powder cannabidiol inhalation: a phase I clinical study. *J Pharm Sci*. (2021) 110:3946–52. doi: 10.1016/j.xphs.2021.08.012

99. Anderson LL, Etchart MG, Bahceci D, Golembiewski TA, Arnold JC. Cannabis constituents interact at the drug efflux pump BCRP to markedly increase plasma cannabidiolic acid concentrations. *Sci Rep*. (2021) 11:14948. doi: 10.1038/s41598-021-94212-6

100. Szaflarski JP, Hernando K, Bebin EM, Gaston TE, Grayson LE, Ampah SB, et al. Higher cannabidiol plasma levels are associated with better seizure response following treatment with a pharmaceutical grade cannabidiol. *Epilepsy Behav*. (2019) 95:131–6. doi: 10.1016/j.yebeh.2019.03.042

101. Pulley ACS, Roberts JA, Lerche NW. Four preanesthetic oral sedation protocols for rhesus macaques (*Macaca mulatta*). *J Zoo Wildl Med*. (2004) 35:497–502. doi: 10.1638/03-092

102. Taylor L, Gidal B, Blakey G, Tayo B, Morrison G. A phase I, randomized, double-blind, placebo-controlled, single ascending dose, multiple dose, and food effect trial of the safety, tolerability and pharmacokinetics of highly purified cannabidiol in healthy subjects. *CNS Drugs*. (2018) 32:1053–67. doi: 10.1007/s40263-018-0578-5

103. Pellesi L, Licata M, Verri P, Vandelli D, Palazzoli F, Marchesi F, et al. Pharmacokinetics and tolerability of oral cannabis preparations in patients with medication overuse headache (MOH)—a pilot study. *Eur J Clin Pharmacol*. (2018) 74:1427–36. doi: 10.1007/s00228-018-2516-3

104. Whalley BJ, Stott C, Gray RA, Jones NA. *Default. The human metabolite of cannabidiol, 7-hydroxy cannabidiol, but not 7-carboxy cannabidiol, is anticonvulsant in the maximal electroshock seizure threshold test (MEST) in mouse*. AES Annual Meeting 2017. (2017). Available at:https://aesnet.org/abstractslisting/the-human-metabolite-of-cannabidiol--7-hydroxy-cannabidiol--but-not-7-carboxy-cannabidiol--is-anticonvulsant-in-the-maximal-electroshock-seizure-threshold-test-(mest)-in-mouse.

105. Koo BS, Lee DH, Kang P, Jeong KJ, Lee S, Kim K, et al. Reference values of hematological and biochemical parameters in young-adult cynomolgus monkey (*Macaca fascicularis*) and rhesus monkey (*Macaca mulatta*) anesthetized with ketamine hydrochloride. *Lab Anim Res*. (2019) 5:7. doi: 10.1186/s42826-019-0006-0

106. Xie L, Xu F, Liu S, Ji Y, Zhou Q, Wu Q, et al. Age- and sex-based hematological and biochemical parameters for *Macaca fascicularis*. *PLoS One*. (2013) 8:64892. doi: 10.1371/journal.pone.0064892

107. Manolio TA, Burke GL, Savage PJ, Jacobs DR, Sidney S, Wagenknecht LE, et al. Sex- and race-related differences in liver-associated serum chemistry tests in young adults in the cardia study. *Clin Chem*. (1992) 38:1853–9. doi: 10.1093/clinchem/38.9.1853

108. Giboney PT. Mildly elevated liver transaminase levels in the asymptomatic patient. *Am Fam Physician*. (2005) 71:1105–10.

109. Tang O, Selvin E, Arends V, Saenger A. Short-term stability of hematologic parameters in frozen whole blood. *J Appl Lab Med*. (2019) 4:410–4. doi: 10.1373/jalm.2018.028357

110. Limpongsa E, Tabboon P, Pongjanyakul T, Jaipakdee N. Preparation and evaluation of directly compressible orally disintegrating tablets of cannabidiol formulated using liquisolid technique. *Pharmaceutics*. (2022) 14:2407. doi: 10.3390/pharmaceutics14112407

111. Abbotts KSS, Ewell TR, Butterklee HM, Bomar MC, Akagi N, Dooley GP, et al. Cannabidiol and cannabidiol metabolites: pharmacokinetics, interaction with food, and influence on liver function. *Nutrients*. (2022) 14:2152. doi: 10.3390/nu14102152

112. Stott CG, White L, Wright S, Wilbraham D, Guy GW. A phase I study to assess the single and multiple dose pharmacokinetics of THC/CBD oromucosal spray. *Eur J Clin Pharmacol*. (2013) 69:1135–47. doi: 10.1007/s00228-012-1441-0

113. Zuardi AW, Rodrigues NP, Silva AL, Bernardo SA, Hallak JEC, Guimarães FS, et al. Inverted U-shaped dose-response curve of the anxiolytic effect of cannabidiol during public speaking in real life. *Front Pharmacol*. (2017) 8:259. doi: 10.3389/fphar.2017.00259

114. Child RB, Tallon MJ. Cannabidiol (CBD) dosing: plasma pharmacokinetics and effects on accumulation in skeletal muscle, liver and adipose tissue. *Nutrients*. (2022) 14:2101. doi: 10.3390/nu14102101

115. Malfait AM, Gallily R, Sumariwalla PF, Malik AS, Andreakos E, Mechoulam R, et al. The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. *Proc Natl Acad Sci*. (2000) 97:9561–6. doi: 10.1073/pnas.160105897

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Glossary

7-COOH-CBD	7-Nor-7-carboxycannabidiol
ALP	Alkaline phosphatase
AUC_{last}	Area under the curve until the last measurement
$AUMC_{last}$	Area under the moment curve until the last measurement
AST	Aspartate aminotransferase
BQL	Below the quantification level
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBD/ArHO	Cannabidiol-/cannabidiolic acid-rich hemp oil
CBG	Cannabigerol
CBGA	Cannabigerolic acid
CBN	Cannabinol
C_{max}	Maximum serum concentration
COOH-THC	11-nor-9-Carboxy-Δ^9-tetrahydrocannabinol
COOH-THC-Glu	11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol-Glucuronide
GGT	Gamma-glutamyl transferase
IM	Intramuscularly
MRT_{last}	Mean residence time until the last measurement
NHP	Nonhuman primate
PK	Pharmacokinetic
R^2	Coefficient of determination
SC	Subcutaneously
$t_{1/2-\lambda z}$	Half-life of the terminal phase
THC	Δ^9-tetrahydrocannabinol;
THCA	Δ^9-tetrahydrocannabinolic acid
T_{max}	Time to maximal serum concentration

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Behavioral observations, heart rate and heart rate variability in horses following oral administration of a cannabidiol containing paste in three escalating doses (part 1/2)

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Cannabidiol (CBD) products have been proposed to exert stress- and anxiety-relieving effects in animals. Despite the increasing popularity of CBD for veterinary use, the available research detailing the effects of CBD in horses is limited. The aim of this study (part 1 of 2) was to analyze stress parameters via behavioral observations and heart rate monitoring in healthy horses following single oral administration of a CBD containing paste in different doses. Study products were two pastes for oral administration, one containing CBD and one containing no active ingredient. Pastes were applied as single administrations in consecutive trials with escalating dosages (doses: 0.2, 1.0, 3.0 mg CBD/kg) to a treatment (trial 1: $n = 3$, trial 2: $n = 3$, trial 3: $n = 5$ horses) and a control group (trial 1: $n = 3$, trial 2: $n = 3$, trial 3: $n = 6$ horses) with minimum wash-out periods of seven days in between. Behavioral parameters were evaluated using video recordings to score the levels of sedation including the horses' reactions to acoustic and visual stimuli. Facial expression was assessed using photographs. Evaluation was based on the previously described facial sedation scale for horses (FaceSed) and the Horse Grimace Scale. For baseline values, identical observations were recorded on the day before each paste administration. Both paste administration and behavioral evaluation were performed double blinded. Cardiac beat-to-beat (R-R) intervals were continuously recorded throughout the trial and assessed using heart rate and heart rate variability parameters. Statistical analysis included comparison between treatment and control group over escalating doses and time points using linear mixed models. The CBD paste was well tolerated, and no side effects were observed. Analysis of sedation scores and facial expressions did not indicate significant differences between treatment and control group over the escalating doses. The heart rate was neither reduced, nor were significant changes in heart rate variability observed compared to the control group. Main limitation of this study is the small sample size. Further research is required to determine adequate doses and indications for the use of CBD products in horses.

KEYWORDS

behavior, CBD, equine, FaceSed, Horse Grimace Scale, sedation score

1 Introduction

Cannabidiol (CBD) belongs to the most well-known compounds of *Cannabis* plants and is gaining increasing attention in the field of veterinary medicine. Unlike Δ^9-tetrahydrocannabinol (THC), CBD does not exhibit psychoactive properties (1, 2) but has been tested for analgesic, anti-inflammatory and anti-convulsant effects in companion animals (3–8). Additionally, the impact of CBD on anxiety and stress relief is currently under investigation. In humans, stress and anxiety are the most common indications for CBD use (9).

Mechanisms of action include various pathways: CBD may act as a ligand on serotonin$_{1A}$ (5-HT$_{1A}$) receptors (10–14) and inhibits the deactivation of endogenous cannabinoids such as anandamide (AEA) (15–17). AEA is a ligand of the endocannabinoid (eCB) system which regulates emotional responses and can reduce anxiety (12, 18, 19). CBD may also influence cannabinoid type 1 (CB$_1$) receptors of the eCB system as an indirect agonist by increasing membrane fluidity and therefore modulating the constitutional activity of CB$_1$ (12, 20, 21).

In humans and rodents, CBD has been reported to decrease heart rate and to show anxiolytic effects (9, 22–25). However, results remain inconsistent, as other studies could not confirm these findings to the same extent (26–29). Further effects of CBD include sedation, which has been reported in humans (30, 31). In dogs, surveys among US veterinarians and pet owners have reported that sedation is a perceived side effect following CBD or hemp supplementation (32–34). It was additionally suggested that CBD supplementation may decrease stress-related aggressive behavior (1). Another study could not identify significant alteration in daily activity or quality of sleep in dogs (35). There are few reports detailing the effect of CBD on equine behavior: One study found a reduction of reactivity without any significant effect on the heart rate (36). Other reports showed no effect of CBD on ataxia, sedation scores or overall equine behavior (37, 38). Two case reports described CBD as an effective treatment for stereotypic behavior such as crib-biting and mechanical allodynia (39, 40). The effect of CBD on horses is of particular interest as all cannabinoids are on the list of prohibited substances issued by the international governing body of equestrian sports (FEI, Fédération Equestre Internationale) due to their assumed psychotropic properties (41).

The aim of this study was to analyze stress levels via behavioral observations and heart rate monitoring in healthy horses following oral administration of a CBD containing paste to further validate equine behavior under the influence of CBD medication. The authors hypothesized that increasing CBD doses would have a moderately calming effect in horses.

2 Materials and methods

2.1 Animals

Twelve Haflinger × Warmblood cross horses, including seven mares and five stallions, were randomly assigned to a treatment or a control group ($n = 6 + 6$). Horses' age varied between 3 to 16 years (median: 11 years) in the treatment group and 10 to 26 years (median: 10.5 years) in the control group. Mares and stallions were housed separately with mares having free paddock access. All horses were fed hay and mineral feed, and spent 8 h a day on pasture. The study was approved by the competent authority for licensing and notification procedures for animal experiments (LAVG) in Brandenburg, Germany (AZ: 2347–12–2021).

2.2 Study products

Study products were two pastes (treatment and control). The treatment paste contained 55% full spectrum CBD plant extract, medium-chain triglyceride (MCT) coconut oil, naturally occurring phytocannabinoids, terpenes, flavonoids and beeswax (TAMACAN XL 55%®, Herosan healthcare GmbH, Austria). The THC content was below 0.2%. The control paste contained MCT oil and beeswax only. The ingredients of both pastes were analyzed, and concentrations of the contents were confirmed by an independent and internationally accredited anti-doping laboratory (Institute of Biochemistry, German Sport University Cologne, Germany). Pastes were labeled "A" or "B" by the manufacturer before shipment to conceal their formulations. People handling the horses, i.e., caretakers and sample takers, were unaware of the horses' group assignment.

2.3 Dose escalation study

The study was divided into three trials with administration of CBD paste in escalating doses (trial 1: 0.2 mg CBD/kg; trial 2: 1 mg CBD/kg; trial 3: 3 mg CBD/kg). Doses were selected based on the manufacturer's recommendation and the current literature (36, 38). The first two trials were performed with three horses in each group ($n = 3$ treatment $+$ 3 control) and close attention was paid to the occurrence of possible side effects. The third trial (3 mg CBD/kg) was subsequently performed with all twelve horses ($n = 6$ treatment $+$ 6 control). The day before each trial, horses were physically examined and a jugular vein catheter was aseptically placed. On the day of trial, the paste (A or B) was orally administered at 6:30 am. For better acceptance, the paste was inserted into a treat. To determine pharmacokinetic parameters of CBD administration in horses, multiple blood and urine samples were taken throughout the trials from all horses (42).

Equine behavior was recorded for the subsequent evaluation of a sedation score by an independent observer at time points 0, 1, 2, 4 and 12 hours (h) after paste administration (Figure 1). The occurrence and the depth of sedation was determined based on the observed position of the horse's head and the reaction to acoustic and visual stimuli (Table 1). Acoustic stimuli included a clicker as it is used for positive reinforcement training as well as the crackling noise of a plastic bag. As a visual stimulus, a pink cloth was attached to a stick and waved in front of the horse's face. Reactions to the stimuli were video recorded. Additionally, photographs were taken for subsequent assessment of the facial expressions. Expressions were rated based on the horse's orbital openings, position of ears, visibility of chewing muscles, position of lips and dilation of nostrils (Table 2).

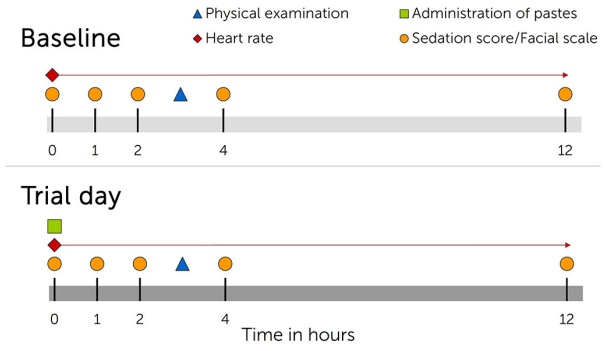

FIGURE 1
Timeline showing interventions for each cannabidiol (CBD) oral medication trial. **Upper panel**, day before trial start. **Lower panel**, trial day. Trials were repeated three times with single administration of escalating CBD doses (0.2 mg CBD/kg BW; 1 mg CBD/kg BW; 3 mg CBD/kg BW) and wash-out periods of minimum seven days in between trials.

Each horse's heart rate (HR) was continuously recorded throughout the trials using a Polar® H10 heart rate sensor (Polar® Electro Oy, Kempele, Finland). The sensor was attached to an electrode belt which spanned around the horse's chest. To enhance skin contact and signal transmission, the coat was trimmed and moisturized with water over the heart base between the 4th and 5th intercostal space where the electrodes were positioned. Each sensor was connected to a mobile device via Bluetooth to document the cardiac beat-to-beat (R-R) intervals with the Polar® Equine App (Version 1.2.1, Polar® Electro, Kempele, Finland).

Repeated physical examination was performed 2–4 h following paste administration, and blood samples were obtained for white blood cell (WBC) count.

Baseline values including recordings of equine behavior and heart rate were obtained in the same pattern as described on the day before each trial for comparative analysis (Figure 1). Trials were divided by wash-out periods of at least seven days.

2.4 Assessment of behavioral observations

Evaluation of the video recordings was based on a previously described sedation score (43). For assessment of the photographs, a facial expression scale was developed based on the facial sedation scale for horses (FaceSed) (44) and the Horse Grimace Scale (45). The described parameters were modified according to the reactions and expressions observed in the study animals (Tables 1, 2). Videos and photographs of each horse were randomly arranged and blinded assessment was performed by one person who was experienced in equine behavior studies but not actively involved in any of the trials. For each horse, stimulus and time point, the five parameters of the sedation score were summed up, resulting in scores ranging from 5 to 20 (Table 1). The scores of the three stimuli were then summed up to a total for each horse and time point, resulting in a total sedation score ranging from 15 to 60. For the facial expression scale, parameters were similarly added up to a possible total sum of 6–18 for each time point and each individual horse. A score of 10 was given when the eyes were open, the ears forward pointing, the chewing muscles moderately present, the lips loosely touching and the nostrils non-dilated (Table 2). High scores represent a deeper relaxation or sedation.

2.5 Assessment of heart rate and heart rate variability

Heart rate (HR) and heart rate variability (HRV) were analyzed using the software Kubios® HRV Standard (ver. 3.5, Kubios® Oy, Kuopio, Finland). Parameters included the mean HR in beats per minute (bpm), the root mean square of successive beat-to-beat differences (RMSSD in milliseconds, ms) and the standard deviation of normal-to-normal beat-to-beat intervals (SDNN, ms). Automatic beat correction was applied to remove artifacts (threshold: very low, 0.3 s). Each recording period was divided into sections of 15 min as previously described (46).

2.6 Statistical analysis

Data were recorded in Microsoft Excel® (Version 2304) and statistical analysis was performed with SPSS® Statistics 27 (IBM®, NY, USA). First, data was analyzed descriptively: The value for each total sedation score and the sedation scores of the three stimuli were displayed in bar charts (mean + standard deviation). For the inductive analysis, the difference between the total sedation score at baseline and during the trial was calculated for each horse and time point (ranging from −45 to +45). Similarly, the differences between score on baseline and trial day were calculated for the facial expression scale (ranging from −12 to +12). The effects of the dose levels on the differences between baseline and trial day of the total sedation score were analyzed using linear mixed models. Individual horses were assigned as subjects, dose levels as fixed effects (reference = control group; trial 1 = 0.2 mg CBD/kg; trial 2 = 1 mg CBD/kg; trial 3 = 3 mg CBD/kg) and time points as random effects (0 h; 1 h; 2 h; 4 h; 12 h). Residuals were visually inspected for normal distribution. The level of significance was $p < 0.05$. For the facial expression scale, the differences between baseline and trial day were calculated and tested for an effect of dose levels using a linear mixed model as described above.

For HR, RMSSD and SDNN parameters, the first eight 15-minute sections (total of two hours) post paste administration were selected for analysis as CBD blood concentrations reached a maximum here (42). To test for an effect of dose levels on the parameters, linear mixed models were calculated as described above.

To identify systematic differences between baseline and trial day values of HR, RMSSD and SDNN within the treatment group over time, linear mixed models for each outcome were calculated with trials (reference = baseline; trial 1 = 0.2 mg CBD/kg; trial 2 = 1 mg CBD/kg; trial 3 = 3 mg CBD/kg) as fixed effects. The following analysis was performed as described above with individual horses

TABLE 1 Sedation score developed for behavioral observations following single oral administration of cannabidiol (CBD) in three escalating doses (0.2 mg CBD/kg; 1 mg CBD/kg; 3 mg CBD/kg), based on the sedation score by Poller et al. (43).

Head position	
1	Lower lip at height of shoulder joint or higher
2	Lower lip between shoulder and olecranon
3	Lower lip between olecranon and carpal joint
4	Lower lip at carpal joint or lower
Reaction to stimulus: head movement	
1	Focus directed toward stimulus, jerky aversion
2	Focus directed toward stimulus, aversion, then refocusing on stimulus
3	Focus directed toward stimulus, slight aversion
4	Indifference/no reaction
Reaction to stimulus: ear movement	
1	Ears pointed, obvious flickering of ears, steady response to stimulus
2	Moderate flickering of one or both ears
3	Slight flickering of one or both ears
4	Indifference/no reaction
Reaction to stimulus: Chewing	
1	Chewing movement is interrupted and does not continue
2	Chewing movement is repeatedly interrupted and recontinued
3	Chewing movement is interrupted once and recontinued
4	Indifference/no interruption of chewing
Reaction to stimulus: body movement	
1	Moving back more than one step, turning away
2	Moving back one step, head jerking
3	Jerking/lifting/averting of head
4	Indifference/no reaction
Total sum for EACH stimulus: 5 - 20	
Total sum for ALL stimuli: 15 - 60	

A total sum was calculated for each stimulus (clicker, bag, cloth) and for all stimuli.

TABLE 2 Facial expression scale developed for behavioral observations following single oral administration of cannabidiol (CBD) paste in three escalating doses (0.2mg CBD/kg; 1mg CBD/kg; 3mg CBD/kg), based on the FaceSed (44) and Horse Grimace Scale (45).

Orbital opening	
2	Eyes completely open
3	Eyes partially open (> 50%)
4	Eyes almost/completely closed (< 50%)
Position of ears	
1	Pinned back
2	Forward pointed, position of attention
3	Asymmetrical; one ear hanging
4	Wide opening between ear tips
Chewing muscles	
1	Strained/obviously present
2	Moderately present
3	Not present
Lips	
1	Strained mouth
2	Loose touching of lips
3	Slight relaxation of one lip
4	Pronounced relaxation/hanging of one lip
Nostrils	
1	Dilated, outer ring clearly visible
2	Non-dilated nostrils
3	Small nostrils, relaxed outer ring
Total sum: 6 - 18	

TABLE 3 Mean ± standard deviation of white blood cell (WBC) count after single oral administration of a cannabidiol (CBD) containing paste in three trials.

Parameter (Ref)	First trial (0.2 mg CBD/kg)	Second trial (1 mg CBD/kg)	Third trial (3 mg CBD/kg)
Control group			
WBC count (5–10 10^9/L)	7.43 ± 0.98	6.88 ± 0.38	7.79 ± 1.28
Number of horses out of Ref (Value out of Ref)	$n = 0/3$	$n = 0/3$	$n = 1/6$ (10.31 10^9/L)
Treatment group			
WBC count (5–10 10^9/L)	10.49 ± 0.68	9.79 ± 1.33	7.97 ± 2.19
Number of horses out of Ref (Value out of Ref)	$n = 1/3$ (11.17 10^9/L)	$n = 1/3$ (11.63 10^9/L)	$n = 1/5$ (11.60 10^9/L)

The number of horses with serum levels outside of the reference range (Ref) are reported for each group.

as subjects, dose levels as fixed effects and time points as random effects.

3 Results

3.1 Animals

The horses' body weight was on average 488 ± 55 kg in the treatment group and 443 ± 56 kg in the control group. During the first two trials, no side effects such as gastrointestinal intolerances were observed following paste application and it was considered safe to proceed with trial three. During trial three, one mare developed signs of a jugular vein thrombophlebitis and was excluded, resulting in five remaining horses in the treatment group to complete trial three ($n = 5 + 6$). Over all trials, the WBC count remained close to reference range with only mild WBC elevation (maximum WBC in the treatment group = 11.63 10^9/L) (Table 3).

3.2 Behavioral observations

3.2.1 Sedation score

For all three trials, graphical illustration of the statistical data using bar charts did not identify a clear trend for higher or lower sedation scores between groups or dose levels (Figure 2, Supplementary Figures S1–S3). During trial 1, overall scores for baseline values ranged from 29.3 ± 1.3 to 40.3 ± 3.9 at all time points in the treatment group. Overall scores for trial day values ranged from 29.5 ± 5.5 to 45.3 ± 2.5 at all time points. In the control group, values ranged between 27.8 ± 5.3 to 34.5 ± 6.3 at baseline and between 23.2 ± 1.0 to 39.9 ± 10.8 on trial day. No trend was observed for values being generally higher or lower at certain time points in either group.

During trial 2, baseline values ranged from 32.0 ± 6.7 to 41.8 ± 8.3 and trial day values from 38.8 ± 10.0 to 44.3 ± 9.9 in the treatment group. All values were higher on trial day than at baseline as exemplified by graphical illustration. In the control group, baseline values were between 28.4 ± 6.2 to 36.8 ± 7.3 and trial day values between 28.8 ± 10.4 to 37.7 ± 10.2. Values were higher on trial day than the corresponding baseline values at time points 2, 4 and 12.

During trial 3, baseline values in the treatment group were between 31.1 ± 5.5 to 37.9 ± 12.2 and trial day values between 29.8 ± 10.8 to 39.2 ± 11.4. In the control group, baseline values ranged from 28.0 ± 6.6 to 41.7 ± 9.9 and trial day values from 31.3 ± 6.7 to 35.4 ± 4.1. No trend was observed for values being generally higher or lower at certain time points in either group.

Linear mixed models with escalating doses as fixed effects did not identify significant differences between the total sum of sedation scores in the treatment and control group [$P(F) = 0.527$]. Even during trial 2, the difference was not significant [$P(F) = 0.180$]. Similarly, the individual scores were not significantly influenced by escalating doses for stimulation with a clicker [$P(F) = 0.196$], crackling of a plastic bag [$P(F) = 0.442$] or waving with the pink cloth [$P(F) = 0.915$]. Estimates for random effects for the total sum were: $\beta = 25.9$ [95% confidence intervals (CI) = 6.7, 100.6; standard error (SE) = 17.9], for clicker: $\beta = 7.7$ (95% CI = 2.9, 20.4; $SE = 3.8$) and for plastic bag: $\beta = 1.3$ (95% CI = 0.0, 126.8; $SE = 3.0$). Random effects were not estimated for visual stimulation with a cloth. For the total sum, 21.7% of variability was accounted to differences between time points. For stimulation with a clicker and plastic bag, time points as random effects were attributed to 32.6 and 4.7% of variability, respectively.

3.2.2 Facial expression scale

Examples for scoring of the facial expressions are shown in Supplementary Table S1. Graphical illustration of sedation scores is shown in Figure 3.

During trial 1, overall scores for baseline values ranged from 10.0 ± 0.0 to 12.0 ± 2.2 at all time points in the treatment group. Overall scores for trial day values ranged from 9.7 ± 0.5 to 10.3 ± 0.5 at all time points. All values were equal or lower on trial day than at baseline. In the control group, baseline values ranged from 8.5 ± 1.5 to 10.7 ± 0.9 and from 10.0 ± 0.0 to 12.7 ± 2.1 on trial day. All values were equal or higher on trial day than at baseline. In this trial, the most notable differences between baseline and trial day were found at time point 1 (treatment group: 12.0 ± 2.2 to 10.3 ± 0.5) and time point 12 (control group: 10.7 ± 0.9 to 12.7 ± 2.1).

During trial 2, baseline values in the treatment group were between 9.8 ± 0.6 to 10.7 ± 0.6 and trial day values between 10.0 ± 0.0 to 10.7 ± 0.5. In the control group, baseline values ranged from 10.0 ± 0.0 to 10.7 ± 0.9 and trial day values from 10.0 ± 0.0 to 10.5 ± 0.7. No trend was observed for values being generally higher or lower at certain time points in either group.

During trial 3, baseline values in the treatment group ranged from 10.0 ± 0.8 to 10.7 ± 0.7 and trial day values from 10.0 ± 0.0 to 10.4 ± 0.5. In the control group, baseline values ranged from 10.0 ± 0.0 to 10.2 ± 0.9 and trial day values from 10.0 ± 0.0 to 10.4 ± 0.8. No trend was observed for values being generally higher or lower at certain time points in either group.

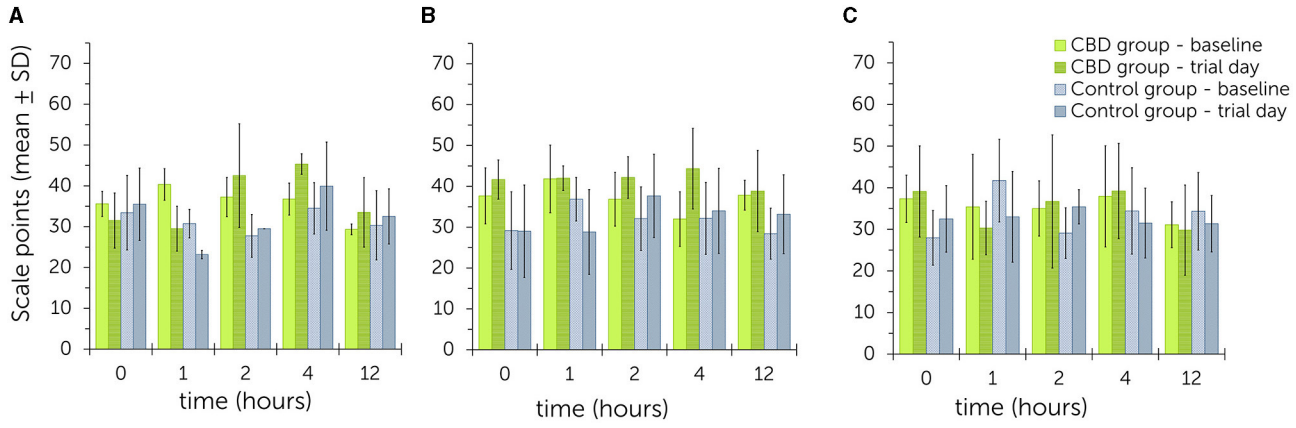

FIGURE 2
Summed up sedation scores after acoustic and visual stimulations (clicker, plastic bag, pink cloth) following single oral administration of cannabidiol (CBD) paste in escalating doses (**A**: 0.2 mg CBD/kg; **B**: 1 mg CBD/kg; **C**: 3 mg CBD/kg) - comparison between values obtained on baseline and trial day for the treatment and control group. Higher scale points relate to a higher level of sedation (Table 1). No significant differences were found between treatment and control group over all three trials.

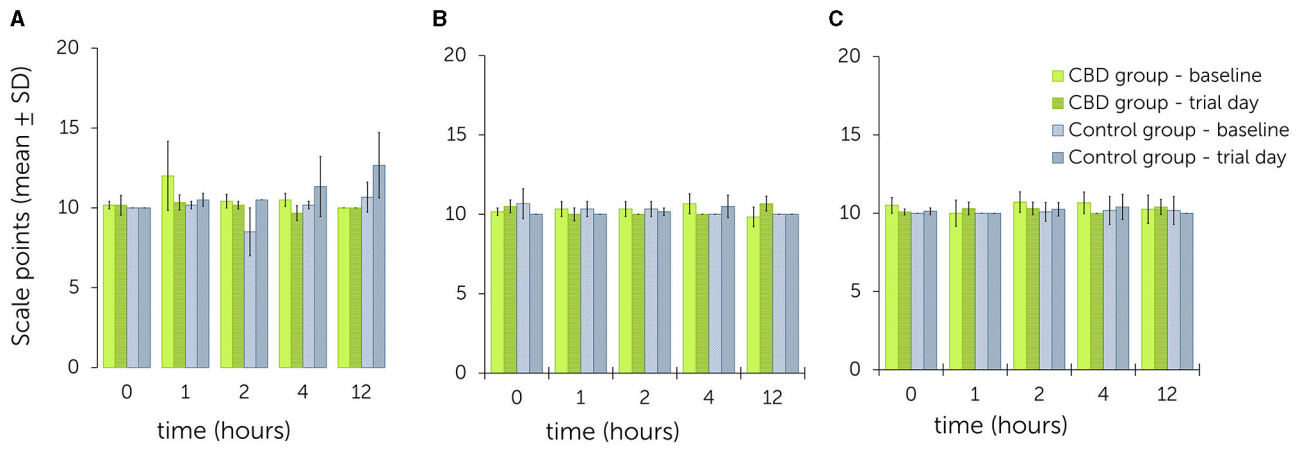

FIGURE 3
Facial expression scale following single oral administration of cannabidiol (CBD) paste in escalating doses (**A**: 0.2 mg CBD/kg; **B**: 1 mg CBD/kg; **C**: 3 mg CBD/kg) - comparison between values obtained on baseline and trial day for the treatment and control group. Higher scale points relate to a higher level of sedation (Table 2).

TABLE 4 **Fixed effects estimates for the comparison of differences (Δ) between score levels reached on a facial expression scale on baseline and trial days [single oral administration of cannabidiol (CBD) paste in three escalating doses (0.2 mg CBD/kg; 1 mg CBD/kg; 3 mg CBD/kg)].**

Parameter	Regression coefficient (β)	95% confidence intervals (CI)	Standard error (SE)	*p*-value
Δ Score levels (facial expression scale)				
Intercept	0.3	0.0, 0.7	0.2	0.077
Control group	Reference			
Trial 1 (0.2 mg CBD/kg)	−0.9	−1.6, −0.1	0.4	0.021
Trial 2 (1 mg CBD/kg)	−0.4	−1.1, 0.4	0.4	0.344
Trial 3 (3 mg CBD/kg)	−0.6	−1.2, 0.0	0.3	0.065

The linear mixed model did not identify a significant effect of escalating CBD doses on the facial expression scale when compared to the control group [$P(F) = 0.080$]. Considering the fixed effects estimates, a significant effect was evident between trial 1 and the control group ($p = 0.021$) (Table 4). The estimate for the random effects was $\beta = 0.1$ (95% CI = 0.0, 27.4; $SE = 0.2$) with 3.3% of variability attributed to differences between time points.

3.3 Heart rate and heart rate variability

3.3.1 Comparison between treatment and control group

Mean HR and HRV values are shown in Table 5. On trial days, the mean HR in the first 2 h post paste administration was between 42.1 ± 8.6 bpm to 45.4 ± 7.5 bpm in the treatment group, and between 41.3 ± 8.2 bpm to 44.4 ± 9.8 bpm in the control group.

RMSSD values ranged between 122.7 ± 48.8 ms and 152.9 ± 36.6 ms in the treatment group, and 137.1 ± 35.4 ms and 151.6 ± 29.3 ms in the control group. For SDNN, mean values were between 105.4 ± 22.8 ms and 163.1 ± 48.4 ms in the treatment group, and between 135.7 ± 64.4 ms and 156.8 ± 49.6 ms in the control group. Graphical representations of mean HR, RMSSD and SDNN are shown in Figures 4–6 (trial days) and Supplementary Figures S4–S6 (baseline).

Statistical analysis using linear mixed models found that doses as fixed effects had no significant impact on HR [$P(F) = 0.139$], RMSSD [$P(F) = 0.104$] and SDNN [$P(F) = 0.202$]. A significant difference could not be identified even between the highest CBD dose (3 mg CBD/kg) and the control group (HR: $p = 0.377$; RMSSD: $p = 0.189$; SDNN: $p = 0.734$) (Table 6).

For HR, the estimate for the random effects was $\beta = 31.5$ (95% CI = 15.1, 65.7; $SE = 11.8$). Differences between time sections are accounted for 44.1% of variability. The RMSSD estimate was $\beta = 607.0$ (95% CI = 262.0, 1406.3; $SE = 260.2$) and 33.2% of variability was attributed to time sections. For SDNN, β was 1107.0 (95% CI = 456.3, 2685.8; $SE = 500.6$). Time sections were associated with 33.7% of variability.

3.3.2 Comparison between baseline and trial day within the treatment group

Mean HR values showed no trend indicating a consistent increase or decrease from baseline to trial day in the treatment group (Table 5). Mean RMSSD and SDNN values showed a consistent increase from baseline to trial day during all trials, except

TABLE 5 Mean ± SD values for HR, RMSSD and SDNN values from the first 2 h after single oral cannabidiol (CBD) paste administration with corresponding baseline values. Due to technical issues, the trial 1 R-R-interval data are partly incomplete.

Parameter	Treatment group – baseline (mean ± SD)	Treatment group – trial day (mean ± SD)	Control group – baseline (mean ± SD)	Control group – trial day (mean ± SD)
HR (bpm)				
Trial 1 (0.2 mg CBD/kg)	30.2 ± 2.9	45.4 ± 7.5	no data	41.4 ± 4.6
Trial 2 (1 mg CBD/kg)	45.3 ± 7.0	43.3 ± 4.1	43.2 ± 7.2	41.3 ± 8.2
Trial 3 (3 mg CBD/kg)	42.6 ± 6.6	42.1 ± 8.6	39.0 ± 4.4	44.4 ± 9.8
RMSSD (ms)				
Trial 1 (0.2 mg CBD/kg)	127.7 ± 51.2	152.9 ± 36.6	no data	151.6 ± 29.3
Trial 2 (1 mg CBD/kg)	112.7 ± 33.8	123.6 ± 30.6	151.3 ± 39.4	137.1 ± 35.4
Trial 3 (3 mg CBD/kg)	113.8 ± 40.0	122.7 ± 48.8	151.0 ± 61.7	140.9 ± 48.2
SDNN (ms)				
Trial 1 (0.2 mg CBD/kg)	140.8 ± 44.6	163.1 ± 48.4	no data	156.8 ± 49.6
Trial 2 (1 mg CBD/kg)	110.1 ± 41.0	105.4 ± 22.8	154.4 ± 71.1	146.0 ± 49.7
Trial 3 (3 mg CBD/kg)	104.6 ± 44.7	131.0 ± 61.1	121.5 ± 38.5	135.7 ± 64.4

SD, standard deviation; HR, heart rate; RMSSD, root mean square of successive R-R interval differences; SDNN, standard deviation of normal-to-normal R-R intervals; bpm, beats per minute; ms, milliseconds.

for a decrease in SDNN values during trial 2 (110.1 ± 41.0 ms to 105.4 ± 22.8 ms).

Examination of the differences between baseline and trial day values identified no significant effect for HR [$P(F) = 0.136$] over all three trials but found significant effects for RMSSD [$P(F) = 0.016$] and SDNN [$P(F) < 0.001$]. Both significant findings can be attributed to trial 1 and trial 3 (Table 7). Estimates for random effects for HR were: $\beta = 13.1$ (95% CI = 5.0, 34.1; $SE = 6.4$), for RMSSD: $\beta = 768.5$ (95% CI = 399.6, 1478.2; $SE = 256.5$) and for SDNN: $\beta = 1052.6$ (95% CI = 537.88, 2060.1; $SE = 360.6$). For HR, RMSSD and SDNN values, differences between time sections are accounted for 22.5%, 40.6% and 39.6% of variability, respectively.

4 Discussion

Investigation of stress parameters in healthy horses, including behavioral observations and heart rate monitoring, following oral administration of a CBD containing paste in escalating doses did not identify consistently significant differences when compared to a control group.

CBD products are marketed for a variety of conditions in animals including improving general wellbeing and having a calming and stress-relieving effect (3–8). Sedation is a reported side effect associated with CBD application in humans and dogs (30–34, 47). To assess sedation in horses, multiple scoring systems have been proposed but are mainly aimed at testing sedatives such as detomidine or acepromazine (43, 48, 49). As levels of sedation in this study were not pronounced and scoring based on established scales did not produce satisfying results, a previously described sedation scale (43) was adjusted to the behavior exhibited by the horses in the current study (37). The dose levels tested in this study (0.2 mg CBD/kg, 1 mg CBD/kg, 3 mg CBD/kg) did not result in any significant difference in sedation scores after acoustic or visual stimulation compared to the control group. This is in agreement with a previous report where sedation levels were scored in horses following CBD administration (37). In this report, pellets containing 150 mg CBD (~ 0.29 mg CBD/kg) were fed over 56 days with no significant difference in sedation levels detected when compared to a control group. In humans, sedation was described as a side effect after daily oral intake of a total of 600 mg CBD over 6 weeks (47). Future studies may investigate whether higher dose administrations lead to more significant signs of sedation in horses.

Photographs were taken to assess the potential influence of CBD on equine facial expression. Existing scoring systems including FaceSed and Horse Grimace Scale (HGS) were modified to suit the purpose of the current report, as CBD administration did not produce sedation levels comparative to those depicted in the FaceSed scale (44, 45). Horses additionally displayed facial expressions described in the HGS, like strained mouth and chewing muscles. As the horses included in the current study did not undergo any painful procedures, similar expressions were interpreted as signs of stress. Expressions related to annoyance, such as pinned-back ears, were also exhibited. Only the modified scores of trial 1 (0.2 mg CBD/kg) were significantly different when compared between treatment and control group ($p = 0.021$). Score levels were higher at baseline than on trial day in the treatment group at time points 1, 2 and 4, whereas score levels in the control group were consistently lower at baseline than on trial day (Figure 3). As this result is the only significant event in this study part and comparisons with higher dose administrations did not produce significant results, its relevance should be interpreted with caution.

CBD reduces anxiety and stress by acting as a direct or indirect agonist on 5-HT_{1A}- and CB_1-receptors (10–14, 20). Stress

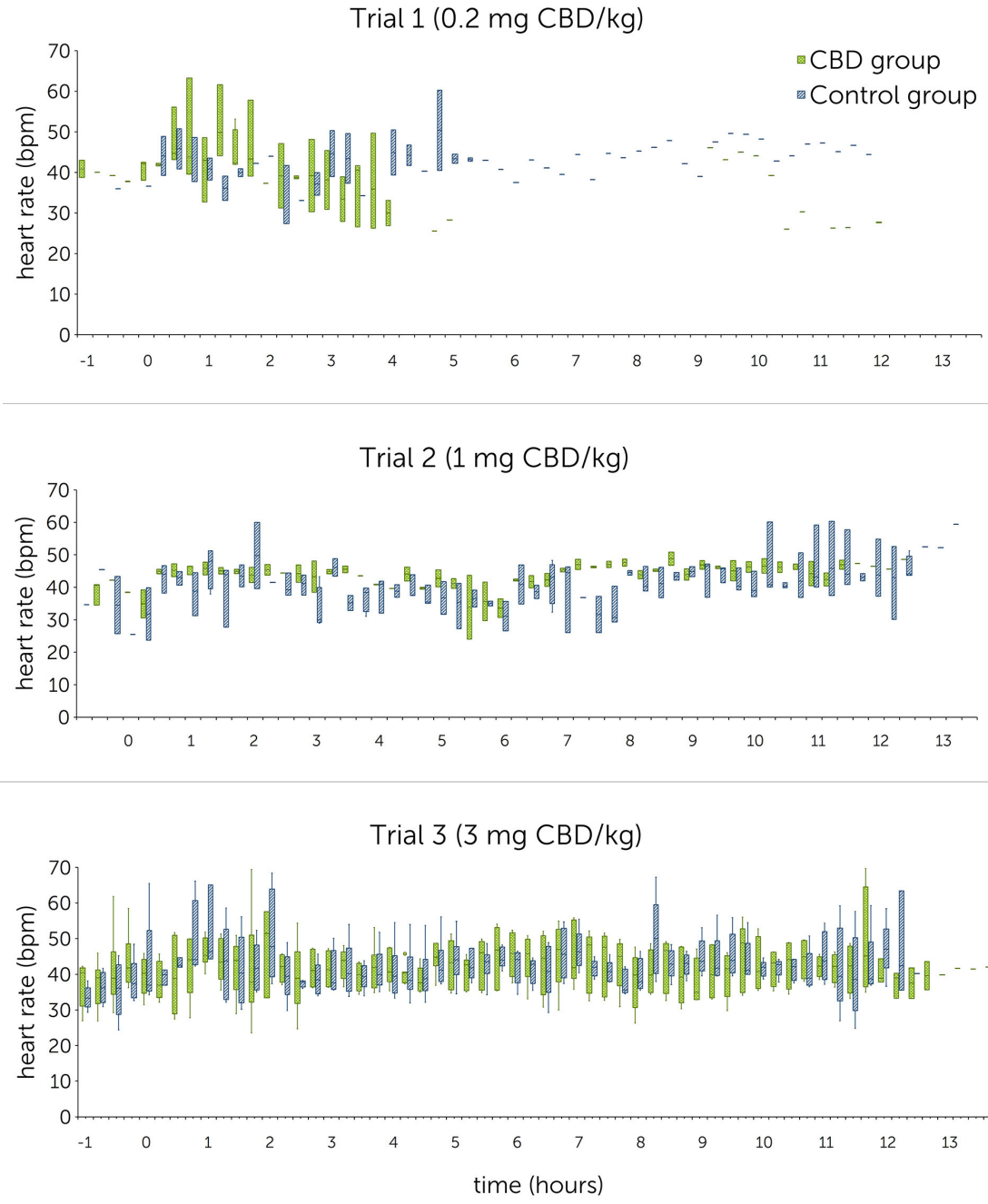

FIGURE 4
Heart rates [beats per minute (bpm)] following single oral administration of cannabidiol (CBD) in three escalating doses (0.2 mg CBD/kg BW; 1 mg CBD/kg BW; 3 mg CBD/kg BW) at time point 0, displayed in 15-min sections over 12 h. Due to technical issues, the trial 1 R-R-interval data are partly incomplete.

levels can be evaluated based on changes of heart rate and heart rate variability in horses (50–53). A comparatively lower HR and increased HRV values (RMSSD and SDNN) indicate an autonomic shift toward a parasympathetic dominance and therefore a reduction of stress (50, 52, 54). In rodents, one-time intraperitoneally injected CBD (10 mg/kg) has been shown to reduce the increase of HR and blood pressure in a stress inducing and fear conditioning setting, suggesting an anxiolytic effect similar to diazepam (24, 55). Another study identified a modest effect of oral CBD (total dose: 30 mg) on resting HR and HRV in humans (29). The relevance for physiological functions with the shown effect is however questionable and should be evaluated with caution as the study design did not include a control group (29). Other studies in horses and dogs showed no influence of CBD on HR or HRV so far: One study in horses found no significant difference in HR during a novel object test between a treatment group fed 100 mg pelleted CBD (~ 0.2 mg CBD/kg) and a control group (36). In dogs, a treatment and a placebo

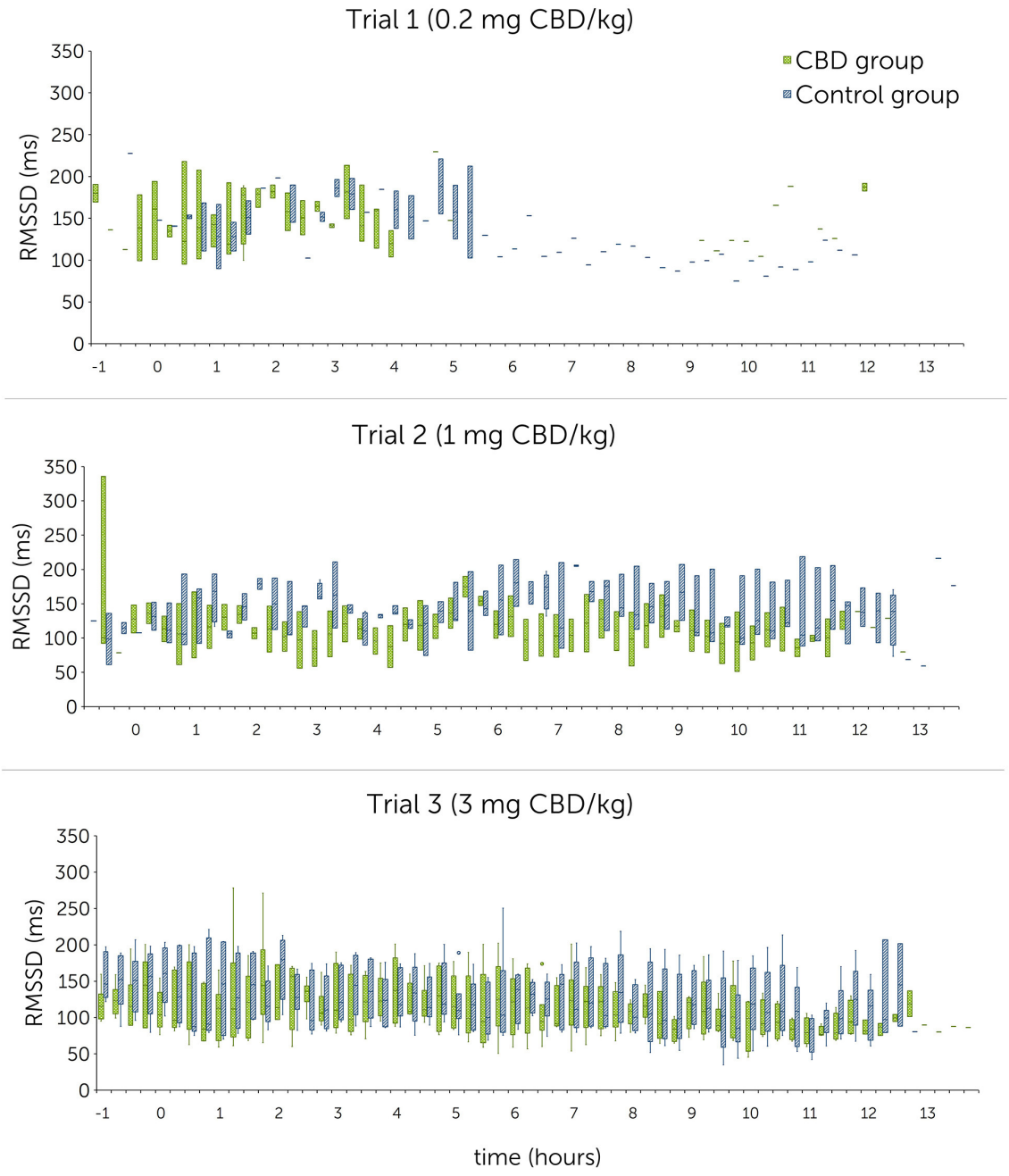

FIGURE 5
Root mean square of successive R-R interval differences (RMSSD) in milliseconds (ms) following single oral administration of cannabidiol (CBD) in three escalating doses (0.2 mg CBD/kg BW; 1 mg CBD/kg BW; 3 mg CBD/kg BW) at time point 0, displayed in 15-min sections over 12 h. Due to technical issues, the trial 1 R-R-interval data are partly incomplete.

group displayed similar HR and HRV values during a stress test. The dose tested here was 4 mg CBD/kg, administered orally every day over a period of 6 months (56). Similarly, dogs treated orally with 1.4 mg CBD/kg showed no significant changes in RMSSD and SDNN following a fear response test (57). To the best of the authors' knowledge, there are no studies investigating the effect of CBD on resting HR and HRV in healthy horses so far. Due to the short interval of stimulation, it was decided not to specifically analyze HR and HRV during sedation scoring including acoustic and visual stimuli in the current study. HR and HRV compared over the first 2 h after paste administration identified non-significant differences between the treatment and control group in all trials. Comparison within the treatment group showed a consistent increase of the RMSSD compared between all three baseline and trial day values with a significant effect identified for trial 1 (0.2 mg CBD/kg) (Table 7). For SDNN, significant increases were detected for trial 1 and trial 3 (3 mg CBD/kg) (Table 7). These results point toward a decreased sympathetic and an increased

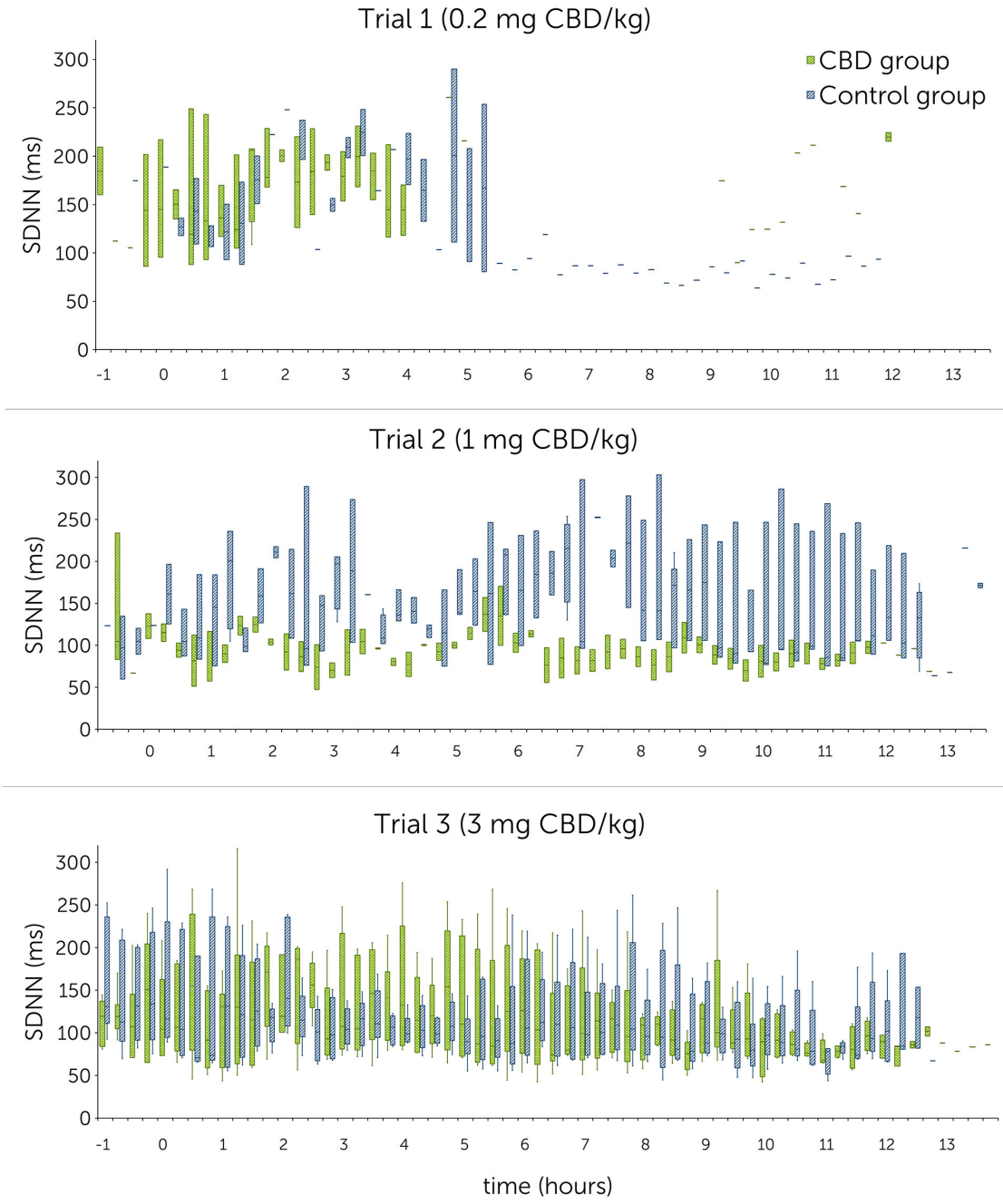

FIGURE 6
Normal-to-normal R-R intervals (SDNN) in milliseconds (ms) following single oral administration of cannabidiol (CBD) in three escalating doses (0.2 mg CBD/kg BW; 1 mg CBD/kg BW; 3 mg CBD/kg BW) at time point 0, displayed in 15-min sections over 12 h. Due to technical issues, the trial 1 R-R-interval data are partly incomplete.

parasympathetic tonus following CBD administration and support the hypothesized relaxing effect of CBD. However, as the 95% confidence intervals are large, results should still be interpreted with caution.

Cannabis and cannabinoids are FEI declared prohibited substances, with CBD and cannabidiolic acid (CBDA) listed as controlled medication, due to their possible psychotropic and analgesic properties (41). In this study, an influence of CBD in escalating dose levels on equine behavioral parameters could not be confirmed, but it cannot be excluded that higher doses or administration over longer time periods would influence a horse's behavior. As horses in the current study were healthy and displayed a calm behavior throughout, the effect of CBD on stressed or anxious horses would be an additional point of interest.

Limitations of this study include the small sample size and the assessment of single administrations of one CBD containing product only. As horses were closely monitored and sedation levels were scored multiple times per day, a habituation effect

TABLE 6 Fixed effects estimates for comparison between treatment and control group of HR, RMSSD and SDNN values from the first 2 h following single oral administration of cannabidiol (CBD) paste in three escalating doses (0.2mg CBD/kg; 1mg CBD/kg; 3mg CBD/kg).

Parameter	Regression coefficient (β)	95% confidence intervals (CI)	Standard error (SE)	*p*-value
HR (bpm)				
Intercept	43.7	41.4, 46.0	1.1	<0.001
Control group	Reference			
Trial 1 (0.2 mg CBD/kg)	2.6	−1.4, 6.5	2.0	0.196
Trial 2 (1 mg CBD/kg)	0.5	−4.1, 5.1	2.3	0.826
Trial 3 (3 mg CBD/kg)	−1.5	−4.8, 1.8	1.7	0.377
RMSSD (ms)				
Intercept	134.6	123.4, 145.8	5.6	<0.001
Control group	Reference			
Trial 1 (0.2 mg CBD/kg)	11.6	−8.3, 31.6	10.1	0.251
Trial 2 (1 mg CBD/kg)	2.9	−20.5, 26.2	11.8	0.809
Trial 3 (3 mg CBD/kg)	−11.0	−27.5, 5.5	8.3	0.189
SDNN (ms)				
Intercept	135.8	120.7, 150.8	7.5	<0.001
Control group	Reference			
Trial 1 (0.2 mg CBD/kg)	18.1	−8.7, 44.9	13.5	0.184
Trial 2 (1 mg CBD/kg)	−12.1	−43.3, 19.1	15.8	0.445
Trial 3 (3 mg CBD/kg)	−3.8	−26.0, 18.4	11.2	0.734

HR, heart rate; RMSSD, root mean square of successive R-R interval differences; SDNN, standard deviation of normal-to-normal R-R intervals; bpm, beats per minute; ms, milliseconds.

TABLE 7 Fixed effects estimates for comparison within the treatment group of HR, RMSSD and SDNN values from the first 2 h between baseline and following single oral administration of cannabidiol (CBD) paste in three escalating doses (0.2mg CBD/kg; 1mg CBD/kg; 3mg CBD/kg).

Parameter	Regression coefficient (β)	95% confidence intervals (CI)	Standard error (SE)	*p*-value
HR (bpm)				
Intercept	42.5	40.6, 44.4	1.0	<0.001
Baseline values	Reference			
Trial 1 (0.2 mg CBD/kg)	3.4	0.3, 6.6	1.6	0.034
Trial 2 (1 mg CBD/kg)	0.9	−2.7, 4.5	1.8	0.627
Trial 3 (3 mg CBD/kg)	−0.4	−2.9, 2.1	1.3	0.766
RMSSD (ms)				
Intercept	118.4	107.2, 120.5	5.6	<0.001
Baseline values	Reference			
Trial 1 (0.2 mg CBD/kg)	25.0	8.8, 41.1	8.2	0.003
Trial 2 (1 mg CBD/kg)	16.6	−1.8, 35.1	9.3	0.077
Trial 3 (3 mg CBD/kg)	7.7	−5.1, 20.5	6.5	0.233
SDNN (ms)				
Intercept	112.4	99.2, 125.6	6.6	<0.001
Baseline values	Reference			
Trial 1 (0.2 mg CBD/kg)	40.1	20.8, 59.4	9.8	<0.001
Trial 2 (1 mg CBD/kg)	3.0	−19.0, 25.1	11.1	0.785
Trial 3 (3 mg CBD/kg)	21.3	6.0, 36.6	7.7	0.007

HR, heart rate; RMSSD, root mean square of successive R-R interval differences; SDNN, standard deviation of normal-to-normal R-R intervals; bpm, beats per minute; ms, milliseconds.

cannot be excluded. Signs of stress or annoyance as evident on the photographs may partially result from repeated testing. However, as treatment and control groups underwent the exact same protocol, the effect of repeated testing was deemed negligible as it was concluded that it would have occurred similarly in both groups.

5 Conclusions

The analysis of stress parameters did not identify consistently significant effects of orally administered CBD on levels of sedation, the resting heart rate or heart rate variability in horses. Escalating doses (0.2 mg CBD/kg to 3 mg CBD/kg) did not result in a significant reduction of the heart rate, or increased sedation or relaxation. Oral administration of CBD containing paste proved to be well-tolerated and did not cause any side effects. Further research is required to determine specific indications for the use of CBD products in horses.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by the authority for licensing and notification procedures for animal experiments (LAVG) in Brandenburg, Germany (AZ: 2347–12–2021). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

FE: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing—original draft. AE: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing—review & editing. KCJ: Formal analysis, Methodology, Software, Validation, Writing—review & editing. NB: Conceptualization, Data curation, Methodology, Project administration, Writing—review & editing. HP: Data curation, Formal analysis, Investigation, Methodology, Writing—review & editing. WB: Conceptualization, Methodology, Project administration, Supervision, Writing—review & editing. CL: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing—review & editing. MW: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing—review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. This study received funding from Herosan healthcare GmbH.

The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication. All authors declare to have full control over the data and no other competing interests.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1305868/full#supplementary-material

References

1. Corsetti S, Borruso S, Malandrucco L, Spallucci V, Maragliano L, Perino R, et al. Cannabis sativa L. may reduce aggressive behaviour towards humans in shelter dogs. *Sci Rep.* (2021) 11:2773. doi: 10.1038/s41598-021-82439-2

2. Casajuana Köguel C, López-Pelayo H, Balcells-Olivero MM, Colom J, Gual A. Constituyentes psicoactivos del cannabis y sus implicaciones clínicas: una revisión sistemática. *Adicciones.* (2018) 30:140–51. doi: 10.20882/adicciones.858

3. Potschka H, Bhatti SF, Tipold A, McGrath S. Cannabidiol in canine epilepsy. *Vet J.* (2022) 12:105913. doi: 10.1016/j.tvjl.2022.105913

4. Corsato Alvarenga I, Panickar KS, Hess H, McGrath S. Scientific validation of cannabidiol for management of dog and cat diseases. *Annu Rev Anim Biosci.* (2023) 11:227–46. doi: 10.1146/annurev-animal-081122-070236

5. Miranda-Cortés A, Mota-Rojas D, Crosignani-Outeda N, Casas-Alvarado A, Martínez-Burnes J, Olmos-Hernández A, et al. The role of cannabinoids in pain modulation in companion animals. *Front Vet Sci.* (2022) 9:1050884. doi: 10.3389/fvets.2022.1050884

6. Mogi C, Yoshida M, Kawano K, Fukuyama T, Arai T. Effects of cannabidiol without delta-9-tetrahydrocannabinol on canine atopic dermatitis: a retrospective assessment of 8 cases. *Can Vet J.* (2022) 63:423–6.

7. McGrath S, Bartner LR, Rao S, Packer RA, Gustafson DL. Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. *J Am Vet Med Assoc.* (2019) 254:1301–8. doi: 10.2460/javma.254.11.1301

8. Gugliandolo E, Licata P, Peritore AF, Siracusa R, D'Amico R, Cordaro M, et al. Effect of cannabidiol (CBD) on canine inflammatory response: an ex vivo study on lps stimulated whole blood. *Vet Sci.* (2021) 8:185. doi: 10.3390/vetsci8090185

9. Moltke J, Hindocha C. Reasons for cannabidiol use: a cross-sectional study of CBD users, focusing on self-perceived stress, anxiety, and sleep problems. *J Cannabis Res.* (2021) 3:5. doi: 10.1186/s42238-021-00061-5

10. Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol Sci.* (2009) 30:515–27. doi: 10.1016/j.tips.2009.07.006

11. Campos AC, Moreira FA, Gomes FV, Del Bel EA, Guimarães FS. Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. *Philos Trans R Soc Lond B Biol Sci.* (2012) 367:3364–78. doi: 10.1098/rstb.2011.0389

12. Blessing EM, Steenkamp MM, Manzanares J, Marmar CR. Cannabidiol as a potential treatment for anxiety disorders. *Neurotherapeutics.* (2015) 12:825–36. doi: 10.1007/s13311-015-0387-1

13. Fogaça MV, Campos AC, Coelho LD, Duman RS, Guimarães FS. The anxiolytic effects of cannabidiol in chronically stressed mice are mediated by the endocannabinoid system: Role of neurogenesis and dendritic remodeling. *Neuropharmacology.* (2018) 135:22–33. doi: 10.1016/j.neuropharm.2018.03.001

14. Fogaça MV, Campos AC, Guimarães FS. "Cannabidiol and 5-HT1A receptors," in *Neuropathology of Drug Addictions and Substance Misuse*, ed V. R. Preedy (Amsterdam: Elsevier) (2016), 749–59.

15. Leweke FM, Piomelli D, Pahlisch F, Muhl D, Gerth CW, Hoyer C, et al. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Transl Psychiatry.* (2012) 2:e94. doi: 10.1038/tp.2012.15

16. Watanabe K, Kayano Y, Matsunaga T, Yamamoto I, Yoshimura H. Inhibition of anandamide amidase activity in mouse brain microsomes by cannabinoids. *Biol Pharm Bull.* (1996) 19:1109–11. doi: 10.1248/bpb.19.1109

17. Di Marzo V, Bisogno T, Petrocellis L de Anandamide: some like it hot. *Trends Pharmacol Sci.* (2001) 22:346–9. doi: 10.1016/S0165-6147(00)01712-0

18. Riebe CJ, Pamplona FA, Kamprath K, Wotjak CT. Fear relief-toward a new conceptual frame work and what endocannabinoids gotta do with it. *Neuroscience.* (2012) 204:159–85. doi: 10.1016/j.neuroscience.2011.11.057

19. McLaughlin RJ, Hill MN, Gorzalka BB, A. critical role for prefrontocortical endocannabinoid signaling in the regulation of stress and emotional behavior. *Neurosci Biobehav Rev.* (2014) 42:116–31. doi: 10.1016/j.neubiorev.2014.02.006

20. McPartland JM, Duncan M, Di Marzo V, Pertwee RG. Are cannabidiol and Δ(9) -tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. *Br J Pharmacol.* (2015) 172:737–53. doi: 10.1111/bph.12944

21. Howlett AC, Scott DK, Wilken GH. Regulation of adenylate cyclase by cannabinoid drugs. Insights based on thermodynamic studies. *Biochem Pharmacol.* (1989) 38:3297–304. doi: 10.1016/0006-2952(89)90628-X

22. Granjeiro EM, Gomes FV, Guimarães FS, Corrêa FM, Resstel LB. Effects of intracisternal administration of cannabidiol on the cardiovascular and behavioral responses to acute restraint stress. *Pharmacol Biochem Behav.* (2011) 99:743–8. doi: 10.1016/j.pbb.2011.06.027

23. Gomes FV, Resstel LB, Guimarães FS. The anxiolytic-like effects of cannabidiol injected into the bed nucleus of the stria terminalis are mediated by 5-HT1A receptors. *Psychopharmacology.* (2011) 213:465–73. doi: 10.1007/s00213-010-2036-z

24. Resstel LB, Tavares RF, Lisboa SF, Joca SR, Corrêa FM, Guimarães FS. 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. *Br J Pharmacol.* (2009) 156:181–8. doi: 10.1111/j.1476-5381.2008.00046.x

25. Crippa JA, Derenusson GN, Ferrari TB, Wichert-Ana L, Duran FL, Martin-Santos R, et al. Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *J Psychopharmacol.* (2011) 25:121–30. doi: 10.1177/0269881110379283

26. Shallcross J, Hámor P, Bechard AR, Romano M, Knackstedt L, Schwendt M. The divergent effects of CDPPB and cannabidiol on fear extinction and anxiety in a predator scent stress model of PTSD in rats. *Front Behav Neurosci.* (2019) 13:91. doi: 10.3389/fnbeh.2019.00091

27. Zieba J, Sinclair D, Sebree T, Bonn-Miller M, Gutterman D, Siegel S, et al. Cannabidiol (CBD) reduces anxiety-related behavior in mice via an FMRP-independent mechanism. *Pharmacol Biochem Behav.* (2019) 181:93–100. doi: 10.1016/j.pbb.2019.05.002

28. Fusar-Poli P, Crippa JA, Bhattacharyya S, Borgwardt SJ, Allen P, Martin-Santos R, et al. Distinct effects of Δ9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Arch Gen Psychiatry.* (2009) 66:95–105. doi: 10.1001/archgenpsychiatry.2008.519

29. Williams NN, Ewell TR, Abbotts KS, Harms KJ, Woelfel KA, Dooley GP, et al. Comparison of five oral cannabidiol preparations in adult humans: pharmacokinetics, body composition, and heart rate variability. *Pharmaceuticals.* (2021) 14:35. doi: 10.3390/ph14010035

30. Dos Santos RG, Guimarães FS, Crippa JA, Hallak JE, Rossi GN, Rocha JM, et al. Serious adverse effects of cannabidiol (CBD): a review of randomized controlled trials. *Expert Opin Drug Metab Toxicol.* (2020) 16:517–26. doi: 10.1080/17425255.2020.1754793

31. Dos Santos RG, Hallak JE, Crippa JA. Neuropharmacological effects of the main phytocannabinoids: a narrative review. *Adv Exp Med Biol.* (2021) 1264:29–45. doi: 10.1007/978-3-030-57369-0_3

32. Kogan LR, Hellyer PW, Robinson NG. Consumers' perceptions of hemp products for animals. *J Am Holistic Vet Med Assoc.* (2016) 42:40–8.

33. Kogan L, Schoenfeld-Tacher R, Hellyer P, Rishniw M. US veterinarians' knowledge, experience, and perception regarding the use of cannabidiol for canine medical conditions. *Front Vet Sci.* (2018) 5:338. doi: 10.3389/fvets.2018.00338

34. Kogan LR, Hellyer PW, Silcox S, Schoenfeld-Tacher R. Canadian dog owners' use and perceptions of cannabis products. *Can Vet J.* (2019) 60:749–55.

35. Morris EM, Kitts-Morgan SE, Spangler DM, Gebert J, Vanzant ES, McLeod KR, et al. Feeding cannabidiol (CBD)-containing treats did not affect canine daily voluntary activity. *Front Vet Sci.* (2021) 8:645667. doi: 10.3389/fvets.2021.645667

36. Draeger A, Thomas E, Jones K, Godwin P, Davis A, Porr S. 81 Cannabidiol in the horse: Effects on movement and reactivity. *J Equine Vet Sci.* (2021) 100:103544. doi: 10.1016/j.jevs.2021.103544

37. St Blanc MP, Chapman AM, Keowen ML, Garza F, Liu C-C, Gray L, et al. Effects of a supplement containing cannabidiol (CBD) on sedation and ataxia scores and health. *J Equine Vet Sci.* (2022) 117:104085. doi: 10.1016/j.jevs.2022.104085

38. Ryan D, McKemie DS, Kass PH, Puschner B, Knych HK. Pharmacokinetics and effects on arachidonic acid metabolism of low doses of cannabidiol following oral administration to horses. *Drug Test Anal.* (2021) 13:1305–17. doi: 10.1002/dta.3028

39. Cunha RZ, Felisardo LL, Salamanca G, Marchioni GG, Neto OI, Chiocchetti R. The use of cannabidiol as a novel treatment for oral stereotypic behaviour (crib-biting) in a horse. *Vet Anim Sci.* (2023) 19:100289. doi: 10.1016/j.vas.2023.100289

40. Ellis KL, Contino EK. Treatment using cannabidiol in a horse with mechanical allodynia. *Equine Vet Educ.* (2021) 33:168. doi: 10.1111/eve.13168

41. Fédération Equestre Internationale. *FEI Clean Sport - Prohibited Substances Database*. (2022). Available online at: https://inside.fei.org/content/anti-doping-rules (accessed June 23, 2022).

42. Eichler F, Pozniak B, Machnik M, Schenk I, Wingender A, Baudisch N, et al. Pharmacokinetic modelling of orally administered cannabidiol and implications for medication control in horses. *Front Vet Sci.* (2023) 10:1234551. doi: 10.3389/fvets.2023.1234551

43. Poller C, Hopster K, Rohn K, Kästner SB. Nociceptive thermal threshold testing in horses - effect of neuroleptic sedation and neuroleptanalgesia at different stimulation sites. *BMC Vet Res.* (2013) 9:135. doi: 10.1186/1746-6148-9-135

44. Oliveira AR de, Gozalo-Marcilla M, Ringer SK, Schauvliege S, Fonseca MW, Esteves Trindade PH, et al. Development and validation of the facial scale (FaceSed) to evaluate sedation in

horses. *PLoS ONE.* (2021) 16:e0251909. doi: 10.1371/journal.pone.0251909

45. Dalla Costa E, Minero M, Lebelt D, Stucke D, Canali E, Leach MC. Development of the Horse Grimace Scale (HGS) as a pain assessment tool in horses undergoing routine castration. *PLoS ONE.* (2014) 9:e92281. doi: 10.1371/journal.pone.0092281

46. Younes M, Robert C, Barrey E, Cottin F. Effects of age, exercise duration, and test conditions on heart rate variability in young endurance horses. *Front Physiol.* (2016) 7:155. doi: 10.3389/fphys.2016.00155

47. Boggs DL, Surti T, Gupta A, Gupta S, Niciu M, Pittman B, et al. The effects of cannabidiol (CBD) on cognition and symptoms in outpatients with chronic schizophrenia a randomized placebo controlled trial. *Psychopharmacology.* (2018) 235:1923–32. doi: 10.1007/s00213-018-4885-9

48. Oliveira AR de, Gozalo-Marcilla M, Ringer SK, Schauvliege S, Fonseca MW, Trindade PH, et al. development, validation, and reliability of a sedation scale in horses (EquiSed). *Front Vet Sci.* (2021) 8:611729. doi: 10.3389/fvets.2021.611729

49. Schauvliege S, Cuypers C, Michielsen A, Gasthuys F, Gozalo-Marcilla M. How to score sedation and adjust the administration rate of sedatives in horses: a literature review and introduction of the Ghent Sedation Algorithm. *Vet Anaesth Analg.* (2019) 46:4–13. doi: 10.1016/j.vaa.2018.08.005

50. Lewinski M. von, Biau S, Erber R, Ille N, Aurich J, Faure J-M, et al. Cortisol release, heart rate and heart rate variability in the horse and its rider: different responses to training and performance. *Vet J.* (2013) 197:229–32. doi: 10.1016/j.tvjl.2012.12.025

51. König von Borstel U, Euent S, Graf P, König S, Gauly M. Equine behaviour and heart rate in temperament tests with or without rider or handler. *Physiol Behav*. (2011) 104:454–63. doi: 10.1016/j.physbeh.2011.05.010

52. Visser EK, van Reenena CG, van der Werf JTN, Schilder MBH, Knaap JH, Barneveld A, et al. Heart rate and heart rate variability during a novel object test and a handling test in young horses. *Physiol Behav.* (2002) 76:289–96. doi: 10.1016/S0031-9384(02)00698-4

53. Visser EK, van Reenen CG, Rundgren M, Zetterqvist M, Morgan K, Blokhuis HJ. Responses of horses in behavioural tests correlate with temperament assessed by riders. *Equine Vet J.* (2003) 35:176–83. doi: 10.2746/0425164037761 14108

54. Lenoir A, Trachsel DS, Younes M, Barrey E, Robert C. Agreement between electrocardiogram and heart rate meter is low for the measurement of heart rate variability during exercise in young endurance horses. *Front Vet Sci.* (2017) 4:170. doi: 10.3389/fvets.2017.00170

55. Resstel LB, Joca SR, Moreira FA, Corrêa FM, Guimarães FS. Effects of cannabidiol and diazepam on behavioral and cardiovascular responses induced by contextual conditioned fear in rats. *Behav Brain Res.* (2006) 172:294–8. doi: 10.1016/j.bbr.2006.05.016

56. Hunt AB, Flint HE, Logan DW, King T, A. single dose of cannabidiol (CBD) positively influences measures of stress in dogs during separation and car travel. *Front Vet Sci.* (2023) 10:1112604. doi: 10.3389/fvets.2023.1112604

57. Morris EM, Kitts-Morgan SE, Spangler DM, McLeod KR, Costa JH, Harmon DL. The impact of feeding cannabidiol (CBD) containing treats on canine response to a noise-induced fear response test. *Front Vet Sci.* (2020) 7:569565. doi: 10.3389/fvets.2020.569565

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Behavioral observations, heart rate and cortisol monitoring in horses following multiple oral administrations of a cannabidiol containing paste (part 2/2)

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As a remedy against stress and anxiety, cannabidiol (CBD) products are of increasing interest in veterinary medicine. Limited data is available describing the actual effectiveness of CBD in horses. The aim of this study (part 2 of 2) was to analyze stress parameters via behavioral observation, heart rate monitoring and assessment of blood and saliva cortisol levels in healthy horses treated repeatedly with a CBD containing paste. Twelve horses were randomly assigned to a treatment or a control group. Two pastes were orally administered in a double-blinded study design, one paste containing CBD and one paste without active ingredient. Both pastes were administered twice daily over 15 days (dose: 3 mg CBD/kg). Behavioral observations were conducted daily using a sedation score and a rating of facial expressions, based on the previously described facial sedation scale for horses (FaceSed) and the Horse Grimace Scale. Blood and saliva samples were obtained regularly to determine cortisol levels throughout the study. Cortisol levels were analyzed by means of liquid chromatography/tandem mass spectrometry (LC/MS/MS). Behavioral observations and cortisol levels were compared between groups. Prior to paste administration, a novel object test was performed and the horses' reaction to loading on a trailer was recorded. Both tests were repeated after 13 days of paste application. Movement patterns such as different gaits during the novel object test were evaluated and an ethogram was designed to assess exhibited behavioral traits. Cardiac beat-to-beat (R-R) intervals were recorded throughout and evaluated using heart rate (HR) and heart rate variability (HRV) parameters. Blood and saliva samples for cortisol analysis were taken before and after the tests. Daily behavioral observations and cortisol levels did not differ between the treatment and the control group. Similarly, analysis of movement patterns, HR, HRV and cortisol levels during the novel object test and trailer test did not identify significant differences between the groups. Regularly administered oral CBD (3 mg/kg BID over 15 days) had no statistically significant effect on behavioral observations, cortisol levels, HR and HRV in horses. Further research is required to establish adequate doses and indications for the use of CBD in horses.

KEYWORDS

behavior, CBD, equine, FaceSed, heart rate variability, Horse Grimace Scale, novel object test, sedation score

1 Introduction

Supplements containing cannabis compounds have been promoted as remedies for the treatment of numerous conditions such as anxiety or osteoarthritis in human and animal patients (1–5). Their popularity has increased in recent years but few scientific studies have investigated the actual effectiveness in animals and specifically horses (6–8). The predominant cannabis compounds include the phytocannabinoids cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC), which is known for its psychoactive properties (9–11). CBD is currently under investigation for its proposed relaxing and anxiolytic effects in humans, rodents and dogs (3, 12–23). CBD interacts directly with the serotonin$_{1A}$ (5-HT$_{1A}$) receptor (1, 24–27) and indirectly with the cannabinoid type 1 (CB$_1$) receptor from the endocannabinoid (eCB) system by inhibiting the deactivation of endogenous cannabinoids (28–30). 5-HT$_{1A}$ receptors and the eCB system regulate stress responses and can exhibit an anxiolytic effect when activated (27, 31–33). The CB$_1$ receptor and its significance as a therapeutic target are currently under investigation (34, 35).

The pharmacological activity of the acidic forms of CBD and THC, cannabidiolic acid (CBDA) and Δ^9-tetrahydrocannabinolic acid (THCA), has been scarcely reported so far (9). CBDA and THCA have been shown to interact with the eCB system with their functionality still under study (36–38). In addition to phytocannabinoids, cannabis plants contain terpenoid and flavonoid contents which are described to exhibit multiple effects, including anti-inflammation or sedation (39).

In the European Union (EU), companies declare their cannabis products for horses as "nutritional supplements" as opposed to medicinal products and are therefore not under regulation by the European Medicines Agency (EMA). To date, there is no authorized cannabis veterinary medicinal product in the EU or North America available (40). The Fédération Equestre Internationale (FEI) has banned all cannabis products due to the exhibition of potentially psychotropic effects (41). Since 2022, CBD is classified as a controlled medication (41).

In horses, options for the assessment of stress-responses include behavioral observations such as sedation scores or facial expression scales (42–46) as well as the analysis of physiological parameters like cortisol levels (47–51), heart rate and heart rate variability (48, 52–54). A common and frequently documented test to evaluate stress or fear in animals is the novel object test (6, 54–57). One report has assessed the effect of CBD in horses using a novel object test with evaluation of reactivity and heart rate after daily feeding of CBD pellets (dose: ~0.2 mg CBD/kg SID) for 6 weeks (6). When compared to a control group, reactivity scores were lower, but no significant difference in heart rate was identified (6).

Transportation and loading on trailers cause stress responses in horses which are reflected in increased heart rates and cortisol levels (58–60). Different training methods or even sedatives can be applied to effectively reduce these stress responses (58–61). No report has documented a potential effect of CBD on equine stress levels during loading on a trailer so far.

The aim of this study was to validate equine behavior and stress reactions including the response to a novel object test and a trailer test via heart rate and cortisol level monitoring in healthy horses following repeated oral administration of CBD containing paste (3 mg CBD/kg BID) for 15 days. The authors hypothesized that regular CBD administrations would have a calming effect in horses.

2 Materials and methods

2.1 Animals and study products

Twelve horses (seven mares and five stallions, Haflinger x Warmblood cross) were enrolled in the study. Horses were randomly assigned to a treatment or a control group ($n = 6 + 6$). Horses' age was 3–16 years (median: 11 years) with an average body weight of 488 ± 55 kg in the treatment group. In the control group, the age was 10–26 years (median: 10.5 years) and the body weight 443 ± 56 kg. This study was designed as a prospective, randomized clinical trial. Study products were two pastes for oral administration, one containing 55% full spectrum CBD plant extract, medium-chain triglyceride (MCT) coconut oil, naturally occurring phytocannabinoids, terpenes, flavonoids and beeswax with a THC content of <0.2% (TAMACAN XL 55%®, Herosan healthcare GmbH, Austria). The second paste lacked an active ingredient and contained MCT coconut oil and beeswax [see part 1/2 for further detail (62)]. Pastes were labeled as "A" or "B" to conceal the formulation. The study was approved by the competent authority for licensing and notification procedures for animal experiments (LAVG) in Brandenburg, Germany (AZ: 2347-12-2021). Animals included had to pass a general physical examination by a licensed veterinarian and had a blood sample analysis including assessment of a complete blood count (CBC), kidney and liver biomarkers prior to study start. Exclusion criteria included irregularities during examination of the circulatory, respiratory and gastrointestinal systems, and signs of pain or inflammation such as fever and high white blood cell counts.

2.2 Multiple dose study

The multiple dose study started following a wash-out period of 25 days after the dose escalation study (62) to ensure a complete elimination of all cannabinoids following previous CBD applications. The day before study start, horses were physically examined, and a jugular vein catheter was aseptically placed. The jugular vein thrombophlebitis of one mare from the previous study part had resolved by this time (62). Serum and urine samples were tested for residual cannabinoid contents from the previous study part.

Throughout the study, physical examination was repeated daily in every horse. Pastes (dose: 3 mg CBD/kg) were administered before feeding every 12 h (6:30 a.m. and 6:30 p.m.) for 15 days. Equine behavioral observations were video recorded daily between 7:30 am and 8:30 am using two acoustic stimuli (clicker and crackling of a plastic bag) and one visual stimulus (waving of a pink cloth). Video length was between 30 s and 60 s. Photographs of the horses' faces were further taken once daily between 8:30 and 9:30 a.m. for assessment of facial expressions. Analysis of facial expressions was performed on one photo per horse and day. Videos and photographs were taken with an Apple iPhone SE® (Apple Inc., CA, United States). Analysis of facial expressions was based on the facial sedation scale for horses (FaceSed) (43) and the Horse Grimace Scale (45). Facial parameters analyzed included orbital opening, position of ears, tension of chewing muscles represented by their visible presence, relaxation of lips and dilation of nostrils (62). Figure 1 shows a timeline of the study.

Blood and saliva samples obtained for assessment of cannabinoid levels (63) were additionally analyzed for cortisol levels. Samples were taken on the day before start of paste administrations (day 0), days 1–4, 8, 15–19, 23, and 30 (Figure 1). To avoid any influence of the circadian rhythm, only samples taken between 8:00 a.m. and 9:00 a.m. were chosen for cortisol analysis. Per each horse, 10 mL of blood was collected into serum separating tubes, stored at room temperature for 30–60 min and centrifuged at 3,000 × g for 10 min. From each tube, 5 mL of serum was then transferred into a fresh tube to be frozen and stored at −20°C. Samples were analyzed per each individual horse. To further analyze cortisol levels, saliva samples were taken with synthetic swabs (Salivette®, SARSTED AG & Co. KG, Nümbrecht, Germany). Swabs were removed from the tube using Gross-Maier Dressing Forceps and inserted into the horse's mouth for approximately 30 s. Two to three swabs were used for each sample. Salivettes® were centrifuged at 1,000 × g for 10 min. Saliva was subsequently transferred into new tubes, frozen and stored at −20°C.

2.3 Novel object test and trailer test

To obtain baseline behavioral values, a novel object test and horses' reactions to loading on a trailer were video recorded 3 days before the start of paste administration. Blood and saliva samples were taken for measurement of cortisol levels immediately prior to the novel object test. A Polar® H10 heart rate sensor (Polar® Electro Oy, Kempele, Finland) was attached to an electrode belt which spanned around the horse's chest. Each horse's coat was trimmed and moisturized with water over the heart base between the 4th and 5th intercostal space to enhance signal transmission. The heart rate sensor was connected to a mobile device via Bluetooth to record cardiac beat-to-beat (R-R) intervals using the Polar® Equine App (Version 1.2.1, Polar® Electro, Kempele, Finland). For the novel object test, an inflatable pool raft (approximately 170 × 80 × 10 cm, yellow pineapple) served as the unknown object. The pool raft was chosen for its bright and large exterior, and to minimize the possible risk of injury for the animals. The test began with horses being led into a round pen (Ø 15 m). The person leading the horse left the round pen and the object was lowered from the ceiling in the center of the round pen (Figure 2). After 10 min, the horse was taken out of the round pen and the object was raised to the ceiling again.

Each horse was subsequently led into a riding hall, where a trailer was parked. Horses were guided directly toward the trailer and up the ramp. If a horse was not willing to walk up the ramp, it was led back in a circle for another attempt (maximum five attempts). A second person was then asked to stand behind the horse and support its guidance toward the trailer. Loading was not enforced by any additional measures. After the tests, blood and saliva samples were obtained for later assessment of cortisol levels.

Both tests were repeated after 13 days of paste administration (Figure 1), as CBD concentrations in serum were expected to have reached a steady state by this time (63). A new pool raft with similar dimensions but differing outer appearance (green turtle) was chosen for the second novel object test. The remainder of the protocol including the setup for loading on a trailer remained the same. All tests were recorded using a video camera (GoPro HERO10®, San Mateo, United States).

2.3.1 Assessment of novel object test

All video recordings were randomized and blinded. Evaluation was performed by one observer who was experienced in equine behavior studies and not aware of the horses' group assignments. For each recording, the time periods spent in different movement patterns were assessed. Movement patterns included sniffing the ground, standing still, moving in each gait (walk, trot, canter) and rolling.

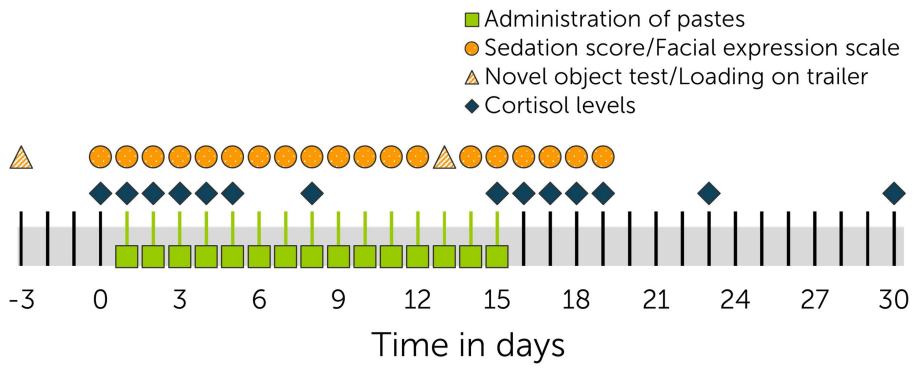

FIGURE 1
Timeline of multiple dose study. Pastes (3 mg CBD/kg and control) were administered twice daily (n = 6 + 6 horses) from days 1 to 15.

FIGURE 2
Novel object test. A pool raft (yellow pineapple) was chosen as the unknown object. The horse is wearing an electrode belt with a heart rate sensor around its chest.

During locomotion in each gait, the number of changes in direction were additionally documented. The horses' reactions to the novel object itself were recorded by taking a note of the time it took a horse to first fixate the object visually, first approach the object and first touch the object.

2.3.2 Assessment of trailer test

Randomized and blinded video recordings were assessed by an observer experienced in equine behavior studies, who was not involved in the previous study parts. Each horse's compliance with entering the trailer was scored on a scale from 0 to 7 for each attempt (Table 1). The attempt with the highest score was selected for statistical analysis.

2.3.3 Ethogram

An adjusted ethogram was developed to evaluate the behavioral traits shown throughout the novel object- and the trailer tests (Table 2). Randomized and blinded video analysis was performed by three observers who were not involved in the previous study parts but specifically trained for equine behavioral assessment. The number of behavioral traits displayed per horse was evaluated. Results of all three assessments were pooled to median values for further analysis.

TABLE 1 Behavioral scoring for trailer test.

Score	
0	Horse stops in front of the ramp
1	One front leg is on the ramp
2	Both front legs are on the ramp (with support)
3	Both front legs are on the ramp (no support)
4	Both front legs are in the trailer (with support)
5	Both front legs are in the trailer (no support)
6	Horse is in the trailer (with support)
7	Horse is in the trailer (no support)

"Support" refers to a second person standing behind the horse to guide it on the trailer.

2.3.4 Assessment of heart rate and heart rate variability

Each cardiac beat-to-beat (R-R) recording was divided into sections of 5 min as previously described (54). Automatic beat correction was applied to remove artifacts (threshold: very low, 0.3 s). Heart rate (HR) and heart rate variability (HRV) including the following parameters: mean HR in beats per minute (bpm), root mean square of successive beat-to-beat differences (RMSSD in milliseconds, ms) and standard deviation of normal-to-normal R-R intervals (SDNN, ms) were evaluated using the software Kubios® HRV Standard (ver. 3.5, Kubios® Oy, Kuopio, Finland).

2.4 Assessment of cortisol levels

Cortisol levels in serum and saliva samples were determined by means of high-performance liquid chromatography/tandem mass spectrometry (LC/MS/MS). Information on the sample preparation/extraction, instrumental conditions, validation, analysis and method validation are summarized in the Supplementary material.

2.5 Statistical analysis

Data were recorded in Microsoft Excel® (Version 2304) and statistical analysis was performed with SPSS® Statistics 27 (IBM®, NY, United States). Data were visually inspected and tested with a Shapiro–Wilk test for normal distribution. Behavioral observations (sedation score, facial expression scale) and cortisol concentrations were analyzed using an analysis of variance (ANOVA) with a Greenhouse–Geisser correction and a general linear model for repeated measures to test for differences between the treatment and the control group over time. Cortisol levels in serum and saliva were further tested for correlation using Spearman's rank correlation coefficient.

For the novel object test and the trailer test, the differences between movement patterns, reactions to the unknown objects,

TABLE 2 Ethogram developed for evaluation of the [†]novel object test and [§]trailer test.

Behavioral trait	Description
Bucking[†]	Fast dynamic movement in which the horse lowers its head, rounds its back and jumps in the air, sometimes leaving the ground with all four legs while kicking with the hindquarters
Cocking hindleg[†]	Horse standing firmly on three legs while one hindleg touches the ground with only the tip of the hoof
Defecating[†]	The horse relieving itself from fecal matter
Digging/scratching[†§]	Standing firmly on three legs while purposefully scratching the ground with the tip of one front hoof
Ear movement[§]	(Independent) flickering of one or both ears
Flehmen response[†]	Stretching the neck and the head upwards while curling the nose and exposing the teeth
Freezing[§]	Freezing of the horse with tense posture and forward gaze
Head tossing[†§]	Abrupt, powerful, short movement of the head and neck sideways or upwards; usually combined with tilting of the head
Licking/chewing[†]	Movement of the jaw that results in opening and closing of the mouth including movement of the tongue
Looking around or behind[§]	Turning the head and neck toward the back without leg movements
Neighing[†§]	The sound of a characteristic noise of a horse with different volumes and voice pitches
Remaining near exit[†]	The horse seeks close proximity to the exit of the round pen and remains there
Rolling[†]	Laying on the ground and demonstration a rolling motion, sometimes tilting over to the other side
Sniffing[†]	Horse lowers the head and sniffs the ground
Sniffing the ramp[§]	Horse lowers the head and sniffs the ramp
Snorting[†§]	Accelerated exhale through the nostrils accompanied by a characteristic flapping sound of the nostrils
Stomping[†]	Lifting of one leg and placing it back down forcefully
Tail swishing[†§]	Short, intense, omnidirectional movement of the tail
Treading on the spot[§]	Lifting and lowering the hooves without forward, backward or sideways movements
Urinating[†]	The horse relieving itself from urine in a characteristic stand
Walking backwards[§]	Stepping backwards
Walking sideways[§]	Stepping sideways

scores for loading on a trailer, ethogram behavioral traits and cortisol levels during the first test (baseline) and after 13 days of paste administration were calculated for each horse. Differences between the treatment and control group were compared using a *t*-test (for normally distributed data) or a Mann–Whitney-U-Test (for not normally distributed data). For the ethogram, intraclass correlation coefficients determined the level of agreement between the observers for each observed behavioral trait. HR, RMSSD and SDNN parameters obtained during the second test were analyzed using an ANOVA to test for differences between the treatment and the control group. Residuals were visually inspected for normal distribution. The level of significance was $p < 0.05$.

3 Results

3.1 Animals

Daily physical examinations of all horses did not identify any side effects such as gastrointestinal intolerances associated with paste application. On the day before study start, no residual cannabinoid contents were detected in serum or urine. Regular blood analyses did not identify significant irregularities in CBC, kidney and liver biomarkers (63). CBD concentrations in serum reached a steady state after 2 days of CBD paste administration with a mean maximum serum concentration (C_{max}) of 38.4 ± 8.9 ng/mL (63).

3.2 Behavioral observations

Mean values for sedation scores ranged from 34.0 ± 5.0 (day 3) to 51.7 ± 1.5 (day 19) in the treatment group, and 39.0 ± 1.5 (day 15) to 56.0 ± 2.0 (day 19) in the control group. For the facial expression scale, values ranged from 9.7 ± 2.0 (day 3) to 12.6 ± 2.3 (day 9) in the treatment group, and 10.3 ± 0.8 (day 0) to 13.8 ± 1.1 (day 1) in the control group (Figure 3). On 12 out of 18 days, values for sedation scores were higher in the control group than in the treatment group. Comparison using an ANOVA with a Greenhouse–Geisser correction showed no significant differences between groups for the sedation score [$F(3.0, 11.9) = 2.3$, $p = 0.127$] and the facial expression scale [$F(1.0, 1.0) = 1.5$, $p = 0.435$]. Due to technical difficulties, videos and photographs of day 13 and 14 were not assessable for scoring.

3.3 Morning cortisol levels

Throughout the course of the multiple dose study, cortisol levels in serum were on average 54.7 ± 18.6 ng/mL in the treatment group

and 62.2 ± 19.2 ng/mL in the control group. For saliva, mean cortisol levels were on average 0.40 ± 0.30 ng/mL in the treatment group and 0.63 ± 0.45 ng/mL in the control group (Figure 4). Differences between groups were tested using an ANOVA with a Greenhouse–Geisser correction and were non-significant for cortisol levels in serum [$F(4.1, 37.0) = 1.7$, $p = 0.171$] and in saliva [$F(1.6, 3.2) = 1.0$, $p = 0.442$] over all days. Correlation between serum and saliva cortisol levels was $r_s = 0.53$ ($p < 0.001$).

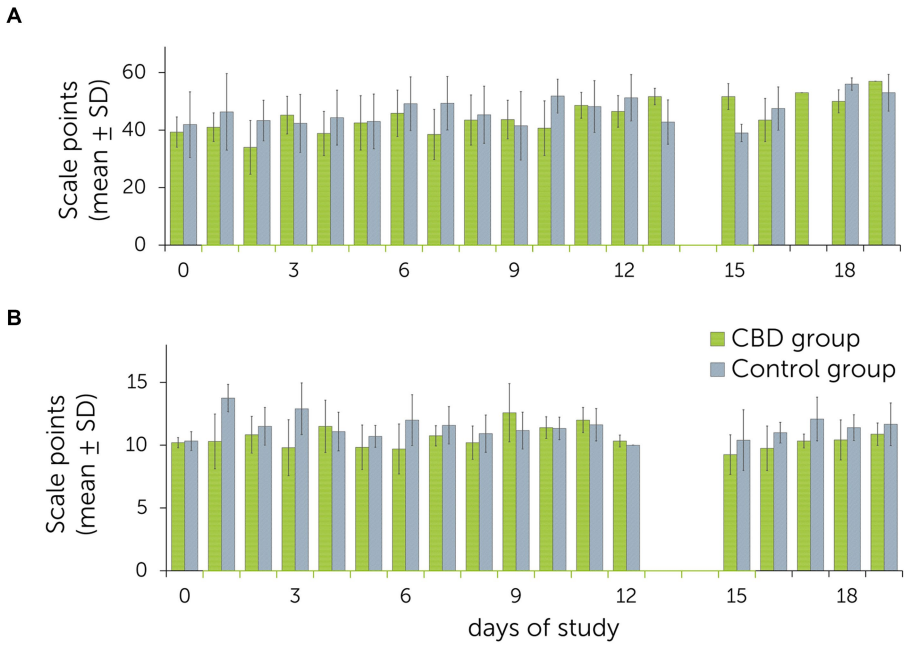

FIGURE 3
Mean ± standard deviations (SD) of behavioral observations obtained during the multiple dose study with daily administration of cannabidiol (CBD) and placebo pastes to a treatment and control group (n = 6 + 6 horses). The treatment group received CBD containing paste from days 1 to 15 (3 mg CBD/kg BID p.o.). **(A)** Summed up sedation scores after acoustic and visual stimulations (clicker, plastic bag, pink cloth). **(B)** Daily facial expression scores. Higher scale points relate to a higher level of relaxation/sedation.

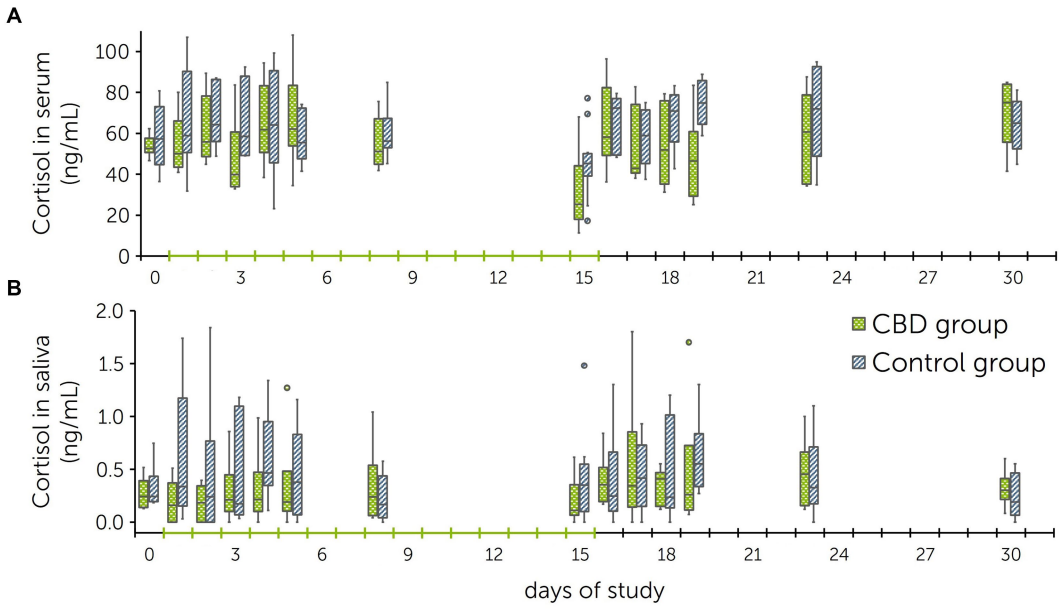

FIGURE 4
Boxplots of cortisol levels in serum **(A)** and saliva **(B)** obtained during the multiple dose study with daily administration of cannabidiol (CBD) and placebo pastes to a treatment and control group (n=6+6 horses). The treatment group received CBD containing paste from days 1 to 15 (3 mg CBD/kg BID p.o.).

3.4 Novel object test and trailer test

3.4.1 Novel object test

The initial reactions to lowering of the pool raft was trotting or galloping alongside the outer parameter of the round pen in all horses. Movements then reduced to walking, standing or sniffing the ground with a subsequent continuation of trotting or galloping in a number of cases. Movement patterns for each individual horse are depicted in Figure 5. The difference between each movement pattern shown during the novel object test before trial start (baseline) and after 13 days of paste administration was calculated for each horse. Comparison of the differences between treatment and control group proved to be non-significant for all movement patterns (sniffing: $p = 0.699$; walking: $p = 0.818$; trotting: $p = 0.818$; galloping: $p = 0.394$; rolling: $p = 0.699$).

During both tests, horses changed direction several times. Differences in the number of changes of direction between before and after treatment ranged from 0 to 4 for each horse in the treatment group and from 1 to 8 for each horse in the control group. There was no significant difference found when compared between groups ($p = 0.485$).

In both novel object tests, all horses first fixated the pool raft visually 1.1–1.4 min after the start with non-significant difference between groups ($p = 0.485$). During the first novel object test (baseline), all horses approached the novel object after approximately 3 min (treatment group: 3.0 ± 1.3 min, control group: 3.0 ± 1.5 min). During the second novel object test, horses in the treatment group first approached the novel object after 4.4 ± 3.4 min and horses in the control group after 1.5 ± 0.5 min. Differences were non-significant ($p = 0.065$). During the baseline novel object test, four horses in each group touched the object. Two horses in the treatment group and four horses in the control group touched the pool raft during the second novel object test. Modes of touching included careful reaching with head and neck, tentative touching, or nibbling. Statistically significant difference was not identified between groups ($p = 0.485$).

3.4.1.1 Novel object test: ethogram

Ten out of fifteen behavioral traits were rated with ICC values of > 0.90. The ICC value for "remaining near exit" was 0.80. "Cocking hindleg" and "stomping" were rated with ICC values between 0.50–0.75, and "licking/chewing" and "snorting" were rated with ICC values < 0.50.

In both groups, the most frequently exhibited trait was "sniffing" (treatment group: median at baseline = 12 times, median after paste administration = 16.5 times; control group: median at baseline = 9.5 times, median after paste administration = 10.5 times). Other behavioral traits (Table 2) were exhibited a median of 0–4 times. Individual stallions showed behavioral traits such as "tail swishing" and "head tossing" up to 18 and 29 times, respectively.

The difference between each behavioral trait exhibited during the baseline test and after paste administration was calculated per horse. Comparison of the differences between groups showed no significant effect [p values ranging from 0.132 ("head tossing") to > 0.999 ("bucking")].

3.4.2 Trailer test

During the baseline test, three horses in the treatment group entered the trailer completely (scores 6 and 7, Table 1), one horse placed both front legs in the trailer (score 4), one horse went as far as putting both front legs on the ramp of the trailer (score 2) and one horse stopped in front of the ramp (score 0). In the control group, two horses entered the trailer (scores 6 and 7), two horses put both front legs in the trailer (scores 4 and 5) and two horses stopped before the ramp (score 0).

After 13 days of paste administration, the scores of six horses (three in each group) did not change (treatment group: scores 7, 7, 0; control group: scores 6, 0, 0). One horse in the treatment group was rated with a higher score (score 2 to 3). Two horses in the treatment group and three horses in the control group scored lower in the second test (treatment group: score 6 to 3, score 4 to 3; control group: score 7 to 6, score 5 to 3, score 4 to 3).

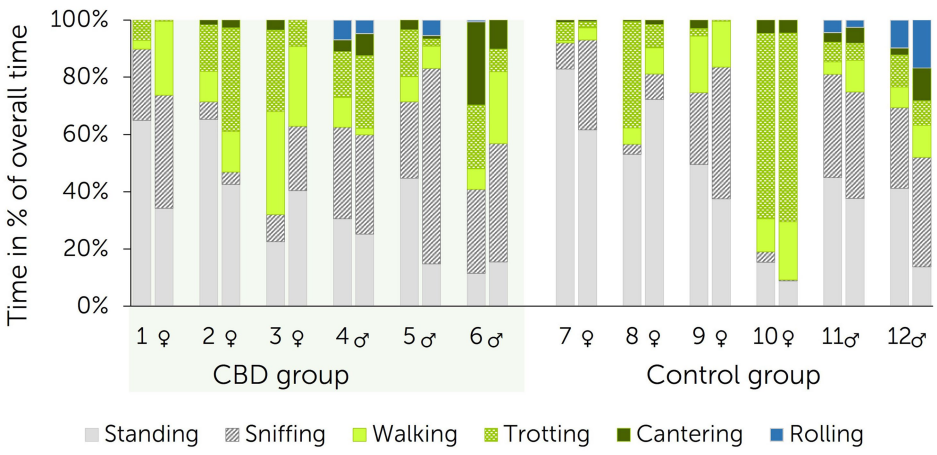

FIGURE 5
Movement patterns during novel object test in direct comparison per individual horse (1–12) between baseline (left bars) and after 13 days of paste administration (right bars) to a treatment and control group ($n = 6 + 6$ horses). The treatment group received a cannabidiol (CBD) containing paste twice daily from days 1 to 15 (3 mg CBD/kg).

For each horse, the differences between scores determined during baseline and after paste administration were calculated with no significant effect when compared between groups ($p = 0.589$).

3.4.2.1 Trailer test: ethogram

Observer agreement using the ICC was rated > 0.90 for six out of twelve behavioral traits. ICC values for "tail swishing," "looking around or behind," and "treading on the spot" were between 0.75 and 0.90. "Ear movement," "freezing" and "snorting" were rated with ICC values of < 0.50.

In both groups, the behavioral trait most frequently observed was "ear movement" during the baseline test (treatment group: median of 5 times; control group: median of 3 times) and after paste administration (both groups: median of 3 times). "Ear movement," "head tossing" and "looking around or behind" was mainly observed in stallions (between 10 and 13 times each). No horse exhibited "digging/scratching." Differences were calculated between the baseline test and after paste administration for each individual horse. Differences were compared between groups using the Mann–Whitney-U-Test with resulting p values ranging from 0.180 ("looking around or behind") to > 0.999 ("digging/scratching," "neighing," "walking sideways").

3.4.3 Heart rate and heart rate variability

Due to technical difficulties, recordings of R-R intervals during the novel object test and the trailer test before study start (baseline) were not available for analysis. It was decided to compare HR and HRV data obtained during the second tests between treatment and control group. The mean values assessed during the novel object test for HR were: 48.6 ± 1.5 bpm, for RMSSD: 93.4 ± 22.1 ms and for SDNN: 87.9 ± 26.3 ms in the treatment group. In the control group, mean values for HR were: 44.9 ± 5.3 bpm, for RMSSD: 113.8 ± 36.5 ms and for SDNN: 113.5 ± 58.9 ms.

During the trailer test, the mean HR was 47.2 ± 3.7 bpm, mean RMSSD was 121.1 ± 21.3 ms and mean SDNN was 118.6 ± 37.6 ms in the treatment group. In the control group, mean values were HR: 46.3 ± 10.7 bpm, RMSSD: 124.2 ± 45.0 ms and SDNN: 132.4 ± 61.0 ms. Analysis using a one-way ANOVA with a Greenhouse–Geisser correction found no statistically significant differences between treatment and control group over both trials for HR: $F(1.5, 12.2) = 1.2$, $p = 0.312$, RMSSD: $F(5, 40) = 1.6$, $p = 0.183$ and SDNN: $F(6, 36) = 1.6$, $p = 0.178$.

3.4.4 Cortisol levels

Serum and saliva samples for cortisol analysis were obtained prior to each novel object test and after each trailer test. Before the first novel object test (baseline), cortisol levels of horses in the treatment group were 44.68 ± 11.08 ng/mL in serum and 0.17 ± 0.09 ng/mL in saliva. After the baseline tests, cortisol levels increased to 68.87 ± 24.95 ng/mL in serum and 0.46 ± 0.38 ng/mL in saliva. Before the second novel object test, serum cortisol levels were 45.22 ± 12.61 ng/mL and saliva cortisol levels 0.15 ± 0.05 ng/mL. After the second trailer test, cortisol levels increased to 47.23 ± 18.27 ng/mL (serum) and 0.35 ± 0.15 ng/mL (saliva) (Figure 6).

Prior to the baseline novel object test, cortisol levels in the control group were 46.28 ± 16.10 ng/mL in serum and 0.26 ± 0.19 ng/mL in saliva. After loading on a trailer, cortisol levels reached 60.87 ± 18.67 ng/mL in serum and 0.20 ± 0.09 ng/mL in saliva. Before the second novel object test, serum cortisol levels were 59.40 ± 25.12 ng/mL and saliva cortisol levels were 0.78 ± 0.48 ng/mL. After the second trailer test, cortisol levels were 61.42 ± 30.30 ng/mL (serum) and 0.50 ± 0.51 ng/mL (saliva) (Figure 6).

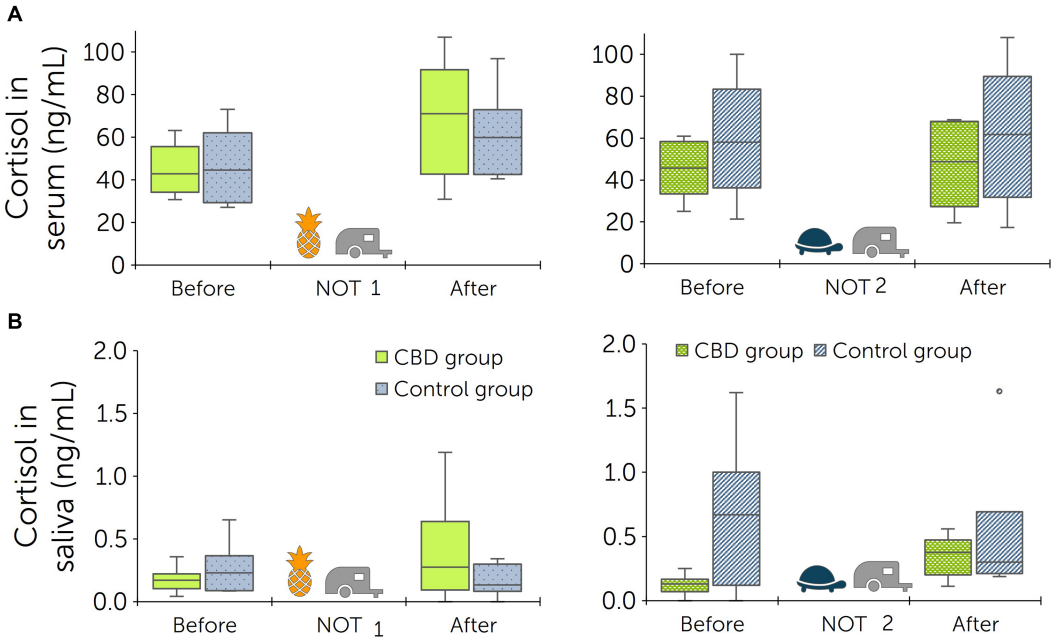

FIGURE 6
Cortisol levels in serum **(A)** and saliva **(B)** before the novel object test (NOT) and trailer test, and immediately after both tests. Tests were performed twice: prior to start of paste administrations (baseline) and following 13 days of paste administrations to a treatment and control group (n = 6 + 6 horses). Pool rafts were used as novel objects [pineapple for the baseline test (NOT 1), turtle for the second test (NOT 2)]. The treatment group received a cannabidiol (CBD) containing paste twice daily from days 1 to 15 (3 mg CBD/kg).

Differences between cortisol levels measured in serum and saliva before and after the tests were calculated for each horse. Comparison of test results from the second tests found a significant difference between groups for cortisol levels in saliva ($p = 0.016$), but not in serum ($p > 0.999$). Within the treatment group, comparison between baseline tests and tests following CBD paste administration showed no significant differences (serum: $p = 0.505$; saliva: $p > 0.999$).

4 Discussion

Regular oral administration of a CBD containing paste at a dose of 3 mg/kg was well-tolerated by all horses in this study. Multiple oral CBD administrations did not have a significant effect on behavioral observations and cortisol monitoring. Parameters investigated in a novel object test and during loading on a trailer did not differ significantly from the control group.

Case reports have described CBD as an effective agent for the treatment of mechanical allodynia, chronic crib-biting and wind-sucking at an oral dose of 0.5 mg CBD/kg BID in horses (64, 65). These reports did not test CBD levels in serum, but previous studies reported maximum CBD concentrations of less than 20 ng/mL in serum following administration of up to 3 mg CBD/kg p.o. (8, 66–71). Two studies found C_{max} levels of 51 ng/mL CBD in serum following oral administration of 2 mg CBD/kg SID for 7 days (67, 70), and C_{max} levels of 55.7 ng/mL CBD in serum following a single oral dose of 10 mg CBD/kg (72). The C_{max} levels of 38.4 ± 8.9 ng/mL in serum reported during the current study (63) are therefore in line with previous reports, and comparatively high (70). In dogs, similar CBD dose levels lead to much higher concentration maxima in serum: one study has shown that the median C_{max} of CBD was 102.3 ng/mL after single oral administration of 2 mg CBD/kg (4). The absorption and retention of CBD in horses seems to be more akin to humans than dogs (70). Single oral intake of 400 mg CBD resulted in a subjective reduction in anxiety in humans with generalized social anxiety disorder (15). However, as no therapeutic serum concentrations for anxiety in humans are available so far, further studies are required to translate administered CBD dose levels to therapeutic serum concentrations.

The facial expression scale used in this study was based on the facial sedation scale for horses (FaceSed) and the Horse Grimace Scale (HGS) (43, 45). Two studies have reported an effective assessment of facial expressions using the HGS to indicate pain levels (73, 74). In the current study, daily behavioral observations of sedation levels using a sedation score and a facial expression scale did not differ significantly between treatment and control group. This assessment is in line with previous studies that found no significant effect on sedation levels following regular CBD pellet feedings (~0.29 mg CBD/kg over 56 days) in horses (7) and oral administration of CBD treats (4.5 mg CBD/kg BID over 21 days) in dogs (18). Reports on US veterinarians and pet owners' perceptions of CBD and hemp use in dogs state that sedation/tiredness were the most commonly observed side effects (75–77). In humans, sedation was reported as a side effect following daily oral intake of 600 mg CBD over 6 weeks (78). As doses were higher in these reports, the question remains whether increased dose levels and therefore increased serum concentrations would lead to a similar effect in horses.

Cortisol is a steroid hormone which is subject to a circadian rhythm. Cortisol levels assessed in previous publications were reported to be highest between 8 am and 12 pm (serum: 25–70 ng/mL; saliva: 0.55–0.70 ng/mL) (50, 79) and are comparable to levels reached in the current study. Depending on the time of day and stress exposure, saliva levels can reach up to 3 ng/mL in horses but usually stay below 1 ng/mL (49, 50, 80). Saliva sampling is a noninvasive, pain-free additional technique to gain more information about cortisol levels (49, 81). Salivary and serum cortisol levels have been reported to have different degrees of correlation ($r_s = 0.32–0.80$) (50, 81). In this study, a moderate correlation was seen between serum and salivary cortisol levels ($r_s = 0.53$) (82). Minor disruptions leading to stress responses can result in deviations from the normal circadian cortisol rhythm and may elevate cortisol levels in blood (50, 79). In this study, no significant effect of CBD on morning cortisol levels was identified.

Novel object tests have been used in a variety of species and can be performed with different unknown objects (54–57) or even unknown horses (Novel horse test) (83). Novel object tests are designed as fear tests and are used to document the intensity of an animal's fearfulness when confronted with the unknown object. As no standard protocol exists, neither regarding the kind of object nor the duration of exposure, scoring of reactions and assessment of additional parameters (such as heart rate) tend to vary. In this study, two novel object tests were performed with similarly sized yet differently colored and shaped objects (pool rafts: yellow pineapple and green turtle) to make the test results comparable and exclude a habituation effect. One report tested habituation to a frightening stimulus (white nylon bag) in 2-year-old colts. It was concluded that the horses were habituated to the stimulus after four training sessions which were all conducted within 1 day (84). As the novel object tests performed in this study were only performed twice and were 16 days apart, habituation was considered to be an unlikely limiting factor. The effect of CBD in horses has been tested in another study using a novel object test following daily oral administration of CBD pellets (~0.2 mg CBD/kg) (6). A significantly lower degree of reactivity compared to a control group was documented (6). A fear response test performed in dogs following oral CBD treatment (1.4 mg CBD/kg) showed no significant effect (85). In agreement with this report, the current study found no significant difference between treatment and control group regarding movement patterns. Reaction times to the novel object differed between groups: during the first novel object test, horses in both groups took about 3 min to first approach the novel object. During the second test, horses in the treatment group took more time to first approach the object (4.4 ± 3.4 min) than horses in the control group (1.5 ± 0.5 min). These differences could suggest that CBD does either not exhibit a fear-reducing effect in the studied dose level, or that CBD has a relaxing effect and reduces the horse's interest in the novel object. Statistical analysis showed that the differences between groups are bordering on significance ($p = 0.065$), which might be biased by the small sample size. Future tests should include larger sample sizes and potentially nervous horses when determining CBD's effect as a fear-reducing or anxiolytic agent.

Loading on a trailer is considered a stressful event for horses (58–60). Different training methods are described to reduce horses' discomfort and anxiety (58–60). In addition to training, sedatives like acepromazine may be used to reduce stress responses (61). Oral CBD (total of 400 mg, single administration) has been reported to subjectively decrease anxiety in humans with generalized social

anxiety disorder (15). The effect of CBD on horses' reactions to loading on a trailer has not been reported yet, but results of this study suggest that it does not increase horses' willingness to enter a trailer at the tested dose level.

Behavioral traits displayed by horses during the novel object- and the trailer test were assessed using a customized ethogram. Behavioral observations may be performed using a software (53) or handwritten lists prepared by one to four independent observers (73, 74, 86). To reduce subjectivity, three observers rated behavioral traits in this study. Most behavioral traits displayed a good (0.75–0.90) to excellent agreement (> 0.90) (87). Behavioral traits with poor agreement (< 0.50) included "ear movement," "freezing," "licking/chewing" and "snorting." Poor scores might be related to an insufficient description of the respective traits, or to the more difficult detection of smaller movements such as "ear movement" or "licking/chewing" especially in combination with other movements when watching a video recording. A wide variety of behavioral traits were assessed including noises ("neighing") and whole body movements ("walking backwards"), as well as behaviors indicative of stress such as "bucking" or "head tossing" (88). No significant differences in displayed behavioral traits were identified between treatment and control group.

Studies investigating heart rate (HR) and heart rate variability (HRV: RMSSD and SDNN) have shown that a decrease in HR and increase in RMSSD and SDNN suggest an autonomic shift toward a parasympathetic dominance and are therefore indicative of the horse's stress levels (48, 54, 89–92). Measurement of HR and HRV is an established tool to evaluate stress responses due to pain or anxiety-inducing events (90, 93–96). Additionally, assessments of HR and HRV have been performed during novel object tests (54–56, 97), and loading on a trailer and subsequent transport (98, 99) in horses. The effect of CBD on HR and HRV has been documented in horses, dogs, humans and rodents with varying results. In horses, HR assessed during a novel object test found no significant effect between a treatment group fed 100 mg pelleted CBD (~0.2 mg CBD/kg) and a control group (6). A stress test performed in dogs similarly found no significant differences in HR and HRV values between a treatment (single oral administration of 4 mg CBD/kg) and a placebo group (100). A second report in dogs equally identified no significant changes in RMSSD and SDNN following a fear response test when treated orally with 1.4 mg CBD/kg (85). In contrast, single intraperitoneal CBD administration in rodents (10 mg CBD/kg) significantly reduced the increase of HR and blood pressure in a stress inducing and fear conditioning setting, suggesting an anxiolytic effect (14, 16). In this study, HR values were higher and RMSSD and SDNN were lower in the treatment than in the control group, indicating a less pronounced parasympathetic state in the treatment group. However, as these differences were statistically non-significant, their relevance is debatable.

Measurement of cortisol concentrations is an established parameter for stress evaluation in horses (49, 51, 81, 92, 99). When comparing the cortisol levels before and after the novel object- and trailer tests, cortisol levels in serum increased to varying degrees (Figure 6). Within the treatment group, the increase was less pronounced after the second round of tests. Statistical analysis showed that this reduction was non-significant. In the control group, salivary cortisol levels had decreased after both test rounds. The difference between treatment and control group was therefore found to be significant ($p = 0.016$). The effect of CBD on cortisol levels has been investigated in humans, dogs and horses with varying results (17, 66, 100–102). After a stress test, dogs that received oral CBD (4 mg CBD/kg) showed significantly lower serum cortisol concentrations than a control group (100). In horses, one study compared cortisol levels between horses that were administered CBD oil and horses receiving olive oil after transportation with no significant findings (66). Studies performed in humans are difficult to compare due to their differing designs and intentions, but have similarly not found a significant effect of CBD on cortisol levels (101, 102).

As all cannabinoids are listed as prohibited substances by the FEI, and CBD is defined as a controlled medication (41), future studies are required to determine what effects oral dosing of CBD exactly exerts in horses, and what dose levels and intervals are needed to achieve these effects. No consistently significant effects on equine behavior were observed in this study.

A small sample size is the main limitation of this study. Further limitations include the missing recordings of R-R intervals during the novel object test and the trailer test before study start (baseline). Consequently, comparison of HR and HRV was carried out between groups following paste administration. Subjects were healthy horses that did not show behavioral problems. Further trials with larger sample sizes are needed to validate the potential effectiveness of CBD in anxious or nervous horses. Future studies may also include more detailed assessments of HRV parameters including the parasympathetic tone activity (PTA) index. Oral dosing using different formulations such as micellar formulation should also be considered (72). Clinical studies as have been performed with dogs (4) are of interest to further assess the potential use of CBD in equine medicine.

5 Conclusion

This study did not detect consistently significant effects of regularly administered oral CBD (3 mg/kg BID over 15 days) on behavioral observations or morning cortisol levels in healthy horses. Horses' reactions to a novel object and loading on a trailer were tested with no significant differences identified between treatment and control group. Parameters assessed included movement patterns, reaction to the novel object, heart rate and heart rate variability, and cortisol levels in serum and saliva. No adverse reactions were observed following multiple administrations of a CBD containing paste. Further research is required to determine adequate indications for the use of CBD products in horses.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by the competent authority for licensing and notification procedures for animal experiments (LAVG) in Brandenburg, Germany (AZ: 2347-12-2021). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

FE: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft. AE: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. MM: Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Writing – review & editing. KCJ: Formal analysis, Methodology, Software, Validation, Writing – review & editing. SW: Formal analysis, Methodology, Software, Validation, Writing – review & editing. NB: Conceptualization, Data curation, Methodology, Project administration, Writing – review & editing. JB: Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – review & editing. MP: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. MT: Methodology, Supervision, Writing – review & editing. WB: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. CL: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. MW: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1305873/full#supplementary-material

References

1. Fogaça MV, Campos AC, Coelho LD, Duman RS, Guimarães FS. The anxiolytic effects of cannabidiol in chronically stressed mice are mediated by the endocannabinoid system: role of neurogenesis and dendritic remodeling. *Neuropharmacology*. (2018) 135:22–33. doi: 10.1016/j.neuropharm.2018.03.001

2. Henson JD, Vitetta L, Quezada M, Hall S. Enhancing endocannabinoid control of stress with Cannabidiol. *J Clin Med*. (2021) 10:10. doi: 10.3390/jcm10245852

3. Moltke J, Hindocha C. Reasons for cannabidiol use: a cross-sectional study of CBD users, focusing on self-perceived stress, anxiety, and sleep problems. *J Cannabis Res*. (2021) 3:5. doi: 10.1186/s42238-021-00061-5

4. Gamble L-J, Boesch JM, Frye CW, Schwark WS, Mann S, Wolfe L, et al. Pharmacokinetics, safety, and clinical efficacy of Cannabidiol treatment in osteoarthritic dogs. *Front Vet Sci*. (2018) 5:165. doi: 10.3389/fvets.2018.00165

5. Della Rocca G, Di Salvo A. Hemp in veterinary medicine: from feed to drug. *Front Vet Sci*. (2020) 7:387. doi: 10.3389/fvets.2020.00387

6. Draeger AL, Thomas EP, Jones KA, Davis AJ, Porr CS. The effects of pelleted cannabidiol supplementation on heart rate and reaction scores in horses. *J Vet Behav*. (2021) 46:97–100. doi: 10.1016/j.jveb.2021.09.003

7. St Blanc MP, Chapman AM, Keowen ML, Garza F, Liu C-C, Gray L, et al. Effects of a supplement containing Cannabidiol (CBD) on sedation and Ataxia scores and health. *J Equine Vet*. (2022) 117:104085. doi: 10.1016/j.jevs.2022.104085

8. Ryan D, McKemie DS, Kass PH, Puschner B, Knych HK. Pharmacokinetics and effects on arachidonic acid metabolism of low doses of cannabidiol following oral administration to horses. *Drug Test Anal*. (2021) 13:1305–17. doi: 10.1002/dta.3028

9. Mechoulam R, Hanuš LO, Pertwee R, Howlett AC. Early phytocannabinoid chemistry to endocannabinoids and beyond. *Nat Rev Neurosci*. (2014) 15:757–64. doi: 10.1038/nrn3811

10. Golombek P, Müller M, Barthlott I, Sproll C, Lachenmeier DW. Conversion of Cannabidiol (CBD) into psychotropic cannabinoids including tetrahydrocannabinol (THC): a controversy in the scientific literature. *Toxics*. (2020) 8:8. doi: 10.3390/toxics8020041

11. Casajuana Köguel C, López-Pelayo H, Balcells-Olivero MM, Colom J, Gual A. Constituyentes psicoactivos del cannabis y sus implicaciones clínicas: una revisión sistemática. *Adicciones*. (2018) 30:140–51. doi: 10.20882/adicciones.858

12. Granjeiro EM, Gomes FV, Guimarães FS, Corrêa FM, Resstel LB. Effects of intracisternal administration of cannabidiol on the cardiovascular and behavioral responses to acute restraint stress. *Pharmacol Biochem Behav*. (2011) 99:743–8. doi: 10.1016/j.pbb.2011.06.027

13. Gomes FV, Resstel LB, Guimarães FS. The anxiolytic-like effects of cannabidiol injected into the bed nucleus of the stria terminalis are mediated by 5-HT1A receptors. *Psychopharmacology*. (2011) 213:465–73. doi: 10.1007/s00213-010-2036-z

14. Resstel LB, Tavares RF, Lisboa SF, Joca SR, Corrêa FM, Guimarães FS. 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. *Br J Pharmacol*. (2009) 156:181–8. doi: 10.1111/j.1476-5381.2008.00046.x

15. Crippa JA, Derenusson GN, Ferrari TB, Wichert-Ana L, Duran FL, Martin-Santos R, et al. Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *J Psychopharmacol (Oxford, England)*. (2011) 25:121–30. doi: 10.1177/0269881110379283

16. Resstel LB, Joca SR, Moreira FA, Corrêa FM, Guimarães FS. Effects of cannabidiol and diazepam on behavioral and cardiovascular responses induced by contextual conditioned fear in rats. *Behav Brain Res*. (2006) 172:294–8. doi: 10.1016/j.bbr.2006.05.016

17. Corsetti S, Borruso S, Malandrucco L, Spallucci V, Maragliano L, Perino R, et al. *Cannabis sativa* L. may reduce aggressive behaviour towards humans in shelter dogs. *Sci Rep*. (2021) 11:2773. doi: 10.1038/s41598-021-82439-2

18. Morris EM, Kitts-Morgan SE, Spangler DM, Gebert J, Vanzant ES, McLeod KR, et al. Feeding Cannabidiol (CBD)-containing treats did not affect canine daily voluntary activity. *Front Vet Sci*. (2021) 8:645667. doi: 10.3389/fvets.2021.645667

19. Shallcross J, Hámor P, Bechard AR, Romano M, Knackstedt L, Schwendt M. The divergent effects of CDPPB and Cannabidiol on fear extinction and anxiety in a predator scent stress model of PTSD in rats. *Front Behav Neurosci*. (2019) 13:91. doi: 10.3389/fnbeh.2019.00091

20. Fusar-Poli P, Crippa JA, Bhattacharyya S, Borgwardt SJ, Allen P, Martin-Santos R, et al. Distinct effects of Δ9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Arch Gen Psychiatry*. (2009) 66:95–105. doi: 10.1001/archgenpsychiatry.2008.519

21. Williams NN, Ewell TR, Abbotts KS, Harms KJ, Woelfel KA, Dooley GP, et al. Comparison of five Oral Cannabidiol preparations in adult humans: pharmacokinetics, body composition, and heart rate variability. *Pharmaceuticals (Basel)*. (2021) 14. doi: 10.3390/ph14010035

22. Zieba J, Sinclair D, Sebree T, Bonn-Miller M, Gutterman D, Siegel S, et al. Cannabidiol (CBD) reduces anxiety-related behavior in mice via an FMRP-independent mechanism. *Pharmacol Biochem Behav*. (2019) 181:93–100. doi: 10.1016/j.pbb.2019.05.002

23. De Gregorio D, McLaughlin RJ, Posa L, Ochoa-Sanchez R, Enns J, Lopez-Canul M, et al. Cannabidiol modulates serotonergic transmission and reverses both allodynia and anxiety-like behavior in a model of neuropathic pain. *Pain*. (2019) 160:136–50. doi: 10.1097/j.pain.0000000000001386

24. Fogaça MV, Campos AC, Guimarães FS. Cannabidiol and 5-HT1A receptors In: *Neuropathology of drug addictions and substance misuse*: Elsevier (2016). 749–59.

25. Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol Sci*. (2009) 30:515–27. doi: 10.1016/j.tips.2009.07.006

26. Campos AC, Moreira FA, Gomes FV, Del Bel EA, Guimarães FS. Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. *Philos Trans R Soc Lond Ser B Biol Sci*. (2012) 367:3364–78. doi: 10.1098/rstb.2011.0389

27. Blessing EM, Steenkamp MM, Manzanares J, Marmar CR. Cannabidiol as a potential treatment for anxiety disorders. *Neurotherapeutics*. (2015) 12:825–36. doi: 10.1007/s13311-015-0387-1

28. Leweke FM, Piomelli D, Pahlisch F, Muhl D, Gerth CW, Hoyer C, et al. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Transl Psychiatry*. (2012) 2:e94. doi: 10.1038/tp.2012.15

29. Watanabe K, Kayano Y, Matsunaga T, Yamamoto I, Yoshimura H. Inhibition of anandamide amidase activity in mouse brain microsomes by cannabinoids. *Biol Pharm Bull*. (1996) 19:1109–11. doi: 10.1248/bpb.19.1109

30. Di Marzo V, Bisogno T, De PL. Anandamide: some like it hot. *Trends Pharmacol Sci*. (2001) 22:346–9. doi: 10.1016/S0165-6147(00)01712-0

31. Riebe CJ, Pamplona FA, Kamprath K, Wotjak CT. Fear relief-toward a new conceptual frame work and what endocannabinoids gotta do with it. *Neuroscience*. (2012) 204:159–85. doi: 10.1016/j.neuroscience.2011.11.057

32. McLaughlin RJ, Hill MN, Gorzalka BB. A critical role for prefrontocortical endocannabinoid signaling in the regulation of stress and emotional behavior. *Neurosci Biobehav Rev*. (2014) 42:116–31. doi: 10.1016/j.neubiorev.2014.02.006

33. Russo EB, Burnett A, Hall B, Parker KK. Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochem Res*. (2005) 30:1037–43. doi: 10.1007/s11064-005-6978-1

34. Kupczyk P, Rykala M, Serek P, Pawlak A, Slowikowski B, Holysz M, et al. The cannabinoid receptors system in horses: tissue distribution and cellular identification in skin. *J Vet Intern Med*. (2022) 36:1508–24. doi: 10.1111/jvim.16467

35. Galiazzo G, Tagliavia C, Giancola F, Rinnovati R, Sadeghinezhad J, Bombardi C, et al. Localisation of cannabinoid and cannabinoid-related receptors in the horse ileum. *J Equine Vet*. (2021) 104:103688. doi: 10.1016/j.jevs.2021.103688

36. McPartland JM, Mac Donald C, Young M, Grant PS, Furkert DP, Glass M. Affinity and efficacy studies of Tetrahydrocannabinolic acid a at cannabinoid receptor types one and two. *Cannabis Cannabinoid Res*. (2017) 2:87–95. doi: 10.1089/can.2016.0032

37. Bolognini D, Rock EM, Cluny NL, Cascio MG, Limebeer CL, Duncan M, et al. Cannabidiolic acid prevents vomiting in Suncus murinus and nausea-induced behaviour in rats by enhancing 5-HT1A receptor activation. *Br J Pharmacol*. (2013) 168:1456–70. doi: 10.1111/bph.12043

38. de Petrocellis L, Ligresti A, Moriello AS, Allarà M, Bisogno T, Petrosino S, et al. Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. *Br J Pharmacol*. (2011) 163:1479–94. doi: 10.1111/j.1476-5381.2010.01166.x

39. McPartland JM, Russo EB. Cannabis and Cannabis extracts. *J Cannabis Therapeutics*. (2001) 1:103–32. doi: 10.1300/J175v01n03_08

40. Briyne N, De HD, Sandler I, Stiles E, Szymanski D, Moody S, et al. Cannabis, Cannabidiol oils and tetrahydrocannabinol-what do veterinarians need to know? *Animals (Basel)*. (2021) 11. doi: 10.3390/ani11030892

41. Fédération Equestre Internationale. *FEI clean sport - prohibited substances database* (2022). Available at: https://inside.fei.org/content/anti-doping-rules.

42. Poller C, Hopster K, Rohn K, Kästner SB. Nociceptive thermal threshold testing in horses - effect of neuroleptic sedation and neuroleptanalgesia at different stimulation sites. *BMC Vet Res*. (2013) 9:135. doi: 10.1186/1746-6148-9-135

43. De OAR, Gozalo-Marcilla M, Ringer SK, Schauvliege S, Fonseca MW, Esteves Trindade PH, et al. Development and validation of the facial scale (face Sed) to evaluate sedation in horses. *PLoS One*. (2021) 16:e0251909. doi: 10.1371/journal.pone.0251909

44. Schauvliege S, Cuypers C, Michielsen A, Gasthuys F, Gozalo-Marcilla M. How to score sedation and adjust the administration rate of sedatives in horses: a literature review and introduction of the Ghent sedation algorithm. *Vet Anaesth Analg*. (2019) 46:4–13. doi: 10.1016/j.vaa.2018.08.005

45. Dalla Costa E, Minero M, Lebelt D, Stucke D, Canali E, Leach MC. Development of the horse grimace scale (HGS) as a pain assessment tool in horses undergoing routine castration. *PLoS One*. (2014) 9:e92281. doi: 10.1371/journal.pone.0092281

46. De OAR, Gozalo-Marcilla M, Ringer SK, Schauvliege S, Fonseca MW, Trindade PH, et al. Development, validation, and reliability of a sedation scale in horses (Equi Sed). *Front Vet Sci*. (2021) 8:611729. doi: 10.3389/fvets.2021.611729

47. König V, Borstel U, Visser EK, Hall C. Indicators of stress in equitation. *Appl Anim Behav Sci*. (2017) 190:43–56. doi: 10.1016/j.applanim.2017.02.018

48. von Lewinski M, Biau S, Erber R, Ille N, Aurich J, Faure J-M, et al. Cortisol release, heart rate and heart rate variability in the horse and its rider: different responses to training and performance. *Vet J*. (2013) 197:229–32. doi: 10.1016/j.tvjl.2012.12.025

49. Peeters M, Closson C, Beckers J-F, Vandenheede M. Rider and horse salivary cortisol levels during competition and impact on performance. *J Equine Vet*. (2013) 33:155–60. doi: 10.1016/j.jevs.2012.05.073

50. Bohák Z, Szabó F, Beckers J-F, Melo de Sousa N, Kutasi O, Nagy K, et al. Monitoring the circadian rhythm of serum and salivary cortisol concentrations in the horse. *Domest Anim Endocrinol*. (2013) 45:38–42. doi: 10.1016/j.domaniend.2013.04.001

51. Becker-Birck M, Schmidt A, Lasarzik J, Aurich J, Möstl E, Aurich C. Cortisol release and heart rate variability in sport horses participating in equestrian competitions. *J Vet Behav*. (2013) 8:87–94. doi: 10.1016/j.jveb.2012.05.002

52. König von Borstel U, Euent S, Graf P, König S, Gauly M. Equine behaviour and heart rate in temperament tests with or without rider or handler. *Physiol Behav*. (2011) 104:454–63. doi: 10.1016/j.physbeh.2011.05.010

53. Visser EK, van Reenen CG, Rundgren M, Zetterqvist M, Morgan K, Blokhuis HJ. Responses of horses in behavioural tests correlate with temperament assessed by riders. *Equine Vet J*. (2003) 35:176–83. doi: 10.2746/042516403776114108

54. Visser EK, van Reenena CG, van der Werf JTN, Schilder MBH, Knaap JH, Barneveld A, et al. Heart rate and heart rate variability during a novel object test and a handling test in young horses. *Physiol Behav*. (2002) 76:289–96. doi: 10.1016/S0031-9384(02)00698-4

55. Christensen JW, Keeling LJ, Nielsen BL. Responses of horses to novel visual, olfactory and auditory stimuli. *Appl Anim Behav Sci*. (2005) 93:53–65. doi: 10.1016/j.applanim.2005.06.017

56. Munsters CC, Visser KE, van den Broek J, van Sloet Oldruitenborgh-Oosterbaan MM. The influence of challenging objects and horse-rider matching on heart rate, heart rate variability and behavioural score in riding horses. *Vet J*. (2012) 192:75–80. doi: 10.1016/j.tvjl.2011.04.011

57. Forkman B, Boissy A, Meunier-Salaün M-C, Canali E, Jones RB. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiol Behav*. (2007) 92:340–74. doi: 10.1016/j.physbeh.2007.03.016

58. Tateo A, Padalino B, Boccaccio M, Maggiolino A, Centoducati P. Transport stress in horses: effects of two different distances. *J Vet Behav*. (2012) 7:33–42. doi: 10.1016/j.jveb.2011.04.007

59. Dai F, Dalla Costa A, Bonfanti L, Caucci C, Di Martino G, Lucarelli R, et al. Positive reinforcement-based training for self-loading of meat horses reduces loading time and stress-related behavior. *Front Vet Sci*. (2019) 6:350. doi: 10.3389/fvets.2019.00350

60. Shanahan S. Trailer loading stress in horses: behavioral and physiological effects of nonaversive training (TTEAM). *J Appl Anim Welf Sci*. (2003) 6:263–74. doi: 10.1207/s15327604jaws0604_1

61. Santos Godoi TL, Villas-Boas JD, Almeida NA, Trigo PI, De AFQ, De MMA. Pharmacopuncture versus Acepromazine in stress responses of horses during road transport. *J Equine Vet*. (2014) 34:294–301. doi: 10.1016/j.jevs.2013.06.008

62. Eichler F, Ehrle A, Jensen KC, Baudisch N, Petersen H, Bäumer W, et al. Behavioral observations, heart rate and heart rate variability in horses following oral administration

of a cannabidiol containing paste in three escalating doses (part 1/2). *Front Vet Sci*. (2023) 10:1305868. doi: 10.3389/fvets.2023.1305868

63. Eichler F, Poźniak B, Machnik M, Schenk I, Wingender A, Baudisch N, et al. Pharmacokinetic modelling of orally administered cannabidiol and implications for medication control in horses. *Front Vet Sci*. (2023) 10:1234551. doi: 10.3389/fvets.2023.1234551

64. Ellis KL, Contino EK. Treatment using cannabidiol in a horse with mechanical allodynia. *Equine Vet Educ*. (2021) 33:33. doi: 10.1111/eve.13168

65. Cunha RZ, Felisardo LL, Salamanca G, Marchioni GG, Neto OI, Chiocchetti R. The use of cannabidiol as a novel treatment for oral stereotypic behaviour (crib-biting) in a horse. *Vet Anim Sci*. (2023) 19:100289. doi: 10.1016/j.vas.2023.100289

66. Turner PL, Guay KA, Jones T, Cohen L, Elwonger F, van Geem R, et al. 113 Cannabidiol supplementation and physiological response in transported horses. *J Anim Sci*. (2022) 100:33. doi: 10.1093/jas/skac028.063

67. Williams MR, Holbrook TC, Maxwell L, Croft CH, Ientile MM, Cliburn K. Pharmacokinetic evaluation of a Cannabidiol supplement in horses. *J Equine Vet*. (2022) 110:103842. doi: 10.1016/j.jevs.2021.103842

68. Yocom AF, O'Fallon ES, Gustafson DL, Contino EK. Pharmacokinetics, safety, and synovial fluid concentrations of single- and multiple-dose Oral administration of 1 and 3 mg/kg Cannabidiol in horses. *J Equine Vet*. (2022) 113:103933. doi: 10.1016/j.jevs.2022.103933

69. Draeger AL, Hoffman LK, Godwin PR, Davis AJ, Porr SA. *Pharmacokinetics of a single feeding of pelleted cannabidiol in horses*. Steeplechase: Murray State University (2020).

70. Schwark WS, Wakshlag JJ. A one health perspective on comparative cannabidiol and cannabidiolic acid pharmacokinetics and biotransformation in humans and domestic animals. *Am J Vet Res*. (2023) 84:1–9. doi: 10.2460/ajvr.23.02.0031

71. Turner SE, Knych HK, Adams AA. Pharmacokinetics of cannabidiol in a randomized crossover trial in senior horses. *Am J Vet Res*. (2022) 83:83. doi: 10.2460/ajvr.22.02.0028

72. Sánchez de Medina A, Serrano-Rodríguez JM, Díez de Castro E, García-Valverde MT, Saitua A, Becero M, et al. Pharmacokinetics and oral bioavailability of cannabidiol in horses after intravenous and oral administration with oil and micellar formulations. *Equine Vet J*. (2023) 55:1094–103. doi: 10.1111/evj.13923

73. Dalla Costa E, Bracci D, Dai F, Lebelt D, Minero M. Do different emotional states affect the horse grimace scale score? A pilot study. *J Equine Vet*. (2017) 54:114–7. doi: 10.1016/j.jevs.2017.03.221

74. Dalla Costa E, Stucke D, Dai F, Minero M, Leach MC, Lebelt D. Using the horse grimace scale (HGS) to assess pain associated with acute laminitis in horses (*Equus caballus*). *Animals (Basel)*. (2016) 6:6. doi: 10.3390/ani6080047

75. Kogan LR, Hellyer PW, Silcox S, Schoenfeld-Tacher R. Canadian dog owners' use and perceptions of cannabis products. *Can Vet J*. (2019) 60:749–55.

76. Kogan L, Schoenfeld-Tacher R, Hellyer P, Rishniw M. US Veterinarians' knowledge, experience, and perception regarding the use of Cannabidiol for canine medical conditions. *Front Vet Sci*. (2018) 5:338. doi: 10.3389/fvets.2018.00338

77. Kogan LR, Hellyer PW, Robinson NG. Consumers' perceptions of hemp products for animals. *J Am Holistic Vet Med Assoc JAHVMA*. (2016):42.

78. Boggs DL, Surti T, Gupta A, Gupta S, Niciu M, Pittman B, et al. The effects of cannabidiol (CBD) on cognition and symptoms in outpatients with chronic schizophrenia a randomized placebo controlled trial. *Psychopharmacology*. (2018) 235:1923–32. doi: 10.1007/s00213-018-4885-9

79. Irvine CH, Alexander SL. Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. *Domest Anim Endocrinol*. (1994) 11:227–38. doi: 10.1016/0739-7240(94)90030-2

80. Aurich J, Wulf M, Ille N, Erber R, Von LM, Palme R, et al. Effects of season, age, sex, and housing on salivary cortisol concentrations in horses. *Domest Anim Endocrinol*. (2015) 52:11–6. doi: 10.1016/j.domaniend.2015.01.003

81. Peeters M, Sulon J, Beckers J-F, Ledoux D, Vandenheede M. Comparison between blood serum and salivary cortisol concentrations in horses using an adrenocorticotropic hormone challenge. *Equine Vet J*. (2011) 43:487–93. doi: 10.1111/j.2042-3306.2010.00294.x

82. Schober P, Boer C, Schwarte LA. Correlation coefficients: appropriate use and interpretation. *Anesth Analg*. (2018) 126:1763–8. doi: 10.1213/ANE.0000000000002864

83. Roelfsema E. Endocrinological and behavioural adaptations to experimentally induced physical stress in horses. *Utrecht University: Thesis*. (2008):234.

84. Christensen JW, Rundgren M, Olsson K. Training methods for horses: habituation to a frightening stimulus. *Equine Vet J*. (2006) 38:439–43. doi: 10.2746/042516406778400574

85. Morris EM, Kitts-Morgan SE, Spangler DM, McLeod KR, Costa JH, Harmon DL. The impact of feeding Cannabidiol (CBD) containing treats on canine response to a noise-induced fear response test. *Front Vet Sci*. (2020) 7:569565. doi: 10.3389/fvets.2020.569565

86. Terry RL, McDonnell SM, van Eps AW, Soma LR, Liu Y, Uboh CE, et al. Pharmacokinetic profile and behavioral effects of gabapentin in the horse. *J Vet Pharmacol Ther*. (2010) 33:485–94. doi: 10.1111/j.1365-2885.2010.01161.x

87. Perinetti G. Sta tips part IV: selection, interpretation and reporting of the intraclass correlation coefficient. *sejodr*. (2018) 5:3–5. doi: 10.5937/sejodr5-17434

88. Kaiser L, Heleski CR, Siegford J, Smith KA. Stress-related behaviors among horses used in a therapeutic riding program. *J Am Vet Med Assoc*. (2006) 228:39–45. doi: 10.2460/javma.228.1.39

89. Lenoir A, Trachsel DS, Younes M, Barrey E, Robert C. Agreement between electrocardiogram and heart rate meter is low for the measurement of heart rate variability during exercise in Young endurance horses. *Front Vet Sci*. (2017) 4:170. doi: 10.3389/fvets.2017.00170

90. Reid K, Rogers CW, Gronqvist G, Gee EK, Bolwell CF. Anxiety and pain in horses measured by heart rate variability and behavior. *J Vet Behav*. (2017) 22:1–6. doi: 10.1016/j.jveb.2017.09.002

91. Borell E von, Langbein J, Després G, Hansen S, Leterrier C, Marchant J, et al. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals -- a review. *Physiol Behav*. (2007) 92:293–316. doi: 10.1016/j.physbeh.2007.01.007

92. Hernández-Avalos I, Mota-Rojas D, Mendoza-Flores JE, Casas-Alvarado A, Flores-Padilla K, Miranda-Cortes AE, et al. Nociceptive pain and anxiety in equines: physiological and behavioral alterations. *Vet World*. (2021) 14:2984–95. doi: 10.14202/vetworld.2021.2984-2995

93. Gehlen H, Faust M-D, Grzeskowiak RM, Trachsel DS. Association between disease severity, heart rate variability (HRV) and serum cortisol concentrations in horses with acute abdominal pain. *Animals (Basel)*. (2020) 10. doi: 10.3390/ani10091563

94. Gehlen H, Loschelder J, Merle R, Walther M. Evaluation of stress response under a standard euthanasia protocol in horses using analysis of heart rate variability. *Animals (Basel)*. (2020) 10:10. doi: 10.3390/ani10030485

95. Rietmann TR, Stuart A, Bernasconi P, Stauffacher M, Auer JA, Weishaupt MA. Assessment of mental stress in warmblood horses: heart rate variability in comparison to heart rate and selected behavioural parameters. *Appl Anim Behav Sci*. (2004) 88:121–36. doi: 10.1016/j.applanim.2004.02.016

96. Rietmann TR, Stauffacher M, Bernasconi P, Auer JA, Weishaupt MA. The association between heart rate, heart rate variability, endocrine and behavioural pain measures in horses suffering from laminitis. *J Vet Med A Physiol Pathol Clin Med*. (2004) 51:218–25. doi: 10.1111/j.1439-0442.2004.00627.x

97. Lee KE, Kim JG, Lee H, Kim BS. Behavioral and cardiac responses in mature horses exposed to a novel object. *J Anim Sci Technol*. (2021) 63:651–61. doi: 10.5187/jast.2021.e51

98. Waran NK, Robertson V, Cuddeford D, Kokoszko A, Marlin DJ. Effects of transporting horses facing either forwards or backwards on their behaviour and heart rate. *Vet Rec*. (1996) 139:7–11. doi: 10.1136/vr.139.1.7

99. Schmidt A, Biau S, Möstl E, Becker-Birck M, Morillon B, Aurich J, et al. Changes in cortisol release and heart rate variability in sport horses during long-distance road transport. *Domest Anim Endocrinol*. (2010) 38:179–89. doi: 10.1016/j.domaniend.2009.10.002

100. Hunt AB, Flint HE, Logan DW, King T. A single dose of cannabidiol (CBD) positively influences measures of stress in dogs during separation and car travel. *Front. Vet. Sci*. (2023) 10:1112604. doi: 10.3389/fvets.2023.1112604

101. Appiah-Kusi E, Petros N, Wilson R, Colizzi M, Bossong MG, Valmaggia L, et al. Effects of short-term cannabidiol treatment on response to social stress in subjects at clinical high risk of developing psychosis. *Psychopharmacology*. (2020) 237:1121–30. doi: 10.1007/s00213-019-05442-6

102. Mongeau-Pérusse V, Rizkallah E, Morissette F, Brissette S, Bruneau J, Dubreucq S, et al. Cannabidiol effect on anxiety symptoms and stress response in individuals with cocaine use disorder: exploratory results from a randomized controlled trial. *J Addict Med*. (2022) 16:521–6. doi: 10.1097/ADM.0000000000000959

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Healthy cats tolerate long-term daily feeding of Cannabidiol

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Cannabidiol (CBD)-containing products are widely commercially available for companion animals, mirroring popularity in human use. Although data on the safety and efficacy of long-term oral supplementation are increasing in dogs, evidence remains lacking in cats. The purpose of these studies was to address gaps in the knowledge around the long-term suitability and tolerance of a tetrahydrocannabinol (THC)-free CBD distillate in clinically healthy cats. The studies were randomized, blinded, and placebo-controlled. The first study supplemented cats with either a placebo oil ($n = 10$) or with 4 mg/kg body weight (BW) CBD in placebo oil ($n = 9$) daily, with a meal, for 4 weeks. The concentration of CBD in plasma was measured over 4 h at d0 (first dose) and again at d14 (after 2 weeks of daily dosing). The second study supplemented cats daily with either placebo oil ($n = 10$) or 4 mg/kg BW CBD in placebo oil ($n = 10$) for a period of 26 weeks. A comprehensive suite of physiological health measures was performed throughout the study at baseline (week 0) and after 4, 10, 18, and 26 weeks of feeding, followed by a 4-week washout sample (week 30). Postprandial plasma CBD time course data, at both d0 and d14, showed a peak plasma CBD concentration at 2 h after the dose. This peak was 251 (95% CI: 108.7, 393.4) and 431 (95% CI, 288.7, 573.4) ng/mL CBD at d0 and d14, respectively, and the area under the curve concentration was higher by 91.5 (95% CI, 33.1, 149.9) ng-h/mL after 2 weeks of supplementation ($p = 0.002$). While in the first study the CBD group displayed increased alanine aminotransferase (ALT; 68.7 (95% CI, 43.23, 109.2) U/L) at week 4 compared to the placebo control group [1.44-fold increase (95% CI, 0.813, 2.54)], statistical equivalence (at 2-fold limits) was found for ALT across the duration of the second, long-term study. All other biochemistry and hematology data showed no clinically significant differences between supplement groups. Data presented here suggest that a THC-free, CBD distillate fed at a dose of 4 mg/kg BW was absorbed into plasma and well tolerated by healthy cats when supplemented over a period of 26 weeks.

KEYWORDS

cannabidiol, cat, safety, CBD, cannabinoids, feline

1 Introduction

Cannabis sativa, also known as hemp, contains hundreds of phytocompounds including cannabidiol (CBD), tetrahydrocannabinol (THC), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), and cannabivarin (CBDV) to name a few (1, 2). These compounds differ in their chemical properties and physiological impacts. CBD is the non-psychotropic, and main, component of *C. sativa*, receiving a wealth of interest over recent years due to its potential for

anti-inflammatory, anti-oxidative, neuroprotective, and anti-anxiety effects (3). As such it has made for a promising candidate in many therapeutic areas such as pain management in osteoarthritis, epilepsy, Alzheimer's disease, multiple sclerosis, and anxiety in humans (3), benefits which may translate to animals (4). CBD, therefore, shows efficacy for a wide variety of conditions which act via numerous pathways linked to the endocannabinoid system (5), and these have been collectively labeled the endocannabinoidome (6), indicating there are several potential modes of action. These include, but are not limited to, G-protein-coupled cannabinoid receptors type 1 and 2 (CB1 and CB2), transient receptor vanilloid type-1 (TRPV1) channel, G-protein-coupled receptor 55 (GPR55) or 119 (GPR119), and peroxisome proliferator-activated receptors (PPAR)α and γ (5). There are also promising effects of CBD in the treatment of neurodevelopmental disorders such as schizophrenia directly and indirectly via dopamine receptors (7). THC, a psychoactive component of *C. sativa*, is found in small quantities (lower than 0.3%) in hemp extracts (3). When present in combination with CBD during companion animal trials, THC is thought to lead to the observation of more severe dose-dependent adverse events (8).

To date, no regulatory body has deemed the current safety and efficacy literature surrounding CBD sufficient for pets (9). Despite this, the use of CBD products in pets has increased as they have gained traction in the human market (10). Recent publications have demonstrated that 4 mg CBD/kg BW per day administered over a 6-month period in dogs is well tolerated (11) and that a single 4 mg/kg BW dose reduces anxiety during a car journey or separation test (12). Another study evaluating the effect of long-term supplementation of a CBD-rich dose in beagles found that it was generally well tolerated (13). However, due to higher frequency of abnormal fecal scores and a higher alkaline phosphatase (ALP), the authors advised extra caution at a daily 10 mg/kg BW dose compared to 5 mg/kg BW (13). In contrast, very little information exists on the safety of CBD for cats, and there is no current literature on its efficacy in the treatment of disorders.

In one feline CBD tolerance study, eight cats were fed capsules containing 2 mg/kg CBD in fish oil (50:50 mix of CBD and CBDA) twice a day for 12 weeks (14). All biochemistry data were found to be within normal ranges with the exception of one cat which had elevated alanine aminotransferase (ALT) during the treatment, and no further information on health of the cat was provided. The authors heavily caveated that the lack of a control group limited the ability to know whether any of these effects were due to the CBD dose, the carrier oil, or other environmental factors (14). Pharmacokinetic data from the same manuscript established that CBD could be detected in serum for up to 8 h. In another recently published study, CBD pharmacokinetics showed a mean peak CBD value of 282 mg/mL at 2 h following a CBD dose of 1.37 mg/kg (15). These cats were dosed twice a day with a paste comprised of mainly CBD and CBDA (6.4 mg/g and 5.3 mg/g, respectively), with THC, THCA, CBG, and CBGA included at 25-fold lower amounts, and meals were fed 1 h after dosing (15). When comparing dog and cat, data suggest that CBD has a lower bioavailability in cats compared to dogs but with a similar half-life (16). Although food is known to increase bioavailability of CBD in humans (17), there have been no postprandial investigations of CBD distillate given in low doses concurrently with a meal in cats to understand whether this finding is translatable. The CBD-containing anti-seizure drug Epidiolex®, when given to fasted and fed cats in a cross-over design study at a dose of 5 mg/kg BW, identified a higher area under the curve and maximum concentration of CBD in the plasma of fed cats (18).

Here, we describe the findings of a 6-month tolerance study of a single daily 4 mg/kg dose of a THC-free, CBD distillate and an additional four-week postprandial plasma CBD time course study in healthy adult cats.

2 Materials and methods

2.1 Animals and husbandry

Two studies were reviewed and approved by the Waltham Animal Welfare and Ethical Review Body and conducted under the authority of the Animals (Scientific Procedures) Act 1986. To ensure suitability for the study, the cats underwent a pre-study health assessment, including a physical examination by a registered Veterinary Surgeon and hematological, plasma biochemical, and urine analysis to confirm the absence of underlying conditions. Cats were housed at the Waltham Petcare Science Institute, grouped in social rooms under routine husbandry conditions and were extensively trained for and habituated to all procedures. Cats were observed within these social rooms for feces and free-catch urine collections. For all blood samples, cats were given topical local anesthesia (1 mL EMLA™ cream 5%; AstraZeneca) prior to either jugular or cephalic blood draws. Where a cephalic sample was obtained, a 22G catheter was positioned, which remained in place for the duration of the sampling period. On the day of sampling, cats were allowed to return to their social rooms and monitored closely for any welfare concerns (i.e., pulling out the catheter or scratching at the jugular area). Cats' health was monitored via weekly (study one: postprandial plasma CBD time course) and fortnightly (study two: tolerance test) physical health and biochemistry, hematology, and urinalysis data reviews with Veterinary Surgeons who were blinded to the groups. Throughout the study, commercial single batch (Royal Canin® Instinctive wet and Royal Canin® Fit-32 dry format) diets were offered in amounts required to maintain an ideal body weight (BW) and body condition score (BCS), assessed according to a 9-point scale. These were used to calculate individual MER (19). The diets underwent nutrient analysis (Eurofins, United Kingdom), and both were confirmed as complete and balanced according to minimum requirements set by the Association of American Feed Control Officials (AAFCO). Water was available *ad libitum*.

Study one, for postprandial plasma CBD time course: 19 healthy adult cats took part in a 4-week study (8 female cats and 11 male cats, age 1.4 to 10.1 years, and weight between 3.42 kg and 5.58 kg).

Study two, to assess long-term tolerance: 20 healthy adult cats took part in a 26-week study, (6 female cats and 14 male cats, age 2.1 to 10.8 years, and weight between 3.39 kg and 5.77 kg). Sixteen of these 20 cats had previously participated in the pharmacokinetic profiling study, with a washout of 9 weeks between studies.

2.2 CBD description and dosing

Hemp-derived distillate and placebo oil were acquired from Kazmira LLC (Colorado, United States). The CBD oil was analyzed by

a third-party laboratory for full-spectrum analysis of cannabinoid content (including CBD and THC), potential contaminants, and potency (Botanacor Laboratories, Colorado, United States). The THC content was below the limit of analytical detection (<0.02 mg/mL), and no other cannabinoids were detected except for trace amounts (estimated at 0.17 mg/mL) of cannabidivarin, below the limit of quantification (0.32 mg/mL). The distillate was diluted with a food-grade sunflower oil and flavored with 1% rotisserie chicken type, natural flavor blend (Apex Flavors, Inc. Maryland, United States) to provide CBD at a final concentration of 43.76 mg/mL. The placebo oil was the food-grade sunflower oil with 1% rotisserie chicken type, natural flavor blend (Apex Flavors, Inc. Maryland, United States). Each cat was provided with 8 g "bolus" of a commercial pate (Purina® Gourmet Gold) food with the supplement incorporated to provide a dose of 4 mg/kg BW (the placebo oil amount was calculated as if it was the concentration of the CBD oil). The bolus was offered once a day, prior to the morning meal, and consumption was recorded and monitored.

2.3 Study design

Both studies were blinded. Cats were randomized and balanced across two parallel treatment groups: CBD and placebo. When balancing the groups, age, sex, and housing location were considered. The cats were then split into two staggers for logistical ease (10 cats per stagger group, 4–6 cats in each treatment group), with a 1-week offset between stagger groups for trial initiation and collection of samples. To accurately dose CBD, cats were weighed weekly.

Cats were fed in a wet food am, dry food pm feeding regimen for 4 weeks before a baseline blood sample was collected for each study, and this feeding pattern was then continued for the duration of the study.

2.3.1 Study one: postprandial plasma CBD time course

Following the collection of an overnight fasted (>14 h) blood sample (max 4.1 mL) on day 0 (first CBD dose) and day 14 (after 2 weeks of daily supplementation), the cats were orally dosed with their CBD or placebo oil, mixed with 1 mL of Sheba® creamy snack (now called Dreamies® Creamy) from a needle-less syringe. These were willingly consumed, and the full dose was administered before the rest of the samples were collected to determine CBD concentrations in the plasma at 1 h, 2 h, and 4 h post-CBD dose and morning meal. On all other days, cats were offered the pate bolus with supplement. Fasted samples were also collected at week 4 without further postprandial sampling.

2.3.2 Study two: long-term tolerance test

Overnight fasted (>14 h) blood samples (max 4.8 mL) were collected at weeks 0, 4, 10, 18, and 26. An additional blood sample (week 30) was collected 4 weeks after supplementation was ceased. A blood sample was collected for a veterinary health check at week 2, and this was not analyzed as part of the trial data set. For logistical reasons, feces and urine samples were collected between 3 and 9 days after blood sampling (at weeks 0, 10, 18, and 26) for urinalysis (urine only) and CBD analysis.

2.4 Measures and analyses

2.4.1 Blood-based measurements

Lithium heparin-treated blood was centrifuged at 2,000 g, and the resulting plasma was used for the determination of standard biochemistry parameters: total protein, albumin, inorganic phosphate, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium, cholesterol, urea, creatinine, triglycerides, magnesium, sodium, potassium, chloride, and glucose, using an AU480 analyzer (Beckman Coulter, United States). EDTA-treated blood was collected for the measurement of standard hematology parameters using a three-part differential automated hematology analyzer (IDEXX ProCyte Dx, Buckinghamshire, United Kingdom). Parameters measured were total leukocyte count, differentiated leukocyte counts as a number and percentage (neutrophils, eosinophils, basophils, lymphocytes, and monocytes), total erythrocyte count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, erythrocyte distribution width, platelet count, and mean platelet volume. EDTA-treated blood was also collected for CBD quantification. Serum clot activated blood was centrifuged and stored at 4°C before analysis by IDEXX Laboratories (United Kingdom), and total bilirubin, gamma-glutamyl transferase (GGT), and fasted bile acids were measured using an AU5800 clinical chemistry analyzer (Beckman Coulter; United States). Additionally at baseline and 26 and 30 weeks, serum clot activated blood was used to evaluate markers of bone turnover: bone-specific alkaline phosphatase (BALP) and carboxy-terminal telopeptide cross-links (CTx) using MicroVue™ BALP ELISA kit (Quidel®, United States) and Serum CrossLaps CTX-I ELISA kit (Immunodiagnostic Systems Limited; United Kingdom), respectively. Both assays were performed according to the manufacturer's instructions on a Synergy HT plate reader (Agilent Technologies, United States) and have sensitivity limits of 0.7 U/L for BALP and 0.02 ng/mL CTX. Reference ranges, where shown, refer to those published by Antech® Diagnostic Laboratories for biochemistry analysis and IDEXX Laboratories (United Kingdom) for complete blood count and liver panel (Bilirubin, GGT and Bile Acids) data.

2.4.2 Urinalysis

Urine was collected (min 3 mL total volume) using a free-catch method with a uripet (Fisher Scientific; United Kingdom), 1 week after the blood sample timepoints, i.e., at weeks 1, 11, 19, and 27. Urine-specific gravity was measured using a refractometer (J.A.K. Marketing Ltd., United Kingdom), and glucose, bilirubin, ketone, specific gravity, blood, pH, protein, urobilinogen, nitrite, and leukocytes were analyzed using the Status Plus Analyzer with Multistix® 10SG urine test strips (Siemens Healthcare Limited; United Kingdom). An aliquot of urine was also processed for CBD quantification.

2.5 Feces collection

A single fresh fecal sample was collected alongside urine at weeks 1, 11, 19, and 27, a core sample was obtained, and CBD levels were quantified.

2.6 CBD extraction and mass spectrometry analysis

Extraction and analysis of CBD in samples were adapted from the method used by Vaughn et al. (20), fully validated in-house (11). In brief, for urine samples, the internal standard (300 µL of 40 ng/mL, CBD-d3) was aliquoted into 100 µL of feline urine and vortexed for 5 s. Samples were centrifuged (13,201 g for 5 min), and 325 µL of the supernatant was aliquoted into a labeled glass amber vial containing 650 µL 0.1% formic acid in water. The sample was vortexed again (5 s) and analyzed as described. For feline fecal samples, the internal standard (750 µL of 300 ng/mL, CBD-d3) was aliquoted into microfuge tubes containing 0.25 g (±0.01 g) of feces and vortexed for 30 min. Samples were centrifuged (2,292 g for 10 min), and 390 µL of the supernatant was aliquoted into a fresh tube containing 780 µL of 0.1% formic acid in water and vortexed (5 s) and centrifuged for a second time (17,968 g for 10 min). The supernatant was aliquoted to a labeled glass amber vial, vortexed again (5 s), and analyzed as described. For plasma, the internal standard (60 µL of 40 ng/mL, CBD-d3) was aliquoted into 20 µL of feline plasma and vortexed for 5 s. Samples were centrifuged (13,201 g for 5 min),and 65 µL of the supernatant was aliquoted into a labeled glass amber vial containing 130 µL 0.1% formic acid in water. The sample was vortexed again (5 s) and analyzed as described.

A liquid chromatograph coupled with triple quadrupole mass spectrometer (Agilent 6460C LC-QQQ-MS, Agilent, United States) was used for analysis. A Kinetex 2.6 µm Phenyl-Hexyl 100A, 50 × 2.1 mm column was used in conjunction with an X3 SecurityGuard ULTRA Cartridge ultra-high performance liquid chromatography (UHPLC) phenyl column guard (Phenomenex, Cheshire, United Kingdom). The mobile phase was delivered at a flow rate of 0.4 mL/min, and the gradient parameters were as follows (solvent A was 0.1% formic acid in ultra-high quality (UHQ) water, and solvent B was 0.1% formic acid in acetonitrile): 0 min: 30% B, 5.3 min: 95% B, 6.3 min: 70% B. The scanning conditions were in multiple reaction monitoring (MRM) mode. Cannabidiol (CBD) and Cannabidiol-D3 (CBD-d3, used as an internal standard) certified reference materials were obtained from Fisher (Loughborough, United Kingdom). Samples were analyzed against a set of 10 linearity standards between 0.25 and 2,000 ng/mL CBD, each prepared with CBD-d3 to a final concentration of 10 ng/mL.

2.7 Statistical analysis

2.7.1 Power analysis

The sample size for this study was determined through *a priori* power analysis by simulation, for the primary measure of ALT. Adult cat ALT measurements from historical data sets were used to estimate the within- and between-cat variance components. Using these variance components, data sets were simulated in the design described for the tolerance study (parallel with 2 treatments, 6 timepoints including baseline) for a range of cat numbers. For each cohort size, 1,000 data sets were simulated, and the analysis and planned comparisons described below were applied to each. The power was calculated as the percentage of the 1,000 data sets where equivalence could be declared at 2-fold limits, using a significance level of 5%, given that no difference between the treatment groups or timepoints had been induced.

The estimated sample size required to achieve 80% power was 12 cats (6 per treatment group). Given the nature of the study and potential for study fatigue, the total sample size was inflated to 20 cats (10 per treatment group). This powering was used for both study 1 and study 2.

2.7.2 Alanine aminotransferase

For ALT, a linear mixed model was fit to the log10 concentration, with treatment group, timepoint (i.e., weeks on trial), and their interaction as categorical fixed effects, and a random intercept for animal. Within each treatment group, comparisons between baseline and each subsequent timepoint were tested, and at each timepoint, a comparison between treatment groups was also tested. All comparisons were tested for equivalence at 2-fold limits using two one-sided tests (TOSTs) at a 5% significance level, adjusted for family-wise error-rate (FWER; using the 'single-step' method of the R package "multcomp" implemented through the glht function). Note that FWER adjustment was made according to the number of contrasts performed, where contrasts are defined as each pair of TOSTs, due to the requirement that both tests were significant to infer equivalence. Significant *p*-values for the tests are reported, alongside the back-transformed estimates of the difference (i.e., fold changes) with 95% confidence intervals in each case. Back-transformed estimates of the mean and FWE-corrected 95% confidence intervals are also provided for each treatment/timepoint.

2.7.3 Secondary measures

For secondary measures, excluding those with insufficient samples (LIH, GGT, PDW, P_LCR) or insufficient sample variability (BCS), linear mixed models were fit with the same fixed and random effect structures as for ALT. Assumptions of normality were assessed through visual inspection of residuals, and, if this assumption was deemed to be violated, the response variable was log10 transformed. Pairwise planned comparisons between groups at each timepoint and between baseline and each subsequent timepoint for each group were tested for differences at a 5% significance level, with multiplicity correction (FWE, 'single-step') applied within but not across models. Significant *p*-values are reported alongside the corresponding difference estimates and 95% confidence intervals in each case. Estimates of the mean and 95% confidence intervals are also provided for each treatment/timepoint. Fasted CBD in plasma, for the CBD group weeks 2 to 26, was modeled with a sole categorical fixed effect of timepoint, a random intercept for animal, plus the incorporation of variance weights by timepoint, due to anticipated differences in plasma CBD variability over time. All values below LOD were imputed. Means and adjusted 95% confidence intervals are reported for modeled data, alongside raw data for all groups and timepoints. Pairwise contrasts are not reported.

2.7.4 CBD concentrations in blood plasma and AUC

Both CBD concentrations in blood plasma (at each timepoint) and area under the curve (AUC calculated per hour and over all 4 h from ingestion) were analyzed as further outcome variables. AUC was calculated using a linear trapezoidal method though custom code implemented in R:

$$AUC = \frac{1}{2}(C_1C_2)(t_2 - t_1)$$

Due to measurements for the placebo group being unanimously below the LOD, data for this group were excluded from these analyses. For AUC, a linear mixed model was fit to data for CBD treatment group only, untransformed, with timepoint (i.e., Weeks 0 vs. 2) as the only fixed effect, and a random intercept for animal. For raw CBD concentration, a linear mixed model was again fit to data for the CBD treatment group only, untransformed, with timepoint (i.e., Week 0 vs. 2), hour (0 vs. 1 vs. 2 vs. 4, categorically coded), and their two-way interaction as fixed effects, plus a random intercept for animal. For the raw CBD model, variance weighting was applied (using the R package 'nlme') due to heteroscedasticity between timepoints (i.e., variance was much lower at hour 0). Pairwise comparisons between timepoints are reported, and FWE-corrected 95% CIs are plotted for visual comparison against the LOD (12.0).

2.7.5 Statistical software and packages

All analyses were performed in R version 4.2.2 (2022-10-31), the R Foundation for Statistical Computing (21). Packages necessary for analysis were lme4 (22), nlme (23), and multcomp (24).

3 Results

3.1 Postprandial plasma CBD time course study

3.1.1 Observations from study

At the 4-week timepoint, three cats were noted as having unusual responses. One cat had blood in urine with no other signs of illness, one cat had asymptomatic high ALT and AST, and the third cat had inappetence, pyrexia, and high ALT and AST. Both cats showing increased ALT and AST were in the CBD group and were subsequently removed from consideration for the long-term study. Veterinary consultation determined the presence of infection in the pyrexic cat. Due to identification of a likely treatment-unrelated cause for this individual's elevated responses, their data were removed from the study prior to statistical analysis. Data recorded from the other two cats were included in the analysis.

3.1.2 Bodyweight and food intake

All cats completing the study remained within 6% of their starting weight. The dosing of the supplement in a small amount of pate food was generally accepted by the cats. Five cats in total did not consume the whole bolus on all offered occasions. Two cats refused the full 8 g pate bolus (containing the supplement): one in the CBD group on one occasion and the other in the placebo group on 2 non-consecutive days. There were 15 partial refusals involving 5 g of pate bolus or less over the study, split between five cats (three in placebo and two in CBD group). The three cats in the placebo group accounted for 13 of the partial refusals.

3.1.3 Postprandial plasma CBD time course after first dose and after 2 weeks of dosing

At d0 (week 0) and week 2, the mean peak CBD concentration in the plasma occurred 2 h after dosing ($p < 0.001$; Figure 1A). The mean fasted (hour 0) CBD concentration was higher at week 2 than d0 ($p < 0.001$; Figure 2A), showing that CBD remains detectable in the plasma for up to 24 h after dosing. Although there were no significant differences postprandially (h 1, 2, and 4) between week 2 and d0 concentrations ($p \geq 0.12$, Figure 2A), area under the curve data, collected over the 4 h sample period, were significantly higher at week 2 (246.9 ng-h/mL, 95% C.I: 188.4, 305.5) than at first dose (155.4 ng-h/mL, 95% C.I: 96.9, 214.0, $p = 0.002$, Figure 2B). Over the full 4 h time course, the AUC data were 621.7 (95% C.I: 387.5, 855.9) and 987.7 ng/mL (95% C.I: 753.5, 1222.0) for first dose and at week 2, respectively. At week 4, the mean fasted CBD concentration was 16.32 (range 10.8 to 28.03) ng/mL (data not shown).

3.1.4 Liver health parameters

For the CBD treatment group, two one-sided tests (TOSTs) failed to verify that the week 4 mean ALT concentration was below the

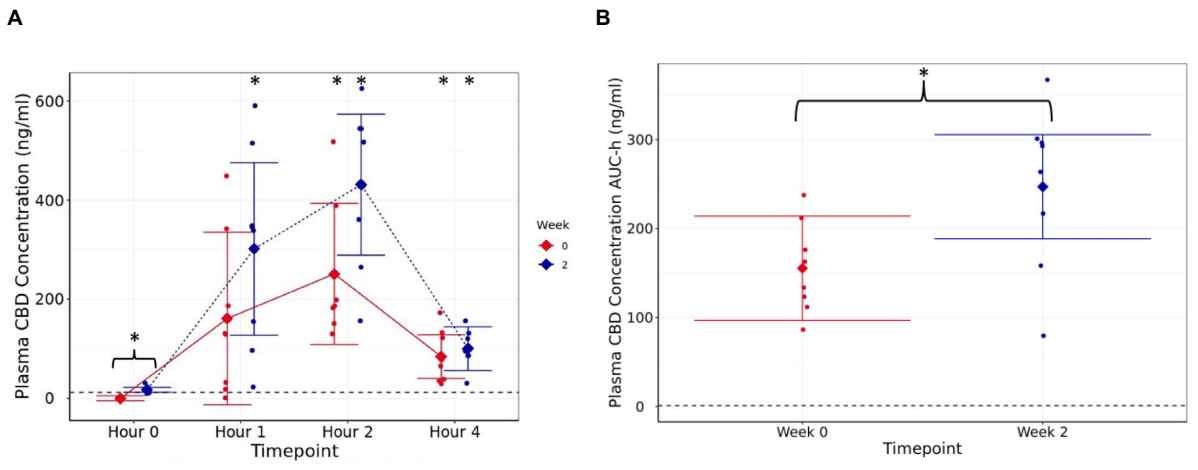

FIGURE 1
Plasma CBD concentration (ng/mL) **(A)** estimated means with 95% confidence intervals, at h 0 (fasted baseline), h 1, 2, and 4 measured at week 0 (d0, first supplement of 4 mg/kg BW, solid line) and week 2 (after 14 days of daily supplementation at 4 mg/kg BW, dotted line), **(B)** area under the curve (per hour) over the 4-h pharmacokinetic period for each timepoint (weeks). Dashed line shows limit of detection. * shows difference between the post-dose timepoint, and h 0 is statistically significant. The presence of a "}" in addition indicates the difference is between week 0 and week 2.

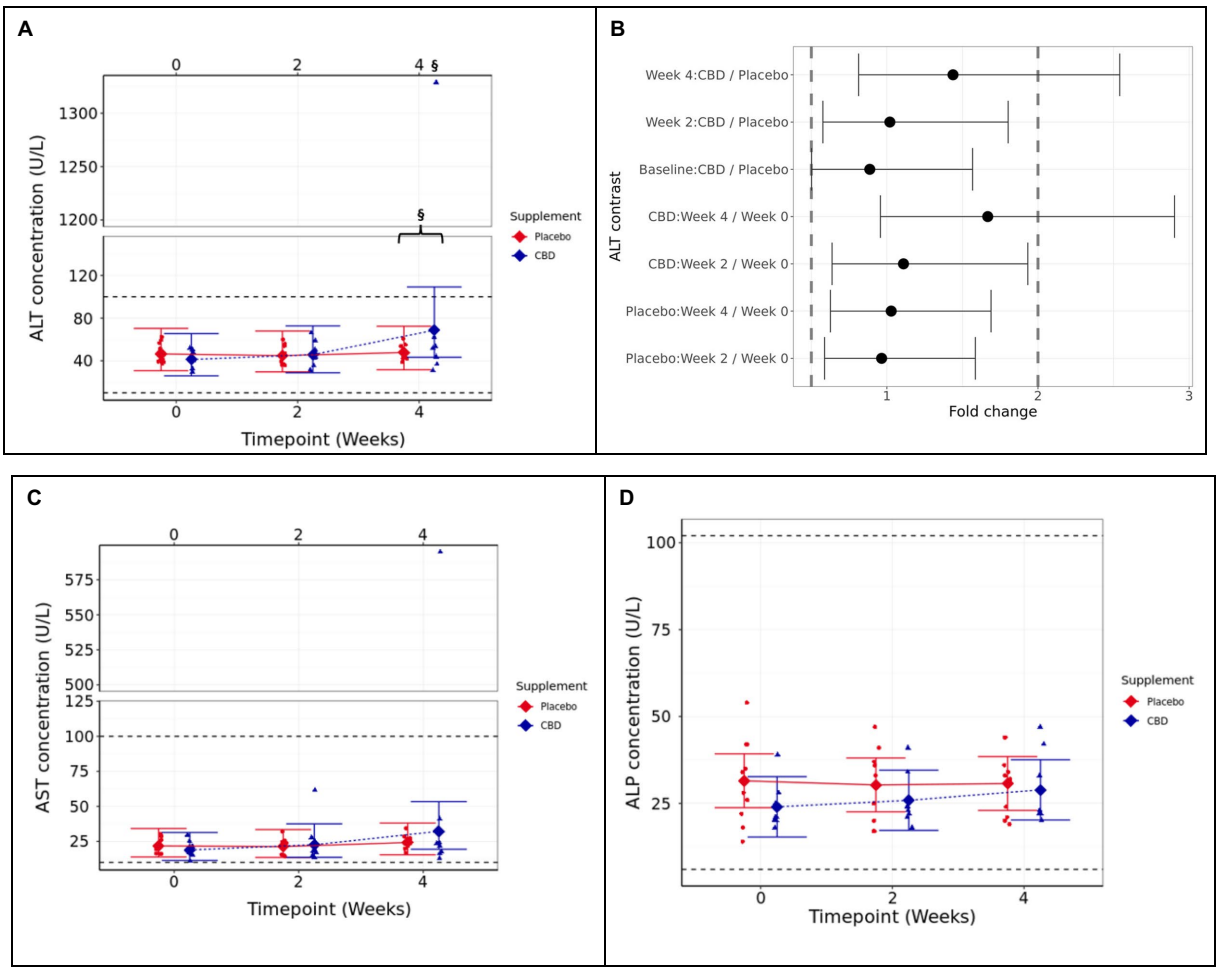

FIGURE 2
Means and 95% confidence intervals (C.I.) for fasted plasma measures over the 4-week study **(A)** ALT (U/L), **(B)** two one-sided test (TOST) fold change contrast plot, **(C)** AST (U/L), and **(D)** ALP (U/L). § indicates that equivalence between the timepoint and week 0 is not supported ($p \geq 0.05$). The presence of a "}" in addition indicates non-equivalence between CBD treatment and placebo groups. Reference ranges included as dashed horizontal lines across the figure. TOST thresholds are shown with dashed vertical lines. Statistical equivalence is indicated by CIs falling entirely within these bounds (true for all contrasts except Week 4: CBD vs. Placebo, and CBD: Week 4 vs. 0). Statistical difference is indicated by CIs falling entirely to the left or right of 1 fold change. Liver health parameters.

upper 2-fold limit compared to either week 0 or the placebo control group ($p \geq 0.388$; Figures 3A,B). The results indicate that cats in the CBD treatment group had higher mean ALT concentration at week 4 (1.667 fold change) compared to week 0 and also compared to placebo control cats (1.438 fold change).

Aspartate aminotransferase (AST) followed a similar trend to ALT data, and the CBD-treated cat that showed extremely high ALT also had a high AST value. However, this did not result in a significant difference between groups at week 4 ($p = 0.81$), nor between week 4 and baseline ($p = 0.112$; Figure 3C). Alkaline phosphatase (ALP) was also found not to significantly differ between treatment groups ($p \geq 0.447$) or over time ($p \geq 0.062$; Figure 3D). Bilirubin and fasted bile acids were not significantly different between groups (Supplementary data S1).

3.2 Long-term tolerance study

3.2.1 Observations and/or removals from study

Three cats in total were removed from trial by week 10: one for consistently poor behavior during sampling (from week 4), one for high ALT (from week 4), and one for inappetence, high fasted bile acids, and high ALT (week 10). Veterinary consultation determined the presence of infection in both cats removed from trial for high ALT: one was from the placebo group and the other from the CBD group. Data from these three cats were incomplete and therefore excluded from statistical analysis.

3.2.2 Bodyweight and food intake

All cats completing the study remained within 11% of their starting weight. The rate of bolus refusal was less than 1.5% of total offerings over the duration of the study. One cat in the CBD group fully refused the bolus on one occasion. In addition, five cats had partial refusals of the bolus: two in the placebo group (1 partial refusal each) and three cats from the CBD group. Two of the cats in the CBD group partially refused on 15 and 7 occasions, respectively, and the other cat partially refused on 1 occasion.

3.2.3 Liver health parameters

Mean values of ALT, the primary study measure, were found to be statistically equivalent at 2-fold limits for each tested pairwise

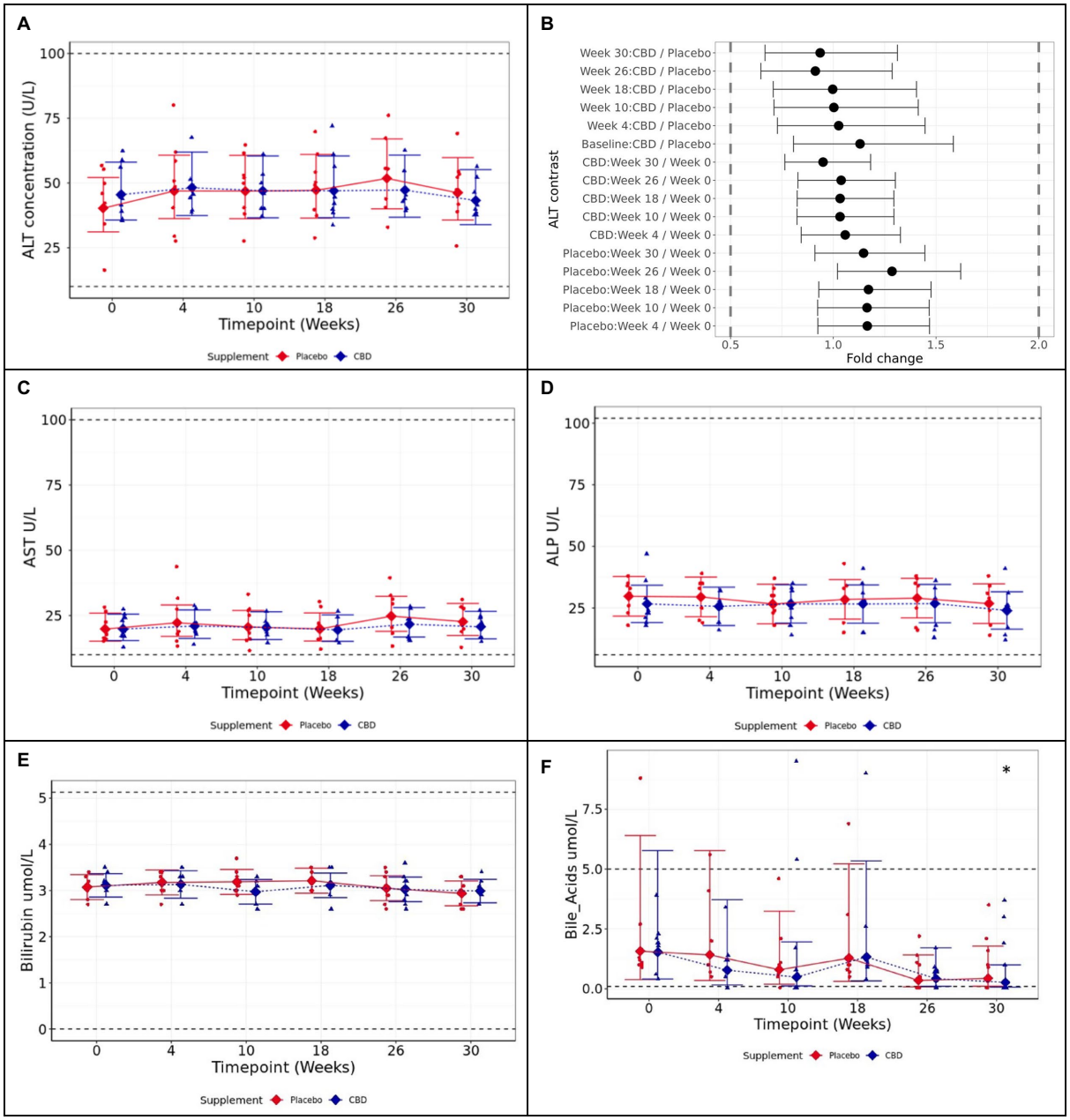

FIGURE 3
Means and 95% confidence intervals (C.I.) fasted plasma measures over the study **(A)** ALT (U/L), **(B)** two one-sided test (TOST) fold change contrast plot, **(C)** AST (U/L), **(D)** ALP (U/L), **(E)** bilirubin (μmol/L), and **(F)** bile acids (μmol/L). * shows difference between the timepoint, and week 0 is statistically significant ($p < 0.05$). Reference ranges included as dashed horizontal lines across the figure. TOST thresholds are shown with dashed vertical lines. Statistical equivalence is indicated by CIs falling entirely within these bounds (true for all contrasts). Statistical difference is indicated by CIs falling entirely to the left or right of 1 fold change.

contrast, both between the placebo and CBD groups over the 26-week supplemented phase ($p < 0.001$), and by comparison with the respective week 0 baseline for each group ($p < 0.001$; Figures 4A,B). AST was found to be significantly increased at week 26 compared to baseline in placebo group cats, although all values remained within physiological reference range ($p = 0.02$; Figure 4C). ALP did not significantly differ over time within either group, or between groups at any timepoint ($p \geq 0.89$; Figure 4D).

Bilirubin did not significantly differ between groups or over the course of the study in either group ($p \geq 0.725$; Figure 4E). Fasted bile acids also did not significantly differ between CBD and placebo-treated groups across the study ($p \geq 0.997$); however, CBD cats showed significantly reduced bile acid concentration at week 30 (washout) compared to baseline ($p = 0.019$; Figure 4F). For other biochemistry, hematology, bone-specific alkaline phosphatase (BALP), and carboxy-terminal telopeptide cross-link (CTx) data, there were no significant differences of clinical importance between the treatment groups. All measures were within normal reference ranges, with the exception of cholesterol, sodium:potassium ratio, mean platelet volume (MPV), and eosinophil count (Supplementary Tables S2, S3).

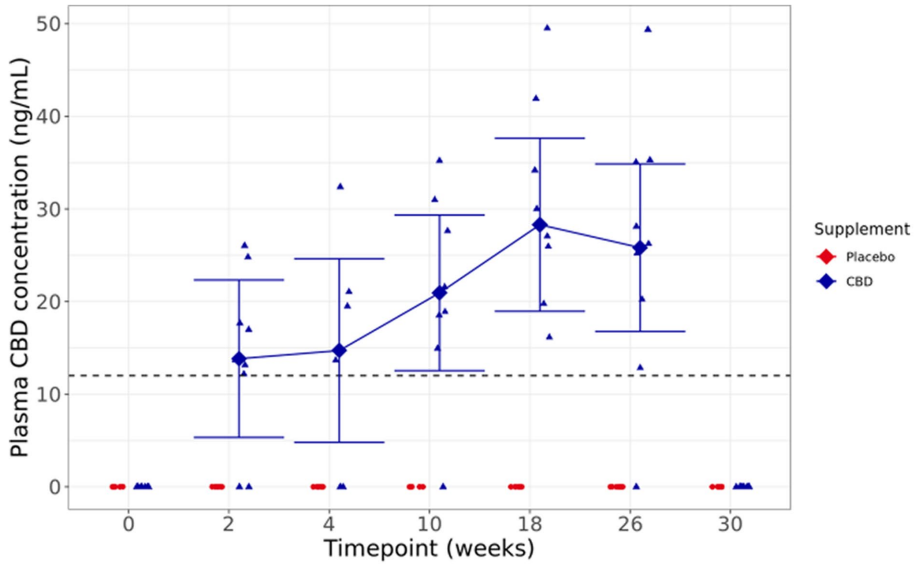

FIGURE 4
Means and within-model adjusted 95% confidence intervals for fasted plasma CBD concentration (ng/ml) over the study duration. Raw data points are plotted on the graph. Dashed line shows limit of detection.

3.2.4 CBD concentration

Visual inspection of fasted plasma CBD levels shows a broad range of concentrations, from below the limit of quantification to 49.52 ng/mL across the supplemented group, with a trend toward increased concentrations over the duration of the study. At the 30-week washout point, 4 weeks after dosing was completed, and there were no detectable levels of CBD in the plasma. One cat did not have detectable CBD concentrations at any sampled timepoint, despite full consumption of the bolus the day before each sampling (Figure 4).

Urine concentrations were all below the limit of detection (data not shown), while feces showed concentrations from 45.51 to 481.24 μg/g when a snapshot concentration was analyzed (data not shown).

4 Discussion

The aim of these studies in healthy cats was 2-fold: first, to evaluate the postprandial time course of plasma CBD concentrations over 4 hours following ingestion of an initial dose, and also after 2 weeks of daily supplementation at 4mg/kg BW; and second to demonstrate tolerance of daily supplementation of 4mg/kg BW over a 26-week period.

Peak mean CBD concentration in plasma was found at 2 h post-dose at both d0 (251 ng/mL) and week 2 (431 ng/mL), and CBD absorption and clearance rates were higher at week 2 compared to the first dose. This conclusion was inferred from the significant increase in AUC coupled with the lack of differences between the postprandial concentrations of plasma CBD. This timing broadly agrees with recent literature (14, 15). Deabold et al. (14) dosed six fasted cats with 2 mg/kg BW of a 50:50 CBD/CBDA mix in fish oil and reported a maximum concentration of 43 ng/mL at 2 h. Rozental et al. (25) also used a fasted protocol to evaluate a CBD isolate in sunflower oil, observing a maximum concentration at 2 h of 17.8 and 61.1 ng/mL for 2.5 and 5.0 mg/kg BW, respectively. Dosing of Epidiolex® at 5 mg/kg BW to fed cats was found to increase the maximum concentration (465.3 ng/mL) and area under the curve (2650.0 h × ng/mL) data when compared to fasted dosing (269.0 ng/mL and 921.0 h × ng/mL, respectively) (18). In our study, the 2 h data of 251 ng/mL (95% CI: 108.7, 393.4) from cats fed a meal with their CBD dose are more similar to those reported by Wang et al. (15) for the first dose in their study at 282 ng/mL (±149.4), where the meal was fed 1 h after the CBD/CBDA paste offering. We observed an AUC upon first dose of 621.7 ng/mL (95% CI: 387.5, 855.9) over our 4 h time course compared to 908.5 ng/mL (±528.1) over 24 h in the literature (15), indicating that there is potentially a higher presence of CBD in the plasma after a dose of CBD/CBDA paste when compared to CBD in sunflower oil and that there is circulating CBD beyond the 4 h mark. When comparing these AUC values, it should be noted that pharmacokinetic software packages have been used by the other groups, likely employing a linear-log trapezoidal method, while our analysis employed a linear trapezoidal method which may overestimate the AUC (26). Given the comparatively short time frame (4 h) of our postprandial sampling, any impact of this overestimate should be limited. When comparing the literature, it is important to understand the compositions of the treatments being used as inclusion of several phytocompounds has been shown to alter the observed effects when compared to single phytocompounds. This observation, named the "entourage effect" by several publications (27–29), describes the potential for other compounds found in hemp, such as THC or CBG, to interact and possibly increase absorption of CBD (30). Both the U.S. Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) have expressed concerns over the limitations of the current literature when it comes to interpreting safety due to the different preparations and extracts, varying concentrations of CBD or other cannabinoids, small sample sizes, and quality of the data (31, 32).

This is the first study in cats to report data on fasted plasma CBD concentrations using uniform dosing over a period of more than a week and after a washout period. Data were highly variable between individuals but showed overall increase in circulating concentrations up to week 18. This high variability between individual cats has been noted in the literature previously (14, 25). A recent theory describes body fat as a contributing factor, suggesting that with increased BCS there is a higher potential for CBD to be held in reservoir within the body (25). Our BCS data were insufficiently broad to assess this. Moreover, due to the semi-qualitative nature of this measurement and potential for assessor bias, the ability to confirm this via BCS is limited and alternative methods of assessing fat content should be considered. Similar trends in plasma CBD concentrations were seen in a comparable study performed in dogs, and additionally the routes of excretion, i.e., via the feces rather than urine, also appear to be consistent (11).

To evaluate whether chronic daily feeding of CBD was safely tolerated by the cats, both groups received regular veterinary examinations, as well as routine assessment of hematology, clinical biochemistry, and urinalysis. With the exception of cholesterol, sodium:potassium ratio, mean platelet volume (MPV), and eosinophil count, all group means for biochemistry and hematology analytes remained within published reference ranges throughout the 26-week study. Differences between groups or over time were transient and not deemed to be of clinical significance during the weekly and fortnightly veterinary reviews.

Across both studies, three cats experienced high ALT with concurrent inappetence and/or general lethargy. These cats were subsequently diagnosed, via abdominal ultrasound as well as blood biochemistry and complete blood count information, with suspected ascending cholangitis (an inflammation of the gall bladder and liver). One cat was in the placebo group and two in the CBD group. It is unknown whether the incidence of cholangitis was higher in the CBD group coincidentally or if the supplement (and/or involvement in the study) contributed through added pressure on the hepatic (and any linked) system and metabolic processes in these cats. The clinical opinion, however, was that CBD itself was unlikely to have caused the infection directly. Literature suggests that the prevalence of cholangitis in cats worldwide is common and cited as the second most common hepatic disease (33) across the four distinct forms of the condition: neutrophilic, lymphocytic, destructive, and chronic (34). The asymptomatic cat from study one was followed beyond study completion, no further complications were noted, and a return to normal range for ALT and AST occurred within 3 weeks. The changes in liver enzymes observed in specific individuals in the present studies, considered together with the variability of plasma CBD measurements, suggest that there are likely to be individual differences in the response of cats to multiple doses of CBD. It is not known whether there is a genetic basis for susceptibility to high ALT (or hypertransaminasemia). When evaluating adverse observations such as hypertransaminasemia potential drug interactions are the focus (35), however, during these studies we controlled access to any potential medications that could interact with CBD to minimize this risk. It is known that cats have a low capacity for hepatic glucuronidation which reduces the capacity for metabolism and excretion of several compounds including non-steroidal anti-inflammatories and CBD (16, 36). This may be a contributing factor to the differences between dog and cat responses to CBD; however, future work to address individual susceptibility could explore metabolomics of CBD absorption and excretion.

In conclusion, THC-free CBD fed at a dose of 4 mg/kg BW was absorbed into plasma and well tolerated when supplemented over 26 weeks in cats. However, caution should be applied, and veterinary checks recommended, if any history of liver issues is known or in the event of suspected concurrent infection. There is also further need for determining efficacy of CBD doses to improve our understanding of CBD and its use in cats.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Waltham Animal Welfare and Ethical Review Body. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JC: Conceptualization, Data curation, Supervision, Writing – original draft. RB: Data curation, Methodology, Project administration, Writing – review & editing. AB: Conceptualization, Writing – review & editing. ZE: Data curation, Formal analysis, Writing – original draft. CN: Formal analysis, Methodology, Writing – original draft. PW: Conceptualization, Supervision, Writing – review & editing. DL: Conceptualization, Supervision, Writing – review & editing. LH: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

JC, RB, AB, ZE, PW, DL, and LH are all employees of Mars Petcare, a manufacturer of pet food. CN was an employee of Mars Petcare during the completion of the trials detailed here.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1324622/full#supplementary-material

SUPPLEMENTARY TABLE S1
Biochemistry, liver panel, BAP and CTX - mean estimates and 95% CIs.

SUPPLEMENTARY TABLE S2
Haematology - mean estimates and 95% CIs.

SUPPLEMENTARY FIGURE S1
Means and 95% confidence intervals (C.I.) fasted plasma measures over the 4 week study a) Bilirubin concentration (μmol/L) and b) bile acids (μmol/L). *Shows difference between the timepoint and week 0 is statistically significant ($p < 0.05$). Reference ranges included as dashed horizontal lines across the figure.

References

1. Merkus FWHM. Cannabivarin and Tetrahydrocannabivarin, two new constituents of hashish. *Nature*. (1971) 232:579–80. doi: 10.1038/232579a0

2. Nahler G. Cannabidiol and contributions of major hemp Phytocompounds to the "entourage effect"; possible mechanisms. *Alter Complement Integr Med*. (2019) 5:1–16. doi: 10.24966/ACIM-7562/100066

3. Hilderbrand RL. Hemp & Cannabidiol: what is a medicine? *Mo Med*. (2018) 115:306–9.

4. De Briyne N, Holmes D, Sandler I, Stiles E, Szymanski D, Moody S, et al. Cannabis, Cannabidiol oils and tetrahydrocannabinol—what do veterinarians need to know? *Animals*. (2021) 11:892. doi: 10.3390/ani11030892

5. Schiano Moriello A, Di Marzo V, Petrosino S. Mutual links between the Endocannabinoidome and the gut microbiome, with special reference to companion animals: a nutritional viewpoint. *Animals*. (2022) 12:348. doi: 10.3390/ani12030348

6. Arturo IF, Fabiana P. Endocannabinoidome In: *Encyclopedia of life sciences*. Wiley: John Wiley & Sons, Ltd. (2018). doi: 10.1002/9780470015902.a0028301

7. Di Bartolomeo M, Stark T, Maurel OM, Iannotti FA, Kuchar M, Ruda-Kucerova J, et al. Crosstalk between the transcriptional regulation of dopamine D2 and cannabinoid CB1 receptors in schizophrenia: analyses in patients and in perinatal Δ9-tetrahydrocannabinol-exposed rats. *Pharmacol Res*. (2021) 164:105357. doi: 10.1016/j.phrs.2020.105357

8. Yu CHJ, Rupasinghe HPV. Cannabidiol-based natural health products for companion animals: recent advances in the management of anxiety, pain, and inflammation. *Res Vet Sci*. (2021) 140:38–46. doi: 10.1016/j.rvsc.2021.08.001

9. Petcare K. A. CBD: regulations and research. (2021). Available at: https://prohibitionpartners.com/2021/03/19/petcare-cbd-regulations-and-research/

10. Greb A, Puschner B. Cannabinoid treats as adjunctive therapy for pets: gaps in our knowledge. *Toxicol Commun*. (2018) 2:10–4. doi: 10.1080/24734306.2018.1434470

11. Bradley S, Young S, Bakke AM, Holcombe L, Waller D, Hunt A, et al. Long-term daily feeding of cannabidiol is well-tolerated by healthy dogs. *Front Vet Sci*. (2022) 9:457. doi: 10.3389/fvets.2022.977457

12. Hunt ABG, Flint HE, Logan DW, King T. A single dose of cannabidiol (CBD) positively influences measures of stress in dogs during separation and car travel. *Front Vet Sci*. (2023) 10:604. doi: 10.3389/fvets.2023.1112604

13. Corsato Alvarenga I, Wilson KM, McGrath S. Tolerability of long-term cannabidiol supplementation to healthy adult dogs. *J Vet Intern Med*. (2023) 27:1–10. doi: 10.1111/jvim.16949

14. Deabold KA, Schwark WS, Wolf L, Wakshlag JJ. Single-dose pharmacokinetics and preliminary safety assessment with use of CBD-rich hemp nutraceutical in healthy dogs and cats. *Animals*. (2019) 9:832. doi: 10.3390/ani9100832

15. Wang T, Zakharov A, Gomez B, Lyubimov A, Trottier NL, Schwark WS, et al. Serum cannabinoid 24 h and 1 week steady state pharmacokinetic assessment in cats using a CBD/CBDA rich hemp paste. *Front Vet Sci*. (2022) 9:895368. doi: 10.3389/fvets.2022.895368/full

16. Schwark WS, Wakshlag JJ. A one health perspective on comparative cannabidiol and cannabidiolic acid pharmacokinetics and biotransformation in humans and domestic animals. *Am J Vet Res*. (2023) 84:1–9.

17. Perucca E, Bialer M. Critical aspects affecting Cannabidiol Oral bioavailability and metabolic elimination, and related clinical implications. *CNS Drugs*. (2020) 34:795–00. doi: 10.1007/s40263-020-00741-5

18. Jukier T, Cruz-Espindola C, Martin D, Boothe DM. Disposition of a single oral dose of a cannabidiol medication in healthy cats. *Front Vet Sci*. (2023) 10:1517. doi: 10.3389/fvets.2023.1181517/full

19. Bjornvad CR, Nielsen DH, Armstrong PJ, McEvoy F, Hoelmkjaer KM, Jensen KS, et al. Evaluation of a nine-point body condition scoring system in physically inactive pet cats. *Am J Vet Res*. (2011) 72:433–7. doi: 10.2460/ajvr.72.4.433

20. Vaughn D, Kulpa J, Paulionis L. Preliminary investigation of the safety of escalating cannabinoid doses in healthy dogs. *Front Vet Sci*. (2020) 7:51. doi: 10.3389/fvets.2020.00051

21. R Development Core Team. *R: a language and environment for statistical computing*. Vienna, Austria: The R Foundation for Statistical Computing (2017).

22. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4 (2015) 67. doi: 10.18637/jss.v067.i01

23. Pinheiro J, Bates D, DebRoy S, Sarkar DTeam RC. Nlme: Linear and nonlinear mixed effects models. (2018).

24. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. *Biometr J*. (2008) 50:346–63. doi: 10.1002/bimj.200810425

25. Rozental AJ, Gustafson DL, Kusick BR, Bartner LR, Castro SC, McGrath S. Pharmacokinetics of escalating single-dose administration of cannabidiol to cats. *J Vet Pharmacol Ther [Internet]*. (2023) 46:25–33. doi: 10.1111/jvp.13100

26. Certara TN (2011). Knowledge Base. Calculating AUC (Linear and Log-linear). Available at: https://www.certara.com/knowledge-base/calculating-auc-linear-and-log-linear/

27. Worth T. Unpicking the entourage effect. *Nature*. (2019) 572:S12–3. doi: 10.1038/d41586-019-02528-1

28. Ferber SG, Namdar D, Hen-Shoval D, Eger G, Koltai H, Shoval G, et al. The "entourage effect": terpenes coupled with cannabinoids for the treatment of mood disorders and anxiety disorders. *Curr Neuropharmacol*. (2019) 18:87–96. doi: 10.2174/1570159X17666190903103923

29. Anand U, Pacchetti B, Anand P, Sodergren MH. Cannabis-based medicines and pain: a review of potential synergistic and entourage effects. *Pain Manag*. (2021) 11:395–03. doi: 10.2217/pmt-2020-0110

30. Anderson LL, Etchart MG, Bahceci D, Golembiewski TA, Arnold JC. Cannabis constituents interact at the drug efflux pump BCRP to markedly increase plasma cannabidiolic acid concentrations. *Sci Rep*. (2021) 11:14948. doi: 10.1038/s41598-021-94212-6

31. Hahn SM, Abernethy A. Better data for a better understanding of the use and safety profile of Cannabidiol (CBD) products. Administration USF& D. (2021). Available at: https://www.fda.gov/news-events/fda-voices/better-data-better-understanding-use-and-safety-profile-cannabidiol-cbd-products

32. EFSA Panel on Nutrition NFAllergens F, Turck D, Bohn T, Castenmiller J, De Henauw S, et al. Statement on safety of cannabidiol as a novel food: data gaps and uncertainties. *EFSA J*. (2022) 20:e07322. doi: 10.2903/j.efsa.2022.7322

33. Griffin S. Feline abdominal ultrasonography: what's normal? what's abnormal? *Liver J Feline Med Surg*. (2019) 21:12–24. doi: 10.1177/1098612X18818666

34. Assawarachan SN, Yodsheewan R, Maneesaay P, Rattanapinyopituk K, Chuchalermporn P, Kongchun A, et al. Feline cyst-like lymphocytic Cholangiohepatitis in a cat: first case report. *Animals (Basel)*. (2022) 12:3278. doi: 10.3390/ani12233278

35. Souza JDR, Pacheco JC, Rossi GN, De-Paulo BO, Zuardi AW, Guimarães FS, et al. Adverse effects of Oral Cannabidiol: an updated systematic review of randomized controlled trials. *Pharmaceutics*. (2022) 14:2598. doi: 10.3390/pharmaceutics14122598

36. Lascelles BDX, Court MH, Hardie EM, Robertson SA. Nonsteroidal anti-inflammatory drugs in cats: a review. *Vet Anaesth Analg*. (2007) 34:228–50. doi: 10.1111/j.1467-2995.2006.00322.x

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Case report: Cannabinoid therapy for discoid lupus erythematosus in a dog

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Discoid lupus erythematosus (DLE) is a common autoimmune skin disease in dogs. Conventional treatments, such as corticosteroids, can be effective but often have side effects. This case report presents a successful use of cannabinoid therapy (CT) in a dog with DLE resistant to conventional treatment. A 2-year-old mixed-breed dog with a history of DLE presented with worsening lesions despite treatment with corticosteroids and other medications. Liver enzymes levels were elevated, indicating corticosteroid-induced side effects. CT with a CBD-rich full spectrum Cannabis oil was initiated. The dosage was gradually adjusted until the minimum effective dose was found. Within a few weeks of starting CT, the dog showed significant improvement in skin lesions and in liver enzymes levels. After 1 year, the dog remains clinically stable on a low dose of full-spectrum CBD-rich oil. No evidence of DLE recurrence was observed. This case suggests that CT may be a viable alternative or complementary therapy for DLE in dogs, particularly for those experiencing adverse effects from conventional treatments. Further research is warranted to confirm the efficacy and safety of CT for DLE management in dogs.

KEYWORDS

discoid lupus erythematosus, cannabinoid therapy, CBD, THC, autoimmune disease, cannabis

1 Introduction

Discoid lupus erythematosus (DLE) is an immune-mediated skin disease that affects dogs of both sexes and breeds from the age of 2. It is caused by the production of antibodies against cellular components (autoantibodies) of the skin and leads to hypersensitivity reactions of type II or III (1). Autoantibodies target healthy skin cell components, particularly nuclear structures and ribosomal proteins, initiating the inflammatory cascade. Pro-inflammatory cytokines, like IL-6 and TNF-alpha, act as amplifiers, recruiting inflammatory cells and boosting their destructive tendencies, leading to tissue damage and visible lesions (2, 3). The Complement cascade also plays a crucial role in DLE as it perforates cell membranes, raising more tissue damage. As a result of the inflammatory cascade present on DLE, free radicals accumulate, amplify the inflammation, and wreak havoc by damaging cells and tissues.

The clinical signs of DLE include depigmentation, hair loss, and redness, which can progress to crusting and ulceration. The lesions are most common on the nose and ears, but

they can also occur on the limbs, genitals, and mouth. The definitive diagnosis of DLE is made by physical examination, medical history, and histopathological examination (2, 4, 5).

Conventional immunosuppressive treatments such as corticosteroids (6) and calcineurin inhibitors (7) can be effective but often have side effects. Cannabinoids represent a novel class of immunomodulating compounds that are being thoroughly studied for diverse inflammatory and auto-immune diseases (8–13). These Cannabis-derived molecules act upon the endocannabinoid system (ECS) of vertebrate animals and utterly aims the maintenance of homeostasis throughout the intracellular environment across all body systems (14). While the exact mechanism of action for cannabinoids in DLE in dogs remains under investigation, their immunomodulatory effects through the endocannabinoid system (ECS) offer a promising explanation for their therapeutic potential. Cannabidiol (CBD) and Tetrahydrocannabinol (THC) inhibit mast cell degranulation, reducing the release of inflammatory mediators like histamine and prostaglandins (15). CBD and THC also downregulates the production of pro-inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha (15), while promoting the release of anti-inflammatory cytokines like IL-10 and promotes the activity of regulatory T cells (Tregs) (16), which suppress the overall immune response and prevent excessive inflammation and scavenge free radicals preventing oxidative stress (17). CBD can inhibit the activation of the Complement cascade, avoiding the recruitment of more inflammatory cells. Known for its analgesic and anti-pruritic effects, THC seems to act through activation of CB1 receptors in sensory neurons, which inhibits the transmission of itch signals to the spinal cord and brain, providing direct relief from scratching and discomfort. THC can suppress the release of the neuropeptide substance P, which contributes to neurogenic inflammation and itch sensation, and thus reduces neurogenic inflammation (18).

This case report presents the successful use of cannabinoid therapy (CT) in a dog with DLE resistant to conventional treatment.

2 Case description

A 2-year-old female mixed-breed dog weighing 25.5 kg and with a body condition score of 5 (on a scale of 1 to 9) was presented to the Veterinary School Clinic (CVE) of the Federal University of Santa Catarina with a previous histopathological diagnosis of discoid lupus erythematosus. The main complaint was epidermal scaling, depigmentation, and crust formation in the nasal bridge region and inside the nostrils. Previously, the dog had been treated with topical tacrolimus (Tacroz® 1 mg, ointment, BID), vitamin E (DrogaVET®, 400 IU, 1 capsule PO, BID), and a sunscreen and hydration lotion (Hydra Reflex® lotion, applied before sun exposure) for 30 days, but no improvement of the lesions was observed.

Upon physical examination, the nasal region presented with 0.8 mm hypopigmented areas, diffuse erythema with erosion, and desquamation. Treatment with 1.5 mg/kg prednisolone (40 mg Eurofarma generic, 1 tablet PO, BID) was initiated for 2 weeks. While the dog exhibited slight improvement upon a one-month follow-up, the lesions persisted (Figure 1A). Corticosteroid therapy was extended for another 2 weeks, unfortunately leading to a worsening of the lesions.

During 7 months, three attempts to reduce the corticosteroid dosage proved unsuccessful. The dog developed behavioral changes, including increased irritability with its housemates, weight gain from 25.5 kg to 36.7 kg, a 44% increase in 7 months, and indications of liver damage. Liver function tests conducted 1 month apart confirmed these concerns, showing elevated levels of alanine aminotransferase (ALT) (160 U/L and 276.6 U/L respectively, reference range: 10–88 U/L) and alkaline phosphatase (FA) (181 U/L and 416 U/L respectively, reference range: 20–156 U/L).

No baseline tests were performed to measure the patient's condition before initiating corticosteroid therapy. Prior to the initial examination, no complaints beyond peeling, depigmentation, and crusting were documented. The emergence of behavioral and weight concerns only occurred following the administration of corticosteroids.

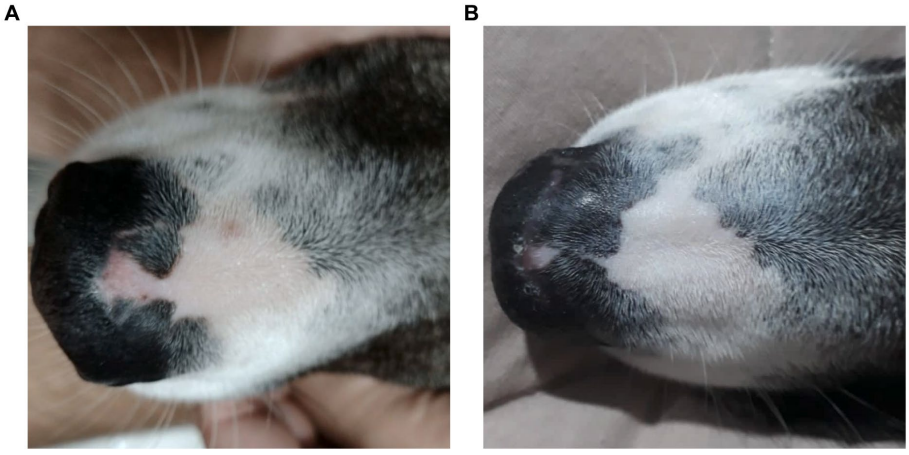

FIGURE 1
A female, mixed-breed dog, 2 years old, presented with epidermal depigmentation, on the nasal bridge. **(A)** Macroscopic image showing depigmentation in the nasal planum prior to treatment with Cannabis oil (date: 05/31/2022). **(B)** Macroscopic image showing the reduction and stabilization of depigmentation in the nasal planum after 1 year and 3 months of treatment with Cannabis oil (date: 09/19/2023).

With corticosteroid therapy no longer an option, the owner, concerned about the dog's well-being, explored alternative treatments. After discussion, it was decided to interrupt corticosteroid therapy and to try cannabinoid therapy (CT) with cannabis derivatives.

The patient was directed to a veterinarian specialist well-versed in cannabinoid therapy. The veterinarian prescribed a full-spectrum oil containing a 2:1 THC:CBD ratio (20 mg/mL THC, 10 mg/mL CBD, and a total of 40 mg/mL, considering other non-identified cannabinoid species). All Cannabis-based products used along this dog's treatment were provided by the following non-governmental medical Cannabis associations in Brazil: Cannabis Sem Fronteiras (CSF), AMA-ME, Alternativa, and Santa Cannabis, to whom we express our gratitude for their timely delivery and generous donation of several Cannabis oils throughout the treatment.

Cannabinoid treatment began with a single drop of the cannabis oil (0.08 mg/kg/day total cannabinoids; AMA-ME) administered orally once daily for 3 days. The dose was then gradually increased every 3 days, progressing from one oral drop once daily to one drop twice daily (0.16 mg/kg/day total cannabinoids), then to two drops twice daily (0.32 mg/kg/day total cannabinoids), and so on, until the optimal dose for symptom control was identified. Interestingly, the owner reported an improvement in the dog's behavior shortly after discontinuing prednisolone and within the first day of receiving the cannabis oil.

Forty days later, the patient returned with ear discomfort. Cytology swabs revealed yeast fungal otitis in the left ear and bacterial otitis with cocci and rods in the right ear. An ear cleaner compound containing 50% panthenol, 20% glycyrrhizic acid, 20% Lactic Acid, 3% mint essential oil and 2% chamomile essential oil (Oto Clean Up®, one spray per ear, once daily for 3 days) and an anti-inflammatory, antibiotic and antifungal otological suspension (49% orbifloxacin, 5.14% mometasone furoate and 5.14% Posaconazole; Posatex®, eight drops per ear, once daily for 6 days, starting after the initial cleaning) were prescribed. While the owner reported administering eight drops of the 2:1 THC:CBD oil (40 mg/mL) twice daily (1.28 mg/kg/day total cannabinoids; AMA-ME), no significant improvement in the skin condition was observed. To address this, the protocol was adjusted to include 10 drops of a full-spectrum CBD-rich oil (50 mg/mL) twice daily (1.96 mg/kg/day total cannabinoids; Alternativa) while reducing the THC-dominant oil (40 mg/mL) to three drops once daily (0.24 mg/kg/day total cannabinoids; AMA-ME). Within a few weeks, the dog exhibited significant improvement in dermatological signs, accompanied by a concurrent improvement in liver function.

Ten days after the previous evaluation the dog returned for a follow-up appointment. The nasal planum lesion continued to shrink, prompting an increase in the CBD-rich oil dose from 10 to 15 drops (2.4 mg/kg/day total cannabinoids; Alternativa). The THC-rich oil dosage remained unchanged at three drops (0.24 mg/kg/day total cannabinoids; CSF). While mild erythema, discharge, and hair loss persisted in the left ear, the prescribed ear solution was continued for another 3 days, leading to complete resolution of the bilateral otitis externa.

Fifteen days later, a follow-up assessment revealed no further reduction in the area of nasal planum depigmentation, though its progression had stabilized. The patient's overall condition had demonstrably improved with weight loss, and the owner reported a return to normal, playful behavior, with no observable signs of discomfort. Both cannabis oils doses were maintained at the previous levels.

Approximately 1 month and a half after the previous consultation, a follow-up revealed the nasal planum lesion to be static, exhibiting no further improvement or deterioration. However, a new lesion characterized by depigmentation and signs of allergic conjunctivitis was identified on the medial aspect of the right nostril. Keravit® eye ointment (topically, twice daily for 5 days) was prescribed. Cannabis therapy remained unaltered, and the patient was advised to minimize sun exposure.

Three months later, the animal returned with no sign of depigmentation on the nasal planum. However, new crusted lesions were observed on the vulva. The affected area was cleaned with chlorhexidine 1% (Asseptcare spray®, BID for 7 days), while the dosages of the cannabis oils were maintained. Although the possibility of a lupus-related lesion was mentioned, no further diagnostic investigation was pursued. A subsequent phone follow-up confirmed complete resolution of the vulvar lesion.

Two months later, the animal returned for a clinical reassessment and annual vaccination. Peripheral blood was collected for a comprehensive evaluation, including biochemical analysis, blood cell count, and an antinuclear antibody (ANA) test. Despite the ongoing treatment, the animal remained clinically stable. The blood count revealed a discrete erythrocytosis, with elevated red blood cell count (8.79×10^6/µl; reference: $5.5–8 \times 10^6$/µl), hemoglobin (20.8 d/dL; reference: 12–18 g/dL), and hematocrit (65.1%; reference: 37–55%). Additionally, anisocytosis, polychromasia, macroplatelets, lipemia and hemolysis were observed in the serum. Notably, ALT levels, while still exceeding the reference range, had decreased to 123.6 U/L (10–88 U/L). Encouragingly, the ANA test result was negative.

Given the ease of access and satisfactory results, the animal's cannabinoid therapy transitioned to a single full-spectrum cannabis oil with a 3:1 CBD:THC ratio (40 mg/mL total cannabinoids, Alternativa). To determine the minimum effective dose, the owner was instructed to gradually taper the medication, reducing the cannabis oil by 1 drop (0.08 mg/kg total cannabinoids) every 3 days and monitoring for any regression in the treatment response. This titration schedule would continue until the optimal maintenance dose was established.

One-year post-diagnosis, the animal maintains clinical stability (Figure 1B) on a twice-daily cannabinoid dose of 0.32 mg/kg/day from Santa Cannabis at the same 3:1 CBD:THC ratio at 40 mg/mL, with a body weight of 26.6 kg, only 1 kilogram above initial measurement.

3 Discussion

Discoid lupus erythematosus (DLE) remains a mysterious foe in dogs. Its origins are shrouded in a mix of genetics, infections, hormones, and sun exposure (1). This autoimmune disease presents as scaly, discolored patches typically on the nose, but sometimes venturing to ears, lips, and beyond (2, 19). Diagnosis involves piecing together the clinical signs, skin tests, and bloodwork (19), though specific autoantibody tests often elude DLE's grasp (4). Differential diagnoses include nasal pyoderma, demodicosis, dermatophytosis, erythematous or foliaceus pemphigus, dermatomyositis, uveodermatological syndrome, solar nasal dermatitis, and nasal

depigmentation (2, 5, 20). Traditional therapies, like corticosteroids and calcineurin inhibitors, hold the fort initially, but often require long-term commitment due to DLE's tendency to return, and carry potential side effects (2, 7). Weight gain and behavior changes were both side effects observed in the present case. The dog showed increased irritability with its housemates and the animal's weight rose 44% from 25.5 kg to 36.7 kg during corticosteroid therapy. One month into cannabinoid treatment, the patient had already reduced weight reaching 32.4 kg (9% less) and ended up at 26.6 kg as of the final observation performed (Figure 2).

The patient's initial presentation included persistent depigmentation and scaling on the nasal plan, along with crusted lesions on the ears and vulva. An episode of otitis externa was also reported. Increased ALT and FA enzymes were observed, relevant to the prolonged use of corticosteroids. However, no prior tests were performed before the start of corticosteroid treatment for comparison purposes. The animal was referred with a positive biopsy for DLE and a negative antinuclear antibody (ANA) test.

The treatment for DLE involves controlling the inflammatory pathways involved in the pathogenesis. Lesions typically respond well to a variety of medications. In most cases, treatment must be continued throughout the animal's life due to frequent relapses after dose reduction, as observed in this case. In mild cases, DLE treatment consists of topical application of corticosteroids such as betamethasone, fluocinolone, or cyclosporine 1 to 2% twice daily (BID) until complete remission, which may take 4 to 6 weeks (3). The frequency of applications is then reduced to a minimum of every 24 or 48 h. Also, topical treatments with calcineurin inhibitors such as tacrolimus ointment 0.1% BID can be used in mild cases (2, 3, 21). Our canine patient, diagnosed with DLE, initially embarked on a journey of corticosteroids and tacrolimus ointment but this was ineffective in controlling the disease-derived lesions. Additionally, tapering proved treacherous, and the liver showed signs of distress. Vitamin E, a potential ally in symptom control, was unfortunately abandoned before its impact could be assessed. In more severe cases, immunosuppressive therapy is indicated. This may include systemic corticosteroids such as prednisone or prednisolone at a dose of 2 mg/kg SID or 1 mg/kg BID orally until lesions are healed, which may take up to 4 weeks, and there can be no improvement at all. Vitamin E or a combination of niacinamide and tetracycline as complementary treatments for the control of signs such as pruritus in DLE have been recommended (3). Cyclosporine is recommended for severe cases of DLE (20), but it is expensive and can be difficult to maintain treatment consistently. The major challenge lies in the search for an effective and safe long-term therapy.

Cannabis derivatives, beyond their pain-relieving prowess, are emerging as potential knights in shining armor against inflammation and immune system overwork in the veterinary world (8, 15, 22–26). Unlike traditional drugs, these compounds dance with the body's own endocannabinoid system (ECS), a master conductor of cellular harmony, homeostasis and diverse functions (27). By influencing the immune response's orchestra, they can silence the pro-inflammatory drums and amplify the anti-inflammatory melodies (15, 28–30). This intricate waltz aligns beautifully with DLE's needs, as evidenced by the improvement seen in our patient.

Veterinarians are interested in the use of cannabinoid compounds derived from the *Cannabis sativa* plant as safe and effective alternatives for the treatment of DLE in dogs. Clinical studies and case reports in animals have shown positive results for the use of these compounds in the treatment of canine osteoarthritis (22–25, 31), epilepsy (31–34), behavioral disorders in dogs (35)and horses (36), and anti-inflammatory effects (37). However, no studies or reports have been conducted so far on the use of cannabis oil in the treatment of DLE in dogs.

As observed, conventional therapy with corticosteroids can be effective, but it is limited by its prolonged use. Cannabinoids, on the other hand, lack significant side effects and are safe for long-term usage (27, 31, 38, 39). Cannabinoid therapy for DLE, still in its infancy, needs meticulous adjustments and individualized doses. This case exemplifies the "Start low, go slow" mantra, where the total daily dose gently ascended from 0.08 mg/kg to 2.64 mg/kg, guided by the patient's unique response. With each careful increase, the aim is to restore the ECS's rhythm and find the perfect melody, the smallest effective dose, for each individual animal, acknowledging the diverse symphony of each ECS (40, 41).

Full-spectrum derivatives of *Cannabis sativa* contain hundreds of cannabinoids, such as THC and CBD, which act on various G protein-coupled receptors, including cannabinoid receptors 1 and 2 (CB1 and CB2), Transient receptor potential vanilloid (TRPV), Transient receptor potential melastatin (TRPM), Transient receptor potential ankyrin (TRPA) and the Peroxisome Proliferator-Activated Receptor (PPAR) (15, 27, 29). These receptors trigger myriad effects on cellular

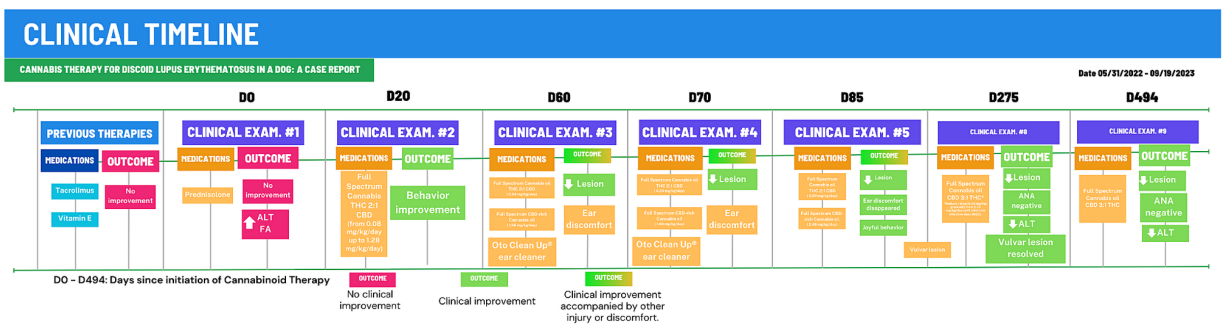

FIGURE 2
Clinical timeline for 494-days of Cannabinoid Therapy (CT). After 275 days of CT, the animal was stabilized and total cannabinoid dose was gradually reduced to 0.38 mg/kg/day. D0, First examination at CVE/UFSC; Outcomes in red boxes, no improvement or worsening; Outcomes um green boxes, clinical improvement.

metabolism including important signaling pathways intrinsic to the immune response, such as AMPc, MAPK/ERK/MEK/FOS/JUN, and PI3/Akt (42) and modulate the cellular environment towards homeostasis and thus resolving inflammatory processes (15, 27, 29, 42).

Cannabinoids have been associated with the modulation of immune function and the inhibition of the release of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interferon alpha, gamma, and beta (IFN-α, IFN-ɣ, and IFN-β), interleukins IL-1β, IL-6, IL-12, IL-23, and regulates the nuclear factor NF-κB (15, 27, 43, 44). Cannabinoids also modulate the activation of T and B lymphocytes, promote the secretion of IL-10 and stimulate regulatory T cells, therefore reducing the inflammatory response (15, 29). CBD inhibits the release of IFN-α selectively via CB2 in plasmacytoid dendritic cells, which are present in high levels in the skin of patients with discoid lupus erythematosus (DLE) (30). The hypersensitivity reactions that occur in DLE are responsible for pro-inflammatory mechanisms that lead to tissue infiltration and damage. Studies have shown that cannabinoids are able to inhibit these processes (45–47). This may explain the improvement seen in the patient in question.

Our yearlong DLE case highlights successful dose titration for an Individualized Cannabinoid Therapy (ICT) approach. The treatment initiated with a daily cannabinoid dose of 0.08 mg/kg. Notable clinical improvements on the nasal planum started to be observed at 0.32 mg/kg/day, prompting a further increase to 2.64 mg/kg/day. Following clinical stabilization, the dose was gradually reduced, achieving the minimum effective dose of 0.32 mg/kg/day total cannabinoids.

Throughout the treatment period, the dog exhibited robust overall well-being, maintained an active and playful disposition, and experienced a stabilization of its dermatological signs. No corticoids were needed during the ICT. This offers initial indications that cannabinoids could potentially serve as a viable and health-conscious alternative to extended therapeutic approaches for DLE in dogs. The quest for definitive answers however continues: rigorous studies are needed to solidify the effectiveness of cannabis derivatives for DLE; the optimal dosage and administration schedule remain a melody waiting to be composed; and long-term safety and efficacy data require further research.

While the song of cannabis therapy for DLE in dogs holds immense promise, we must continue listening closely, gathering more evidence, and refining the tune. This case report adds its verse to the growing chorus, paving the way for future research and potentially offering a new rhythm of hope and a haven from the long-term reign of corticosteroids for dogs battling this challenging disease.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the studies involving animals in accordance with the local legislation and institutional requirements because this is a case report. The dog was ongoing clinical treatment at our university and we hereby describe the positive outcome of the cannabis treatment performed. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

MS: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. BC: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. EA: Conceptualization, Data curation, Investigation, Supervision, Validation, Writing – review & editing, Resources, Writing – original draft. MP: Conceptualization, Investigation, Supervision, Validation, Writing – review & editing, Data curation, Formal analysis, Project administration.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2024.1309167/full#supplementary-material

References

1. Nastri Gouvêa F, Santos Pennacchi C, Vieira Fernandes Ferreira A, de Almeida P, Luna Alves APP. Discoid lupus erythematosus in dog. *Veterinária Notícias*. (2022) 28:1–4. doi: 10.14393/VTN-v28n1-2022-62645

2. Olivry T, Linder KE, Banovic F. Cutaneous lupus erythematosus in dogs: a comprehensive review. *BMC Vet Res*. (2018) 14:132. doi: 10.1186/s12917-018-1446-8

3. Kurtdede A, Alic Ural D. Guzel Ondokuz Mayıs Üniversitesi M. Cutaneous lupus erythematosus in a dog. Available at: https://www.researchgate.net/publication/287763990

4. Pereira P, Cristina Barini Nunes A, Alves J, Cunha DO, Acb N, Medvep PJ. Lúpus eritematoso discoide (LED)-Relato de caso em um canino SRD Discoid lupus erythematosus-Case report in a dog SRD. *Alergologia Veterinária*. (2014) 3:390–3.

5. Banovic F, Linder KE, Uri M, Rossi MA, Olivry T. Clinical and microscopic features of generalized discoid lupus erythematosus in dogs (10 cases). *Vet Dermatol*. (2016) 27:488–e131. doi: 10.1111/vde.12389

6. Griffin CE, Stannard AA, Ihrke PJ, Ardans AA, Cello RM, Bjorling DR. Canine discoid lupus erythematosus. *Vet Immunol Immunopathol*. (1979) 1:79–87. doi: 10.1016/0165-2427(79)90009-6

7. Banovic F, Robson D, Linek M, Olivry T. Therapeutic effectiveness of calcineurin inhibitors in canine vesicular cutaneous lupus erythematosus. *Vet Dermatol*. (2017) 28:493–e115. doi: 10.1111/vde.12448

8. Nagarkatti P, Pandey R, Rieder SA, Hegde VL, Nagarkatti M. Cannabinoids as novel anti-inflammatory drugs. *Future Med Chem*. (2009) 1:1333–49. doi: 10.4155/fmc.09.93

9. Croxford JL, Yamamura T. Cannabinoids and the immune system: potential for the treatment of inflammatory diseases? *J Neuroimmunol*. (2005) 166:3–18. doi: 10.1016/j.jneuroim.2005.04.023

10. Shao K, Stewart C, Grant-Kels JM. Cannabis and the skin. *Clin Dermatol*. (2021) 39:784–95. doi: 10.1016/j.clindermatol.2021.05.006

11. Banach D, Ferrero P. Cannabis and pathologies in dogs and cats: first survey of phytocannabinoid use in veterinary medicine in Argentina. *J Cannabis Res*. (2023) 5:39. doi: 10.1186/s42238-023-00209-5

12. Lima MG, Tardelli VS, Brietzke E, Fidalgo TM. Cannabis and inflammatory mediators. *Eur Addict Res*. (2021) 27:16–24. doi: 10.1159/000508840

13. Atalay S, Jarocka-karpowicz I, Skrzydlewskas E. Antioxidative and anti-inflammatory properties of cannabidiol. *Antioxidants*. (2020) 9:21. doi: 10.3390/antiox9010021

14. Cital S, Kramer K, Hughston L, Gaynor JS eds. *Cannabis therapy in veterinary medicine*. Cham: Springer International Publishing (2021).

15. Anil SM, Peeri H, Koltai H. Medical Cannabis activity against inflammation: active compounds and modes of action. *Front Pharmacol*. (2022) 13:908198. doi: 10.3389/fphar.2022.908198

16. Holloman BL, Nagarkatti M, Nagarkatti P. Epigenetic regulation of cannabinoid-mediated attenuation of inflammation and its impact on the use of cannabinoids to treat autoimmune diseases. *Int J Mol Sci*. (2021) 22:7302. doi: 10.3390/ijms22147302

17. Khaksar S, Bigdeli M, Samiee A, Shirazi-zand Z. Antioxidant and anti-apoptotic effects of cannabidiol in model of ischemic stroke in rats. *Brain Res Bull*. (2022) 180:118–30. doi: 10.1016/j.brainresbull.2022.01.001

18. Henry RJ, Kerr DM, Finn DP, Roche M. For whom the endocannabinoid tolls: modulation of innate immune function and implications for psychiatric disorders. *Prog Neuro-Psychopharmacol Biol Psychiatry*. (2016) 64:167–80. doi: 10.1016/j.pnpbp.2015.03.006

19. Ferreira TC, Pinheiro ADN, Leite AKRM, Guedes RFM, Pinheiro DCSN. Pathogenesis, biomarkers and immunotherapy in autoimmune skin diseases in dogs and cats. A review. *Rev Bras de Saude e Prod Anim*. (2015) 9:299–319. doi: 10.5935/1981-2965.20150030

20. Ataide W, Silva V, Ferraz H, Amaral A, Romani A. Lúpus Eritematoso Discoide Em Cães. *Encicl Biosf*. (2019) 16:995–1009. doi: 10.18677/encibio_2019a81

21. Messinger L, Strauss T, Jonas L (2017). A randomized, double-blinded placebo controlled crossover study evaluating 0.03% tacrolimus ointment monotherapy in the treatment of discoid lupus erythematosus in dogs. Available at: www.symbiosisonline.orgwww.symbiosisonlinepublishing.com

22. Gamble LJ, Boesch JM, Frye CW, Schwark WS, Mann S, Wolfe L, et al. Pharmacokinetics, safety, and clinical efficacy of Cannabidiol treatment in osteoarthritic dogs. *Front Vet Sci*. (2018) 5:165. doi: 10.3389/fvets.2018.00165

23. Brioschi FA, di Cesare F, Gioeni D, Rabbogliatti V, Ferrari F, D'Urso ES, et al. Oral transmucosal cannabidiol oil formulation as part of a multimodal analgesic regimen: effects on pain relief and quality of life improvement in dogs affected by spontaneous osteoarthritis. *Animals*. (2020) 10:1–14. doi: 10.3390/ani10091505

24. Verrico CD, Wesson S, Konduri V, Hofferek CJ, Vazquez-Perez J, Blair E, et al. A randomized, double-blind, placebo-controlled study of daily cannabidiol for the treatment of canine osteoarthritis pain. *Pain*. (2020) 161:2191–202. doi: 10.1097/j.pain.0000000000001896

25. Mejia S, Duerr FM, Griffenhagen G, McGrath S. Evaluation of the effect of Cannabidiol on naturally occurring osteoarthritis-associated pain: a pilot study in dogs. *J Am Anim Hosp Assoc*. (2021) 57:81–90. doi: 10.5326/JAAHA-MS-7119

26. Rieder SA, Chauhan A, Singh U, Nagarkatti M, Nagarkatti P. Cannabinoid-induced apoptosis in immune cells as a pathway to immunosuppression. *Immunobiology*. (2010) 215:598–605. doi: 10.1016/j.imbio.2009.04.001

27. Copas G, Amazonas E, Brandon S. The pharmacology of cannabinoids In: S Cital, K Kramer, L Hughston and JS Gaynor, editors. *Cannabis therapy in veterinary medicine*. Cham: Springer International Publishing (2021). 17–59.

28. Robaina Cabrera CL, Keir-Rudman S, Horniman N, Clarkson N, Page C. The anti-inflammatory effects of cannabidiol and cannabigerol alone, and in combination. *Pulm Pharmacol Ther*. (2021) 69:102047. doi: 10.1016/j.pupt.2021.102047

29. de Lorimier L-P, Hazzah T, Amazonas E, Cital S. Cannabinoids in oncology and immune response In: S Cital, K Kramer, L Hughston and JS Gaynor, editors. *Cannabis therapy in veterinary medicine*. Cham: Springer International Publishing (2021). 231–69.

30. Luz-Veiga M, Azevedo-Silva J, Fernandes JC. Beyond pain relief: a review on Cannabidiol potential in medical therapies. *Pharmaceuticals*. (2023) 16:155. doi: 10.3390/ph16020155

31. Alvarenga IC, Panickar KS, Hess H, Mcgrath S. Scientific validation of Cannabidiol for Management of dog and cat diseases. *Annu Rev Anim Biosci*. (2023) 11:227–46. doi: 10.1146/annurev-animal-081122

32. McGrath S, Bartner LR, Rao S, Packer RA, Gustafson DL. Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. *J Am Vet Med Assoc*. (2019) 254:1301–8. doi: 10.2460/javma.254.11.1301

33. Potschka H, Bhatti SFM, Tipold A, McGrath S. Cannabidiol in canine epilepsy. *Vet J*. (2022) 290:105913. doi: 10.1016/j.tvjl.2022.105913

34. De Risio L, Stabile F, São Braz B, Garcia GA. Safety and efficacy of cannabidiol-cannabidiolic acid rich hemp extract in the treatment of refractory epileptic seizures in dogs. Available at: https://www.epidiolex.com/sites/default/files/pdfs/VV-MED-

35. Corsetti S, Borruso S, Malandrucco L, Spallucci V, Maragliano L, Perino R, et al. *Cannabis sativa* L. may reduce aggressive behaviour towards humans in shelter dogs. *Sci Rep*. (2021) 11:2773. doi: 10.1038/s41598-021-82439-2

36. Cunha RZ, Felisardo LL, Salamanca G, Marchioni GG, Neto OI, Chiocchetti R. The use of cannabidiol as a novel treatment for oral stereotypic behaviour (crib-biting) in a horse. *Vet Anim Sci*. (2023) 19:100289. doi: 10.1016/j.vas.2023.100289

37. Gugliandolo E, Licata P, Peritore AF, Siracusa R, D'amico R, Cordaro M, et al. Effect of cannabidiol (Cbd) on canine inflammatory response: an ex vivo study on lps stimulated whole blood. *Vet Sci*. (2021) 8:185. doi: 10.3390/vetsci8090185

38. de Andrade DF, Gewehr JLH, de Almeida EA. Safety and efficacy of the therapeutic use of Cannabis-based products in the treatment of dogs: an integrative review. *Cannabis Cannabinoid Res*. (2022) 7:736–44. doi: 10.1089/can.2021.0172

39. Lima TDM, Santiago NR, Alves ECR, Chaves DSDA, Visacri MB. Use of cannabis in the treatment of animals: a systematic review of randomized clinical trials. *Anim Health Res Rev*. (2022) 23:25–38. doi: 10.1017/S1466252321000189

40. MacCallum CA, Russo EB. Practical considerations in medical cannabis administration and dosing. *Eur J Intern Med*. (2018) 49:12–9. doi: 10.1016/j.ejim.2018.01.004

41. Sideris A, Lauzadis J, Kaczocha M. The basic science of cannabinoids. *Anesth Analg*. (2024) 138:42–53. doi: 10.1213/ANE.0000000000006472

42. López-Cardona AP, Sánchez-Calabuig MJ, Beltran-Breña P, Agirregoitia N, Rizos D, Agirregoitia E, et al. Exocannabinoids effect on in vitro bovine oocyte maturation via activation of AKT and ERK1/2. *Reproduction*. (2016) 152:603–12. doi: 10.1530/REP-16-0199

43. Shrivastava A, Kuzontkoski PM, Groopman JE, Prasad A. Cannabidiol induces programmed cell death in breast cancer cells by coordinating the cross-talk between apoptosis and autophagy. *Mol Cancer Ther*. (2011) 10:1161–72. doi: 10.1158/1535-7163.MCT-10-1100

44. Preet A, Qamri Z, Nasser MW, Prasad A, Shilo K, Zou X, et al. Cannabinoid receptors, CB1 and CB2, as novel targets for inhibition of non-small cell lung cancer growth and metastasis. *Cancer Prev Res*. (2011) 4:65–75. doi: 10.1158/1940-6207.CAPR-10-0181

45. Preteroti M, Wilson ET, Eidelman DH, Baglole CJ. Modulation of pulmonary immune function by inhaled cannabis products and consequences for lung disease. *Respir Res*. (2023) 24:95. doi: 10.1186/s12931-023-02399-1

46. Nagarkatti P, Miranda K, Nagarkatti M. Use of cannabinoids to treat acute respiratory distress syndrome and cytokine storm associated with coronavirus disease-2019. *Front Pharmacol*. (2020) 11:589438. doi: 10.3389/fphar.2020.589438

47. Maayah ZH, Ferdaoussi M, Alam A, Takahara S, Silver H, Soni S, et al. Cannabidiol suppresses cytokine storm and protects against cardiac and renal injury associated with Sepsis. *Cannabis Cannabinoid Res*. (2022). doi: 10.1089/can.2022.0170

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Pharmacokinetic of two oral doses of a 1:20 THC:CBD *cannabis* herbal extract in cats

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Objective: To determine the pharmacokinetics (PK) of two oral doses of a *Cannabis* herbal extract (CHE) containing 1:20 THC:CBD in 12 healthy Domestic Shorthair cats.

Methods: Single-dose PK were assessed after oral administration of CHE at low or high dose (2 mg CBD + 0.1 mg THC, or 5 mg CBD + 0.25 mg THC per kg bw, respectively; $n = 6$ per group) in fasting cats. Blood samples were drawn up to 48 h following CHE administration. Plasma samples were analyzed for CBD, THC, and metabolites 6-OH-CBD, 7-OH-CBD, 11-OH-THC, and THC-COOH using a previously validated LC–MS/MS method.

Results: CBD and THC were quickly absorbed (mean T_{max} of 2.4–2.9 h). Maximum plasma concentrations (C_{max}) ranged from 36–511 ng/mL and 6.8–61 ng/mL for CBD and THC, respectively. Elimination was initially rapid for both CBD and THC, though a prolonged elimination phase was noted for CBD in some cats ($T_{1/2\,\lambda}$ up to 26 h). Dose-adjusted C_{max} and $AUC_{0\text{-last}}$ values were not statistically significantly different ($p > 0.05$) between dose groups indicating CBD and THC concentrations increased in a manner proportional (linear) to the dose. Dose-adjusted THC C_{max} and $AUC_{0\text{-last}}$ were significantly higher than the corresponding dose-adjusted CBD parameters ($p < 0.01$). Low concentrations of the metabolite 6-OH-CBD were quantified but metabolites 7-OH-CBD, 11-OH-THC, and THC-COOH were not detected in any plasma samples. Inter-individual variance was notable. Salivation shortly after dosing was observed in two cats in the high dose group; these animals had substantially lower cannabinoid concentrations than other cats in this group. No adverse clinical signs (including vomiting, change in mentation or other neurological signs) were noted.

Clinical significance: Although cats did not display adverse effects after administration of a single oral dose of 1:20 THC:CBD CHE formulation at 2 or 5 mg CBD/kg bw, observed plasma concentrations were highly variable but generally lower than in dogs receiving the same dose and formulation. Administration of CHE in the fasting state may not optimize CBD absorption, and oral dosing may be challenging when administering an oil-based CHE in some cats.

KEYWORDS

cannabinoids, CBD -cannabidiol, THC -tetrahydrocannabinol, pharmacokinetics, relative bioavailability, feline

Introduction

Phytocannabinoids are compounds derived from plants, *Cannabis sativa* and *Cannabis indica*. Although used in traditional medicine for centuries, medical use of cannabinoids has received increased medical interest with discovery of the endocannabinoid system (1) and its recent legalization in many countries. Cannabinoids bind allosterically to cannabinoid receptors (CB1 and CB2) that are widely distributed throughout the mammalian body (1), making them an attractive therapy for a variety diseases and conditions in both human and veterinary medicine. However, the mechanism of action is not fully understood, and the extent of its therapeutic properties is currently under investigation. There are over 120 cannabinoids, the most common being delta-9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD). THC is widely recognized for its psychotropic effects and thus its toxicity has been extensively studied in both humans and animals; but the drug has also demonstrated to be effective at decreasing symptoms of nausea and vomiting in human patients receiving chemotherapy (2). CBD has been gaining attention as a nutraceutical due to its ability to provide therapeutic benefits without impairing cognition. It has been recognized to effectively reduce seizure severity and frequency in children and young adults (3, 4) and appears promising for similar use in anticonvulsant-resistant epileptic animals (5). Furthermore, CBD has been demonstrated to have anti-inflammatory and immunomodulating properties in models using canine and equine whole blood (6, 7).

When considering potential benefits of their use in veterinary medicine, understanding the pharmacokinetics of cannabinoids in animals is crucial for developing rational dosing regimens. Oral bioavailability of CBD appears to be low in cats, possibly due to its lipophilic structure and potential for first-pass metabolism (8). Differences in pharmacokinetic parameters have been observed both within and between species (9). Felines appeared to have generally lower maximum CBD plasma concentrations compared to canines, implying species-specific factors which affect bioavailability (10). Fasting versus fed states, cannabinoid ratios (CBD:THC), and dose also appeared to alter CBD pharmacokinetics (9). There are limited published studies evaluating CBD pharmacokinetics in cats (10–14), and even fewer using formulations containing known quantities of both CBD and THC (13, 14). With many cannabinoid products available to animal owners, each with unique chemical makeup, understanding potential interactions between cannabinoids and the resulting impact on plasma concentrations is essential.

The objective of this study is to determine the cannabinoid-plasma concentrations in cats for two different doses of a 20:1 CBD:THC cannabis herbal extract (CHE) previously evaluated in dogs (15). This study aims to develop a rational dosing regimen suitable for use in clinical trials evaluating the safety and efficacy of Cannabis herbal extracts (CHE) in cats.

Materials and methods

Cats

This study was approved by the Usask Animal Research Ethics Board (Animal Use Protocol 20,210,019). Twelve Domestic Shorthair (DSH) cats (four castrated males and eight spayed females) housed at the WCVM Animal Care Unit were used in this study. Ages ranged from 0.75–9 years and weighed 3.34–6.91 kg at the start of study. Health was assessed via history, physical examination, and complete blood count and chemistry profiles prior to study initiation, all cats were considered in good health. The standard diet was a nutritionally balanced commercial cat food offered twice daily in individual cat feeders, however food was withheld from cats for 12 h prior to dosing and offered again 2 h post-dose.

Test item

CBD-enriched *Cannabis* herbal extract (CHE) with nominal concentrations of 20 mg CBD and 1 mg THC per mL in olive oil base (CanniMed) was provided from a licensed cannabis producer (Aurora Cannabis Inc.). All necessary regulatory approvals for experimental use of this CHE in cats was granted by Health Canada (Experimental Studies Certificate and Cannabis research exemption) prior to study initiation. A certificate of analysis was submitted by Aurora Cannabis Inc. for the batch of CanniMed used in the study.

Pharmacokinetic (PK) study design

Cats were stratified by weight and sex, then randomly assigned to low (2 mg CBD + 0.1 mg THC/kg) or high (5 mg CBD + 0.25 mg THC/kg) dose groups ($n = 6$ per dose group). All cats were fasted for 12 h prior to the planned dosing time. On the dosing day, indwelling cephalic vein catheters were placed and 1–1.5 mL whole blood collected as a Time 0 sample. CHE dose volumes were based on Day −1 body weights and ranged from 0.33–0.69 mL (low dose) and 0.89–1.58 mL. Oral dose administration was performed using 1 mL or 3 mL syringes placed on the back of the cat's tongue, followed by holding the cat's mouth closed for 10–20 s or until swallowing was noted. Starting 2 h after dosing, and throughout the rest of the blood collection period, cats had free access to their normal diets via automated microchip feeders. During the intensive blood collection phase (first 8 h after dosing), treated cats were confined to a single room with free access to food and water, in order to facilitate regular blood collection and supervision. Cats were monitored post-dose for any adverse events (AEs) such as head shaking, hypersalivation, or vomiting.

Whole blood samples (2.0–2.5 mL) were taken via the catheters at the following nominal times; 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 32, and 48 h. The catheters were flushed with saline regularly to prevent clots from lodging. When taking samples, the first 0.5 mL of blood was discarded to prevent sample dilution from the saline flush. If the catheters became dislodged, kinked, or plugged, the blood sample was collected by jugular or alternate limb cephalic venipuncture. All blood samples were collected in labeled lithium heparin tubes and immediately refrigerated. Actual collection times were recorded for each sample. Whole blood samples were centrifuged at 1200 x G for 10 min. Plasma was separated via pipette into 200 µL aliquots and stored in Eppendorf Protein Lo-Bind microcentrifuge tubes and frozen at −80°C for up to 12 months prior to analysis.

Adverse event and neurological assessment

Throughout the first 12 h of the pharmacokinetic study phase, cats were directly monitored for signs of vomiting, salivation, diarrhea,

changes in mentation, hyperesthesia, or any other physical or neurological abnormality.

Plasma sample preparation and LC–MS/MS analysis

The analytical method used for cannabinoid analysis in feline plasma was a version of an assay previously validated for canine plasma (15). Assay limit of detection (LOD) was 0.98 ng/mL for all analytes measured (CBD, THC, 6-OH-CBD, 7-OH-CBD, 11-OH-THC, and THC-COOH). Lower limit of quantification (LLOQ) was 1.97 ng/mL for all analytes except 7-OH-CBD (3.91 ng/mL).

Pharmacokinetic analysis

Plasma concentration versus time data was analyzed for each cat using non-compartmental modeling (Phoenix WinNonLin, Certara, Princeton, NJ, United States). Final PK parameters were expressed as mean ± SD. Maximum plasma concentration (C_{max}) and time to maximum concentrations (T_{max}) were assessed by visual inspection of the C-T curves. Determination of the log-linear terminal rate constant, λ_z, was based on the terminal slope (typically the last 4 quantifiable plasma samples, from 12–48 h post-dose) of the natural logarithmic C-T curve using linear regression analysis. However, for some cats with prolonged quantifiable plasma concentrations, an intermediate (β-phase) rate constant was determined from samples between T_{max} and 12 h. The β-phase half-life ($T_{1/2,\beta}$) and terminal elimination (λ_z) half-life ($T_{1/2,\lambda z}$) were calculated as ln2/β and ln2/λ_z, respectively. The area under the C-T curve from 0 h to the last quantifiable plasma concentration ($AUC_{0\text{-last}}$) was determined using the linear trapezoidal rule. In order to compare PK parameters between the low and high dose groups, C_{max} and $AUC_{0\text{-last}}$ were dose-normalized (divided by dose administered). Apparent volume of distribution (V_d/F) and apparent clearance (Cl_S/F) were also derived.

Statistical analysis

Dose-normalized C_{max} and $AUC_{0\text{-last}}$ values for CBD and THC were compared between low and high dose groups with a two-sample *T*-test (Graphpad Prism 9.3, GraphPad Software, La Jolla, CA). Dose-normalized C_{max} and $AUC_{0\text{-last}}$ were also compared between THC and CBD using a two-sample *T*-test. A p value of <0.05 was defined as the cutoff for statistical significance. Due to limited numbers of observations and variation in assessment procedures between dose groups, statistical evaluation of the neurological evaluations and adverse events was considered inappropriate; only incidence of findings is reported.

Results

CHE formulation

A single batch of CHE (CanniMed) was used for the entirety of this study and contained 19.5 mg CBD and 1.0 mg THC per mL (nominal concentrations of 20 and 1.0 mg/mL, respectively). Elemental impurities, mycotoxins, or pesticides were either not detected or not quantifiable.

Dose administration and adverse events

Administration of the CHE was generally well tolerated in the cats. To ensure swallowing of the CHE following dose administration, the cat's mouth was held closed for 10–20 s or until swallowing was visualized to ensure swallowing. However, two cats in the high dose group had moderate hypersalivation within 2–10 min of dosing (see Figure 1). Following analysis of plasma samples by LC–MS/MS, it was noted that these two cats had substantially lower cannabinoid concentrations than the other cats in the high dose group. It is presumed that these cats swallowed only a fraction of the administered dose and may have expelled the remainder in the saliva or while licking their lips during salivation. However, because there was no way to verify or quantify that these cats did not receive the entire dose, their plasma concentrations were included in the data analysis. Cats were intensively monitored for the first 8 h after dosing; no cat vomited, regurgitated, or coughed up any CHE during this time. Due to demonstration of hyperesthesia in dogs after use of the same CHE doses and formulation (15), cats were carefully evaluated for any potential neurological or behavioural changes. No neurological abnormalities were observed and the cats did not exhibit sedation or altered mentation.

Pharmacokinetic results

Mean ± SD plasma concentrations of CBD and THC by dose group are shown in Table 1. Variance within each dose group was very high, with CV% exceeding 100% at some time points. Mean plasma concentration versus time curves for CBD and THC in both dose groups are shown in Figure 2. In the high dose (5 mg CBD/kg bw) group, all 6 cats had plasma CBD concentrations above the limit of quantification (1.97 ng/mL) at all study time (including 48 h post-dose). For cats in the low dose (2 mg CBD/kg bw) dose group, CBD concentrations were only quantifiable up to 24 h post-dose. THC concentrations were only quantifiable (> 1.97 ng/mL) up to 4–12 h after dosing. The CBD metabolite 6-OH-CBD was quantifiable sporadically at various time points from 0.5–12 h post-dose, with the highest single concentration observed of 16.8 ng/mL. Other cannabinoid metabolites included in the assay (7-OH-CBD, 11-OH-THC, and THC-COOH) were not detectable in any plasma samples.

CBD and THC pharmacokinetic (PK) parameters are shown in Table 2. PK parameters were not derived for 6-OH-CBD due to the limited number of quantifiable concentrations observed. Dose-adjusted C_{max} and AUC_{last} (i.e., C_{max} or $AUC_{0\text{-last}}$/dose) values were not statistically significantly different between the low and high dose groups for either CBD or THC. However, when the dose-adjusted parameter (C_{max} or AUC_{last}) results were combined for both dose groups and compared between cannabinoids, the THC dose-adjusted parameter was statistically significantly higher than the CBD dose-adjusted parameter ($p < 0.01$). Apparent volume of distribution (V_d/F) and apparent clearance (Cl_S/F) were calculated but not reported due to the unknown bioavailability (*F*) and high variance observed.

FIGURE 1
Cat demonstrating hypersalivation immediately following administration of a single 5 mg CBD/kg bw oral dose of 1:20 THC:CBD CHE formulation.

TABLE 1 Mean ± S.D. concentrations of CBD and THC in plasma for fasted Domestic Shorthair cats administered a single dose of CHE ($n = 6$ per dose group).

Time (h)	Low dose (2 mg CBD + 0.1 mg THC/kg bw)			High dose (5 mg CBD + 0.25 mg THC/kg bw)		
	CBD (ng/mL)	THC (ng/mL)	THC:CBD ratio	CBD (ng/mL)	THC (ng/mL)	THC:CBD ratio
0.5	7.1 ± 6.4 (5)	ND	–	24.9 ± 28.4	4.3 ± 3.0	0.17
1.0	19.6 ± 21.9	6.3 ± 1.3 (2)	–	81.9 75.3	11.8 ± 9.2 (4)	0.14
1.5	37.6 ± 27.9	6.4 ± 4.1	0.32	165.1 ± 135.2	20.7 ± 15.7	0.13
2.0	52.1 ± 24.9	8.8 ± 4.1	0.17	223.8 ± 188.1	24.3 ± 22.8	0.11
3.0	93.6 ± 90.4	15.6 ± 13.2	0.17	187.4 ± 157.4	29.4 ± 20.4	0.16
4.0	54.6 ± 46.3	10.7 ± 9.2	0.20	143.8 ± 113.3	24.9 15.3	0.17
6.0	19.6 ± 11.6	4.7 2.4	0.24	55.8 ± 47.6	13.3 ± 10.3	0.24
8.0	9.3 ± 3.1	2.5 ± 0.5 (2)	0.26	47.6 ± 55.5	8.4 ± 6.9 (4)	0.18
12.0	4.9 ± 2.1	ND	–	19.9 ± 16.9	4.4 ± 1.3 (2)	–
24.0	2.4 ± 0.3 (2)	ND	–	7.7 ± 3.1	ND	–
32.0	BLOQ	ND	–	5.2 ± 2.2	ND	–
48.0	BLOQ or ND	ND	–	3.0 ± 0.7 (6)	ND	–

Numbers in parentheses indicate number of quantifiable plasma concentrations at the time point. BLOQ, below limit of quantification (LLOQ = 1.97 ng/mL), ND, not detectable (LOD = 0.98 ng/mL), NA, not available.

Discussion

There is limited and sometimes conflicting information regarding pharmacokinetics and pharmacodynamics of cannabinoids in feline medicine, which makes recommending specific dose regimens challenging for veterinarians. Delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are two of the 120 cannabinoids discovered from the Cannabis plants that have been studied most extensively, particularly in recent years. This study investigated the pharmacokinetics of CBD and THC after administration of a single dose of 1:20 THC:CBD cannabis herbal extract in fasting cats, with plasma concentrations collected over a 48-h period. The CHE doses selected for this study (2 or 5 mg CBD; 0.1 or 0.25 mg THC/kg bw) were based on those used for the same formulation in dogs which resulted in minimal adverse effects (15).

The PK parameters derived in this study was broadly similar to others evaluating cannabinoid PK in cats. Oral CBD administration in fasting cats has consistently demonstrated rapid absorption, with published mean T_{max} values typically reported around 2 h (10–13). Mean CBD T_{max} values in this study were 2.4 and 2.5 h for the 2 and 5 mg CBD/kg bw doses, respectively. Previously published studies in cats noted rapid CBD elimination half-life values of 1.5–4 h (10, 11, 13), but the elimination half-lives were typically much longer in this study (up to a mean of 17.1 h in the 5 mg/kg dose group). This difference may be attributable to the dose regimen and plasma sampling schedule. Another study (12) using higher CBD doses (e.g., 5 mg/kg and up) resulting in quantifiable plasma concentrations up to 48 h, noted a similarly prolonged terminal elimination phase (λ_z) and thus longer elimination half-lives. Similar differences in CBD

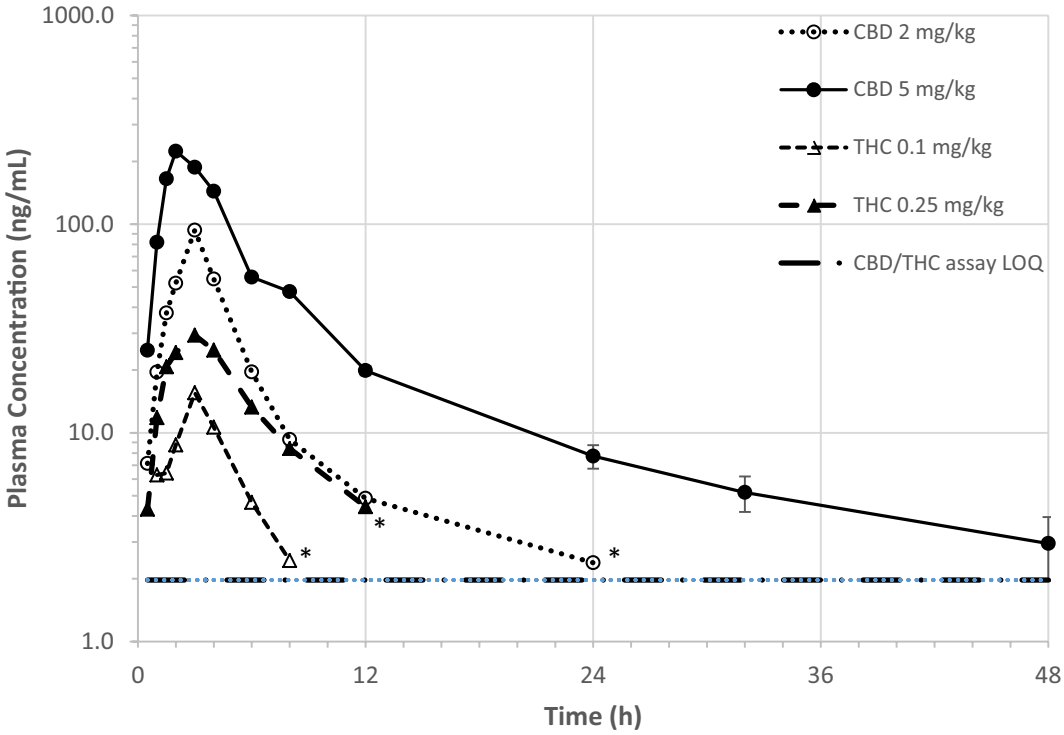

FIGURE 2
Mean plasma CBD and THC concentrations over time in fasted cats (n = 6/dosing group) receiving a single oral dose of 1:20 THC:CBD CHE formulation at 2 or 5 mg CBD/kg bw. Variance (s.d.) not shown due to overlapping error bars. *Mean value based on only 2 quantifiable concentrations.

TABLE 2 Mean (SD) cannabinoid PK parameters in fasted Domestic Shorthair cats (n = 6/dose group) receiving a single oral dose of 1:20 THC:CBD CHE at 2 or 5 mg CBD/kg bw.

Cannabinoid	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/mL)	Dose-Adj. C_{max} (ng/mL per mg/kg dose)[a]	$T_{1/2\ \beta}$ (h)[b]	$T_{1/2\ \lambda}$ (h)[c]	$AUC_{0\text{-last}}$ (ng*h/mL)	Dose-Adj. $AUC_{0\text{-last}}$ (ng*h/mL per mg/kg)[a]
CBD	2	2.4 (0.7)	111.2 (79.0)	55.6 (39.5)	2.9 (2.0)	7.7 (1.0)	344 (183)	170.8 (91.4)
	5	2.5 (0.8)	214.1 (182.8)	42.8 (36.6)	2.5 (0.4)	17.1 (5.8)	1,293 (970)	258.6 (194.0)
Combined CBD dose groups				49.2[A] (36.9)	Combined CBD dose groups			214.7[A] (151.7)
THC	0.1	2.9 (1.6)	17.1 (12.0)	170.6 (120.3)	2.4 (1.5)	NA	52.5 (32.0)	525.4 (320.3)
	0.25	2.7 (0.8)	27.9 (21.9)	111.8 (87.8)	2.0 (0.6)	NA	147 (117)	587.3 (465.8)
Combined THC dose groups				141.2[B] (105.0)	Combined THC dose groups			556.3[B] (378.3)

[a]Dose-adjusted value (parameter value divided by mg/kg dose).
[b]T1/2 β phase, from Tmax – 12 h post-dose.
[c]T1/2 λ (terminal elimination) phase, from 12–48 h post-dose.
T_{max}, time to maximum concentration, C_{max}, maximum concentration, AUC, area under the plasma concentration versus time curve, T1/2, half-life. CBD and THC statistical analysis: Differing alphabetical superscripts in each column indicate statistically significant ($p < 0.05$) differences between mean values (two-sample T-test). NA, not applicable.

elimination half-lives have been noted in canine studies using sampling schedules beyond 24 h (15). Despite the relatively long terminal elimination half-life for CBD in the 5 mg/kg dose group, the likelihood of clinically-relevant bioaccumulation occurring with repeated daily dosing appears to be low. The rapid $T_{1/2\beta}$ (from T_{max} to 24 h) for both CBD and THC (values comparable to the elimination half-life values reported in other animal studies), leaves very low cannabinoid concentrations when the terminal elimination phase begins.

Peak CBD plasma concentrations (C_{max}) and overall exposure (AUC) in cats appear to vary considerably between studies. Wang et al. (13) used a similar dose (1.37 mg CBD/kg bw) and cannabinoid ratio (1:27 THC:CBD) as those used in this study, yet the mean C_{max} in that study was substantially higher than after administration of 2 mg CBD/kg in this study (282.0 ± 149.4 ng/mL compared with 111.2 ± 79.0 ng/mL, respectively). The cats in both studies were fasted; however, the vehicle for cannabinoid delivery was different (food-based paste versus olive oil-based cannabis herbal extract). Another study administering a pure CBD formulation (11) to fasting cats at dose of 5 mg/kg bw reported comparable mean C_{max} and AUC values (269 ± 334 ng/mL; 921 ± 1,003 ng*h/mL) cats administered the same dose in this study (214.2 ± 182.8 ng/mL; 1,293 ± 970 ng*h/mL). Finally,

other recent studies (10, 12) using comparable CBD doses reported mean C_{max} and AUC values that were approximately 20–50% of the values determined in this study.

Cannabinoids are lipophilic and are considered to have poor oral bioavailability, but display increased absorption in fed rather than fasting states in humans (16, 17). A recent crossover study (11) in cats demonstrated similar results with CBD exposure in the fed state being statistically significantly higher than in the fasting state. Other factors impacting oral bioavailability may include the presence of additional cannabinoids (such as THC) in the formulation. While the cannabinoid combination leading to a pharmacodynamic synergism (so-called "entourage effect") has been postulated in humans (4, 18) and animals (19, 20), the potential for THC to modulate CBD pharmacokinetic properties (such as bioavailability or clearance) cannot be ruled out. For example, another study in cats (14) using cannabis formulations with varying ratios of CBD and THC demonstrated significantly higher plasma CBD concentrations when combined with THC at a 1.5:1 CBD:THC ratio, compared to a 25:1 ratio.

As expected, the plasma concentrations and exposure of CBD and THC were elevated in the high dose group (5 mg CBD/kg bw) compared to the lower dose (2 mg CBD/kg bw). The increase in C_{max} and $AUC_{0\text{-}last}$ for both CBD and THC was roughly proportional to the increase in dose (2.5 fold higher). After standardizing by the dose administered (C_{max}/dose and $AUC_{0\text{-}last}$/dose), there were no statistically significant differences between the two dose groups for either parameter, either for CBD or THC. This suggests linear kinetics over the dose range utilized in this study, and is consistent with results from another recent cannabinoid PK study in cats utilizing a larger dose range (12). However, it was readily apparent that although the formulation used was a 1:20 THC:CBD extract (i.e., the THC dose comprised only 5% of the CBD dose), THC plasma concentrations were consistently higher than 5% of the CBD concentrations (Table 1). When dose-adjusted PK parameters from both dose groups are combined, the C_{max} and AUC_{last} for THC were statistically significantly higher than the CBD parameters (Table 2). Increased dose-adjusted THC plasma concentrations (relative to dose-adjusted CBD concentrations) were also demonstrated in other cannabinoid studies in cats using varying ratios of THC and CBD (13, 14), and in our previous canine trial (15) using the identical formulation as this study. The reason for the (relatively) elevated THC concentrations compared with CBD is not immediately obvious. THC may have increased relative bioavailability, or decreased systemic clearance, compared to CBD in cats. For example, a cannabinoid study in rats hypothesized that CBD inclusion may lead to saturation of cytochrome P450 enzymes or transmembrane proteins, thus reducing the metabolism or transport of THC (20). Alternatively, it may be that at the 20-fold difference between THC (0.1 and 0.25 mg/kg) and CBD (2 and 5 mg/kg) doses used in this study, the kinetics are not linear. If so, comparisons of "dose-adjusted" PK parameters between THC and CBD would not be valid. Further studies would be necessary to assess if this suspected THC "overperformance" (relative to CBD) in cats is consistent across varying CBD:THC ratios and doses.

Although multiple CBD and THC metabolites were included in the analytical method, only the CBD metabolite 6-OH-CBD was quantifiable at any sampling times. The other metabolites (7-OH-CBD, 11-OH-THC, and COOH-THC) were not detected in any samples. Another feline cannabinoid PK study did detect low concentrations of 11-OH-THC in feline plasma (14), but had administered significantly higher THC doses than in this study. Analytical methods used in most other previously published feline cannabinoid PK studies did not include cannabinoid metabolites. However, based on results from this study it is unlikely that such metabolites would have been detected.

The CBD and THC plasma concentrations from this feline study were generally lower than those observed in a previous canine study (15) using the same 1:20 THC:CBD formulation (CanniMed) and doses (2 and 5 mg CBD/kg bw). Lower cannabinoid concentrations could be due to species-specific pharmacokinetics in cats, such as inherently decreased absorption or increased rate of clearance compared to dogs. An alternative explanation is that technical challenges associated with oral administration oil-based extracts in cats may also be a factor in the reduced plasma concentrations. Quite simply, it is generally more difficult to administer oral substances to cats than to dogs. Study investigators ensured that the entire dose was administered into the cat's oral cavity, and waited for visual confirmation of swallowing before releasing the cats mouth. However, cats are notorious for "spitting up" oral medications which they conceal in their oral cavity, and it could not be confirmed that all cats swallowed the entire CHE dose. While no cat regurgitated or vomited after dosing, two cats (both in the high dose group) experienced excessive salivation within a couple minutes of dosing and had substantially lower cannabinoid concentrations than the other four cats in this dose group. Any oil-based CHE retained in the oral cavity may have prompted the cat to salivate, and subsequently been expelled from the mouth. However, while these two cats clearly hypersalivated, loss of cannabinoids in the saliva cannot be confirmed and therefore the results from these cats were not excluded from the analysis.

Challenges with oral dosing of felines may also contribute to the high degree of variance (S.D.) in PK parameters in each dose groups. CBD and THC plasma concentrations varied dramatically between individual cats in the same dose group, a finding observed in similar feline cannabinoid PK studies (5, 11). Alternatively cats may simply have inherently high inter-individual variability (or intra-individual variability after multiple doses) in cannabinoid kinetics. Such variance makes developing a therapeutic dosing regimen difficult. Cannabinoid therapeutic drug monitoring is typically not available for veterinary patients, and thus the veterinarian must dose empirically and adjust based on clinical response.

In summary, fasting cats administered a single oral dose of a 1:20 THC:CBD oral extract at 2 or 5 mg CBD/kg demonstrated no significant adverse effects and plasma concentrations generally comparable to other published studies in cats. CBD and THC concentrations increased in a linear fashion over the dose range, but THC concentrations were significantly higher than CBD concentrations when adjusted for dose administered. Plasma concentrations were highly variable between individual cats in the same dose group. While the dose regimens used in this study appear suitable for use in future feline clinical studies, veterinarians should not expect uniform responses when administering the same CHE dose to different cats.

Data availability statement

The datasets presented in this article are not readily available because proprietary information relating to cannabis formulations

used cannot be disclosed. Requests to access the datasets should be directed to AC, al.chicoine@usask.ca.

Ethics statement

The animal study was approved by University of Saskatchewan Animal Research Ethics Board, adhering to the Canadian Council on Animal Care guidelines for humane animal use (Animal Use Protocol Number 20210019). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CL: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. KM: Data curation, Investigation, Writing – original draft. MM-P: Investigation, Writing – original draft. SV: Formal analysis, Methodology, Writing – review & editing. JA: Conceptualization, Methodology, Writing – review & editing. AC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Corsato Alvarenga I, Panickar KS, Hess H, McGrath S. Scientific validation of cannabidiol for management of dog and cat diseases. *Annu Rev Anim Biosci*. (2023) 11:227–46. doi: 10.1146/annurev-animal-081122-070236

2. Orr LE, McKernan JF. Antiemetic effect of delta 9-tetrahydrocannabinol in chemotherapy-associated nausea and emesis as compared to placebo and compazine. *J Clin Pharmacol*. (1981) 21:76S–80S. doi: 10.1002/j.1552-4604.1981.tb02578.x

3. Devinsky O, Cross JH, Wright S. Trial of Cannabidiol for drug-resistant seizures in the Dravet syndrome. *N Engl J Med*. (2017) 377:699–700. doi: 10.1056/NEJMc1708349

4. Lattanzi S, Brigo F, Trinka E, Zaccara G, Cagnetti C, Del Giovane C, et al. Efficacy and safety of Cannabidiol in epilepsy: a systematic review and meta-analysis. *Drugs*. (2018) 78:1791–804. doi: 10.1007/s40265-018-0992-5

5. Rozental AJ, Weisbeck BG, Corsato Alvarenga I, Gustafson DL, Kusick BR, Rao S, et al. The efficacy and safety of cannabidiol as adjunct treatment for drug-resistant idiopathic epilepsy in 51 dogs: a double-blinded crossover study. *J Vet Intern Med*. (2023) 37:2291–300. doi: 10.1111/jvim.16912

6. Turner S, Barker VD, Adams AA. Effects of Cannabidiol on the in vitro lymphocyte pro-inflammatory cytokine production of senior horses. *J Equine Vet*. (2021) 103:103668. doi: 10.1016/j.jevs.2021.103668

7. Gugliandolo E, Licata P, Peritore AF, Siracusa R, D'Amico R, Cordaro M, et al. Effect of Cannabidiol (CBD) on canine inflammatory response: an ex vivo study on LPS stimulated whole blood. *Vet Sci*. (2021) 8:185. doi: 10.3390/vetsci8090185

8. Lucas CJ, Galettis P, Schneider J. The pharmacokinetics and the pharmacodynamics of cannabinoids. *Br J Clin Pharmacol*. (2018) 84:2477–82. doi: 10.1111/bcp.13710

9. Schwark WS, Wakshlag JJ. A one health perspective on comparative cannabidiol and cannabidiolic acid pharmacokinetics and biotransformation in humans and domestic animals. *Am J Vet Res*. (2023) 84:1–9. doi: 10.2460/ajvr.23.02.0031

10. Deabold KA, Schwark WS, Wolf L, Wakshlag JJ. Single-dose pharmacokinetics and preliminary safety assessment with use of CBD-rich hemp nutraceutical in healthy dogs and cats. *Animals*. (2019) 9:832. doi: 10.3390/ani9100832

11. Jukier T, Cruz-Espindola C, Martin D, Boothe DM. Disposition of a single oral dose of a cannabidiol medication in healthy cats. *Front Vet Sci*. (2023) 10:1181517. doi: 10.3389/fvets.2023.1181517

12. Rozental AJ, Gustafson DL, Kusick BR, Bartner LR, Castro SC, McGrath S. Pharmacokinetics of escalating single-dose administration of cannabidiol to cats. *J Vet Pharmacol Ther*. (2023) 46:25–33. doi: 10.1111/jvp.13100

13. Wang T, Zakharov A, Gomez B, Lyubimov A, Trottier NL, Schwark WS, et al. Serum cannabinoid 24 h and 1 week steady state pharmacokinetic assessment in cats using a CBD/CBDA rich hemp paste. *Front Vet Sci*. (2022) 9:895368. doi: 10.3389/fvets.2022.895368

14. Kulpa JE, Paulionis LJ, Eglit GM, Vaughn DM. Safety and tolerability of escalating cannabinoid doses in healthy cats. *J Feline Med Surg*. (2021) 23:1162–75. doi: 10.1177/1098612X211004215

15. Chicoine A, Illing K, Vuong S, Pinto KR, Alcorn J, Cosford K. Pharmacokinetic and safety evaluation of various Oral doses of a novel 1:20 THC:CBD cannabis herbal extract in dogs. *Front Vet Sci*. (2020) 7:583404. doi: 10.3389/fvets.2020.583404

16. Taylor L, Gidal B, Blakey G, Tayo B, Morrison G. A phase I, randomized, double-blind, placebo-controlled, single ascending dose, multiple dose, and food effect trial of the safety, tolerability and pharmacokinetics of highly purified Cannabidiol in healthy subjects. *CNS Drugs*. (2018) 32:1053–67. doi: 10.1007/s40263-018-0578-5

17. Lunn S, Diaz P, O'Hearn S, Cahill SP, Blake A, Narine K, et al. Human pharmacokinetic parameters of orally Administered Delta (9)-tetrahydrocannabinol capsules are altered by fed versus fasted conditions and sex differences. *Cannabis Cannabinoid Res*. (2019) 4:255–64. doi: 10.1089/can.2019.0037

18. Pamplona FA, da Silva LR, Coan AC. Potential clinical benefits of CBD-rich cannabis extracts over purified CBD in treatment-resistant epilepsy: observational data meta-analysis. *Front Neurol*. (2018) 9:759. doi: 10.3389/fneur.2018.00759

19. Wakshlag JJ, Schwark WS, Deabold KA, Talsma BN, Cital S, Lyubimov A, et al. Pharmacokinetics of Cannabidiol, Cannabidiolic acid, Delta 9-tetrahydrocannabinol. *Front vet sci*. (2020) 7:505. doi: 10.3389/fvets.2020.00505

20. Klein C, Karanges E, Spiro A, Wong A, Spencer J, Huynh T, et al. Cannabidiol potentiates Delta (9)-tetrahydrocannabinol (THC) behavioural effects and alters THC pharmacokinetics during acute and chronic treatment in adolescent rats. *Psychopharmacology*. (2011) 218:443–57. doi: 10.1007/s00213-011-2342-0

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Safety study of cannabidiol products in healthy dogs

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The tolerability of different cannabinoids given orally to dogs was evaluated in a randomized, non-blinded, negative controlled, parallel design 90-day repeat dose study with a 14-day recovery period. Healthy beagles (16 males and 16 females) were randomized into four treatment groups and treated with either medium chain triglyceride oil as the control or one of the following: broad spectrum cannabidiol, broad spectrum cannabidiol with cannabigerol, or broad spectrum cannabidiol with cannabidiolic acid at 5 mg total cannabinoids/kg body weight/day. Animals were observed daily with detailed clinical examinations conducted weekly. Animals were monitored for an additional 2 weeks after dosing. Body weights, food consumption and clinical pathology evaluations were included in the study. Cannabinoids were well tolerated when healthy male and female beagles were dosed for 90 consecutive days. Annual post-market surveillance data for hemp-derived supplement products sold for use in dogs from 2010 to 2023 (partial year) shows that the rate per 1 million administrations sold is 2.10 for adverse events and 0.01 for serious adverse events. Based on the results of this study, other published studies, and data from extensive post-market surveillance, hemp-derived cannabinoids are well tolerated in healthy dogs at a dose of 5 mg/kg body weight/day.

KEYWORDS

cannabinoid, canine, CBD, CBDA, CBG, cannabis, hemp, NASC

1 Introduction

Cannabinoid products derived from *Cannabis sativa* L., specifically hemp (defined in U.S. Code of Federal Regulations Title 7 Part 1437.3 "Hemp" as *C. sativa* containing <0.3% Δ9-tetrahydrocannabinol (THC)), are increasing in use for both humans and their pets. Consumer and veterinary surveys indicate use in pets is notable and likely to grow and consumer understanding of the products they are giving their pets is low. Alvarenga et al. (1) collected data from 1,238 survey participants (mostly in the US) via a website that pools participants specifically for online research. They reported that 28.8% ($n = 356$) of respondents indicated they currently give or had given their pet cannabidiol (CBD) or cannabis product and 51.4% ($n = 882$) indicated they would be interested in giving their pet a CBD or cannabis product. Of the respondents who were currently giving or had given a supplement, CBD isolate was the most commonly identified product (100% CBD, 25.8%, $n = 92$). Broad spectrum (described as 0% THC, 16.6%, $n = 59$) and full spectrum (includes THC, 15.2%, $n = 54$) were also commonly used. However, many survey respondents did not know the purity or composition (42.4%, $n = 151$) (1). There are gaps in information for veterinarians as well and the veterinary community is not fully equipped to council clients on CBD use for their pets. In an anonymous survey of 2,130 US veterinarians in 2018, approximately one third (35%)

said they "did not know much" about the therapeutic effects of hemp/CBD, and 43.7% of respondents indicated they "did not know much" about the toxic effects of hemp/CBD products. The majority (86.4%) of respondents agreed or strongly agreed that therapeutic use and toxicity of hemp/CBD should be researched (2).

The body of evidence for safety of cannabinoid product use in dogs, from both consumer reports and scientific studies, is small but growing. Conversely, the risk of cannabis toxicosis in pets increases with the increasing availability of consumer products, therefore further research on the safety and effectiveness of cannabis products is warranted (3, 4). Information regarding the safety of hemp extracts and isolated hemp cannabinoids from preclinical studies in rodents can be utilized to determine the safety of these extracts, however additional information is required from studies conducted in dogs to adequately determine the safe doses to utilize (5–7). Pharmacokinetic data from recent studies in dogs is available for broad spectrum CBD (8, 9), purified CBD (10), CBD/ cannabidiolic acid (CBDA) (11–13), and cannabigerol (CBG) with cannabigerolic acid (CBGA) (14), as well as delivery methods other than oral (15–17), CBD with THC in a 1:20 THC: CBD ratio (18), and Sativex® (19).

Duration of use, product form, and vehicle (e.g., oil-based extracts) for delivery of cannabinoids have been explored. Alvarenga et al. (9) completed a long-term (36 week) study of broad-spectrum CBD (95% of cannabinoid profile) in a medium chain triglyceride (MCT) vehicle. The authors reported that CBD accumulated in dogs over time as the half-life tripled by the 18-week mark and stayed at that level until 36 weeks, and this effect was proportional to the dose (9). Deabold et al. (8) gave doses of 2 mg CBD/CBDA mix/kg bodyweight (bw)/day to fasted dogs ($n = 6$) over a 12-week period in a chew format. The authors noted that delivery in a chew resulted in a shorter retention time and half-life than an infused oil (8). Wakshlag et al. (11) determined the pharmacokinetics of three different forms of an infused oil containing equal amounts CBD and CBDA and small amounts of THC and tetrahydrocannabinolic acid (THCA). They determined that a vehicle of 25% sunflower lecithin increased the absorption of CBDA and THCA, demonstrating that the vehicle has the potential to affect the safety profile (11).

Oral dosing with cannabinoids in dogs is generally well tolerated. Di Salvo et al. (20) summarized 19 tolerability studies with CBD or CBD/CBDA. Of the two studies that extended beyond 12 weeks duration, one used a CBD-only distillate at approximately 4 mg/kg bw/day and the other used a highly purified CBD (Epidiolex) at up to 100 mg/kg bw/day. Five studies of 12 weeks duration using CBD or CBD/CBDA products were also summarized. Common side effects noted were increase in ALP activity, GI symptoms, somnolence, and ataxia. No serious side effects were noted (20).

This study in healthy male and female beagles given a daily treatment dose for 90 consecutive days evaluates broad spectrum CBD, broad spectrum CBD with CBDA, and is the first to our knowledge to include broad spectrum CBD with CBG in a long-term tolerability study. Given the volume of consumer products sold annually, data from well controlled studies with defined safety endpoints and doses relative to industry use are imperative to understand the risk associated with cannabinoid use in dogs. It is expected that doses of 5 mg/kg bw/day will have no adverse effects in healthy beagles. The current study adds to the available literature evaluating the tolerability of broad-spectrum cannabinoid products in healthy dogs in a fed state.

2 Materials and methods

2.1 Study conduct

This study was conducted by ClinVet USA LLC, an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility which conforms to the guidelines set forth in the National Research Council Guide for the Care and Use of Laboratory Animals (8th Edition, 2011). All procedures were designed in accordance with the principles of the USDA Animal Welfare Act (7 USC § 2,131–2,159) as well as U.S. Code of Federal Regulations Title 9, Part 3. The study protocol was approved by the Institutional Animal Care and Use Committee.

2.2 Animals

Thirty-two intact healthy beagle dogs (16 males and 16 females), with an average age of 18.4 months ± 6.7 (range 11 to 32 months) and weighing an average of 9.9 kg ± 1.2 (range 8.2 to 12.8 kg) at study start, were included in this study. Female dogs were checked for pregnancy prior to inclusion in the study. See section 3.4 Assessment for further information on health assessments. All dogs were housed individually in stainless steel cages, which were cleaned daily and sanitized at least bi-weekly. An acclimation period of 14 days in the housing room was provided. All animals had access to visual, auditory, and olfactory contact during the study. Dogs were exercised with their respective treatment groups and sexes outside of their cages during daily husbandry duties. A 12-h light/dark cycle was maintained throughout the study. All dogs were fed Parable Agriculture Custom 30–22 Dog Food, from Pro-Pet, LLC (dry food) in a daily ration with *ad libitum* water. Animals were dosed daily for 90 days and were then observed for an additional 14 days without dosing. At the end of the study, the animals were returned to the testing facility colony.

2.3 Study design

This study was a randomized, non-blinded, negative controlled, parallel group design. The dogs were randomized by block design into 4 groups. Four sex-balanced groups were created by ranking females ($n = 16$) by decreasing weight, males ($n = 16$) by increasing weight, and blocking the animals into 8 groups of 4 dogs. Within blocks, the dogs were allocated randomly to the treatment groups. Each treatment group was given one daily oral dose of: broad spectrum CBD (test article (TA) 1; group 2), CBD + CBG combination (TA2; group 3), CBD + CBDA combination (TA3; group 4), or MCT oil (Control; group 1) for 90 days. The dogs were fasted overnight after removal of any remaining daily ration and received a normal ration in the morning prior to dosing. Dogs were dosed when in a fed state and doses were delivered orally via syringe.

The daily dose of the test materials was 5 mg of total cannabinoids/kg bw and the volume of the control MCT oil was correlated with the volume dosed in the treatment groups. TA1 was CBD of 80–90% purity, manufactured by Open Book Extracts, Roxboro, NC. TA2 was CBD + CBG in a 1:2 ratio, manufactured by Open Book Extracts. TA3 was CBD + CBDA in a 1:1 ratio, manufactured by KND Labs, Lakewood, CO (Table 1). The control article was MCT oil sourced

TABLE 1 Composition of cannabinoid test articles (TA) used in the 90-day repeat dose study.

Test article	TA1: CBD	TA2: CBD + CBG	TA3: CBD + CBDA
Lot #	BFG-000030-220505	BFG-000031-220505	KND 1:1-CBD/A-MCT-595
CBD (mg/g)	37.01	13.52	17.1100
CBG (mg/g)	0.99	24.56	0.7356
CBDA (mg/g)	ND	ND	18.5308
CBDV (mg/g)	0.13	0.08	ND
CBN (mg/g)	ND	ND	0.5885
CBGa (mg/g)	ND	ND	ND
CBC (mg/g)	0.29	0.11	0.5347
THC (mg/g)	ND	ND	ND
Total cannabinoids (mg/g)	38.42	38.26	35.545
Total terpenes (mg/g)	2.080	0.740	0.0125
Residual solvents	ND	ND	ND*
Heavy metals (μg/g)	ND	ND	<LOQ (0.05)
Pesticides	ND	N1	ND
Microbials	ND	ND	ND

CBC, Cannabichromene; CBD, Cannabidiol; CBDA, Cannabidiolic acid; CBDV, Cannabidivarin; CBG, Cannabigerol; CBGa, Cannabigerolic acid; CBN, Cannabinol; LOQ, Limit of Quantitation; ND, Not detected; THC, Tetrahydrocannabinol.
*With the exception of acetonitrile which was present at 73.0 μg/g (Limit = 5,000 μg/g).

from coconut or palm kernel, manufactured by Jedwards International, Inc., Braintree, MA.

2.4 Assessments

Clinical examinations were performed on all animals during acclimatization (between day −14 and − 1), and days 14, 28, 56, 90 and 104. Clinical examination included but was not limited to vital signs, mucous membranes, eyes, motility, lymph nodes, abdominal palpations, thoracic auscultation, skin condition, behavior, reproductive system, respiratory, cardiac, gastrointestinal, and urinary systems. All animals were also observed twice daily for habitus, color of urine, color and consistency of feces, salivation, vomiting, skin lesions, and obvious change in general condition. Body weights were measured on days −8, −1, and weekly throughout the study. Adverse events (AE) were considered to be any observation that was unfavorable or unintended and occurred anytime during the dosing period (after day 0). Serious adverse events (SAE) were defined as AE that were fatal or life threatening.

Food consumption was determined by weighing food prior to and after feeding each animal daily from day −7 through the end of the study. Blood samples were collected into serum separator tubes (2 mL whole blood), sodium citrate tube (2.7 mL whole blood), and EDTA tube (1.0 mL whole blood) from fasted animals on days −9, 14, 28, 56, 90 and 104 for clinical pathology. Serum from the separator tube was allowed to sit at room temperature for 1 h prior to separation. Plasma from the sodium citrate tube was separated after centrifuging for 10 min at room temperature, then plasma was separated and frozen at −60°C to −90°C before transport to the laboratory. The EDTA tube was not processed. Analyses included hematology, serum chemistry, and coagulation parameters. Hematology parameters were erythrocytes, hemoglobin, leukocytes, MCH, MCHC, MCV, PCV, and platelet count. Serum chemistry parameters were ALT, albumin, ALP, amylase, AST, calcium, chloride, cholesterol, creatine kinase, creatinine, globulin, GGT, glucose, LDH, magnesium, phosphate, potassium, sodium, total protein, and urea nitrogen. Serum chemistry analyses were performed using a Roche Cobas c501 (Roche Diagnostics, Indianapolis, IN, USA) and hematology analyses were completed with a Siemens Advia 2120i (Siemens Medical Solutions USA, Inc. Malvern, PA USA). Coagulation parameters were prothrombin time, fibrinogen, and activated thromboplastin time. Coagulation parameters were analyzed using a Diagnostica Stago STA Compact Max (Diagnostica Stago S.A.S., France). Urine was collected via passive collection in the morning on days −8 /−7, 28, 90 and 104. Urine samples were analyzed for turbidity, specific gravity, pH, protein, glucose, ketones, blood, and bilirubin using a Siemens Clinitek Advantus (Siemens Medical Solutions USA, Inc. Malvern, PA USA). All samples were sent for analysis on the day of collection and analyzed within 1 day.

2.5 Statistical analysis

Statistical analysis procedures were based on International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) Guideline GL43: Target Animal Safety for Veterinary Pharmaceutical Products. Baseline data was considered the last non-missing value for each parameter prior to dosing. Individual hematology and serum chemistry parameters were reported with descriptive statistics: mean, SD, coefficient of variation, geometric mean, median, minimum, maximum, and number of observations (n) in that treatment group. For identifying parameter values that warrant further clinical review, a reference range was defined as the minimum and maximum values for each parameter at baseline across all groups of dogs in the current

study. Because the primary intent of this study was to evaluate tolerability of each formulation, the magnitude of changes from baseline (CFB) to each of the post-administration days were calculated for hematology and serum chemistry parameters. If a parameter for any individual on any post-administration day fell outside the reference range, the CFB for the treatment group was compared to the CFB for the control group, and the CFB within the treatment group was checked for significance. If all the above were statistically significant, further clinical review was completed. Within each treatment, post-administration values were compared to baseline by means of ANOVA with animal and observation time as effects for all laboratory parameters. Between-treatment comparisons of CFB on each post-administration day were performed using a linear mixed model with TA administration as fixed effect and randomization block as random effect. The results of all other measured or observed parameters (clinical examinations, general observations, bw, and food consumption) are reported descriptively and tabulated when appropriate. The level of significance for all formal tests was set at 5% and all tests were two-sided. SAS version 9.4 was used for all statistical analyses.

3 Results

All animals completed the study, and no somnolence, AE or SAE were reported during the study. Sporadic hypersalivation was reported in some animals in the CBD + CBG and CBD + CBDA treatments but these were not deemed to be an AE. In all groups, abnormal and incidental findings were reported in some animals during the daily visual examinations or the more detailed clinical examinations. These were deemed to be unrelated to test or control article exposure and did not negatively impact the results of the study. The most common abnormal observation was diarrhea. There were no statistically significant differences in bw between control and treatment groups at the start of the study and all groups had a higher mean bw on day 104 as compared to the baseline values at the start of the study (Supplementary Table S1). The majority of animals consumed their daily ration each day and differences in mean food consumption within all groups were sporadic and not considered to be an adverse finding (Supplementary Table S2). Clinical pathology data (including hematology and serum chemistry) is presented as mean ± SD for each time point tested. There were statistically significant within-treatment group changes reported in some hematology (Supplementary Table S3) and clinical chemistry (Supplementary Table S4) parameters evaluated as compared to the baseline value. The majority of the changes reported in the hematology parameters evaluated were either transient, had no concurrent clinical signs or correlating changes in other related clinical pathology parameters, or were within reference ranges and were not considered to be a clinically relevant adverse effect of test material treatment. On day 14, one animal receiving the CBD + CBDA treatment had a hemoglobin value slightly below the reference range and on day 56, one animal in the same treatment group had a WBC value which was slightly above the reference range. These changes were not considered clinically relevant due to the transient and /or isolated nature of the changes.

At each time point, the CFB was calculated and comparisons between control and treatment groups with respect to CFB were carried out to determine significance. Statistically significant changes in the mean CFB values of a number of clinical chemistry parameters were reported in the cannabinoid treatment groups compared to the mean CFB values of the control group (Supplementary Table S5). These changes were of a low magnitude and/or transient and/or were within the reference ranges and/or had no correlating changes in related parameters and were therefore determined to be of no clinical relevance.

Some of the changes in clinical chemistry parameters in individual animals were outside of the reference ranges and are discussed. On day 14, one animal in the CBD + CBDA treatment group had a urea value which was slightly below the reference range. One animal in the CBD + CBG treatment group had a potassium value which was slightly below the reference range on study days 14 and 28, while one animal in the CBD + CBDA treatment group had a potassium value which was slightly lower than the reference range on study day 28 only. One animal in the CBD + CBDA treatment group had an iron value lower than the reference range on study day 28 and a sodium level which was slightly above the reference range on study day 56. Two animals showed an increased chloride value which was above the reference range on study day 56 in the CBD + CBDA treatment group. Sodium was elevated to levels above the reference range in one animal in the CBD + CBDA treatment group on study day 56 and in a different animal in the same group on study day 90 as well as in one animal in the CBD + CBG treatment group on study day 90 as well. On study day 104, creatinine kinase values were found to be sporadically elevated above the reference range including in 2 animals in the control group. On study day 104, albumin in one animal in the CBD + CBG treatment group was slightly below the reference range. Given the low magnitude of the changes seen in the clinical chemistry parameters, the transient nature and lack of corresponding clinical or clinicopathological changes, the changes described were considered to be of no clinical relevance.

Changes in ALT, ALP and GGT were reported during the study. For the treatment groups, the CFB was compared to the CFB for the control group for the specific study day (Table 2). The only statistically significant change in GGT CFB values was reported on study day 28 in the CBD treatment group which decreased less than the concurrent controls. Mean CFB for ALP values showed an increase from baseline and were significantly higher in the CBD treatment group on study days 28, 56 and 90 and the CBD + CBDA treatment group on study day 56 as compared to the mean CFB values in the control group, which decreased from baseline. Within these groups, all values for ALP were within the laboratory reference ranges (range 7–115 U/L) with the exception of one animal in the CBD treatment group on study days 28 (314 U/L), 56 (227 U/L) and 90 (205 U/L) and one animal in the CBD + CBDA treatment group on study day 56 (123 U/L) in which the values were above the reference range. Of the values which were outside of the upper reference range for the single animal in the CBD treatment group, two of the three were below a twofold increase and the remaining value peaked at 314 U/L on study day 28 but then decreased in each of the following evaluations and was within the reference range following the 14-day recovery period. The value for the only other animal with a value above the reference range occurred on study day 56 and was below a twofold increase, and the values were within the reference range at the next evaluation on study day 90. Mean CFB ALT values for all treatment groups decreased from baseline, whereas in the control group, values decreased from baseline on day 14 and increased from baseline at all other time points. This resulted in a significant CFB

TABLE 2 Baseline and mean change from baseline (CFB) serum ALT, ALP, and GGT results for healthy beagles treated orally with medium chain triglyceride (MCT) oil (Control; $n = 8$) or 5 mg/kg body weight/day of CBD ($n = 8$), CBD + CBG ($n = 8$) or CBD + CBDA ($n = 8$) for 90 days, followed by 14 days without dosing.

Study day	Control	CBD	CBD + CBG	CBD + CBDA
ALT (U/L)				
−9 (Baseline)	33.3 ± 9.7	39.8 ± 13.8	42.4 ± 12.8	40.6 ± 15.1
	Change from baseline			
14	−3.1 ± 9.0	−3.5 ± 12.0	−8.9 ± 6.6	−8.8 ± 6.0
28	1.4 ± 3.5	−4.5 ± 11.0	−7.0 ± 5.5*	−3.9 ± 3.4
56	0.6 ± 5.0	−5.1 ± 10.2	−7.3 ± 8.3*	−6.3 ± 5.1
90	4.0 ± 3.8	−3.6 ± 10.0*	−2.9 ± 8.9	−5.0 ± 5.6*
104	3.3 ± 2.9	2.3 ± 12.2	−7.1 ± 9.8*	−2.6 ± 5.2
ALP (U/L)				
−9 (Baseline)	23.9 ± 10.6	31.1 ± 10.5	28.8 ± 9.6	31.1 ± 7.0
	Change from baseline			
14	−2.9 ± 4.3	21.5 ± 40.5	0.3 ± 3.1	22.0 ± 45.1
28	−1.4 ± 2.3	56.5 ± 89.5*	4.8 ± 5.7	31.6 ± 37.6
56	−2.3 ± 3.7	41.8 ± 58.5*	6.1 ± 8.2	34.8 ± 26.4*
90	−0.6 ± 3.3	38.5 ± 52.1*	5.9 ± 9.4	19.6 ± 22.4
104	1.3 ± 3.7	10.9 ± 17.1	9.6 ± 22.9	6.6 ± 8.9
GGT (U/L)				
−9 (Baseline)	2.5 ± 1.3	2.0 ± 0.9	2.5 ± 0.9	2.4 ± 1.4
	Change from baseline			
14	−0.5 ± 1.3	0.1 ± 1.7	0.4 ± 0.7	−0.3 ± 1.5
28	−1.8 ± 1.2	−0.1 ± 1.0*	−0.6 ± 1.2	−0.6 ± 1.5
56	0.0 ± 0.9	0.9 ± 1.5	0.4 ± 1.4	0.3 ± 1.7
90	0.3 ± 0.9	0.9 ± 0.8	0.4 ± 0.9	0.3 ± 2.1
104	0.1 ± 1.1	0.6 ± 1.4	−0.8 ± 0.9	−0.6 ± 1.4

Data are reported as mean ± SD for baseline and mean change from baseline ± SD of serum chemistry parameters on each assessment day. Between-group comparisons with respect to CFB were carried out to compare control and cannabinoid groups to determine significance.
*CFB is significantly different from control group on the same assessment day (row) ($p < 0.05$).
U/L, Units per liter.

in the CBD + CBG treatment group on study days 28, 56 and 104, as compared to the control group, however none of the mean values were outside of the reference range at any time (Supplementary Table S4).

There were statistically significant changes in some of the coagulation parameters evaluated however all measured values were within the reference ranges except for one animal in the CBD treatment group which had an elevated fibrinogen value which was deemed to be clinically irrelevant (Supplementary Table S6). Urine was collected prior to dosing and then on study days 28, 90 and 104 and no clinically relevant changes were reported in any treatment groups as compared to controls. Specific gravity and urine pH are summarized in Supplementary Table S7. No significant abnormalities were recorded for any urinalysis parameters evaluated. All animals were returned to the Test Site colony at the end of the study.

4 Discussion

In the current study, daily exposure to CBD, CBD + CBG and CBD + CBDA at 5 mg/kg bw of total cannabinoids for 90 consecutive days was well tolerated. The significant changes seen in some clinical pathology parameters were transient, within reference ranges, of low magnitude, present in a small number of animals or sporadic in nature and all were considered not to be clinically relevant. Biological variability is discussed in Flatland et al. where the authors concluded that a single clinical value needs to be interpreted within three aspects of variation – individual, group, and analytical method (21). In the current study, review for clinical relevance was determined using a reference range set by the baseline values of the animals in the study as previously described. If a parameter for any individual on any post-administration day fell outside this reference range, the CFB for the treatment group was compared to the CFB for the control group, and the CFB within the treatment group was checked for significance. If all the above were statistically significant, further clinical review was completed. Following this method, individual and group variation is accounted for via reference range determination and by placing emphasis on the CFB as indicative of a treatment-related change but only if the treatment group CFB was different from the control group CFB for any parameter. Analytical variation is not applicable in this study as all measurements were made under the same conditions as part of a research study, and not in a clinical setting where variation between equipment, staff, etc. could be notable.

The results from this study correlate with other studies conducted with CBD in healthy dogs which have concluded that CBD, CBG, and CBD with CBDA is well tolerated. Bradley et al. (22) conducted a randomized, placebo-controlled, blinded study with broad-spectrum CBD in healthy dogs. The CBD treated dogs received 4 mg/kg bw/day for 6 months without any adverse effects on health and wellbeing. A transient elevation in ALP was reported in approximately half of the CBD treated dogs which returned to baseline at the end of the 4-week recovery period. Bone ALP was evaluated to determine the tissue source of the ALP and was significantly elevated as compared to controls at the end of 26 weeks of treatment with a significant and strong positive correlation between ALP and bone ALP. Based on these and other results, the authors concluded that the increased ALP was not a clinically relevant biomarker of impaired liver health in healthy dogs following CBD treatment (22). Vaughn et al. (23) also evaluated the safety of CBD in healthy dogs in a 28-day repeat dose trial. In the randomized, blinded, placebo-controlled study, the healthy dogs received either a placebo or 1, 2, 4 or 12 mg CBD/kg bw/day which was well tolerated. All reported AE were mild and self-limiting and occurred in all groups, including the placebo group. Increased serum ALP above the upper reference limit was reported in the 2, 4 and 12 mg/kg bw/day groups which began to decrease after 2 weeks of dosing, but these animals did not have any concomitant increases in other hepatic markers. As with the current study, hypersalivation was seen with greater frequency in the CBD treated groups but this was not considered to be a SAE in either study (23). Deabold et al. (8) evaluated the safety and adverse effects of a CBD containing hemp product in healthy dogs over a 12-week dosing period. The dogs were given 2 mg CBD/kg bw/day and serum chemistry and hematology evaluations showed no clinically relevant changes during the study (8).

In a study by Amstutz et al. (14), CBG and CBGA was trialed in fed and fasted dogs ($n = 6$ intact male beagles) at 2 mg/kg bw twice daily for 2 weeks. The fasted state was tested initially for two-weeks, followed by a two-week washout and then treatment was given in the fed state for two-weeks. On the first day of treatment in both states, a 24-h pharmacokinetic analysis of serum cannabinoids was completed. The authors reported that there were no statistically significant differences in pharmacokinetic parameters between fed and fasted states, however, they note that the serum concentration of CBG tended to be higher in the fasted state. Serum ALP decreased in both fed and fasted states by week 2, which is contrary to other studies of cannabinoids. The authors suspect this may be related to differences in the effect of CBG and CBD on cytochrome P450 although no further evidence is discussed. The only AE reported was vomiting from one dog during the fasting phase with no other clinical symptoms. The authors concluded that CBG and CBGA at 2 mg/kg bw twice daily was well tolerated in fed and fasted healthy beagle dogs (14). In the current study, the test item contained CBD + CBG in a 1:2 ratio. Although not clinically relevant, the CBD and CBD/CBDA treatment groups each showed at least one measurement that was statistically different from the control group for ALP, whereas the CBD/CBG group did not differ from the control group.

Two studies evaluated CBD/CBDA mixed cannabinoid products. Tittle et al. (13) evaluated the pharmacokinetics of a CBD/CBDA extract that also included a low level of THC/THCA when dosed in oil verses a gel capsule. Beagles (7 male and one female) were dosed at 2 mg/kg bw twice daily with food. The initial treatment was the cannabinoid product in a capsule. Pharmacokinetic parameters were measured over 24 h on the first day of dosing and over a subsequent 7 days, followed by a 2-week washout period before the next treatment (cannabinoids in oil) and pharmacokinetic measurements for 7 days. No safety end points were assessed, however, AE noted were mild and included vomiting, diarrhea, licking, and head shaking. Vomiting and diarrhea was observed in three dogs during the washout period as well (13). Wakshlag et al. (11) evaluated a CBD/CBDA product that contained a small amount of THC and THCA, as well as measurable CBGA and cannabichromene. The intent of this study was to evaluate two different oil vehicles and a soft chew format with 2-week treatments followed by 3-week washout periods, resulting in a 12-week trial. Six intact female beagles were dosed at 2 mg/kg bw (oil) or 2.0–2.3 mg/kg bw (soft chew) of CBD/CBDA twice daily. Safety end points measured included ALP, AST, and ALT, albumin, total bilirubin, cholesterol, and glucose. No changes were observed in these parameter during treatment or between successive treatments, and no abnormalities in behavior or health were reported during the trial (11). A key difference in the test item for these studies compared to the current study was the presence of THC at a low level, however, like the current study, no AE related to administration of CBD/CBDA were reported.

Several studies evaluated the clinical efficacy of CBD and other cannabinoids in disease and behavioral conditions. The endpoints evaluated in these studies may not be specifically targeted towards safety, but they can provide some valuable tolerability information regardless. For example, studies have been conducted to evaluate the analgesic effect of CBD in dogs with spontaneous osteoarthritis at varying dose levels and durations, as well as in dogs who recently underwent orthopedic surgery. The dogs received up to 5 mg/kg bw orally for 4 weeks following surgery, which was shown to be well tolerated (12, 24–27). Treatment with CBD-CBDA was evaluated for efficacy on refractory epileptic seizures, intractable idiopathic epilepsy, atopic dermatitis, and immune response (28–31). The effect of CBD on behavioral conditions such as aggression towards animal shelter staff, separation anxiety and car travel, noise-induced fear, and voluntary activity was also evaluated (32–35). In all studies, few minor or zero AE were reported and no SAE that could be attributable to treatment were reported.

In the United States, "pet supplements," also called Dosage Form Animal Health Products, are unapproved animal drugs and available to consumers either through State-level regulations or enforcement discretion by the FDA (3). Products containing CBD are sold in substantial numbers and post-market surveillance data supports the safety of cannabinoids given orally. In 2022, there were 274,129,622 administrations, in dogs, of hemp and hemp derivative products sold, as determined by the National Animal Supplement Council (NASC). The NASC is a 501(c) (6) non-profit trade association that represents most of the industry selling products containing hemp, hemp derived compounds as well as cannabinoids in the US. The NASC requires all member companies marketing products to enter product information, upload product labels and to report AE monthly through its Adverse Event Reporting System (NAERS™) which is a powerful tool for post-market surveillance. Individual companies are also required to record, report, and evaluate AE monthly. Both serious and non-serious AE are reported in the NAERS™ system. Each AE is evaluated and given a risk score using the NASC Adverse Event reporting form, which is also maintained in the NAERS™ system.

In the NAERS™ system, AE and SAE are defined as follows:

- **Adverse Event:** "An Adverse Event is a type of Complaint where a patient has suffered any negative physical effect or health problem that MAY be connected to or associate with use of the product."
- **Serious Adverse Event:** "An Adverse Event with a transient incapacitating effect (i.e., rendering the animal unable to function normally for even a short period of time, such as with a seizure) or non-transient (i.e., permanent) health effect. Transient vomiting or diarrhea do not constitute Serious Adverse Events. A purported Serious Adverse Event requires follow-up with a veterinarian. A layperson diagnosis does not constitute a Serious Adverse Event."

Data from each company is aggregated, statistically processed, and compiled into an Ingredient Risk Report which provides information relating the ingredient(s) to reported AE, both serious and non-serious. The event rates are reported based on the number of administrations in each container sold and unit data is updated quarterly.

Data from NASC Members' products was also used in determining the dosing level used in the current study, 5 mg/kg bw of dogs, which is based on actual products currently in the marketplace. Based on the information from the Ingredient Risk Report, the straight mean and weighted mean doses for all hemp and hemp derivative products were determined to be 6.97 and 9.91 mg/kg bw. Comparatively, the straight mean and weighted mean doses for all CBD products were 0.83 mg/kg and 0.67 mg/kg (maximum 2.10 mg/kg). This provides important information that is difficult to ascertain from consumer surveys. Alvarenga et al. (1) reported that when asked about dose, survey respondents gave empiric answers of volume without concentration or missing a measuring unit, making analysis and reporting unfeasible (1).

The use of CBD in Dosage Form Animal Health Products has been growing; however, the safety of longer-term use has been questioned and deemed to be lacking (8, 22). A recent review of the current literature available for CBD use in dogs documented 19 tolerability studies, 10 pharmacokinetic studies with oral CBD products, seven clinical trials for efficacy in pain control, three for epilepsy, three for behavioral disorders, and three for skin diseases (20) The limitations of this body of evidence are that the number of types of extracts, the study population, and the duration of use are constrained by necessity. Post-market surveillance of AE and SAE in the NAERS™ database assists in the safety evaluation of CBD through real-world use data and supports the conclusion of the aforementioned studies that CBD products are well tolerated.

The information collected from the NAERS™ system report for all products containing hemp and hemp derived compounds shows that the overall report rate per million administrations sold from 2010 to 2023 (as of November 20th, 2023) for AE and SAE in dogs is 2.19 and 0.01, respectively, from over one billion administrations (Table 3). When limited to products specifying CBD, the total administrations in dogs for 2015–2023 (as of November 18, 2023) were 86,081,473, with AE and SAE rates of 1.61 and 0.02 per million administrations, respectively.

For interpretation of these results, it is important to note that regulatory restrictions on label statements affect the classification of dosage form animal health products in the NAERS™ system as the input classification is determined by the producer's or retailer's label. A product containing CBD may be labelled only as hemp or may include a qualifier such as broad-spectrum or full-spectrum, and not all products labelled as hemp contain CBD. NASC provides guidance to their membership that a broad-spectrum hemp extract contains

TABLE 3 National Animal Supplement Council (NASC) Ingredient Risk Report for hemp and hemp-derived compounds in dogs as of November 20, 2023.

Year	Adverse events (report rate/million administrations sold)	Serious adverse events (report rate/million administrations sold)	Administrations sold[a]
2010	0.00	0.00	25,016
2011	0.00	0.00	29,098
2012	0.00	0.00	104,421
2013	11.74	0.00	255,642
2014	0.00	0.00	543,023
2015	0.00	0.00	894,762
2016	0.00	0.00	1,755,993
2017	0.12	0.00	8,124,015
2018	0.50	0.00	40,395,501
2019	0.87	0.00	115,607,342
2020	2.26	0.00	190,065,703
2021	2.09	0.02	293,080,512
2022	2.24	0.03	274,129,622
2023[b]	3.16	0.02	152,536,208
Grand Total	**2.19**	**0.01**	**1,077,546,857**

Events are divided into adverse events and serious adverse events and reported based on the number of administrations in each container sold.

[a] Number of administrations sold is assumed to be a close approximation to administrations consumed.

[b] Usage data for 2023 is incomplete.

"some or all of the compounds found naturally occurring in the plant, where THC has been processed to levels less than 0.3%" and a full-spectrum extract contains "all compounds found naturally occurring in the plant including, but not limited to, terpenes, cannabinoids and THC, where the cultivar's THC level are grown or diluted to be less than 0.3%." Administrations reported for broad-spectrum hemp products for dogs were 84,306,219 and for full-spectrum hemp products for dogs were 287,828,119. The hemp and hemp derivatives report is inclusive of AE for broad-spectrum and full spectrum hemp products, but the AE and SAE rates when calculated separately from the larger category are similar. Broad-spectrum hemp products had an AE and SAE rate of 2.40 and 0.02 per million administrations, respectively, and full-spectrum products had an AE and SAE rate of 2.83 and 0.03 per million administrations, respectively. Effectively, total administrations calculated for hemp and hemp-derivatives overestimates the post-market exposure of dogs to CBD products, and CBD total administrations underestimates the post-market exposure. Based on this information, it is reasonable to surmise that the rate of AE is between 1.6 and 2.8 per million administrations.

It is also important to separate animal health product AE from acute toxicosis due to marijuana (*Cannabis sativa* L. with a THC content higher than 0.3% by dried weight; defined in 21 CFR 1308.11) products intended for human consumption. Howard-Azzeh et al. analyzed factors influencing cannabis poisoning of dogs in the United States between 2009 and 2014 (4). The authors reported that an average of 1.12% of all calls to the Animal Poison Control Center were due to cannabis consumption and concluded that as cannabis products became more available for human consumption, the rate of poisoning in dogs increased.

The low AE rate reported in the NAERS™ system is supported by consumer survey data (1). Of respondents who had given their pet CBD, 45.3% indicated that they observed no side effects. The remaining side effect options included lethargy and sleepiness as the most common (24.2%, $n = 116$ each, participants could choose more than one answer). Other side effects were each indicated by <2% of respondents. In 2016, a similar survey of pet owners in the US via a link on a pet hemp product company website reported that 58.8% of survey respondents ($n = 631$) were currently using a hemp product for their dog. In this survey, pet owners reported sedation as the most common significant effect (53/278 respondents reporting sedation as "significant effect" vs. 4/278 reporting as "no effect," however 190/278 reported this effect as "NA or do not know"). Although other side effects were reported, the authors reported that expense and ineffectiveness were the most common reasons for discontinuation of a product (36).

Variation in hemp product composition and quality could be responsible for differences in efficacy and safety. Botanical extracts prepared from hemp contain a number of phytochemicals including cannabinoids and terpenes, the levels of which can vary between extracts and can have a number of potential bioactivities (37). Extracts are susceptible to issues with product quality such as failure to follow good manufacturing practices, poor quality control, failure to screen for heavy metals, contamination from other plant products, etc. In a recent analysis of pet-specific cannabinoid products, CBD concentrations ranged from 0 to 66 mg/mL (including only oil delivery forms), which represented 0–154% of the label claim concentration. In addition, CBDA was found at high levels in two products (38). While quality control issues are outside the intent of this study, the lack of standardized products is a major hurdle for evaluating the safety of CBD products and supports the necessity of post-market surveillance.

Post market surveillance data and systems that provide continued vigilance are critical to monitor the risk of cannabinoid product use in animals. Even the most well defined and carefully conducted clinical studies cannot duplicate all possible scenarios or potential negative occurrences due to the use of products in the broader marketplace. The current study utilizes clinically relevant doses in a tolerability study to provide supportive baseline data for the evaluation of cannabinoids in domestic dogs. A limitation of this study is that only a single dose level is used for each product, although the dose level was chosen to be representative of real-world use of cannabinoid supplements in dogs. For clinicians and pet owners, information on the tolerability of different cannabinoids combinations can support informed use of these products. This study contributes to a data set demonstrating the safety of cannabinoids, which can be used to support future research in client-owned animals.

The results of the current study indicate that CBD, CBD + CBG and CBD + CBDA at the ratios and doses utilized were well tolerated when healthy male and female beagles were dosed for 90 consecutive days. These clinically determined conclusions are also supported by data from NAERS™ which is the most advanced system in the world for these types of products given to companion animals (specifically animals not intended for use in the human food chain).

Based on the data available it would be the conclusion of the authors that the substances do not pose significant risk to dogs in long-term use.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by the Institutional Animal Care and Use Committee of ClinVet USA LLC, an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

WB: Conceptualization, Funding acquisition, Resources, Writing – review & editing. MD: Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. KV: Project administration, Writing – review & editing. JK-N: Writing – original draft.

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Conflict of interest

MD, KV, and JK-N received compensation from NASC for their activities in the completion of the study and writing the manuscript.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2024.1349590/full#supplementary-material

References

1. Alvarenga IC, MacQuiddy B, Duerr F, Elam LH, McGrath S. Assessment of cannabidiol use in pets according to a national survey in the USA. *J Small Anim Pract*. (2023) 64:513–21. doi: 10.1111/jsap.13619

2. Kogan L, Schoenfeld-Tacher R, Hellyer P, Rishniw M. US veterinarians' knowledge, experience, and perception regarding the use of Cannabidiol for canine medical conditions. *Front Vet Sci*. (2019) 5:1–11. doi: 10.3389/fvets.2018.00338

3. De Briyne N, Holmes D, Sandler I, Stiles E, Szymanski D, Moody S, et al. Cannabis, Cannabidiol oils and tetrahydrocannabinol—what do veterinarians need to know? *Animals*. (2021) 11:1–19. doi: 10.3390/ani11030892

4. Howard-Azzeh M, Pearl DL, Swirski A, Ward M, Hovdey R, O'Sullivan TL, et al. The impact of state Cannabis legislation, county-level socioeconomic and dog-level characteristics on reported Cannabis poisonings of companion dogs in the USA (2009–2014). *PLoS One*. (2021) 16:e0250323. doi: 10.1371/journal.pone.0250323

5. Marx TK, Reddeman R, Clewell AE, Endres JR, Beres E, Vertesi A, et al. An assessment of the genotoxicity and subchronic toxicity of a supercritical fluid extract of the aerial parts of hemp. *J Toxicol*. (2018) 2018:1–26. doi: 10.1155/2018/8143582

6. Dziwenka M, Coppock R, McCorkle A, Palumbo E, Ramirez C, Lerner S. Safety assessment of a hemp extract using genotoxicity and Oral repeat-dose toxicity studies in Sprague-Dawley rats. *Toxicol Rep*. (2020) 7:376–85. doi: 10.1016/j.toxrep.2020.02.014

7. Dziwenka M, Dolan L, Mitchell J. Toxicological safety of Voho hemp oil; a supercritical fluid extract from the aerial parts of hemp. *PLoS One*. (2021) 16:e0261900. doi: 10.1371/journal.pone.0261900

8. Deabold KA, Schwark WS, Wolf L, Wakshlag JJ. Single-dose pharmacokinetics and preliminary safety assessment with use of Cbd-rich hemp nutraceutical in healthy dogs and cats. *Animals*. (2019) 9:1–13. doi: 10.3390/ani9100832

9. Alvarenga IC, Gustafson D, Banks K, Wilson K, McGrath S. Cannabidiol plasma determination and pharmacokinetics conducted at beginning, middle and end of long-term supplementation of a broad-Spectrum hemp oil to healthy adult dogs. *Front Vet Sci*. (2023) 10:1–7. doi: 10.3389/fvets.2023.1279926

10. Della Rocca G, Paoletti F, Conti MB, Galarini R, Chiaradia E, Sforna M, et al. Pharmacokinetics of Cannabidiol following single Oral and Oral Transmucosal Administration in Dogs. *Front Vet Sci*. (2022) 9:1–9. doi: 10.3389/fvets.2022.1104152

11. Wakshlag JJ, Schwark WS, Deabold KA, Talsma BN, Cital S, Lyubimov A, et al. Pharmacokinetics of Cannabidiol, Cannabidiolic acid, Δ9-tetrahydrocannabinol, Tetrahydrocannabinolic acid and related metabolites in canine serum after dosing with three Oral forms of hemp extract. *Front Vet Sci*. (2020) 7:1–12. doi: 10.3389/fvets.2020.00505

12. Gamble LJ, Boesch JM, Frye CW, Schwark WS, Mann S, Wolfe L, et al. Pharmacokinetics, safety, and clinical efficacy of Cannabidiol treatment in osteoarthritic dogs. *Front Vet Sci*. (2018) 5:1–9. doi: 10.3389/fvets.2018.00165

13. Tittle DJ, Wakshlag JJ, Schwark WS, Lyubimov A, Zakharov A, Gomez B. Twenty-four hour and one-week steady state pharmacokinetics of cannabinoids in two formulations of Cannabidiol and Cannabidiolic acid rich hemp in dogs. *Med Res Arch*. (2022) 10:1–10. doi: 10.18103/mra.v10i7.2907

14. Amstutz K, Schwark WS, Zakharov A, Gomez B, Lyubimov A, Ellis K, et al. Single dose and chronic Oral Administration of Cannabigerol and Cannabigerolic Acid-Rich Hemp Extract in fed and fasted dogs: physiological effect and pharmacokinetic evaluation. *J Vet Pharmacol Ther*. (2022) 45:245–54. doi: 10.1111/jvp.13048

15. Bartner LR, McGrath S, Rao S, Hyatt LK, Wittenburg LA. Pharmacokinetics of Cannabidiol administered by 3 delivery methods at 2 different dosages to healthy dogs. *Can J Vet Res*. (2018) 82:178–83.

16. Polidoro D, Temmerman R, Devreese M, Charalambous M, Ham LV, Cornelis I, et al. Pharmacokinetics of Cannabidiol following intranasal, Intrarectal, and Oral Administration in Healthy Dogs. *Front Vet Sci*. (2022) 9:1–8. doi: 10.3389/fvets.2022.899940

17. Samara E, Bialer M, Mechoulam R. Pharmacokinetics of Cannabidiol in dogs. *Drug Metab Dispos*. (1987) 16:469–72.

18. Chicoine A, Illing K, Vuong S, Pinto KR, Alcorn J, Cosford K. Pharmacokinetic and safety evaluation of various Oral doses of a novel 1:20 THC: CBD Cannabis herbal extract in dogs. *Front Vet Sci*. (2020) 7:1–12. doi: 10.3389/fvets.2020.583404

19. Fernandez-Trapero M, Perez-Diaz C, Espejo-Porras F, de Lago E, Fernandez-Ruiz J. Pharmacokinetics of Sativex® in dogs: towards a potential cannabinoid-based therapy for canine disorders. *Biomol Ther*. (2020) 10:1–8. doi: 10.3390/biom10020279

20. Di Salvo A, Conti MB, Della Rocca G. Pharmacokinetics, efficacy, and safety of Cannabidiol in dogs: an update of current knowledge. *Front Vet Sci*. (2023) 10:1–15. doi: 10.3389/fvets.2023.1204526

21. Flatland B, Baral RM, Freeman KP. Current and emerging concepts in biological and analytical variation applied in clinical practice. *J Vet Intern Med*. (2020) 34:2691–700. doi: 10.1111/jvim.15929

22. Bradley S, Young S, Bakke AM, Holcombe L, Waller D, Hunt A, et al. Long-term daily feeding of Cannabidiol is well-tolerated by healthy dogs. *Front Vet Sci*. (2022) 9:1–15. doi: 10.3389/fvets.2022.977457

23. Vaughn DM, Paulionis LJ, Kulpa JE. Randomized, placebo-controlled, 28-day safety and pharmacokinetics evaluation of repeated Oral Cannabidiol Administration in Healthy Dogs. *Am J Vet Res*. (2021) 82:405–16. doi: 10.2460/ajvr.82.5.405

24. Verrico CD, Wesson S, Konduri V, Hofferek CJ, Vazquez-Perez J, Blair E, et al. A randomized, double-blind, placebo-controlled study of daily Cannabidiol for the treatment of canine osteoarthritis pain. *Pain*. (2020) 161:2191–202. doi: 10.1097/j.pain.0000000000001896

25. Klatzkow S, Davis G, Shmalberg J, Gallastegui A, Miscioscia E, Tarricone J, et al. Evaluation of the efficacy of a Cannabidiol and Cannabidiolic acid rich hemp extract for pain in dogs following a Tibial plateau leveling osteotomy. *Front Vet Sci*. (2023) 9:1–10. doi: 10.3389/fvets.2022.1036056

26. Brioschi FA, Di Cesare F, Gioeni D, Rabbogliatti V, Ferrari F, D'Urso ES, et al. Oral Transmucosal Cannabidiol oil formulation as part of a multimodal analgesic regimen: effects on pain relief and quality of life improvement in dogs affected by spontaneous osteoarthritis. *Animals*. (2020) 10:1–14. doi: 10.3390/ani10091505

27. Kogan L, Hellyer P, Downing R. The use of Cannabidiol-rich hemp oil extract to treat canine osteoarthritis-related pain: a pilot study. *Sci Rep*. (2020) 58:35–45.

28. Garcia GA, Kube S, Carrera-Justiz S, Tittle DJ, Wakshlag JJ. Safety and efficacy of Cannabidiol-Cannabidiolic acid rich hemp extract in the treatment of refractory epileptic seizures in dogs. *Front Vet Sci*. (2022) 9:1–12. doi: 10.3389/fvets.2022.939966

29. McGrath S, Bartner LR, Rao S, Packer RA, Gustafson D. Randomized blinded controlled clinical trial to assess the effect of Oral Cannabidiol Administration in Addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. *J Am Vet Med Assoc*. (2019) 254:1301–8. doi: 10.2460/javma.254.11.1301

30. Loewinger M, Wakshlag JJ, Bowden D, Peters-Kennedy J, Rosenberg A. The effect of a mixed Cannabidiol and Cannabidiolic acid based oil on client-owned dogs with atopic dermatitis. *Vet Dermatol*. (2021) 33:329–e77. doi: 10.1111/vde.13077

31. Morris EM, Kitts-Morgan SE, Spangler DM, McLeod KR, Suckow MA, Harmon DL. Feeding treats containing Cannabidiol (Cbd) did not Alter canine immune response to immunization with a novel antigen. *Res Vet Sci*. (2022) 143:13–9. doi: 10.1016/j.rvsc.2021.12.012

32. Corsetti S, Borruso S, Malandrucco L, Spalluci V, Maragliano L, Perino R, et al. May reduce aggressive behaviour towards humans in shelter dogs. *Sci Rep*. (2021) 11:1–10. doi: 10.1038/s41598-021-82439-2

33. Hunt ABG, Flint HE, Logan DW, King T. A single dose of Cannabidiol (Cbd) positively influences measures of stress in dogs during separation and car travel. *Front Vet Sci*. (2023) 10:1–18. doi: 10.3389/fvets.2023.1112604

34. Morris EM, Kitts-Morgan SE, Spangler DM, McLeod KR, Costa JHC, Harmon DL. The impact of feeding Cannabidiol (Cbd) containing treats on canine response to a noise-induced fear response test. *Front Vet Sci*. (2020) 7:1–13. doi: 10.3389/fvets.2020.569565

35. Morris EM, Kitts-Morgan SE, Spangler DM, Gebert J, Vanzant ES, McLeod KR, et al. Feeding Cannabidiol (Cbd)-containing treats did not affect canine daily voluntary activity. *Front Vet Sci*. (2021) 8:1–10. doi: 10.3389/fvets.2021.645667

36. Kogan L, Hellyer P, Robinson NG. Consumers' perceptions of hemp products for animals. *Sci Rep*. (2016) 42:40–8.

37. Andre CM, Hausman JF, Guerriero G. *Cannabis Sativa*: the Plant of the Thousand and one Molecules. *Front Plant Sci*. (2016) 7:1–17. doi: 10.3389/fpls.2016.00019

38. Wakshlag JJ, Cital S, Eaton SJ, Prussin R, Hudalla C. Cannabinoid, terpene, and heavy metal analysis of 29 over-the-counter commercial veterinary hemp supplements. *Vet Med*. (2020) 11:45–55. doi: 10.2147/vmrr.S248712

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Pharmacokinetics and tolerability of single-dose enteral cannabidiol and cannabidiolic acid rich hemp in horses (*Equus caballus*)

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The pharmacokinetics and tolerability of cannabinoids and their metabolites were determined in eight horses after enteral administration of a commercial CBD/CBDA-rich hemp oil product. Each horse was administered 2 mg/kg or 8 mg/kg CBD/CBDA or no treatment in a randomized cross-over design. Serial serum samples collected over 48 h were analyzed by high performance liquid chromatography with tandem mass spectrometry. Plasma chemistry analysis was performed at 0 h and 24 h. Vital parameters, pedometry, and blinded mentation and gait evaluations were recorded at intervals up to 24 h. Manure production and gastrointestinal transit time were tracked for 48 h after oil administration. The median maximal concentration of CBD and CBDA were 5.2 and 36.95 ng/mL in the 2 mg/kg group, respectively; and 40.35 and 353.56 ng/mL in the 8 mg/kg group. The median half-life of elimination was not calculated for the 2 mg/kg CBD treatment due to lack of time points above the lower quantifiable limit beyond the Cmax while it was 7.75 h in the 8 mg/kg group. CBDA absorption was biphasic. Pharmacokinetic parameters for tetrahydrocannabinol, tetrahydrocannabinolic acid, cannabigerolic acid, and 7-carboxy cannabidiol are also reported. No significant differences in any of the measured tolerability parameters were demonstrated between treatment groups. Single-dose enteral administration of CBD/CBDA-rich hemp extract up to 8 mg/kg does not appear to produce neurologic, behavioral, or gastrointestinal effects in horses.

KEYWORDS

cannabidiol, pharmacokinetic, horse, cannabidiolic acid, activity, gastrointestinal

1 Introduction

The endocannabinoid system is a complex yet highly conserved cell signaling pathway found in all chordates, consisting of endocannabinoids, cannabinoid receptors, and metabolizing enzymes (1). Endocannabinoids are eicosanoids synthesized from the polyunsaturated fatty acids present in the lipid membranes of all cells. The most prevalent of

the six identified endocannabinoids are anandamide and 2-arachidonoylglycerol, which serve as ligands for the G-protein coupled receptors CB_1 and CB_2 (2). These receptors are found throughout the body, with CB1 highly concentrated in the central nervous system and CB2 found more in other organ systems and the immune system, where they play diverse roles from regulating appetite, neuronal action potentials to mediating immune responses. Endocannabinoids are metabolized rapidly in the synapse by the enzymes fatty acid amide hydrolase and monoacylglycerol lipase (3).

Phytocannabinoids are chemicals found in *Cannabis sativa* that share structural similarity to the endocannabinoids and may interact with cannabinoid receptors or enzymes in the endocannabinoid metabolic pathway. At least 113 different phytocannabinoids have been identified in *Cannabis*; the most abundant across strains are tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), cannabidivarinic acid (CBDVA), and cannabichromenic acid (CBCA), which during extraction are often decarboxylated to the neutral cannabinoids cannabidiol (CBD), Δ^9-tetrahyrocannbinol (THC), cannabigerol (CBG), cannabidivarin (CBDV), and cannabichromene (CBC) (4). Phytocannabinoids act as agonists, inverse agonists or antagonists with cannabinoid receptors, metabolic enzymes, and other receptors/channels such as those in the transient receptor potential cation channel family (5, 6). Some phytocannabinoids, including CBD, show promise in therapeutic applications in veterinary species (7–11).

CBD is one of the most abundant phytocannabinoids extracted from *Cannabis sativa*, accounting for as much as 40% of the plant's extract (12). While CBD has low affinity for CB_1 and CB_2 cannabinoid receptors (13), it functions as an endocannabinoid modulator by inhibiting fatty acid amide hydrolase and anandamide reuptake, as well as other arachidonic acid metabolizing enzymes (14). A variety of additional mechanisms of action make CBD an interesting candidate as an analgesic agent. CBD activates and desensitizes transient receptor potential vanilloid 1 (TRPV1) channels (5), found in abundance in central nervous system pain pathways. CBD also inhibits glutamate release and suppresses the production of tumor necrosis factor-alpha (15, 16). CBD may work synergistically with other analgesic agents, such as opioids, inhibiting their metabolism via the cytochrome p450 system (17). CBDA can work at similar receptor systems as CBD and is a selective inhibitor of cyclooxygenase-2, a key enzyme involved in inflammation (18). In recent years, interest in cannabinoid medicine has increased rapidly, among both the medical and veterinary communities and the general public, spurred by the drawbacks of conventional analgesic medications such as opioids and non-steroidal anti-inflammatory drugs (19, 20). Cannabinoids have been promoted in both human and veterinary medicine as alternatives or adjuncts to conventional medications in the treatment of pain, seizures, and a variety of other disorders (9, 10). Oral CBD formulations are already marketed to and used by horse owners, often without veterinary oversight. However, only a few published studies exist on the pharmacokinetics, pharmacodynamics, or safety of cannabinoids in horses (21–26). Due to the scarcity of published data in horses, clinical recommendations have often relied on anecdotal experience and extrapolation of data from other species. Oral nutraceuticals show significant inter-species differences in oral bioavailability, potentially leading to significant over- or underdosing when extrapolating doses from one species to another (27). CBD isolates and CBD rich hemp products available for horses come in a wide range of concentrations, formulations, and purity, but few evidence-based guidelines exist for their administration. In addition, CBD, THC, and their metabolites are subject to regulatory control under certain racing jurisdictions and competition horse associations. Further research may demonstrate CBD's utility as an adjunctive analgesic agent. Unfortunately, no such research can proceed meaningfully without a basic knowledge of the pharmacokinetic, tolerability and pharmacodynamics of cannabinoids in full spectrum hemp extracted products which may be different from isolates of cannabinoids (28).

The primary aim of this study was to determine the basic enteral non-compartmental pharmacokinetics of a single dose of CBD/CBDA-rich full spectrum hemp oil in fit, exercised horses similar to what has been done in dogs at 2 and 8 mg/kg (9). The secondary aims of this study were to evaluate if the tested doses of CBD/CBDA produced adverse effects on clinical neurologic status, gastrointestinal transit time, clinicopathologic variables, or altered spontaneous ambulation in horses confined to stalls.

2 Materials and methods

2.1 CBD/CBDA full spectrum oil

CBD/CBDA-rich hemp oil was administered by nasogastric tube in a proprietary oil formulation (Hemp CBD + CBDA Oil, ElleVet Sciences, ME, USA). Compositional analysis of the oil was performed by liquid chromatography by a certified ISO/IEC 17025 third-party laboratory (ProVerde Laboratories, MA, USA). The oil contained 27.73 mg/mL CBD, 34.10 mg/mL CBDA, 1.32 mg/mL Δ^9-THC, 1.27 mg/mL THCA, 0.35 mg/mL CBG, 0.89 mg/mL CBGA, 1.1 mg/mL CBC, and trace levels of CBDV and CBN. The oil contained no tetrahydrocannabivarin (THCV), Δ^8-tetrahydrocannabinol (Δ^8-THC), or exo-tetrahydrocannabinol (exo-THC). The oil product used passed all mycotoxin, heavy metal, microbial, pesticide and solvent contamination tests and complies with USDA certified hemp GMP protocols for hemp production.

2.2 Animals

Eight healthy Thoroughbred horses from a dedicated research herd, two castrated males and six intact females, 3–10 years of age, weighing 526.5 ± 33.4 kg and of ideal body condition were included in the study. Horses were trained for at least 2 months on a high-speed treadmill (Mustang 2200, Graber AG, Switzerland) to achieve fitness representative of Thoroughbred racehorses prior to commencing the study. The standard training regimen was 0.6 km at 4 m/s then 2 km at 8 m/s and 0.6 km at 4 m/s 3 days per week. Prior to each trial horses had to demonstrate adequate fitness by running 1.6 km at 13.5 m/s with warm-up and cool-down of 0.96 km at 4 m/s. Following the fitness test, heart rate was monitored every 5 min and must be below 50 beats per minute within 40 min of concluding the test for the horse to prove fitness. The standard training regimen was maintained during the washout periods but horses were not exercised during the 48-h data collection window for each trial. No concurrent medications or supplements were permitted during the data collection or intervening washout periods. Horses were housed in matted, 13.4 m^2 stalls on data

collection days and returned to their normal outdoor pastures during the intervening washout periods. During the study period, horses were allowed free access to water and coastal Bermuda hay and fed a proprietary pelleted feed once per day in the morning, approximately 2 h prior to treatment. All experimental protocols were reviewed and approved by the University of Florida Institutional Animal Care and Use Committee (protocol 201808925).

2.3 Animal treatments and blood sampling

The study was conducted in three 48-h trials in a randomized cross-over design, each separated by a minimum 2-week washout period during which time the horses were returned to their normal pastures. Each horse completed a no treatment control (water only) trial as well as a 2 mg/kg and 8 mg/kg CBD/CBDA-rich oil dose trial. The dose was calculated based on body weight and a total combined CBD/CBDA concentration in the oil formulation of 62.0 mg/mL (28 mg/mL CBD + 34 mg/mL CBDA). Approximately 2 h after feeding on the first morning of each trial, barium spheres were delivered in approximately 2 L of water via nasogastric tube. Then, in the treatment groups, CBD/CBDA rich hemp oil (Ellevet Sciences, Portland, ME) was delivered via nasogastric tube to ensure accuracy in dosing. The nasogastric tube was flushed again with approximately 2 L of water to flush in residual oil. Nasogastric tubes, water buckets, and funnels were designated as CBD/CBDA treatment or no treatment throughout the study to ensure there was no risk of contamination of control treated horses with CBD/CBDA oil.

Blood was collected by jugular venipuncture into 10 mL red top tubes immediately prior to CBD/CBDA oil administration and at 0.5, 1, 1.5, 2, 3, 4, 8, 12, 24, and 48 h after administration. Blood samples were allowed to coagulate for 1 h and then centrifuged, and the serum was separated into new tubes and stored at −80°C until analysis.

2.4 Cannabinoids analysis in horse serum by LC–MS/MS

Analysis was performed using an exploratory (fit-for-purpose) method for measurement of thirteen cannabinoids and their metabolites at the Toxicology Research Laboratory, University of Illinois at Chicago. The reference standards for CBD and CBDA were obtained from Restek Corporation (Bellefonte, PA); all other reference and internal standards were obtained from Cerilliant Corporation (Round Rock, TX). Cannabinoids (CBD, CBDA, THC, THCA, CBN, CBC, CBG, and CBGA) and their metabolites (11-OH-THC, 7-OH-CBD, 7-COOH-CBD, COOH-THC, and COOH-THC-Glu) concentration in horse serum was determined using high performance liquid chromatography with tandem mass spectrometry (LC–MS/MS) (Nexera X2 and MS 8050, Shimadzu Corp., Kyoto, Japan).

Horse serum (40 µL) was mixed with 20 µL of internal standards (100 ng/mL of CBD-d3, THC-d3, THCA-d3, 7-COOH-CBD-d3, 7-OH-CBD-d5, 11-OH-THC-d3, COOH-THC-d9, and COOH-THC-Glu-d3 in 50% methanol) in a 96 well plate. Proteins were precipitated and compounds were extracted by adding 100 µL of ice-cold acetonitrile to the samples, then vortexing for 1–2 min and centrifuging at 4,000 rpm (2,300 *g*) for 10 min at 4°C. Supernatants (80 µL) were mixed with 80 µL of water in a different 96 well plate, and centrifuged again. The processed samples (10 µL) were injected into Waters Atlantis T3 HPLC column (3 µm 2.1 × 50 mm) with a guard cartridge (Waters VanGuard Atlantis T3) coupled to LC–MS/MS. The column was equilibrated with mobile phase A (0.1% formic acid in water) and mobile phase B (acetonitrile) at 50% B. The compounds were eluted by a linear gradient from 50% B to 95% B over 6 min, and then held at 95% B for 1 min. Subsequently, the column was re-equilibrated at initial composition for 1 min. Flow rate was 0.3 mL/min. The autosampler and column temperature were set at 4 and 30°C, respectively. The compounds were detected in electrospray ionization positive and/or negative mode as described in the Supplementary Table 1. Interface voltage was 4 kV and −3 kV, respectively. Interface, desolvation line, and heat block temperature were 300, 200, and 400°C, respectively. Nebulizing, heating, and drying gas flow were 2.7, 5, and 5 L/min, respectively.

Concentrations of cannabinoids were calculated by LabSolutions software (Shimadzu Corp., Kyoto, Japan) using a quadratic calibration curve with $1/c^2$ weighing based on relative response (peak area of cannabinoids/peak area of internal standards). The calibration curve range, lower limit of detection and quantitation in horse serum is shown in Supplementary Table 1 and assay accuracy and precision is shown in Supplementary Table 2.

Pharmacokinetic analysis examining maximal serum concentration as ng/mL(C_{max}), time of maximal absorption in hours (T_{max}), time half-life elimination in hours ($T_{0.5\ elim}$), serum concentration area under the curve as ng*hr/mL ($AUC_{0->t}$) and mean residence time in hours (MRT), using a pharmacokinetic software package (PK Solutions 2.0, Montrose, CO) for all measurable cannabinoids with sufficient data points for evaluation.

2.5 Gastrointestinal transit time

During each trial, each horse was administered 200 0.125 cm-diameter barium-filled low-density polyethylene plastic resin balls (Precision Plastic Ball Company, IL, USA) by nasogastric tube at time 0, as previously described by Sano et al. (29). Every 6 h for 48 h total, all manure in the stall was collected, sealed in a plastic bag, and weighed. At the conclusion of each trial, all plastic bags were radiographed, and the number of barium-filled spheres present in each manure collection was counted (Table 1).

2.6 Pedometry

To quantify independent movement by the horses, pedometers were placed on the right front and hind pastern. The pedometers (Omron Healthcare, Inc., Hoffman Estates, IL) were used for activity monitoring of foals in a study reported by Grubb et al. (30). The pedometers were secured on the dorsal aspect of the pastern by creating a pocket made of gauze and elastic tape (Elastikon, Johnson & Johnson, NJ, USA), so that each pedometer would stay in place over the course of the study. Measurements were collected starting at time point zero after the baseline neurological evaluation. Subsequent readings were documented before and after gait evaluation at 0.5, 1, 2, 4, 12, and 24 h after treatment so that steps due to forced activity during the gait exam could be subtracted from the total step count.

TABLE 1 Median (95% CI) pharmacokinetic parameters after enteral dosing of CBD/CBDA-rich hemp oil in horses using a non-compartmental model.

Analyte	C_{max}	T_{max}	$T_{0.5Elim}$	AUC	MRT
CBD (2 mg/kg)	5.2 (2.93–8.95)	3.0 (1.52–5.48)	NA	44 (26.52–66.34)	NA
CBD (8 mg/kg)	40.35 (27.70–52.17)	8.0 (4.16–10.34)	8.3 * (6.37–8.93)	501.5 (343.25–687.50)	12.6 (10.29–14.16)
THC (8 mg/kg)	6.65 (5.52–8.25)	6.0 (3.54–9.96)	NA	98.0 (70.74–121.51)	NA
THCA (2 mg/kg)	7.65 (5.32–14.47)	1.25 (0.97–2.63)	10.6 ** (8.58–18.21)	127.0 (100.73–185.3)	12.45 (11.42–13.78)
7-COOH-CBD (2 mg/kg)	133.75 (89.71–169.89)	12.0 (7.87–19.14)	NA	4,450.5 (3206.9–6520.6)	71.4 (57.34–100.44)
7-COOH-CBD (8 mg/kg)	777.5 (630.1–857.4)	12.0 (7.87–19.14)	NA	27,874 (22,851-32,108)	97.1 (73.73–136.82)

Maximum plasma concentration (ng/mL), C_{max}; time at maximum plasma concentration (hours), T_{max}; elimination half-life (hours), $T_{0.5Elim}$; area under the curve (h*ng/mL), AUC; mean residence time (hours), MRT. *$T_{0.5\,elim}$ is based on 6/8 horses with three points beyond Cmax; ** $T_{0.5\,elim}$ is based on 7/8 horses with three points beyond Cmax. NA – not applicable due to being a metabolite or horse number for proper analysis being less than 6 horses in the cohort.

2.7 Vital parameters, hematology and blood chemistry

Horses were weighed on a calibrated scale prior to each trial. Heart rate and respiratory rate were recorded at time 0, 0.5, 1, 2, 4, 12, and 24 h. Blood samples were collected by jugular venipuncture into 10 mL EDTA and heparinized tubes prior to each trial and submitted for hematology and plasma chemistry analysis, respectively, to ensure general health. Heparinized blood samples were collected again 24 h after nasogastric intubation with control or either CBD/CBDA oil treatment for repeat plasma chemistry analysis.

2.8 Mentation and gait scoring

Mentation and gait exams were video recorded at times 0, 0.5, 1, 2, 4, 12, and 24 h and reviewed in randomized order by a board-certified large animal internal medicine specialist blinded to the treatment, trial number, and time point. Each exam consisted of observing the horse from a distance undisturbed in the stall, during approach of the handler and interaction with the horse, walking the horse in a straight line viewed from the front, back and either side, turning the horse in tight circles in either direction, and backing the horse. Horses were assigned mentation scores as follows: 0 (bright and alert, normal, appropriate responsiveness to stimuli and environment); 1 (lethargy, somewhat blank facial expression with slight drooping of the ears and eyelids, sluggish responsiveness to stimuli, and reduced voluntary activity); 2 (stupor, stands in one place with the head held low, responds only to strong stimuli); 3 (semi-coma, stuporous and recumbent); and 4 (coma, recumbent and does not respond to any stimulus). The reviewer also provided a "Yes" or "No" response to indicate hyperesthesia, excitability, or ataxia in each video.

2.9 Statistics

Data were statistically evaluated using Statistix 10.0 (Analytical Software, Tallahassee, FL). Continuous variables (heart rate, respiratory rate, pedometry data, manure production in kilograms, barium ball recovery (%), pharmacokinetic parameters, and plasma chemistry parameters) were assessed for normality using a Shapiro–Wilk test. Physiologic variables (heart rate and respiratory rate) were compared between treatment groups using a repeated measures ANOVA. Pedometry, plasma chemistry, and mentation and gait score data were compared using a Kruskal-Wallis test. Manure production and cumulative barium ball recovery by time and treatment were evaluated by factorial ANOVA.

3 Results

3.1 Pharmacokinetics

No cannabinoids were detected in any of the baseline blood samples. Pharmacokinetic parameters could not be determined for CBC, CBCA, CBG, 11-OH, CBN, or 7-OH-CBD due to falling below the quantitation limits for all samples in both groups. THC and CBGA were measured in only a few samples in the 2 mg/kg CBD/CBDA group; all others were below the limit of quantification. CBD serum concentrations were near the lower limit of quantitation (1–2 ng/mL) in 5 of the horses and below the limit of quantitation in 2 horses by 12 h when treated with 2 mg/kg CBD/CBDA treatment. The final horse only had 3 time points with CBD concentration above the lower limit of quantitation thereby only allowing for reporting of Cmax, Tmax and AUC. When treated with 8 mg/kg CBD/CBDA the CBD concentration in the serum was at the lower limit of quantitation (1–2 ng/mL) at 48 h with 4 horses being in that range and 4 horses being below. Due to a later than expected T max (8 h) $T_{0.5\,elim}$ and MRT were based on 6 of 8 horses with sufficient data points for analysis and are thus reported. The median maximal concentration of CBD was 40.35 ng/mL and the median half-life of elimination of CBD was 7.75 h in the 8 mg/kg group. The mean concentration-time curve for CBD in all horses at 2 and 8 mg/kg is represented in Figures 1A,B at the respective concentrations.

THC was only quantifiable in four horses in the 2 mg/kg dose group, with quantifiable concentrations at 1–5 timepoints between 1 and 8 h after dosing with concentrations being near the lower limit of quantification at 1–2 ng/mL. THC was measured in all horses in the 8 mg/kg dose group, with a median maximum concentration of 6.65 ng/mL occurring at 6 h. Time to maximum concentration was highly variable, from 2 h in one horse to 12 h in 2 horses. All but one horse had quantifiable THC in the serum at 1.5 h, and all horses were at or near the lower limit of quantification (1.0–2.6 ng/mL) with only one horse being below the limit of quantification at 24 h, and no horses had measurable THC by 48 h. The mean concentration-time curves for THC are represented in Figure 1D for the 8 mg/kg treatment and are not represented for the 2 mg/kg time point as 4 of 8 horses had no measurable THC at any time point with only 4 horses showing between 1 and 2 ng.

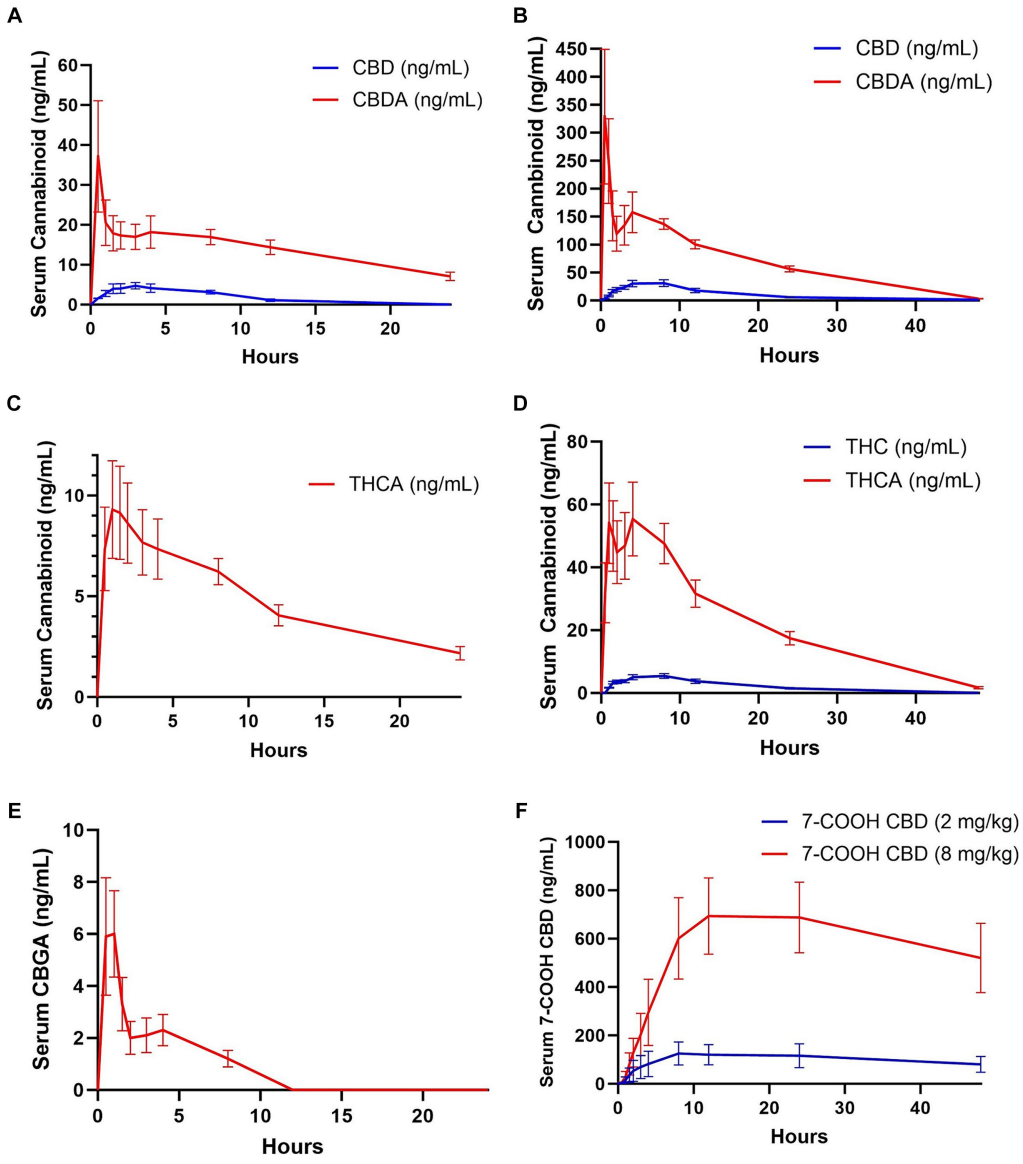

FIGURE 1
Mean ± standard error analyte concentrations in horses after enteral administration of a single dose of a full spectrum CBD/CBDA-rich hemp oil at 2 or 8 mg/kg doses in a randomized cross-over design. **(A)** CBD and CBDA at 2 mg/kg dose (n = 8), **(B)** CBD (n = 8) and CBDA (n = 8) at 8 mg/kg dose **(C)** THCA at 2 mg/kg dose (n = 8) **(D)** THC (n = 8) and THCA (n = 8) at 8 mg/kg dose **(E)** CBGA at 8 mg/kg dose (n = 8), and **(F)** 7-COOH-CBD at 2 and 8 mg/kg dose (n = 8).

CBDA was measurable for pharmacokinetic assessment in both the 2 and 8 mg/kg group however due to a biphasic curve a Tmax1/Tmax 2 and Cmax1 and Cmax 2 are reported as well as AUC and MRT (Table 2). THCA was measurable for pharmacokinetic assessment in 7 of 8 horses in the 2 mg/kg group following typical pharmacokinetic modeling allow for calculations of all parameters including MRT and $T_{0.5\ elim}$, while when horses were treated with 8 mg/kg they displayed atypical biphasic Cmax1 and Cmax2 pharmacokinetics in 5 of the 8 horses and are thus reported in Table 2, similar to CBDA results. The mean concentration-time curves for CBDA, THCA, and CBGA for all horses are represented in Figures 1A–E, respectively.

The major metabolite 7-COOH-CBD Cmax was at 12 h for both the 2 mg/kg and 8 mg/kg group at 133.7 mg/mL and 777.5 ng/mL, respectively. The AUC for 7-COOH CBD at 8 mg/kg was 27,874 ng/mL which was 50 fold greater than CBD suggesting very rapid metabolism of CBD (Table 2 and Figure 1F).

3.2 Gastrointestinal transit time

The mean cumulative manure production over 48 h was 36.9 (SD 9.9) kg in the control group, 38.7 (SD 4.8) kg in the 2 mg/kg group, and 39.1 (SD 9.4) kg in the 8 mg/kg group (Figure 2A). The mean recovery of barium spheres over 48 h was 50.5% (SD 14.6) in the control group, 55.2% (SD 10.3) in the 2 mg/kg group, and 50.1% (SD 14.4) in the 8 mg/kg group (Figure 2B). Neither manure production nor gastrointestinal transit time were significantly different between any of the groups.

3.3 Pedometry

The median, quartile, and range of the number of steps recorded by each horse's pedometer during the study periods are represented in Figure 3. The median number of forelimb and hindlimb steps recorded by each horse's pedometers was not significantly different between treatment groups.

3.4 Vital parameters, hematology and blood chemistry

All horses were determined to be healthy with no clinically relevant abnormalities on pre-trial hematology and plasma chemistry. There were no significant differences in heart rate or respiratory rate between groups at any time before or following treatments. There were no significant differences between pre-nasogastric intubation time 0 samples and 24-h post samples for any analyte on plasma chemistry in the control group. Median blood urea nitrogen was significantly decreased at 24 h post-dosing (17.5 mg/dL, 95% CI 16.0–18.7) in the 2 mg/kg CBD group compared to the pre-dosing time 0 sample (20.5 g/dL, 95% CI 18.0–21.8; $p = 0.016$). Median glucose was significantly increased at 24 h post-dosing (108 mg/dL, 95% CI 97.8–121.9) in the 8 mg/kg CBD group compared to the pre-dosing time 0 sample (90 mg/dL, 95% CI 85.9–97.4; $p = 0.003$). Median bicarbonate was significantly decreased at 24 h post-dosing (31 mEq/L, 95% CI 29.9–31.1) in the 8 mg/kg CBD group compared to pre-dosing time 0 sample (32 mEq/L, 95% CI 31.2–32.3; $p = 0.0018$). There were no significant differences for any plasma chemistry variable between treatments at each time-point (Supplementary Tables 3, 4).

3.5 Mentation and gait scoring

Mentation scores did not differ between the control and treatment groups at any time, and no horse was assigned a mentation score greater than 1 across groups. None of the horses in any treatment group demonstrated hyperesthesia, excitability, or ataxia at any time during the study based on blinded video assessment.

4 Discussion

Pharmacokinetic variables for CBD found in this study were comparable to those previously reported in horses, with a relatively short T_{max} and a highly variable C_{max} and elimination half-life (21–26). Though the present study administered CBD oil by nasogastric tube, these C_{max} values are similar to previously reported C_{max} in horses administered oral doses; Ryan et al. reported a C_{max} of 6.14 ng/mL with a 2 mg/kg dose (21), and Yocom et al. reported a C_{max} of 4.3 ng/mL with a 1 mg/kg oral dose and 19.9 ng/mL with a 3 mg/kg oral dose (22). However, the C_{max} reported in the present study is dramatically lower than that reported by Williams et al. who administered 2 mg/kg CBD in a pelleted formulation at the time of feeding and reported a C_{max} of 51 ± 15 ng/mL, after 7 days of dosing which would equate to a dose of 4 mg/kg of our CBD/CBDA rich hemp oil, providing 2 mg/kg

TABLE 2 Median (95% CI) pharmacokinetic parameters of CBDA and THCA after enteral dosing of CBD/CBDA-rich hemp oil in horses using a non-compartmental model.

Analyte	C_{maxP1}	T_{maxP1}	C_{maxP2}	T_{maxP2}	AUC	MRT
CBDA (2 mg/kg)	20.05 (4.14–69.76)	0.5 (0.24–1.13)	17.7 (10.12–28.97)	8.0 (4.53–10.05)	410.5 (289.53–560.97)	12.8 (11.49–14.14)
CBDA (8 mg/kg)	312.2 (92.67–614.45)	0.5 (0.43–1.07)	164.45 (115.88–251.54)	4.0 (3.54–7.21)	3,353 (2,753.3–4,006.7)	13.1 (11.03–15.77)
THCA (8 mg/kg)*	76.3 (16.03–121.75)	1.3 (0.97–4.43)	59.3 (38–43-89.97)	4.0 (3.83–7.57)	1,130 (789.2–1,336.72)	14.4 (13.05–15.55)

Maximum plasma concentration of peak 1 (ng/mL), C_{maxP1}; time of maximum plasma concentration of peak 1 (hours), T_{maxP1}; maximum plasma concentration of peak 2 (ng/mL), C_{maxP2}; time of maximum plasma concentration of peak 2 (hours), T_{maxP2}; area under the curve (h*ng/mL), AUC; mean residence time (hours), MRT. *THCA C_{maxP2} and T_{maxP2} are data from 5 horses as 3 did not show a "double peak."

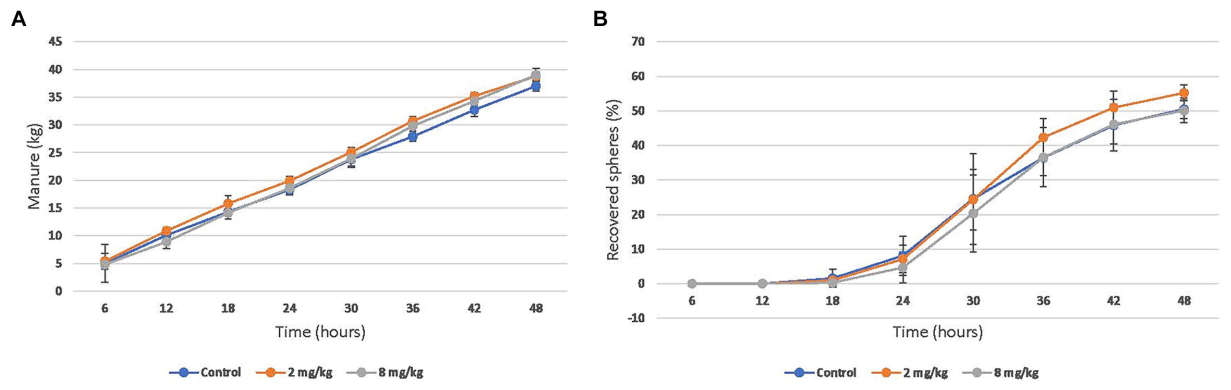

FIGURE 2
Mean (95% CI) cumulative **(A)** Manure production and **(B)** barium sphere recovery after enteral administration of a single dose of CBD/CBDA rich hemp oil to stall-confined horses ($n = 8$) using placebo (water), 2 mg/kg or 8 mg/kg in a randomized cross-over design.

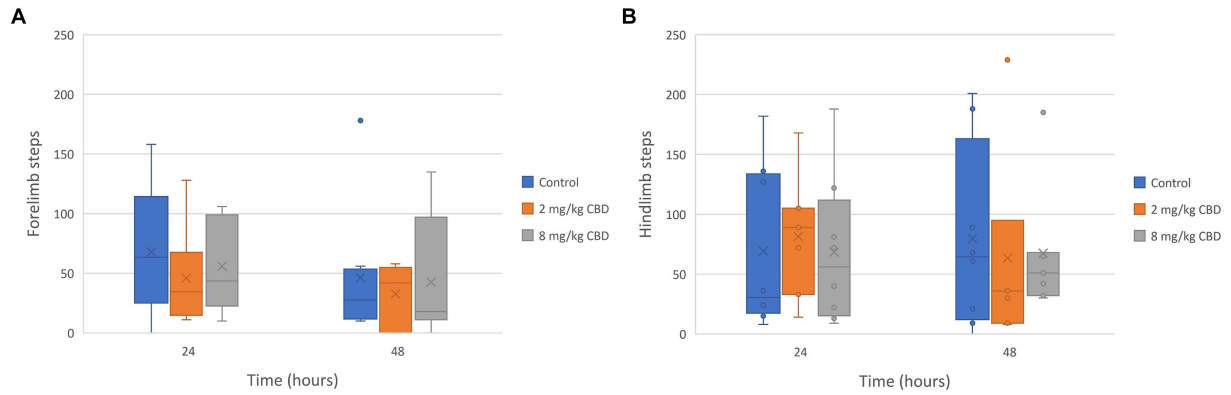

FIGURE 3
(A) Forelimb and **(B)** hindlimb steps taken after enteral administration of a single dose of CBD/CBDA-rich hemp oil to stall-confined horses ($n = 8$) at 2 mg/kg or 8 mg/kg in a randomized cross-over design.

of CBD suggesting some level of tissue accumulation being possible with chronic administration (23). Eichler and colleagues showed long term dosing of 3 mg/kg twice daily resulting in Cmax concentrations of only 12 ng/mL after 12 h, while after 2 weeks serum nadir and peak concentrations were between 8 and 50 ng/mL range (26). Twelve senior horses treated with a single oil based 2 mg/kg dose of CBD displayed an average Cmax of approximately 19 ng/mL which is similar to prior results, yet slightly higher, suggesting that age may play a role in metabolism of CBD (24). Sanchez and colleagues studied naked oil preparations of CBD and micellar forms of CBD at 10 mg/kg to assess absorption over 12 h suggesting that AUC for both formulation were similar, yet Cmax was higher in the micellar form of CBD again suggesting that form of CBD deliver may also influence absorption kinetics (25). These data indicate a difference in the bioavailability of the supplements used and/or significantly enhanced absorption when CBD is administered concurrently with feed or as part of the pelleted ration and that bioaccumulation is tissue may occur with CBD in horses. In humans, the intake and composition of a meal has been shown to significantly affect CBD absorption (31, 32). Overall, enteral absorption of CBD in this oil-based formulation was noticeably lower in horses than has previously been reported in dogs. While a 2 mg/kg dose in dogs produced a median peak plasma concentration of 102 ng/mL and nearly 600 ng/mL at the 8 mg/kg dose (9), the median peak plasma concentration in horses was 5.2 ng/mL at the 2 mg/kg dose and only 40.3 ng/mL at the 8 mg/kg dose, therefore more sensitive assays may be required for individual cannabinoids that are detected at the lower limit of quantification for precise results, particularly in the lower dosing.

The present study also evaluated additional cannabinoids, and peak plasma concentrations of CBDA were much higher than those of CBD. Approximately half of the CBD administered in this study was in the form of CBDA (approximately 1 mg/kg in our CBD/CBDA rich hemp dose of 2 mg/kg). These data suggest CBDA may be more bioavailable than CBD in horses. CBDA itself has been associated with antinociceptive and antihyperalgesic effects in rodent models (33, 34), although these effects have yet to be demonstrated in horses. There is very little clinical data on CBDA and its nocioceptive effects, a recent study evaluating CBDA-rich hemp in cows suggested that providing CBDA rich hemp may mitigate stress and make cows more comfortable (11). Also notable are the much higher concentrations of 7-COOH-CBD found in horses in this study in comparison to previous findings in dogs. Dogs administered a 2 mg/kg dose of CBD/CBDA-rich hemp oil in a similar oil-based formulation achieved a Cmax of 13 ± 2 ng/mL and AUC of 159 ± 6 ng/mL, compared to a mean Cmax of 129.8 ± 40 ng/mL and AUC of 4,863 ± 1656.9 ng/mL in horses in the present study. This difference suggests that horses have a higher metabolic capacity for CBD than dogs and cats (35). Our data is very consistent with other studies showing that the major metabolite of CBD in horses is 7-COOH-CBD when dosed at 1–2 mg/kg showing T max in the 8–12 h time range with AUC ranging from 4,000 to 11,500 h*ng/mL with Cmax being between 307 at 2 mg/kg dosing and 85 ng/mL at 1 mg/kg dosing (21, 24).

CBDA, THCA, and CBGA demonstrated biphasic absorption, with two concentration peaks that were more pronounced at the high dose. This "double peak phenomenon" seen with enteric administration of some drugs has been attributed to separate sites of absorption in the gastrointestinal tract, with the absorption limit of the first site determining the magnitude of the second absorption peak (36–38). This phenomenon could also be attributed to enterohepatic recirculation, delayed gastric emptying, or a feeding time phenomenon (39). Double-peak phenomenon is relatively common in horses, and has been demonstrated for phenylbutazone, trimethoprim-sulphachlorpyridazine, and other orally-administered drugs (39, 40).

The doses of CBD evaluated in this study were well-tolerated, with no observable alterations in mentation, activity in stalls or gastrointestinal transit effects. Few statistically significant changes in blood chemistry parameters were noted 24 h after a single administration of 2 mg/kg or 8 mg/kg CBD, and the values fell within normal reference ranges and were not large enough differences to be clinically relevant. However, further research is needed to elucidate the effects of longer-term administration of cannabinoids in this species. While this study only evaluated single doses, Gamble et al. reported increases in alkaline phosphatase in dogs in the fourth week of daily CBD administration (9). Similarly, increases in alanine transaminase consistent with drug-induced liver injury have been reported in healthy human adults after 2–4 weeks of CBD exposure (41). Horses administered CBD demonstrated increases in gamma-glutamyl transferase, aspartate transaminase, and sorbitol dehydrogenase after 6 weeks of administration in a previous study,

returning to normal 10 days after discontinuation (22). However, the potential for chronic cannabinoid exposure to cause liver dysfunction or other side effects in horses is currently unknown.

Feed and water intake was free choice in all treatment groups and was not measured. However, no significant differences were observed in either cumulative manure production or in gastrointestinal transit time, and may have affected our pharmacokinetics. Further research may investigate whether the timing and availability of feed and water have an effect on the enteric bioavailability of cannabinoids in horses.

The study and adoption of cannabinoid products in veterinary medicine continues to be face legal hurdles, even though hemp production and distribution of products containing less than 0.3% THC are federally legal in the United States. State regulations may vary considerably between jurisdictions, but a majority of states allow hemp production, distribution and sales. The oil used in the present study contained approximately 0.13% Δ^9-THC and 0.13% THCA. In final analysis, the major metabolite associated with psychotropic activity, 11-OH-THC, was undetectable. Serum concentrations of THC reached a mean C_{max} of nearly 7 ng/mL with no adverse events, suggesting that the THC levels are safe with this acute dosing. THCA concentrations using similar dosing were nearly 10-fold higher, further supporting that acidic forms of cannabinoids are absorbed better than their decarboxylated forms. Fortunately, THCA is non-psychotropic and thought to be neuroprotective (42).

Given the variability between products, data on the pharmacokinetics of specific formulations is critical information for veterinarians as CBD, THC, and their metabolites are regulated by many racing jurisdictions and competition horse associations. While the product tested falls well within legal levels of THC, this compound is a Class 1 substance under the Association of Racing Commissioners International (ARCI) and a banned substance under the Federation Equestre Internationale (FEI), meaning that no detectable levels are acceptable. All horses in the 8 mg/kg CBD/CBDA group and 2 horses in the 2 mg/kg CBD group had detectable levels of THC in serum. The ARCI also classifies CBD as a Class 2 substance, for which no detectable levels are acceptable, while the FEI classifies CBD as a controlled and specified substance. It is of note, that while THC, CBD and CBDA are rapidly cleared, the half-life of elimination of 7-COOH CBD is long at 52 h for 2 mg/kg CBD and 71.9 h for 8 mg/kg CBD following a single dose. Additionally, it is important to point out that, to date, there has been no formal studies showing efficacy for any indication in horses in placebo blinded studies with the only study on equine behavior being negative, and one case report suggesting alleviation of cribbing behavior (43, 44). Further studies are needed utilizing proper dosing intervals as it is becoming evident that horses may require increased dosing compared to other species for pharmacodynamic effects and that considering the superior absorption of CBDA that further research on CBDA is needed.

5 Conclusion

CBD concentrations following enteral administration of CBD oil were low and elimination relatively rapid, while CBDA appeared to be the predominant cannabinoid present with potential therapeutic benefit in horses provided a CBD/CBDA-rich hemp oil. No adverse effects were encountered; however, this was a single dose study. The results of this study can be used to guide bodyweight dosing and dosing interval in future multi-dose studies. Further evaluation of therapeutic effects as well as potential adverse effects in multi-dose studies in horses are warranted.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by University of Florida Insitutional Animal Care and Use Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

AT: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. TM: Conceptualization, Data curation, Funding acquisition, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. AZ: Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. BG: Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. AL: Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. WS: Formal analysis, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. MM: Methodology, Writing – review & editing. DP: Conceptualization, Writing – review & editing. AB: Data curation, Project administration, Writing – original draft, Writing – review & editing. JW: Conceptualization, Methodology, Writing – review & editing.

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Conflict of interest

JW and WS are paid consultants of Ellevet Sciences.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2024.1356463/full#supplementary-material

References

1. Elphick MR. The evolution and comparative neurobiology of endocannabinoid signalling. *Philos Trans R Soc Lond Ser B Biol Sci*. (2012) 367:3201–15. doi: 10.1098/rstb.2011.0394

2. Howlett AC. The cannabinoid receptors. *Prostaglandins Other Lipid Mediat*. (2002) 68-69:619–31. doi: 10.1016/S0090-6980(02)00060-6

3. Basavarajappa BS. Critical enzymes involved in endocannabinoid metabolism. *Protein Pept Lett*. (2007) 14:237–46. doi: 10.2174/092986607780090829

4. di Marzo V, Piscitelli F. The endocannabinoid system and its modulation by phytocannabinoids. *Neurotherapeutics*. (2015) 12:692–8. doi: 10.1007/s13311-015-0374-6

5. Costa B, Giagnoni G, Franke C, Trovato AE, Colleoni M. Vanilloid TRPV1 receptor mediates the antihyperalgesic effect of the nonpsychoactive cannabinoid, cannabidiol, in a rat model of acute inflammation. *Br J Pharmacol*. (2004) 143:247–50. doi: 10.1038/sj.bjp.0705920

6. Galiègue S, Mary S, Marchand J, Dussossoy D, Carrière D, Carayon P, et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem*. (1995) 232:54–61. doi: 10.1111/j.1432-1033.1995.tb20780.x

7. Brioschi FA, di Cesare F, Gioeni D, Rabbogliatti V, Ferrari F, D'Urso ES, et al. Oral transmucosal cannabidiol oil formulation as part of a multimodal analgesic regimen: effects on pain relief and quality of life improvement in dogs affected by spontaneous osteoarthritis. *Animals*. (2020) 10:1505. doi: 10.3390/ani10091505

8. Coelho JC, Duarte N, Bento da Silva A, Bronze MR, Mestrinho LA. Placebo-controlled trial of daily oral cannabidiol as adjunctive treatment for cats with chronic gingivostomatitis. *Animals*. (2023) 13:2716. doi: 10.3390/ani13172716

9. Gamble L-J, Boesch JM, Frye CW, Schwark WS, Mann S, Wolfe L, et al. Pharmacokinetics, safety, and clinical efficacy of cannabidiol treatment in osteoarthritic dogs. *Front Vet Sci*. (2018) 5:165. doi: 10.3389/fvets.2018.00165

10. McGrath S, Bartner LR, Rao S, Packer RA, Gustafson DL. Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. *J Am Vet Med Assoc*. (2019) 254:1301–8. doi: 10.2460/javma.254.11.1301

11. Kleinhenz MD, Weeder M, Montgomery S, Martin M, Curtis A, Magnin G, et al. Short term feeding of industrial hemp with a high cannabidiolic acid (CBDA) content increases lying behavior and reduces biomarkers of stress and inflammation in Holstein steers. *Sci Rep*. (2022) 12:3683. doi: 10.1038/s41598-022-07795-z

12. Campos AC, Moreira FA, Gomes FV, del Bel EA, Guimarães FS. Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. *Philos Trans R Soc Lond Ser B Biol Sci*. (2012) 367:3364–78. doi: 10.1098/rstb.2011.0389

13. Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ9-tetrahydrocannabinol, cannabidiol and Δ9-tetrahydrocannabivarin. *Br J Pharmacol*. (2008) 153:199–215. doi: 10.1038/sj.bjp.0707442

14. Bisogno T, Hanuš L, de Petrocellis L, Tchilibon S, Ponde DE, Brandi I, et al. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol*. (2001) 134:845–52. doi: 10.1038/sj.bjp.0704327

15. Gobira PH, Vilela LR, Gonçalves BDC, Santos RPM, de Oliveira AC, Vieira LB, et al. Cannabidiol, a *Cannabis sativa* constituent, inhibits cocaine-induced seizures in mice: possible role of the mTOR pathway and reduction in glutamate release. *Neurotoxicology*. (2015) 50:116–21. doi: 10.1016/j.neuro.2015.08.007

16. Suryavanshi SV, Kovalchuk I, Kovalchuk O. Cannabinoids as key regulators of inflammasome signaling: a current perspective. *Front Immunol*. (2021) 11:613613. doi: 10.3389/fimmu.2020.613613

17. Court MH, Mealey KL, Burke NS, Jimenez TP, Zhu Z, Wakshlag JJ. Cannabidiol and cannabidiolic acid: preliminary in vitro evaluation of metabolism and drug-drug interactions involving canine cytochrome P-450, UDP-glucuronosyltransferase, and P-glycoprotein. *Vet Pharmacol Ther*. (2024) 47:1–13. doi: 10.1111/jvp.13403

18. Takeda S, Misawa K, Yamamoto I, Watanabe K. Cannabidiolic acid as a selective Cyclooxygenase-2 inhibitory component in Cannabis. *Drug Metab Dispos*. (2008) 36:1917–21. doi: 10.1124/dmd.108.020909

19. Philpot LM, Ebbert JO, Hurt RT. A survey of the attitudes, beliefs and knowledge about medical cannabis among primary care providers. *BMC Fam Pract*. (2019) 20:17. doi: 10.1186/s12875-019-0906-y

20. Kondrad E, Reid A. Colorado family physicians' attitudes toward medical marijuana. *J Am Board Family Med*. (2013) 26:52–60. doi: 10.3122/jabfm.2013.01.120089

21. Ryan D, McKemie DS, Kass PH, Puschner B, Knych HK. Pharmacokinetics and effects on arachidonic acid metabolism of low doses of cannabidiol following oral administration to horses. *Drug Test Anal*. (2021) 13:1305–17. doi: 10.1002/dta.3028

22. Yocom AF, O'Fallon ES, Gustafson DL, Contino EK. Pharmacokinetics, safety, and synovial fluid concentrations of single-and multiple-dose oral administration of 1 and 3 mg/kg cannabidiol in horses. *J Equine Vet*. (2022) 113:103933. doi: 10.1016/j.jevs.2022.103933

23. Williams MR, Holbrook TC, Maxwell L, Croft CH, Ientile MM, Cliburn K. Pharmacokinetic evaluation of a cannabidiol supplement in horses. *J Equine Vet*. (2022) 110:103842. doi: 10.1016/j.jevs.2021.103842

24. Turner SE, Knych HK, Adams AA. Pharmacokinetics of cannabidiol in a randomized crossover trial in senior horses. *Am J Vet Res*. (2022) 83:ajvr.22.02.0028. doi: 10.2460/ajvr.22.02.0028

25. Sánchez de Medina A, Serrano-Rodríguez JM, Díez de Castro E, García-Valverde MT, Saitua A, Becero M, et al. Pharmacokinetics and oral bioavailability of cannabidiol in horses after intravenous and oral administration with oil and micellar formulations. *Equine Vet J*. (2023) 55:1094–103. doi: 10.1111/evj.13923

26. Eichler F, Poźniak B, Machnik M, Schenk I, Wingender A, Baudisch N, et al. Pharmacokinetic modelling of orally administered cannabidiol and implications for medication control in horses. *Front Vet Sci*. (2023) 10:1234551. doi: 10.3389/fvets.2023.1234551

27. Deabold KA, Schwark WS, Wolf L, Wakshlag JJ. Single-dose pharmacokinetics and preliminary safety assessment with use of CBD-rich hemp nutraceutical in healthy dogs and cats. *Animals*. (2019) 9:832. doi: 10.3390/ani9100832

28. Anderson LL, Etchart MG, Bahceci D, Golembiewski TA, Arnold JC. Cannabis constituents interact at the drug efflux pump BCRP to markedly increase plasma cannabidiolic acid concentrations. *Sci Rep*. (2021) 11:14948. doi: 10.1038/s41598-021-94212-6

29. Sano H, Martin-Flores M, Santos LCP, Cheetham J, Araos JD, Gleed RD. Effects of epidural morphine on gastrointestinal transit in unmedicated horses. *Vet Anaesth Analg*. (2011) 38:121–6. doi: 10.1111/j.1467-2995.2010.00588.x

30. Grubb TL, Kurkowski D, Sellon DC, Seino KK, Coffey T, Davis JL. Pharmacokinetics and physiologic/behavioral effects of buprenorphine administered sublingually and intravenously to neonatal foals. *J Vet Pharmacol Ther*. (2019) 42:26–36. doi: 10.1111/jvp.12715

31. Crockett J, Critchley D, Tayo B, Berwaerts J, Morrison G. A phase 1, randomized, pharmacokinetic trial of the effect of different meal compositions, whole milk, and alcohol on cannabidiol exposure and safety in healthy subjects. *Epilepsia*. (2020) 61:267–77. doi: 10.1111/epi.16419

32. Silmore LH, Willmer AR, Capparelli EV, Rosania GR. Food effects on the formulation, dosing, and administration of cannabidiol (CBD) in humans: a systematic review of clinical studies. Pharmacotherapy: the journal of human pharmacology and drug. *Therapy*. (2021) 41:405–20. doi: 10.1002/phar.2512

33. Rock EM, Limebeer CL, Parker LA. Effect of cannabidiolic acid and Δ9-tetrahydrocannabinol on carrageenan-induced hyperalgesia and edema in a rodent model of inflammatory pain. *Psychopharmacology*. (2018) 235:3259–71. doi: 10.1007/s00213-018-5034-1

34. Vigli D, Cosentino L, Pellas M, de Filippis B. Chronic treatment with Cannabidiolic acid (CBDA) reduces thermal pain sensitivity in male mice and rescues the hyperalgesia in a mouse model of Rett syndrome. *Neuroscience*. (2021) 453:113–23. doi: 10.1016/j.neuroscience.2020.09.041

35. Wang T, Zakharov A, Gomez B, Lyubimov A, Trottier NL, Schwark WS, et al. Serum cannabinoid 24 h and 1 week steady state pharmacokinetic assessment in cats using a CBD/CBDA rich hemp paste. *Front Vet Sci*. (2022) 9:895368. doi: 10.3389/fvets.2022.895368

36. Godfrey KR, Arundel PA, Dong Z, Bryant R. Modelling the double peak phenomenon in pharmacokinetics. *IFAC Proc*. (2009) 42:127–32. doi: 10.3182/20090812-3-DK-2006.0001

37. Weitschies W, Bernsdorf A, Giessmann T, Zschiesche M, Modess C, Hartmann V, et al. The talinolol double-peak phenomenon is likely caused by presystemic processing after uptake from gut lumen. *Pharm Res*. (2005) 22:728–35. doi: 10.1007/s11095-005-2588-5

38. Mirfazaelian A, Mahmoudian M. A simple pharmacokinetics subroutine for modeling double peak phenomenon. *Biopharm Drug Dispos*. (2006) 27:119–24. doi: 10.1002/bdd.492

39. Baggot J. Bioavailability and bioequivalence of veterinary drug dosage forms, with particular reference to horses: an overview. *J Vet Pharmacol Ther*. (1992) 15:160–73. doi: 10.1111/j.1365-2885.1992.tb01003.x

40. Duijkeren E, Vulto AG, van Oldruitenborgh-Oosterbaan MMS, Kessels BG, van Miert AS, Breukink HJ, et al. Pharmacokinetics of trimethoprim/sulphachlorpyridazine in horses after oral, nasogastric and intravenous administration. *J Vet Pharmacol Ther*. (1995) 18:47–53. doi: 10.1111/j.1365-2885.1995.tb00550.x

41. Watkins PB, Church RJ, Li J, Knappertz V. Cannabidiol and abnormal liver chemistries in healthy adults: results of a phase I clinical trial. *Clin Pharmacol Ther*. (2021) 109:1224–31. doi: 10.1002/cpt.2071

42. Nadal X, del Río C, Casano S, Palomares B, Ferreiro-Vera C, Navarrete C, et al. Tetrahydrocannabinolic acid is a potent PPARγ agonist with neuroprotective activity. *Br J Pharmacol*. (2017) 174:4263–76. doi: 10.1111/bph.14019

43. Eichler F, Ehrle A, Machnik M, Jensen KC, Wagner S, Baudisch N, et al. Behavioral observations, heart rate and cortisol monitoring in horses following multiple oral administrations of a cannabidiol containing paste (part 2/2). *Front Vet Sci*. (2024) 10:1305873. doi: 10.3389/fvets.2023.1305873

44. Cunha RZ, Felisardo LL, Salamanca G, Marchioni GG, Neto OI, Chiocchetti R. The use of cannabidiol as a novel treatment for oral stereotypic behaviour (crib-biting) in a horse. *Vet Anim Sci*. (2023) 19:100289. doi: 10.1016/j.vas.2023.100289

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Pharmacokinetics behavior of four cannabidiol preparations following single oral administration in dogs

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Cannabidiol (CBD) is a natural phytochemical agent and one of the most abundant found in *Cannabis sativa*. It is known to exhibit pharmacological properties on various condition such as relieving-inflammation, pain, epilepsy, and anxiety effect. There has been an increasing trend globally in the use of CBD as a supplement in pets. Consequently, there are various CBD products being marketed that are specifically available for pets. Veterinarians and pet owners are concerned that following ingestion, different CBD formulations may result in a CBD level circulating in the blood that may affect the safe use and efficacy of CBD in pets. Several pharmacokinetics studies in animals have been mainly conducted with an oily form of CBD. To date, there is a lack of data regarding direct comparisons in animals among the CBD plasma kinetic profiles from an oral administration of the various preparation forms. Therefore, the current study evaluated and compared the plasma CBD levels from a single oral administration using four different CBD preparations—liquid (an oil-based form, a nanoemulsion form, or a water-soluble form) or a semi-solid form (as CBD mixed in a treat) in dogs. In total, 32 healthy, crossbreed dogs were randomly assigned into 4 groups and treated according to a 1-period, 4-treatment parallel-design. The three liquid forms were dosed at 5mg/kg body weight, while the single semi-solid form was given at 50mg/treat/dog. The results showed that the CBD plasma profile from the administration of a water-soluble form was comparable to that of the oil-based group. The nanoemulsion-based form tended to be rapidly absorbed and reached its peak sooner than the others. However, the CBD in all preparations reached the maximum plasma concentration within 3h post-dose, with an average range of 92–314μg/L. There were significant differences among certain parameters between the liquid and semi-solid forms. This was the first study to provide pharmacokinetics data regarding CBD in water soluble, nanoemulsion-based, and semi-solid forms for dogs as companion animals. The current data should facilitate the scrutiny of CBD plasma profiles based on different formulations via an oral route in dogs.

KEYWORDS

pharmacokinetics, CBD, cannabidiol, hemp, dog, cannabis

Introduction

Since discovering the endocannabinoids system and its receptor in the late 1960s, there have been extensive studies to understand the associated mechanisms, functions, and chemical interrelationships (1, 2). Cannabinoids are chemical compounds, mainly produced by *Cannabis sativa L.*, that reportedly interact with the endocannabinoids system and exert a biological effect in mammals (1). There are more than 90 compounds in 10 subclasses that have been classified as phytocannabinoids (2). Among these, cannabidiol (CBD), a non phychotropic component of cannabis, has been of interest for its potential use to cure diseases and improve the quality of animal life (3). There have been numerous publications on the *in vitro* and *in vivo* pharmacological effects of CBD in humans and animals, such as anti-inflammatory, analgesic, dermatological and immunomodulation properties (2–5). Specifically, early study of administering CBD in dogs has demonstrated its potential in the alleviation of pain and the clinical symptom of osteoarthritis (6). Recently, it was reported that giving a low dose of CBD in conjunction with an analgesic protocol in horses showed satisfactory pain relief with improved quality of animal life (7). Clinically, the efficacy has been described of CBD to reduce the frequency and severity of seizure in an epileptic dog (8, 9). In addition, CBD has been shown to be useful as adjunctive treatment to relief pruritus in a dog with atopic dermatitis (10).

Despite CBD having substantial therapeutic potential in animals, its pharmacokinetics (PK) profiles in companion animals, especially dogs, have yet to be clearly described (11). CBD is a chemically lipophilic molecule with poor and variable absorption (12). Oral bioavailability of CBD in dogs has been reported to be lower than 20% (13). Notably, it was hypothesized that the first-pass metabolism is one of major concerns regarding the low bioavailability of CBD via oral administration (14). Therefore, there has been much interest to increase the CBD plasma level and to identify alternative routes and different dosage forms of administration.

Several dosage forms of CBD have been studied in animals (11), such as liquid oil-based, capsules, soft chew (15–17), microencapsulated oil beads and transdermal cream (18), intranasal, and as a suppository (19). Nowadays, there are variety of CBD products available for pets, with growing consumption in the global market (11, 20). CBD oil-based preparation is one of most common forms consumed orally by pets and its kinetics behavior has been studied (11). However, the highly lipophilic property of oil-based products affects the CBD level for optimal biological effect, since it has low aqueous solubility and bioavailability. Other CBD options have been developed to improve CBD solubility and delivery into blood circulation and target tissue, such as water-soluble and nanoemulsion forms (5, 21–23). Comparison of the PK profiles of different water-soluble and oil-based preparations has been studied in humans and has confirmed the influence of CBD preparation on its bioavailability (5). Pharmacokinetics describe the time-course concentration of a drug throughout the body and can be utilized as an interpretive and predictive tool of exogenous chemical behavior. The fate of any drug may change based on the site of administration, formulation, and dosage. The PK profiles of different dosage forms in target animals should be studied by taking into consideration the various factors that affect the plasma CBD level.

The scope of the current study was to determine the optimum CBD level using GC-TQ/MS, with the main aim to evaluate the CBD plasma kinetic profiles in mature crossbreed dogs. For this purpose, the study investigated a single-dose CBD administration of four different dosage forms CBD infused in an oil base (OM), a nanoemulsion base (NM), a water-soluble base (WM), and a semi-solid form as a treat (CM). The current investigation should provide insights that are relevant to prudent use and practice on CBD delivery and to efficacy strategies via oral delivery in dogs.

Materials and methods

Chemical and CBD preparation

The CBD standard was purchased from Cerliliant® (product code: 13956–29-1). Certified CBD powder was obtained from Salus Bioceutical (Thailand) Co., Ltd. with purity greater than 99%, as reported by a certified test laboratory third party. HPLC and LC/MS grades of acetonitrile and methanol were purchased from Labscan Co. Ltd. (Bangkok, Thailand).

CBD in oil-based was prepared by dissolving CBD powder in natural virgin coconut oil (100% cold-pressed). In brief, 1.5 g of CBD isolated powder were weighed into a volumetric flask and dissolved in 30 mL of oil and dispersed using a magnetic stirrer on a warm plate at approximately 45°C for 30 min.

The nanoemulsion formulation was not the main objective in this study. Therefore, a test of the potential of a nanoemulsion for CBD delivery was performed following a method developed in-house for oral herbal oil formulations. In short, an oil-in-water nanoemulsion was prepared using a high-pressure homogenization technique (15,000 psi, 5 cycles), comprising oil droplets with diameters in the range 150–200 nm. The nanoemulsion was achieved by mixing an aqueous phase (comprising purified water, propylene glycol, sodium EDTA, paraben concentrate, Tween 80) and an oil phase (comprising CBD, short and medium chain triglycerides, alpha-tocopherol, and Span 80).

The water-based CBD comprised 20% CBD water-soluble powder in a modified blended starch of corn and tapioca as an emulsifier. Briefly, 7.5 g of CBD water-soluble powder were added to a 50 mL volumetric flask. Then, 30 mL of purified water was added and mixed using a magnetic stirrer for 15 min to achieve a final concentration of 50 mg/mL. Notably, prior to administering liquid forms to each animal, the CBD concentration of each preparations was re-assayed using HPLC and the volume was corrected where necessary for a 5 mg/kg dosage. In short, the HPLC- DAD (Thermo Scientific™ Vanquish™ Core HPLC systems) in-house validation method for quantification of CBD showed linearity over the range 0.01–0.4 mg/L, with a coefficient of determination ≥0.999 and a LLOQ of 0.01 mg/L. The percentage values for precision and accuracy were within 3.60–4.18% and 95.6–102.4%, respectively.

The CBD in treat form was prepared by mixing small pieces of the ingredients (corn, rice bran, coconut oil and water). Then, the mixed result was individually loaded to provide CBD oil at 50 mg/treat. All treat samples were placed in an oven at 100°C for 30 min. To prove the CBD level in the treat, 10 treat samples of the same batch were sampled, with the results showing that the CBD level in all samples was at the expected concentration with a standard deviation of less than 1.8%. All CBD preparations were kept in well-sealed containers

and placed in a refrigerator (4°C) before being used for animal ingestion within 7 days.

Animals and ethical considerations

The study was performed in accordance with the permit from the Committee for the Approval of Animal Care and Use for Scientific Research of the Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand (approval number ACKU 62-VET-058).

In total, 32 healthy, crossbreed intact dogs with individually numbered identification (aged 1–5 years, weight 11–23 kg) were equally randomized into 4 parallel design treatment groups (4 males and 4 females in each group). The animals had not been treated with any medication during the previous 4 weeks and were acclimatized for at least 14 days prior to treatment. The animals were housed in separate kennels, with the housing conditions and animals being managed in accordance with the standard of operation of the University. Physical examination, clinical observation, hematology, and blood chemistry were carried out during acclimatization. All animals were fasted overnight before dosing. Any indications of relevant clinical signs or adverse events were observed twice daily for 3 days pre- and post-treatment.

Dosing design

A single oral dose of CBD in liquid forms: for an oil base (OM), a nanoemulsion base (NM), a water-soluble base (WM), was given to each fasted animal based on the animal's actual body weight (BW) with the target being 5 mg/kg BW in individually adjusted dosage volumes. In the semi-solid form (CM), each serving contained 50 mg of CBD per dog that was given by hand directly into the month of the fasted animal for self-ingestion with a tray underneath that collected any spilled pieces of test item, which were then reinserted into the animal's mouth cavity and the animal was carefully observed to ensure all the treat had been swallowed.

Specimens and collection

Blood samples were collected and stored in a tube containing lithium heparin via cephalic or saphenous venipuncture with a no. 22" IV-catheter at the following time points: −1 day, 0, 30 min, and then 1, 2, 3, 6, 10, 24, and 30 h after a single oral ingestion. Following collection, the samples were immediately placed on ice and protected from direct light until centrifugation. After centrifugation at 3,000 × *g* for 10 min at a controlled temperature of 4°C, plasma was collected in laboratory-coded, labeled aliquots. Then, the aliquots were transported in an ice-pack box to be frozen at −80°C in a dark cover box pending analysis within 65 days.

Quantitative measurement of plasma-containing CBD

The groups of eight animals per treatment were studied for their plasma concentration-time profiles of CBD using an in-house, validated gas chromatography method modified from previously described (24). In brief, 100 µL of plasma sample were extracted with 400 µL of methanol and then triple-vortexed at 2,200 rpm for 10 min. Samples were centrifuged at 10,000 × *g* for 10 min at 4°C. A portion of the 100 µL of supernatant was transferred into a 2 mL GC glass vial. An internal standard using myristic acid-D27 in hexane (1 µmol/mL) was added for 20 µL. The mixture was dried at 60°C for 2 h, then added with 50 µL dichloromethane, and dried again for 30 min to remove the residual water. For the trimethylsilylation reaction, a modified method was performed following an assay described (24, 25). In brief, 50 µL of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), containing 1% of trimethylchlorosilane, were added into each mixture and incubated at 37°C for 30 min. The derived samples were cooled at room temperature and transferred into glass vials with micro-inserts and capped immediately for analysis. Each sample was analyzed using gas chromatography triple quadrupole tandem mass spectrometry (GC-TQ/MS; GC 7890B/MSD 7000D; Agilent Technologies; United States) coupled to a PAL3 auto sampler system (CTC Analytics AG; Switzerland).

An injection volume of 2 µL of the derived samples was analyzed using the GC-TQ/MS in split mode with an injector temperature of 250°C, a split ratio of 10:1, with a DB-5MS UI column (30 m, 0.25 mm i.d.; Agilent Technologies; United States). Helium was used as the carrier gas with a constant flow rate of 1.0 mL/min. The GC oven was programmed with an initial oven temperature of 60°C, then ramped from 60°C to 325°C at the rate of 10°C/min, and held for 10 min. The transfer line, ion source, and quadrupole were set at 325°C, 240°C, and 180°C, respectively. The mass spectrometer was operated in dynamic multiple reaction monitoring (dMRM) mode and detected for transition at m/z 389.9 > 301.2 m/z for CBD and 312.0 > 119.9 > 73 m/z for myristic acid-D27. To achieve acceptable precision and accuracy for CBD quantification, the derivatized samples were limited to 30 samples (injections) a day with a proper moisture removal procedure. All samples were stored at <10°C using a PAL3 Peltier stack and tray to ensure the stability of the targeted compounds. In addition, the mass detector tuning and calibration curve were performed daily before commencing the new sequence operation. Data were acquired using the MassHunter software (version 10.0; Agilent Technologies; United States) based on three replicates to calculate the mean and the standard error. The calibration curves were determined using CBD at different concentrations in the range 1–800 ng/mL in plasma with myristic-d27 acid as an internal standard (co-efficient of determination = 0.9999). The quantitative analyses were performed using the Agilent MassHunter software (version 10.0; Agilent Technologies; United States) and exported into the Excel software (Microsoft Corp.; United States) for further data processing.

Pharmacokinetic evaluation

The pharmacokinetic parameters in this study were evaluated following a typical model-independent approach using non-compartmental analysis (NCA). NCA was performed using the R software [version 4.3.2; R Core Team, (2022-10-31)], focusing on the key parameters of maximum plasma concentration (C_{max}), time to maximum concentration (T_{max}), area under the curve to the last quantifiable time-point (AUC_{0-t}), area under the curve extrapolated to

infinity ($AUC_{0\text{-}inf}$), terminal phase elimination rate constant (Ke), apparent volume of distribution during the terminal phase (Vz/F), apparent clearance (CL/F) after non-intravenous administration (assuming that the ratio of clearance to bioavailability is constant without IV comparison), mean residence time extrapolated to infinity (MRT_{inf}), and elimination half-life ($T_{1/2}$). The dose normalized C_{max} and AUC parameters were calculated to facilitate the assessment of dose proportionality. The relative bioavailability values were calculated following dose-normalization using $AUC_{(another\ form)}/AUC_{(OM\ form)} \times 100$.

Statistical analysis

Microsoft Excel and GraphPad Prism version 9.5.1 (733) for Windows (GraphPad Software; United States) were used to calculate descriptive and inferential statistical analyses (where applicable), including all outcome calculation data of the middle of a dataset (median values), measure of central tendency (average; mean), variability (standard deviation, standard error mean) and figures. Normality distribution was determined using the Shapiro–Wilk test, while differences between groups for the $AUC_{0\text{-}t}$, C_{max}, T_{max}, and Ke parameters were analyzed using a Brown-Forsythe ANOVA following a *post hoc* Dunnett's T3 multiple comparisons test. Non-normality distributions for the $T_{1/2}$, $AUC_{0\text{-}inf}$, Vz/F, CL/F and MRT_{inf} parameters were tested using the Kruskal-Wallis with a *post hoc* Dunn-Bonferroni test to achieve pairwise multiple comparison data. A value of $p \leq 0.05$ was defined as significant.

Results

This study was conducted to investigate the pharmacokinetics of CBD in crossbreed intact dogs following a single dose. An in-house validation of the GC-TQ/MS method for fortified dog plasma was achieved with an instrument detection limit at 0.05 μg/L and a LLOQ of 1 μg/L blood plasma, with satisfactory intra-day precision based on coefficient of variation and accuracy results in the ranges 5.8–10.8% and 85.2–110.3%, respectively. Inter-day precision and accuracy were in the ranges 9.8–10.1% and 92.6–102.45%, respectively.

The treatment involved the dogs receiving one of either a single dose of liquid form CBD infused in an oil base (OM), a nanoemulsion base (NM), or a water-soluble base (WM), or of semi-solid form as treat (CM). All animals completed this experiment with no adverse clinical events occurring during the study. At the studied dose, there were no signs of serious gastrointestinal or nervous disorders in the dogs during and post dose. A single intake of each serving contained 50 mg CBD for each dog in the CM group. The data set of dose-normalized C_{max} and AUC parameters were compared to other groups. Notably, only one dog in the CM group appeared to produce more saliva than usual when chewing, but recovered soon after ingestion. The root cause of this was not identified. The PK parameters, using non-compartmental analysis, of CBD in the 4 preparations following a single oral administration to overnight fasted dogs are summarized in Table 1. The plasma concentrations of CBD (mean ± SEM) for each time point of all groups were calculated and are presented as a semi-log graph in Figure 1. Certain PK parameters were statistically significant, as shown in Figure 2. Indeed, following the pharmacokinetic estimation, a dog in the OM group and two dogs in each of the remaining groups were excluded in the subsequent descriptive summary due to insufficient data points in the elimination phase. In addition, the excluded data resulted in inaccurate estimation of the Ke, $T_{1/2}$, Vz/F, and extrapolation of $AUC_{0\text{-}inf}$. It could also affect calculations of MRT and CL/F, as they are calculated using $AUC_{0\text{-}inf}$.

Following dose-normalization, the results showed no significant difference between the C_{max} of the CBD in the plasma after administration of all groups. However, the highest C_{max} of the CBD in the plasma (314.30 ± 81.09 μg/L) was obtained from administration of the WM group, while the lowest C_{max} of the CBD in the plasma was in the CM group (92.29 ± 21.45 μg/L).

The values for the mean $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ of the OM formulation were 1432.06 ± 208.38 and 1494.14 ± 209.87 (μg/L*h), which displayed the highest extent of CBD exposure compared to the other treatments. The CM group provided the lowest extent of CBD in the plasma of around 296.05 ± 41.22 and 313.84 ± 41.92 (μg/L*h) for the mean of last quantifiable time-point and the curve to infinite time, respectively. The relative bioavailability levels after dose-normalization of the other formulations comparing to the OM formulation were 80.9, 59.5, and 34.8% for the WM, NM and CM groups, respectively.

TABLE 1 PK parameters (mean ± SEM) of CBD following single oral dose administration of one of four different dosage forms.

Pharmacokinetic parameter	OM (n = 7)			NM (n = 6)			WM (n = 6)			CM (n = 6)			p-value
$AUC_{0\text{-}t}$ (μg/L*h)	1432.06	±	208.38	853.29	±	188.83	1158.98	±	317.83	296.05	±	41.22	0.0431[a]
$AUC_{0\text{-}inf}$ (μg/L*h)	1494.14	±	209.87	935.19	±	200.42	1308.98	±	378.85	313.84	±	41.92	0.0381[a]
C_{max} (μg/L)	270.10	±	31.88	175.35	±	28.19	314.30	±	81.09	92.29	±	21.45	0.1329[a]
T_{max} (h)	3.21	±	0.82	2.00	±	0.37	2.58	±	0.80	2.83	±	0.70	0.6584
$T_{1/2}$ (h)	8.47	±	1.31	10.19	±	1.35	10.23	±	4.05	9.56	±	1.01	0.4796
Ke (1/h)	0.10	±	0.02	0.08	±	0.01	0.11	±	0.03	0.08	±	0.01	0.4467
Vz/F (L/kg)	55.94	±	19.80	93.93	±	15.01	64.48	±	12.88	141.75	±	20.30	0.0199
CL/F (L/h/kg)	4.00	±	0.85	7.00	±	1.80	5.59	±	1.31	10.54	±	1.61	0.0381
MRT_{inf} (h)	8.96	±	0.27	9.77	±	1.11	10.69	±	3.66	8.14	±	0.75	0.5032

[a]A value of $p \leq 0.05$ was defined as significant and indicated the data with dose-normalization where applicable.
CBD infused in an oil base (OM), a nanoemulsion base (NM), a water-soluble base (WM), or a semi-solid form (CM) in the OM, NM, WM and CM groups, respectively, where p values are based on *post hoc* multiple comparisons using Brown-Forsythe ANOVA and otherwise (underlined) using non-parametric Kruskal-Wallis test.

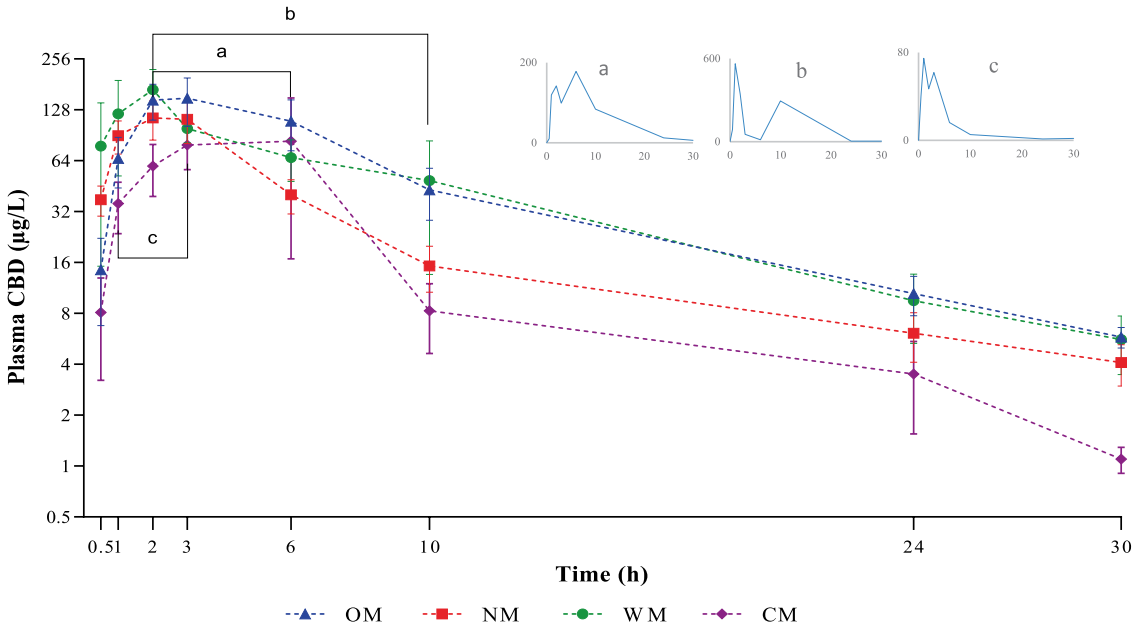

FIGURE 1
Graphical representation of semi-logarithmic scale of CBD plasma levels (mean ± SEM) after single oral dose administration of one of four different dosage forms: CBD infused in an oil base (OM), a nanoemulsion base (NM), a water-soluble base (WM), or a semi-solid form (CM). Line bar and sub-figures with letter a, b and c indicate time-points where secondary-peaks were observed in one dog of the OM, WM and CM groups, respectively.

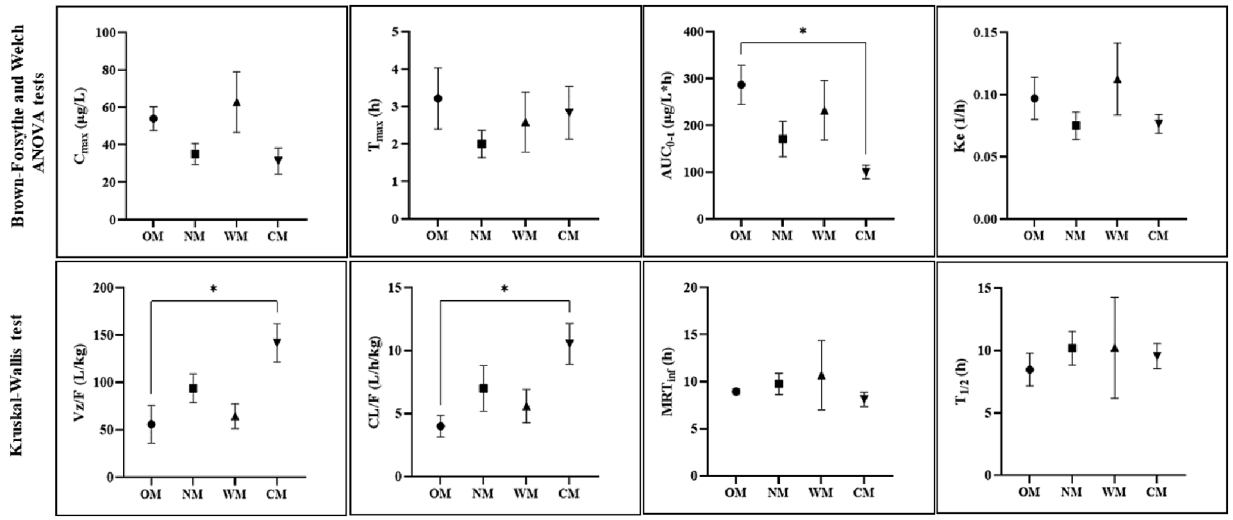

FIGURE 2
Representative PK parameters (mean ± SEM) for four different forms: CBD in an oil base (OM), a nanoemulsion base (NM), a water-soluble base (WM), a semi-solid form (CM), with significance indicated by $*p < 0.05$ (dose-normalized: mg/kg, C_{max} and AUC parameters).

Among the preparations, there was rapid absorption in the NM group, with peak plasma concentrations occurring within 2 h after ingestion. The CBD plasma concentrations of the liquid forms reached a peak over 100 µg/L within 6 h in all dogs. In contrast, only three of the eight dogs in the CM group achieved a C_{max} over 100 µg/L. The exposure using the semi-solid dosage form (CM) represented by the AUC was much lower than for the other dosage forms.

The $T_{1/2}$ of plasma CBD in the OM group was 8.47 ± 1.31 h, which was shorter than for the others but not significantly different. At 30 h post-dose, CBD was detectable in all dogs in the OM group but it was not detected in 1 out of the 8 dogs in each of the NM, WM, and CM groups. All the MRT_{inf} had a similar level range (8.14–10.69 h). There were significant differences between the liquid and semi-solid forms for certain parameters (AUC_{0-t}, Vz/F, and CL/F). As a result, the kinetic profiles of the CBD in the liquid forms were relatively similar, particular for the OM and WM groups. As such, the results demonstrated that the main PK parameters of the CBD within liquid forms were not as

straightforward as anticipated. The impact of the dosage form is covered in the discussion below.

Discussion

Utilizing cannabis-related products to achieve favorable health impacts is rapidly increasing following its legalization in some parts of the world. In June 2022, Thailand removed marijuana from its narcotics list and became the first Asian nation to approve cannabis for medicinal and industrial use (26). Cannabis use is of interest not only in human medicine, but also in veterinary medicine, where extensive research is warranted to better understand the behavior and impact of the drug after administration in animals, since interspecies differences are a main factor influencing PK variation (27). Dogs have been the main companion animal species studied; however, the published PK studies have mainly been on oil-based CBD (11, 15, 28).

The current study was designed to explore the PK patterns of CBD in the plasma from different preparation forms—liquid (the OM, NM, and WM groups) and semi-solid (the CM group)—following a single oral administration in overnight fasted crossbreed dogs. It is known that CBD is a lipophilic compound with limited absorption into circulating blood (12). Several reports on the PK of CBD in dogs have studied CBD in oil-based formulations, microencapsulated-beads, chewable soft capsules, and soft gel capsules (11, 15–18). However, comparison data of oral CBD profiles in companion animals with different formulations are scarce. To the best knowledge of the authors, this is the first report on CBD in nanoemulsion and water-soluble forms in dogs. In addition, according to the limited available information on CBD in semi-solid form, this study has presented plasma CBD behavior following snack-as-treat ingestion in dogs.

Variation in the PK pattern of plasma CBD arises from an extensively first-pass metabolism and its low aqueous solubility that leads to poor bioavailability and a poor biological effect (12, 14). Commonly, inconsistent and variable systemic drug exposure are affected by multiple factors, including route of administration, dosage form, dose range, and health and feeding status. It has been noted that differences in the study design, including animal signalment (breed, sex, age) and status, sampling time point, and determination method, may affect PK outcomes; therefore, it is inappropriate to directly compare those estimated PK parameters between various experiments (29).

The oral bioavailability of CBD in dogs has been estimated to be in range 13–19% (13). Improving CBD delivery into the blood stream and its efficacy via an oral route is challenging to achieve a therapeutic response. Specifically, numerous studies have been conducted with various developed CBD preparations to increase the oral bioavailability and PK evaluation in dogs (11, 15–18).

The current results indicated there were no significant differences between all PK profiles across the CBD delivered in liquid form. However, comparison of the CBD in liquid forms against the semi-solid form identified differences in the Vz/F, CL/F, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf}$ parameters. The current findings showed that the CBD behavior profiles in the WM group were comparable to those in the OM group. Although the highest C_{max} was in the WM group, it was a noticeably high variation, including for the MRT_{inf}, $T_{1/2,}$ and AUC parameters. This might indicate that the estimated rate of absorption in water-soluble dosage form had larger bioavailability variation than for the oil-based form. Coincidentally, another study reported that a water-based formulation of CBD, which had a similar composition to the WM prepared in the current study also reported statistically comparable PK parameters in human plasma compared to that of the CBD in human plasma after oral administration of the oil-based formulation of the CBD (5).

There has been a wide range reported of the CBD maximum concentration following oral administration of oil-based CBD formulations (11). Compared to another experiment, in which there was oil-based administration at the same dose in fasted dogs, the C_{max} value in the OM group in the current report was about twice that of the earlier report (30). In contrast, another experiment that involved drug administration to fed dogs with an equal adjusted dose of CBD-infused oil had a C_{max} that was around twice that of the current study (18).

Several factors influence the bioavailability and disposition of CBD, resulting in relatively high intra- and inter-individual variability in the PK profiles. Co-consumption of CBD with food, particularly in a fat meal, may alter the rate and extent of absorption. It has been reported that in humans, CBD plasma levels increased when concomitantly administered with food or in a fed state (31, 32). Likewise, positive food effects have been associated with increased maximum systemic exposure without affecting the $AUC_{0\text{-}t}$ in dogs; however, higher C_{max} and AUC levels were observed in one out of three fasted dogs (30). Contrary to these results, Vaughn et al. (20) argued that overnight fasting with dogs might enhance the systemic absorption of CBD. In rabbits, it has been reported that feeding decreased systemic CBD absorption (33). In fact, fasted-fed variability is affected by various factors, such as the physiological condition, demographic and genetic factors, chemical, and formulation-related factors (34).

A single dose administration in the current study presented fluctuations of CBD plasma concentrations both within and between groups. Recently, it has been suggested that giving CBD twice daily may reduce the variation in plasma concentration (28). In addition, the maximum CBD plasma concentration has been reported to increase in a dose-dependent manner but some studies seemed not to be linear (16, 28–30).

Notably, the current findings corroborated the phenomenon of the so called 'secondary peak' as it was found in one dog in every group except the NM group. At first, this was considered as a possible error in the sample preparation or related to the laboratory process; however, it was confirmed following double checking and determining with different instruments of detection. The explanation for this phenomenon is not yet clearly understood. The double peak of CBD found in the plasma has been suggested to have been caused by a combination mechanism, such as enterohepatic recycling and intestinal lymphatic absorption (35). In addition, the CBD secondary peak has been reported in dogs given a medium (5 mg/kg) or high (10 mg/kg) dose rather than a low (2 mg/kg) dose, with coprophagia being one possible explanation (29).

However, the absence of a secondary peak of CBD in the dog plasma after orally taking NM form may have been due to the small size of the oil droplets in the nanoemulsion. Consequently, they had a larger surface area and so were efficiently exposed to the intestinal lipase at its binding sites (36). Therefore, the CBD in the oil droplets of the nanoemulsion was absorbed after the oil was digested and rapidly transformed into the primary derivatives (CBD-7-COOH and

OH-7-CBD) through a first-pass metabolism process that resulted in a rapid decrease in the CBD concentration in the plasma (37). Finally, the content of the plasma CBD in the NM form could not be detected as a second peak, as occurred for CBD in the other dosage forms.

In all four dosage forms, the CBD was rapidly absorbed with mean maximum plasma concentrations occurring in the range 2–3.2 h post-dose. This was in agreement with other reports, where the times to reach the maximum plasma concentration were within 1–4 h, with no effect of dose amount or duration of exposure (6, 15, 16, 29).

The current results showed that the extent and rate of CBD systemic exposure in the OM group was highly absorbed. The lowest extent of absorption for the CM formulation compared to the other formulations was confirmed by the relative bioavailability value. The low plasma levels of the CM group could have been due to the low oral bioavailability of the semi-solid formulation, considering that the AUC was significantly lower than for the oil-based formulation (OM group), with the value for Vz/F and CL/F being significant higher than for the OM group. CBD degradation from the treats snack in the CM group following the heat process in preparation was ruled out because the CBD concentration was re-checked prior to being given to the dogs. Unlike the semi-solid form preparation in the current experiment, another study found that CBD in a soft chew format had high absorption with a delayed time to reach its peak, confirming that differently formulated preparations affect the PK outcome (15). As such, liquid and solid forms may alter the rate of absorption and total bioavailability.

Notably, CBD in the NM group followed by the WM group peaked sooner than the CBD in the OM group, which may support the rapid onset of an effect. The effect of nanoemulsion based on rapid oral absorption of the CBD found in the current study was consistent with the results reported by Yen et al. (38), who found that an andrographolide-loaded nanoemulsion was rapidly absorbed via the gastrointestinal tract because the surfactant molecules in the formulation (Tween 80 and Span 80) could suppress the function of P-gp, which inhibited drug secretion by the P-gp-mediated efflux process in the intestinal tissue. Therefore, the shorter time to reach the CBD peak from oral administration of the NM group may have been due to the effect of these particular surfactants.

In fact, oral drug absorption depends on the conditions in the gastro-intestinal tract. Consequently, it was possible that fasting the animals overnight in the current study might have shortened the time to peak concentration for the NM and WM forms. The interaction of PK properties and physiological features in the empty gastro-intestinal tract, including enteric epithelium and influx-efflux transporters, may have hindered this phenomenon; however, the mechanism has not yet been well elucidated.

Currently, there is a lack of research into using a nanoparticle-based approach with different techniques and routes of application to enhance CBD uptake (12). Development and commercial scale production of cannabinoid-loaded nanoemulsions have been highlighted to improve the absorption rate and efficacy for therapeutic purposes (39). The current findings showed that CBD in a nanoemulsion-based formulation tended to achieve rapid absorption, avoiding any fluctuations in kinetic behavior. A similar finding was recently reported, whereby a nanoemulsifying-CBD formulation had a shorter time to reach C_{max} compared to CBD in an oil-based form (35). In addition, it has been mentioned that the nanoemulsion formulated may have improved the rate and variability of absorption (40). However, notably, different CBD nanocarriers delivered different C_{max} and T_{max}, outcomes at a particular site of action (21).

Little information is available on CBD volume distribution in dogs. The larger values of the apparent volume of distribution in the liquid forms in the current study seem to suggest the CBD was more likely retained in the body than circulated in the blood. The current results had a Vz/*F* value nearly triple that of an oil-based treatment with the same dose in another study (28). In humans, it is evident that CBD is rapidly distributed throughout the tissues resulting in very high volume of distribution (41). Notably, the nanoemulsion-based treatment had a significantly higher volume of distribution compared to the semi-solid form. The modifying mode of delivery in the nanocarrier formulation may have enhanced the dispersion of CBD throughout the body. Clinically, CBD could be administered in multiple doses over several days up to a month for a therapeutic effect (18). Thus, the tissue distribution ratio should be considered of the CBD dispersed among physiological tissue and accumulated in parts of the body (20). There should be further study of the biodistribution of different CBD preparations within therapeutic sites of interest in the target animal.

Despite scarce scientific information, the use of CBD in dogs has been of broad interest to owners, based on anecdotal evidence of its therapeutic benefits. Notably the current study was conducted with a non-Beagle breed which may not be directly comparable to reported studies involving a Beagle breed. However, based on visual assessment, the crossbreed dog PK parameters of CBD did not show any significant differences from those in the study conducted with Beagle dogs (11, 28). The crossbreed dog population is estimated at 31–53% in the United States, Germany, and the UK (42). In Thailand, based on domestic survey data of pet owners, crossbreed dogs constitute approximately 29%. A limitation of the current study was the small number of crossbreed dogs with only 8 per group. An another limitation is that less frequently in blood sample collection after 10 h post-dose which may cause insufficient data points in the elimination phase. Since there is a wide range of crossbreed dogs, with undoubtedly differences in response to drug behavior, it is unclear whether the plasma concentrations in the current study can be considered representative of the general population of crossbreed or different-sized dogs. The differences in the CBD absorption rate and its metabolic action across dogs has resulted in high variability in plasma concentrations (20). The interpretation and practical use of the available pharmacokinetic data from the current single-dose study should be further investigated. Nonetheless, the novelty of this study is the generation of data relevant to different CBD forms and its behavior in dogs.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal studies were approved by The Committee for the Approval of Animal Care and Use for Scientific Research of the

Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand (approval number ACKU 62-VET-058). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

SL: Writing – review & editing, Validation, Project administration, Formal analysis, Data curation. NP: Writing – review & editing, Formal analysis. AP: Writing – review & editing, Validation, Formal analysis. RA: Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. NT: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Silver RJ. The endocannabinoid system of animals. *Animals*. (2019) 9:686. doi: 10.3390/ani9090686

2. Andre CM, Hausman J-F, Guerriero G. *Cannabis sativa*: the plant of the thousand and one molecules. *Front Plant Sci*. (2016) 7:19. doi: 10.3389/fpls.2016.00019

3. Corsato Alvarenga I, Panickar KS, Hess H, McGrath S. Scientific validation of Cannabidiol for management of dog and cat diseases. *Annu Rev Anim Biosci*. (2023) 11:227–46. doi: 10.1146/annurev-animal-081122-070236

4. Coelho MPRC, Leme FDOP, Moreira FA, Branco SEMT, Melo MM, de Melo EG. Current review of hemp-based medicines in dogs. *J Vet Pharmacol Ther*. (2021) 44:870–82. doi: 10.1111/jvp.13016

5. Williams NNB, Ewell TR, Abbotts KSS, Harms KJ, Woelfel KA, Dooley GP, et al. Comparison of five Oral Cannabidiol preparations in adult humans: pharmacokinetics, body composition, and heart rate variability. *Pharmaceuticals*. (2021) 14:35. doi: 10.3390/ph14010035

6. Gamble LJ, Boesch JM, Frye CW, Schwark WS, Mann S, Wolfe L, et al. Pharmacokinetics, safety, and clinical efficacy of Cannabidiol treatment in osteoarthritic dogs. *Front Vet Sci*. (2018) 5:165. doi: 10.3389/fvets.2018.00165

7. Interlandi C, Tabbì M, Di Pietro S, D'Angelo F, Costa GL, Arfuso F, et al. Improved quality of life and pain relief in mature horses with osteoarthritis after oral transmucosal cannabidiol oil administration as part of an analgesic regimen. *Front Vet Sci*. (2024) 11:11. doi: 10.3389/fvets.2024.1341396

8. McGrath S, Bartner LR, Rao S, Packer RA, Gustafson DL. Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. *J Am Vet Med Assoc*. (2019) 254:1301–8. doi: 10.2460/javma.254.11.1301

9. Potschka H, Bhatti SFM, Tipold A, McGrath S. Cannabidiol in canine epilepsy. *Vet J*. (2022) 290:105913. doi: 10.1016/j.tvjl.2022.105913

10. Loewinger M, Wakshlag JJ, Bowden D, Peters-Kennedy J, Rosenberg A. The effect of a mixed cannabidiol and cannabidiolic acid based oil on client-owned dogs with atopic dermatitis. *Vet Dermatol*. (2022) 33:329–e77. doi: 10.1111/vde.13077

11. Di Salvo A, Conti MB, della Rocca G. Pharmacokinetics, efficacy, and safety of cannabidiol in dogs: an update of current knowledge. *Front Vet Sci*. (2023) 10:1204526. doi: 10.3389/fvets.2023.1204526

12. Reddy TS, Zomer R, Mantri N. Nanoformulations as a strategy to overcome the delivery limitations of cannabinoids. *Phytother Res*. (2023) 37:1526–38. doi: 10.1002/ptr.7742

13. Samara E, Bialer M, Mechoulam R. Pharmacokinetics of cannabidiol in dogs. *Drug Metab Dispos*. (1988) 16:469–72.

14. della Rocca G, Paoletti F, Conti MB, Galarini R, Chiaradia E, Sforna M, et al. Pharmacokinetics of cannabidiol following single oral and oral transmucosal administration in dogs. *Front Vet Sci*. (2023) 9:1104152. doi: 10.3389/fvets.2022.1104152

15. Wakshlag JJ, Schwark WS, Deabold KA, Talsma BN, Cital S, Lyubimov A, et al. Pharmacokinetics of cannabidiol, cannabidiolic acid, 19- tetrahydrocannabinol, tetrahydrocannabinolic acid and related metabolites in canine serum after dosing with three oral forms of hemp extract. *Front Vet Sci*. (2020) 7:505. doi: 10.3389/fvets.2020.00505

16. Vaughn DM, Paulionis LJ, Kulpa JE. Randomized, placebo-controlled, 28-day safety and pharmacokinetics evaluation of repeated oral cannabidiol administration in healthy dogs. *Am J Vet Res*. (2021) 82:405–16. doi: 10.2460/ajvr.82.5.405

17. Tittle DJ, Wakshlag JJ, Schwark WS, Lyubimov A, Zakharov A, Gomez B. Twenty-four hour and one-week steady state pharmacokinetics of cannabinoids in two formulations of Cannabidiol and Cannabidiolic acid rich hemp in dogs. *Med Res Archiv*. (2022) 10:7. doi: 10.18103/mra.v10i7.2907

18. Bartner LR, McGrath S, Rao S, Hyatt LK, Wittenburg LA. Pharmacokinetics of cannabidiol administered by 3 delivery methods at 2 different dosages to healthy dogs. *Can J Vet Res*. (2018) 3:178–83.

19. Polidoro D, Temmerman R, Devreese M, Charalambous M, Ham LV, Cornelis I, et al. Pharmacokinetics of cannabidiol following intranasal, intrarectal, and oral administration in healthy dogs. *Front Vet Sci*. (2022) 9:899940. doi: 10.3389/fvets.2022.899940

20. Vaughn D, Kulpa J, Paulionis L. Preliminary investigation of the safety of escalating cannabinoid doses in healthy dogs. *Front Vet Sci*. (2020) 7:51. doi: 10.3389/fvets.2020.00051

21. Muresan P, Woodhams S, Smith F, Taresco V, Shah J, Wong M, et al. Evaluation of cannabidiol nanoparticles and nanoemulsion biodistribution in the central nervous system after intrathecal administration for the treatment of pain. *Nanomedicine*. (2023) 49:102664. doi: 10.1016/j.nano.2023.102664

22. Nakano Y, Tajima M, Sugiyama E, Sato Vilasinee H, Sato H. Development of a novel Nano-emulsion formulation to improve intestinal absorption of Cannabidiol. *Med Cannabis Cannabinoids*. (2019) 2:35–42. doi: 10.1159/000497361

23. Hossain KR, Alghalayini A, Valenzuela SM. Current challenges and opportunities for improved Cannabidiol solubility. *Int J Mol Sci*. (2023) 24:14514. doi: 10.3390/ijms241914514

24. Andrenyak DM, Moody DE, Slawson MH, O'Leary DS, Haney M. Determination of Δ-9-tetrahydrocannabinol (THC), 11-hydroxy-THC, 11-nor-9-carboxy-THC and Cannabidiol in human plasma using gas chromatography-tandem mass spectrometry. *J Anal Toxicol*. (2017) 41:277–88. doi: 10.1093/jat/bkw136

25. Fiehn O. Metabolomics by gas chromatography-mass spectrometry: combined targeted and untargeted profiling. *Curr Protoc Mol Biol*. (2016) 114:30.4.1. doi: 10.1002/0471142727.mb3004s114

26. Kalayasiri R, Boonthae S. Trends of cannabis use and related harms before and after legalization for recreational purpose in a developing country in Asia. *BMC Public Health*. (2023) 23:911. doi: 10.1186/s12889-023-15883-6

27. Toutain P-L, Ferran A, Bousquet-Mélou A. Species differences in pharmacokinetics and pharmacodynamics In: F Cunningham, J Elliott and P Lees, editors. *Comparative and veterinary pharmacology*. Berlin, Heidelberg: Springer Berlin Heidelberg (2010). 19–48.

28. Corsato Alvarenga I, Gustafson D, Banks K, Wilson K, McGrath S. Cannabidiol plasma determination and pharmacokinetics conducted at beginning, middle and end of long-term supplementation of a broad-spectrum hemp oil to healthy adult dogs. *Front Vet Sci*. (2023) 10:1279926. doi: 10.3389/fvets.2023.1279926

29. Chicoine A, Illing K, Vuong S, Pinto KR, Alcorn J, Cosford K. Pharmacokinetic and safety evaluation of various Oral doses of a novel 1:20 THC:CBD Cannabis herbal extract in dogs. *Front Vet Sci*. (2020) 7:583404. doi: 10.3389/fvets.2020.583404

30. Doran CE, McGrath S, Bartner LR, Thomas B, Cribb AE, Gustafson DL. Drug-drug interaction between cannabidiol and phenobarbital in healthy dogs. *Am J Vet Res*. (2022) 83:86–94. doi: 10.2460/ajvr.21.08.0120

31. Taylor L, Gidal B, Blakey G, Tayo B, Morrison G. A phase I, randomized, double-blind, placebo-controlled, single ascending dose, multiple dose, and food effect trial of the safety, tolerability and pharmacokinetics of highly purified cannabidiol in healthy subjects. *CNS Drugs*. (2018) 32:1053–67. doi: 10.1007/s40263-018-0578-5

32. Millar SA, Maguire RF, Yates AS, O'Sullivan SE. Towards better delivery of cannabidiol (CBD). *Pharmaceuticals*. (2020) 13:219. doi: 10.3390/ph13090219

33. Rooney TA, Carpenter JW, KuKanich B, Gardhouse SM, Magnin GC, Tully TN. Feeding decreases the oral bioavailability of cannabidiol and cannabidiolic acid in hemp oil in New Zealand white rabbits (*Oryctolagus cuniculus*). *Am J Vet Res*. (2022) 83:6. doi: 10.2460/ajvr.22.01.0006

34. Rangaraj N, Sampathi S, Junnuthula V, Kolimi P, Mandati P, Narala S, et al. Fast-fed variability: insights into drug delivery, molecular manifestations, and regulatory aspects. *Pharmaceutics*. (2022) 14:1807. doi: 10.3390/pharmaceutics14091807

35. Kok LY, Bannigan P, Sanaee F, Evans JC, Dunne M, Regenold M, et al. Development and pharmacokinetic evaluation of a self-nanoemulsifying drug delivery system for the oral delivery of cannabidiol. *Eur J Pharm Sci*. (2022) 168:106058. doi: 10.1016/j.ejps.2021.106058

36. Salvia-Trujillo L, Martín-Belloso O, McClements DJ. Excipient Nanoemulsions for improving Oral bioavailability of bioactives. *Nano*. (2016) 6:17. doi: 10.3390/nano6010017

37. ElSohly MA, Shahzadi I, Gul W. Absorption and bioavailability of novel UltraShear Nanoemulsion of Cannabidiol in rats. *Med Cannabis Cannabinoids*. (2023) 6:148–59. doi: 10.1159/000534473

38. Yen CC, Chen YC, Wu MT, Wang CC, Wu YT. Nanoemulsion as a strategy for improving the oral bioavailability and anti-inflammatory activity of andrographolide. *Int J Nanomedicine*. (2018) 13:669–80. doi: 10.2147/IJN.S154824

39. Leibtag S, Peshkovsky A. Cannabis extract nanoemulsions produced by high-intensity ultrasound: formulation development and scale-up. *J Drug Deliv Sci Tech*. (2020) 60:101953. doi: 10.1016/j.jddst.2020.101953

40. Azmi NAN, Elgharbawy AAM, Motlagh SR, Samsudin N, Salleh HM. Nanoemulsions: factory for food. *Pharma Cosmet Process*. (2019) 7:617. doi: 10.3390/pr7090617

41. Solinas M, Massi P, Cantelmo AR, Cattaneo MG, Cammarota R, Bartolini D, et al. Cannabidiol inhibits angiogenesis by multiple mechanisms. *J Med Chem*. (2012) 63:12137–55. doi: 10.1021/acs.jmedchem.0c00724

42. Turcsán B, Miklósi Á, Kubinyi E. Owner perceived differences between mixed-breed and purebred dogs. *PLoS One*. (2017) 12:e0172720. doi: 10.1371/journal.pone.0172720

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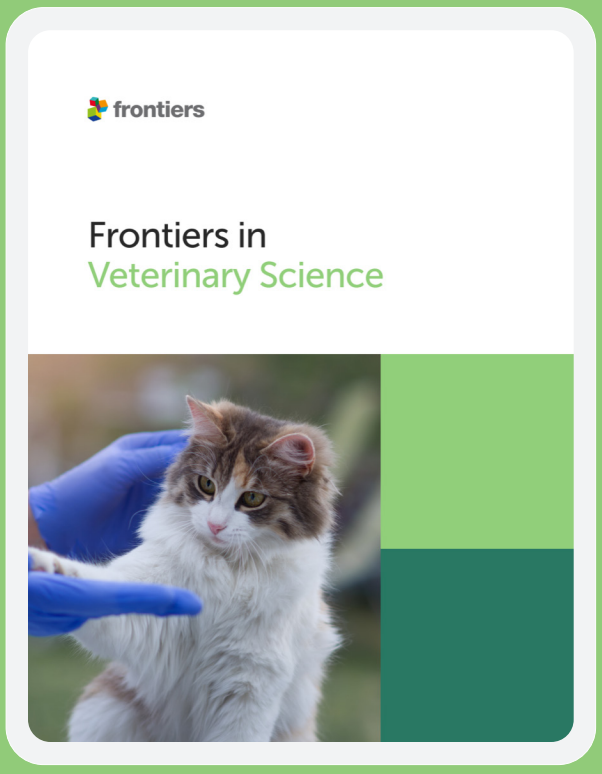

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