MEDICINAL CANNABIS: EVOLUTION OF THERAPEUTIC USE, FUTURE APPROACHES AND OTHER IMPLICATIONS

EDITED BY: Paola Brusa, Francesca Baratta, Massimo Collino and Shimon Ben -Shabat

PUBLISHED IN: Frontiers in Pharmacology

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-83250-163-4
DOI 10.3389/978-2-83250-163-4

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

MEDICINAL CANNABIS: EVOLUTION OF THERAPEUTIC USE, FUTURE APPROACHES AND OTHER IMPLICATIONS

Topic Editors:
Paola Brusa, University of Turin, Italy
Francesca Baratta, University of Turin, Italy
Massimo Collino,University of Turin, Italy
Shimon Ben -Shabat, Ben-Gurion University of the Negev, Israel

Citation: Brusa, P., Baratta, F., Collino, M., Ben -Shabat, S., eds. (2022). Medicinal Cannabis: Evolution of Therapeutic Use, Future Approaches and Other Implications. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83250-163-4

Table of Contents

frontiers | Frontiers in Pharmacology

TYPE Editorial
PUBLISHED 25 August 2022
DOI 10.3389/fphar.2022.999068

OPEN ACCESS

EDITED AND REVIEWED BY
Heike Wulff,
University of California, Davis,
United States

*CORRESPONDENCE
Francesca Baratta,
francesca.baratta@unito.it

SPECIALTY SECTION
This article was submitted to Experimental Pharmacology and Drug Discovery,
a section of the journal
Frontiers in Pharmacology

RECEIVED 20 July 2022
ACCEPTED 25 July 2022
PUBLISHED 25 August 2022

CITATION
Brusa P, Baratta F, Collino M and Ben -Shabat S (2022), Editorial: Medicinal Cannabis: Evolution of therapeutic use, future approaches and other implications.
Front. Pharmacol. 13:999068.
doi: 10.3389/fphar.2022.999068

Editorial: Medicinal *Cannabis*: Evolution of therapeutic use, future approaches and other implications

Paola Brusa[1], Francesca Baratta[1]*, Massimo Collino[2] and Shimon Ben -Shabat[3]

[1]Department of Drug Science and Technology, University of Turin, Torino, Italy, [2]Department of Neurosciences Rita Levi Montalcini, University of Turin, Turin, Piedmont, Italy, [3]Department of Clinical Biochemistry and Pharmacology, Ben-Gurion University of the Negev, Beersheba, Southern, Israel

KEYWORDS

***Cannabis*, formulations, effectiveness, medical use, clinical trials**

Editorial on the Research Topic
Medicinal *Cannabis*: Evolution of Therapeutic Use, Future Approaches and Other Implications

Cannabis has been used in even the oldest traditional medicines available. In the last century, negative attention has prevailed regarding the psychotropic and abuse potential. For this reason, *Cannabis* has been banned and declared illegal in many countries. In recent years, however, there has been a more in-depth evaluation of the legalization of cannabinoids for medical use in several countries following heightened media attention and reports of effectiveness, although not always thoroughly backed up by scientific evidence. The official introduction of pharmaceutical-grade *Cannabis* inflorescences for medicinal purposes has allowed physicians and pharmacists, to prescribe and prepare several *Cannabis* preparations legally. Such products are currently being administered to patients without their efficacy being evaluated in controlled studies: for each patient the composition and route of administration may differ. In addition, many advanced administration systems have been developed or are still under development, but few clinical trials have been completed.

In this context, this Research Topic focused on the in-depth analysis of the legal, technological and pharmacological aspects related to the medical use of *Cannabis*-based formulations.

Anil et al. have directed their research specifically on the activity of *Cannabis* for medical use in the context of inflammatory processes. Although activities in this area are plausible, the high number of active molecules produced by *Cannabis* and simultaneously administered through the extractive products normally used in therapy, has not yet made it possible to identify their specific mechanisms of action. Once the modalities of action of

the active molecules have been clarified, it might be of interest to use purified mixtures to obtain a more significant activity potentially (Anil et al.).

Specific literature reviews were then done for some pathologies such as when Xin et al. investigated the potential therapeutic effect of CBD in bone diseases. Even in this case, further studies are needed to evaluate the benefits and risks of cannabinoids' use (Xin et al.).

A large part of the clinical research relating to *Cannabis* for medical use concerns its use in the context of diseases of the central nervous system. Ortiz et al. examined evidence supporting the therapeutic utility of cannabinoids for treating neurodegenerative diseases, pain, mood disorders, and substance use disorders. Important considerations were also made on the methods of formulation and the routes of administration (Ortiz et al.). Lacroix et al. Also considered *Cannabis* in neurological disorders stressing that currently most of the scientific data supports the potential therapeutic use of *Cannabis* but, as much as patients request it, the knowledge is still too little in-depth. It is therefore certainly urgent to manage clinical trials to provide stronger and safer evidence (Lacroix et al.).

Procaccia et al. discussed how phytocannabinoid profiles differed between plants according to chemovar types and examined the main factors influencing the accumulation of secondary metabolites in the plant, including genotype, growing conditions, processing, storage and the delivery route; the authors highlighted how these factors make the use of *Cannabis* in therapy highly complex (Procaccia et al.).

In addition to the more well-known compounds such as THC and CBD, *Cannabis* produces over 120 other phytocannabinoids. The use of THC is associated with acute psychotropic effects that could potentially be avoided considering that minor cannabinoids and their chemical counterparts could offer the same potential benefits without the same adverse effects. In this regard, Walsh et al. reviewed the literature to provide an overview of the endocannabinoid system, phytocannabinoid biosynthesis and a discussion on molecular pharmacology. Potential therapeutic uses of minor cannabinoids underlining that future studies will have to rigorously evaluate these compounds' risk/benefit *ratio* (Walsh et al.).

The interest in molecules other than cannabinoids such as terpenes is certainly relevant. This interest has grown even greater since the possibility of an "entourage" effect between the active molecules of *Cannabis* has been postulated. Accordingly, Finlay et al. in their study examined whether some terpenes acted directly on cannabinoid receptors. From the results obtained, it was not possible to exclude the existence of an entourage effect. Still, this cannot be linked to a direct action of the terpenes on the cannabinoid receptors. However, the pharmacological mechanism underlying this substances activity remains to be thoroughly investigated (Finlay et al.).

Maayah et al. pointed out that full-spectrum Cannabis extracts have been used in clinical trials to treat various diseases. However, despite their efficacy, their potential use in therapy may be limited by possible behavioural side effects. These researchers then successfully worked on experimental animals to identify a panel of blood metabolites predicting behavioural effects (Maayah et al.).

Pennypacker et al. have evaluated whether the products available on the market in the United States of America are consistent in the concentration of cannabinoids, with the literature indications for use in therapy. Overall, the results of this study have been defined by the authors as alarming as current product offerings do not reflect scientific evidence (Pennypacker et al.).

In the regulatory context MacPhail et al. have analysed the trend of prescriptions in Australia over the last 5 years, noting a substantial increase in prescriptions over time that does not actually reflect a worsening of the pathological conditions of the population but rather a greater prescription linked to greater knowledge and acceptance of this type of therapy (MacPhail et al.).

As regards the use in therapy of medical Cannabis, the current regulations have been analysed by Baratta et al. in those countries where clinical studies have recently been conducted. The results of the trials have been crossed with the pathologies for which the current legislation provides that it is possible to prescribe *Cannabis* allowing relevant considerations (Baratta et al.).

From all the publications collected, it is clear that there is a great interest in the enormous potential of *Cannabis* in the medical field but also a widespread awareness of the extreme need to conduct in-depth research that clarifies the mechanisms of action of the quantity of components present in the phytocomplex of this plant species.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 25 March 2020
doi: 10.3389/fphar.2020.00359

Terpenoids From Cannabis Do Not Mediate an Entourage Effect by Acting at Cannabinoid Receptors

David B. Finlay[1], *Kathleen J. Sircombe*[1], *Mhairi Nimick*[1], *Callum Jones*[2] *and Michelle Glass*[1]*

[1] Department of Pharmacology and Toxicology, University of Otago, Dunedin, New Zealand, [2] Soma Group, Dunedin, New Zealand

The entourage effect was a proposed explanation for biological observations that endocannabinoid ligand activities can be modified by other lipids released from cells at the same time. An increasing volume of anecdotal reports and interest in the plant have provoked research into the activity of minor chemical constituents of the plant—including volatile terpenoids such as myrcene, α- and β- pinene, β-caryophyllene, and limonene. However, to date, no clear interaction has been identified. The current study was designed to determine whether terpenes in the cannabis plant have detectable receptor-mediated activity, or modify the activity of Δ^9-tetrahydrocannabinol, cannabidiol, or the endocannabinoid 2-arachidonylglycerol at the cannabinoid receptors. In addition, we have utilized a standard radioligand binding paradigm with ability to detect orthosteric and allosteric interactions of test compounds. With the possible exception of a weak interaction of β-caryophyllene with CB2, no data were produced to support the hypothesis that any of the five terpenes tested (either alone or in mixtures) have direct interactions with CB1 or CB2, as the binding of radioligand ([^{3}H]-CP55,940), Δ^9-tetrahydrocannabinol, and cannabidiol were unaltered by the presence of terpenes. Similarly, terpene functional effects were also not detected, either alone or in combination with Δ^9-tetrahydrocannabinol, cannabidiol, or 2-arachidonoylglycerol. This study adds to the evidence that the putative entourage effect cannot be explained by direct effects at CB1 or CB2.

Keywords: cannabis, cannabinoid, terpenoid, terpene, entourage effect, signaling, binding

OPEN ACCESS

Edited by:
Luc Zimmer,
Université Claude Bernard Lyon 1, France

Reviewed by:
Alessia Ligresti,
Institute of Biomolecular Chemistry (ICB), France
Eileen Denovan-Wright,
Dalhousie University, Canada

***Correspondence:**
Michelle Glass
michelle.glass@otago.ac.nz

Specialty section:
This article was submitted to Experimental Pharmacology and Drug Discovery, a section of the journal Frontiers in Pharmacology

Received: *08 November 2019*
Accepted: *10 March 2020*
Published: *25 March 2020*

Citation:
Finlay DB, Sircombe KJ, Nimick M, Jones C and Glass M (2020) Terpenoids From Cannabis Do Not Mediate an Entourage Effect by Acting at Cannabinoid Receptors. Front. Pharmacol. 11:359. doi: 10.3389/fphar.2020.00359

INTRODUCTION

Cannabinol (CBN) was the first cannabinoid from the cannabis plant for which a structure was identified (Cahn, 1933). Cannabidiol (CBD) was identified a few years later (Adams et al., 1940a), and the same research group later came close to identifying the structure of tetrahydrocannabinols, in a study involving isomerization of CBD (Adams et al., 1940b). Shortly after, tetrahydrocannabinols were isolated from cannabis resin (Wollner et al., 1942)—though it was more than 20 years before chemical analytical methods were adequate for resolving the final

structure of the main psychoactive component of cannabis, (−)Δ^9-tetrahydrocannabinol (Δ^9-THC; Gaoni and Mechoulam, 1964).

Meanwhile, Loewe was also the first to observe pharmacological differences between cannabinoids (Loewe, 1946), in a study differentiating Δ^9-THC and a synthetic hexyl analog, from CBD: the former, but not the latter, caused catalepsy and central excitation (with some additional species differences). In the years since, at least 489 different compounds (ElSohly and Slade, 2005), including at least 113 cannabinoids (Aizpurua-Olaizola et al., 2016), have been identified from cannabis. The most abundant of these are Δ^9-THC and CBD (Aizpurua-Olaizola et al., 2016; Scherma et al., 2018). Δ^9-THC acts as a partial agonist at type 1 cannabinoid receptors (CB1), which are found mostly in the central and peripheral nervous system and mediate the intoxicating effects for which cannabis is well known (reviewed in Pertwee, 2008a; Pertwee, 2008b). It also acts at type 2 cannabinoid receptors (CB2), which are most highly expressed in immune cells (reviewed in Turcotte et al., 2016). In general, many of the effects of CBD are thought to occur through non-cannabinoid receptor mechanisms (Turner et al., 2017). However, CBD has been demonstrated to bind to CB2 at high (micromolar) concentrations (Pertwee, 2008b)—although this is also controversial, as some evidence suggests that at much lower concentrations than this, CBD may behave as an inverse agonist at CB2 and an antagonist (Thomas et al., 2007) or allosteric modulator (Laprairie et al., 2015) of CB1.

More recently, interest has also turned to the biological activity of the less abundant, "minor" phytocannabinoids and phytoterpenoids, and their ability to produce an "entourage effect". This phenomenon was first described for endogenous glycerol esters (Ben-Shabat et al., 1998), when 2-linoleoylglycerol and 2-palmitoylglycerol were found to increase the on-target affinity and efficacy of the endogenous cannabinoid 2-arachidonoylglycerol (2-AG), with which they co-occur, in spleen—yet without detectable direct interaction with the cannabinoid receptors themselves (though these data were not shown). Similar observations have been described for *N*-palmitoylethanolamide and *N*-oleoylethanolamide (which are co-synthesized with anandamide) and may potentiate anandamide-induced relaxation of arteries (Ho et al., 2008).

Since the publication of the Ben-Shabat et al. study, the term "entourage effect" has been co-opted to refer to the idea that whole cannabis possesses greater therapeutic potential than its individual components (Russo, 2011; Worth, 2019), with many websites suggesting that terpenes can modify the high produced by Δ^9-THC (e.g., https://www.heylocannabis.com/post/what-are-terpenes). Terpenoids are commonly found in plants (Gershenzon and Dudareva, 2007), and at least 120 have been found in cannabis (ElSohly and Slade, 2005)—of which some of the most commonly referenced appear to include linalool, myrcene, limonene, β-caryophyllene, and α- and β-pinene. Previous work has suggested that β-caryophyllene may act as a CB2 agonist (Gertsch et al., 2008), though subsequent studies have questioned this (Santiago et al., 2019).

Evidence for cannabis-derived terpenoids having entourage activity is also sparse. A very recent study has attempted to examine the six terpenoids referred to above for potential entourage activity at cannabinoid receptors. When used either alone or in combination to stimulate AtT-20 cells expressing CB1 or CB2, Δ^9-THC-induced hyperpolarization was unaffected (Santiago et al., 2019)—indeed no GIRK channel-related modulatory effects were detected in this molecular study for any of the terpenes. In a related GIRK assay paradigm, receptor desensitization was also unaffected (Santiago et al., 2019).

The current study aimed to clarify the putative molecular activity of five terpenoids of interest acting specifically (on-target) through CB1/CB2, in a canonical activity pathway (cAMP) which can capture receptor effects with high sensitivity. Effects on orthosteric ligand binding were also included in the study design, as in addition to detecting orthosteric interactions this assay has been shown to be very sensitive to allosteric modulation of CB1 (Ahn et al., 2012; Ignatowska-Jankowska et al., 2015).

TABLE 1 | Terpene purity specifications and calculated molarity (True Terpenes, OR, USA).

Terpene	Density (g/ml)	Purity	Concentration (M)
Myrcene	0.794	97.6%	5.69
α-Pinene	0.859	99.3%	6.26
β-Pinene	0.860	98.2%	6.20
β-Caryophyllene	0.908	91.0%	4.04
Limonene	0.841	99.1%	6.12

MATERIALS AND METHODS

Drugs

All terpenes were purchased from True Terpenes (Portland, OR). Terpene molarities were calculated from the density and purity specified on the supplier's technical data sheets (**Table 1**). Terpenes were diluted to 10 mM in DMSO (Sigma Aldrich, St Louis, MO, USA), and DMSO content was kept consistent in all assays at 1:1,000. Terpenes were assessed in assays separately, and in three different mixtures (**Table 2**): commercial analysis of multiple cannabis variants indicate huge variability in terpenoid formulations between strains (*e.g.,* www.weedmd.com/terpene-profiles), and these mixtures were therefore intended to capture some of this variability.

Δ^9-THC was purchased as resin from THC Pharma GmbH (Frankfurt, Germany), CBD was purchased from Tocris (Bristol, UK), and 2-AG was purchased from Cayman Chemical Company (Ann Arbour, MI). Each was constituted in absolute ethanol at 31.6 mM, and diluted (in vehicle) as required so that the final ethanol content in assays was 1:1,000. Forskolin was purchased from Cayman Chemical Company, and prepared in DMSO at 31.6 mM. All compounds were ≥98% purity, with the exception of THC which was ≥ 95%.

Radioligand Binding Assays

Competition displacement radioligand binding assays were performed as previously described (Finlay et al., 2017). In

TABLE 2 | Constitution of terpene mixtures.

Terpene	Mixture 1 (%)	Mixture 2 (%)	Mixture 3 (%)
Myrcene	40	30	50
α-Pinene	20	17	23
β-Pinene	15	13	17
β-Caryophyllene	20	35	5
Limonene	5	5	5
Total	**100**	**100**	**100**

brief, HEK cells expressing either human CB1 receptors N-terminally tagged with preprolactin signal sequence (pplss) and 3x haemagglutinin (3HA) epitopes (Finlay et al., 2017) or human CB2 receptor N-terminally tagged with 3HA (Grimsey et al., 2011) were harvested in 5 mM EDTA in PBS, and "P2" membranes were prepared in sucrose buffer as previously described (Finlay et al., 2017). Protein content was estimated using a BioRad (Hercules, CA) DC protein assay (modified Lowry assay). For binding assays, radioligand ([^{3}H]-CP55,490, PerkinElmer, Waltham, MA, USA), non-radiolabelled drugs, and P2 membrane preparations were diluted in binding buffer (50 mM HEPES pH 7.4, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 2 mg/ml NZ-origin BSA, MP Biomedicals, Santa Ana, CA, USA) and dispensed into 96-well, polypropylene V-well plates (Hangzhou Gene Era Biotech Co Ltd, Zhejiang, China) in a final reaction volume of 200 µl (membranes were dispensed last). Final radioligand concentration was 1 nM, and protein content was 3 µg/point for pplss-3HA-hCB1 HEK membranes, and 2 µg/point for 3HA-hCB2 HEK membranes.

When all components had been dispensed, the plate was sealed and incubated for 1 h at 30°C. During the incubation, a 96 well harvest plate (GF/C filters, 1.2 µm pores) was treated with 0.1% w/v branched polyethyleneimine (PEI; Sigma Aldrich) in water. Immediately prior to washing, PEI was washed through the filters using a vacuum manifold (Pall Corporation, Port Washington, NY) and all wells were washed once with ice cold wash buffer (50 mM HEPES pH 7.4, 500 mM NaCl, 1 mg/ml BSA. Equilibrated binding mixture was then transferred to the harvest plate under vacuum, and samples washed through. Binding wells were rinsed once with wash buffer and transferred to the harvest plate, and then wells were washed three more times with 200 µl of wash buffer. The plate was then removed, and filters allowed to dry overnight.

The next day, the plate bottom was sealed, and 50 µl of Ultima Gold XR scintillation fluid (PerkinElmer) was dispensed to each well. The plate top was then sealed, and the plate was loaded into a 96 well "rigid" cassette and loaded into a Wallac MicroBeta2® TriLux Liquid Scintillation Counter (PerkinElmer). Scintillation was detected after a 30 min delay, for 2 mins per well. Counts were corrected for detector efficiency. Data were then exported and analyzed in GraphPad Prism v8 (GraphPad Software Inc., La Jolla, CA, USA), and presented normalized to total binding ([^{3}H]-CP55,940 alone; 100%), and maximum displacement (binding in the presence of 10 µM Δ^9-THC).

Functional Assay: Cyclic AMP Signaling

Cellular cAMP was measured using a commercially available BRET assay (CAMYEL), as previously described (Jiang et al., 2007; Cawston et al., 2013). In brief, HEK cells expressing either N-terminally tagged 3HA-tagged hCB1 (first reported in Cawston et al., 2013) or HA-3TCS-hCB2 (first reported in Cawston et al., 2015) were seeded in 10 cm cell culture dishes, and cultured overnight in high glucose DMEM (Hyclone, GE Healthcare, Chicago, IL) supplemented with 10% fetal bovine serum. Cells were then 40–60% confluent, and were transfected with pcDNA3L-His-CAMYEL encoding the CAMYEL biosensor (cAMP sensor with YFP-Epac-RLuc). Transfection was performed by combining 30 µg linear PEI (Polysciences, Warrington, PA, USA) from stock at 1 mg/ml, with 5 µg of CAMYEL plasmid, in a total volume of 500 µl of 150 mM sterile NaCl. Transfection mixture was incubated for 10 mins, then culture medium was replaced and the transfection mixture was dispensed. Dishes were returned to the incubator and cultured overnight. Cells were then lifted with 0.05% trypsin/EDTA (Gibco Thermo Fisher Scientific, Waltham, MA, USA), and seeded at high density (60,000 cells per well) in white 96 well CulturPlates (PerkinElmer) which had been pre-treated with 0.05 mg/ml high molecular weight poly-D-lysine (Sigma) in PBS, to increase adherence.

On assay day, well contents were aspirated with a strip vacuum (Integra Biosciences, Hudson, NH, USA), and wells were washed once with PBS to remove traces of phenol red. Wells were serum starved for 35 mins prior to stimulation in "assay medium"—phenol-free, high glucose DMEM (Hyclone) supplemented with 1 mg/ml BSA and 10 mM HEPES pH 7.4 (Gibco, Thermo). Drugs were prepared at 10x concentration during serum starvation. Five minutes prior to stimulation, Rluc substrate coelenterazine-H (Prolume, Pinetop, AZ, USA; prepared as 5 mM stock in absolute ethanol) was dispensed to the wells to be assayed (final concentration 5 µM). Forskolin, cannabinoid agonists, CBD, and terpenes (or their vehicles, as relevant) were each prepared and mixed together in a dispensing plate. At the start of the assay run, drugs were transferred into assay wells with a multichannel and immediately loaded into a pre-warmed (37°C) plate reader. CAMYEL biosensor emission signals were detected in a LUMIstar Omega plate reader (BMG Labtech, Ortenberg, Germany), using simultaneous detection BRET1 filters (475/30 and 535/30 nm) over a period of approximately 20 mins.

Inverse BRET ratios (460/535 nm) were plotted in GraphPad Prism against time. These data were analyzed by "area under the curve" (AUC) and normalized to a matched basal (vehicle alone, 0%) and 5 µM forskolin (100%) conditions. All terpenes, 2-AG, and CBD were applied at concentrations of 10 µM, Δ^9-THC was used at a concentration of 1 µM. Each individual experiment was carried out in duplicate and repeated at least three times.

Statistics

All statistical tests were performed in GraphPad Prism v8, and entailed 1-way ANOVAs followed by Holm-Šídák tests when tested means were found to be statistically significantly different overall. Tests were run separately for each receptor. For binding data, tests were performed for total binding versus each terpene alone, and when terpenes were screened in combination with other drugs (Δ^9-THC or CBD) then tests were performed for

each matched condition (i.e., Δ^9-THC+terpene was compared to Δ^9-THC; CBD+terpene was compared to CBD). Significant differences in figures of binding data (**Figures 1** and **2**) are denoted by an asterisk (*, $p < 0.05$).

For cAMP data, tests were performed for forskolin alone, and with each other drug (2-AG, Δ^9-THC, CBD, terpene, or terpene mixture). Separate tests were performed for effects in assays involving drug combinations. In these cases, post-testing was performed to compare paired matches of conditions with and without terpenes (*e.g.* Fsk+CBD was compared with Fsk+CBD +Myrcene, *etc.*). Note that statistical significance for cAMP data are not shown in **Figure 3** or **4**—this was in order to avoid confusion about which pairs of conditions were significantly different. Instead, important results are referred to in-text.

RESULTS

Radioligand Binding Assays

At concentrations of 10 μM (given in **Figures 1** and **2** as log molar, −5), none of the terpenes tested significantly altered the binding of [^{3}H]-CP55,940 in membranes containing CB1 (**Figure 1A**). Similarly, in CB2-containing membranes (**Figure 1B**), four of the five terpenes alone did not alter radioligand binding. The exception to this was β-caryophyllene, which displaced [^{3}H]-CP55,940 to a modest extent (approximately 25% of specific binding). No condition altered binding sufficiently to justify a full curve.

To test an entourage-related concept that terpenes may act by modifying the binding of other ligands (particularly those also from the cannabis plant, CBD and Δ^9-THC), the terpenes and/or terpene mixtures (**Table 2**) were tested for their ability to alter displacement of the radioligand by both of these drugs. CBD displaced the radioligand in both CB1- and CB2-containing membranes, as expected (reviewed in Pertwee, 2008b)—[^{3}H]-CP55,940 binding decreased to mean 59.70% and 20.26% of specific binding, respectively. However, no terpene or terpene mixture significantly altered CBD displacement of the radioligand in either membrane (**Figures 2A, B**). Similarly, no significant difference in displacement of the radioligand by Δ^9-THC was induced by terpene mixtures for CB1 (**Figure 2C**), while at CB2 (**Figure 2D**) the combination of Δ^9-THC with mixture 1 slightly but significantly decreased displacement ([^{3}H]-CP55,940 binding was increased from 0% to 8.26% of the window, reflecting a small reduction in displacement by Δ^9-THC).

Functional Assay: Cyclic AMP Signaling

Signaling responses to the known cannabinoid agonists 2-AG and Δ^9-THC were as expected; both significantly inhibited cAMP production induced by 5 μM forskolin at both CB1 (**Figure 3**, $p < 0.05$) and CB2 (**Figure 4**, $p < 0.05$). As these agonists were included as matched controls in assays for each of the five terpenes tested, five determinations were obtained for each 2-AG and Δ^9-THC in each of three independent assay replicates (n=15). At CB1, mean inhibition of the forskolin response by 2-AG and Δ^9-THC was 42.4% (± 1.7%) and 40.7% (± 1.6%), respectively (**Figures 3A–F**). Full concentration-response curves were performed for Δ^9-THC in the CB1 cell line, producing a mean pEC50 of 8.50 (± SEM 0.05, n=3).

In the CB2 cell line, the extents of inhibition differed more, with 2-AG driving 41.7% (± 1.3%) inhibition of the forskolin response, but Δ^9-THC appearing much lower efficacy—just 20.5% (± 1.6%) of the forskolin response was inhibited (**Figures 4A–F**). The Δ^9-THC pEC50 determined in the CB2 cell line was 7.80 (± SEM 0.06, n=3). The effects of CBD at CB1 and CB2 also differed, having no significant effect at CB1, but acting as an inverse agonist at CB2, consistent with a previous report (Thomas et al., 2007), here driving a significant increase in cAMP of 27.0% (± 2.9%) above forskolin alone.

None of the five terpenes screened, either alone or in mixtures, modified cAMP signaling significantly through either CB1 (**Figure 3**) or CB2 (**Figure 4**). Statistical tests to determine this were performed by a single 1-way ANOVA for each cell line, using multiple comparisons to allow comparisons of paired conditions—i.e., each orthosteric ligand with terpene *versus* a matched condition in absence of terpene.

An additional Δ^9-THC condition was also included, to determine whether 10 μM of any terpene would modify the cAMP signaling of an approx. EC50 concentration of Δ^9-THC at

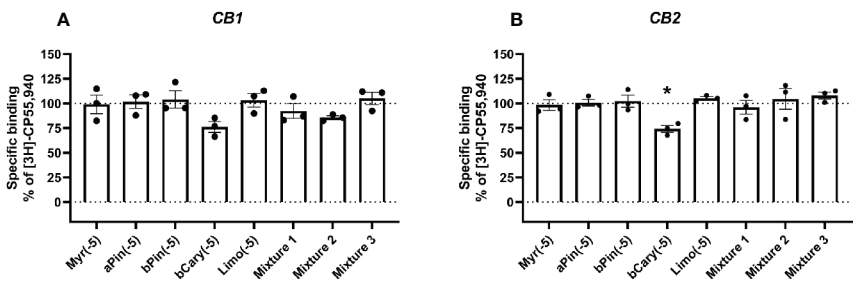


FIGURE 1 | Specific binding of [3H]-CP55,940, with displacement by terpenes (10 μM) in membranes containing hCB1 **(A)** or hCB2 **(B)**. Binding data in all plots are normalized to total [3H]-CP55,940 binding in the absence of displacer (100%), and in the presence of 10 μM THC (0%). Data are means ± SEM of three independent determinations. *$p < 0.05$.

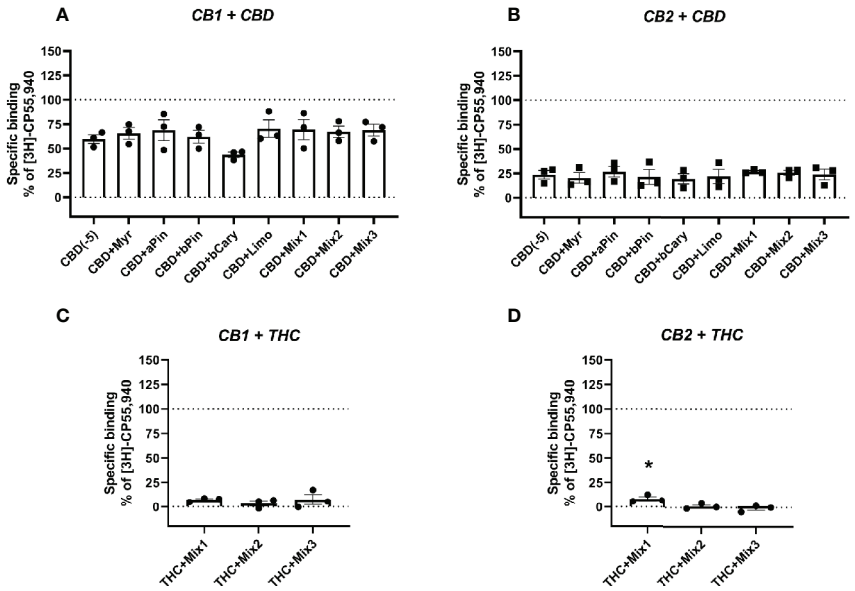


FIGURE 2 | Specific binding of [^{3}H]-CP55,940, with displacement by CBD **(A, B)** or THC **(C, D)** in the presence and absence of terpenes (all at 10 μM) in membranes containing hCB1 **(A, C)** or hCB2 **(B, D)**. Binding data are normalized to total [3H]-CP55,940 binding in the absence of displacer (100%), and in the presence of 10 μM THC (0%). Data are means ± SEM of three independent determinations. *$p < 0.05$.

either CB1 (3.16 nM) or CB2 (30 nM). The purpose of this condition was to capture terpene-induced alterations to the potency of the Δ^9-THC response in each cell line. However, consistent with the data at higher Δ^9-THC concentrations, no change in cAMP signaling was observed in the presence of any of the five terpenes at either CB1 (**Figure 3G**) or CB2 (**Figure 4G**). For results from both CB1 and CB2 cell lines, 1-way ANOVAs were performed but no differences in means were found in the tested conditions.

DISCUSSION

Overall, these data do not support the idea that any of the five terpenes tested in this study contribute to a putative entourage effect directly through the cannabinoid receptors. β-Caryophyllene was found to bind weakly to CB2 alone, but no other functional or binding effects were detected for the terpenes alone or in combination with CBD, or cannabinoid agonists 2-AG and Δ^9-THC. CBD is increasingly becoming a focus of therapeutic studies due to positive results in a series of childhood epilepsy clinical trials (Devinsky et al., 2017; Laux et al., 2019), yet its mechanism of action remains unclear, with over 65 putative molecular targets identified (Bih et al., 2015). We were therefore interested to investigate whether the terpenes could enhance its activity or affinity for cannabinoid receptors, providing a mechanism for interaction with the endocannabinoid system. In this study we confirmed low affinity interactions with CB1 and CB2, as previously reported (reviewed in Pertwee, 2008b). The extent of displacement observed in this study (at 10 μM concentrations) are consistent with Ki values in the low micromolar range reported for CB2, and >10 μM for CB1 (McPartland et al., 2007). In the cAMP assay, CBD showed inverse agonist activity at CB2, again consistent with previous studies (Thomas et al., 2007), but no activity was detected at CB1. The terpenes did not modify either the binding or the functional response of CBD at either receptor.

Radioligand binding experiments can detect both direct orthosteric interactions with a receptor, and in many cases, (including for CB1) allosteric modulation (Ahn et al., 2012; Ignatowska-Jankowska et al., 2015). The assay design used here provides detection of displacement (such as observed for the orthosteric ligands, 2-AG and Δ^9-THC; **Figures 1** and **2**) or enhancement of binding (as seen for all current positive and negative allosteric modulators of CB1, Price et al., 2005; Ahn et al., 2012; Ignatowska-Jankowska et al., 2015). In this light, the lack of binding modulation by the terpenes (excluding β-caryophyllene at CB2) suggests a lack of both orthosteric and allosteric modulation of binding. Significant alterations in radioligand binding by a terpenoid were detected for β-caryophyllene alone (which significantly displaced the radioligand at CB2, **Figure 1B**) and the combination of 10 μM Δ^9-THC with mixture 1, also at CB2 (**Figure 2D**), where the terpene mixture apparently reduced displacement of the radioligand by Δ^9-THC. While this may provide some evidence of terpene effects on binding, it is weak because of the small effect size.

The general lack of terpenoids effects on binding is not sufficient to completely rule out allosteric effects on function, as binding and functional modulation are separate in theory

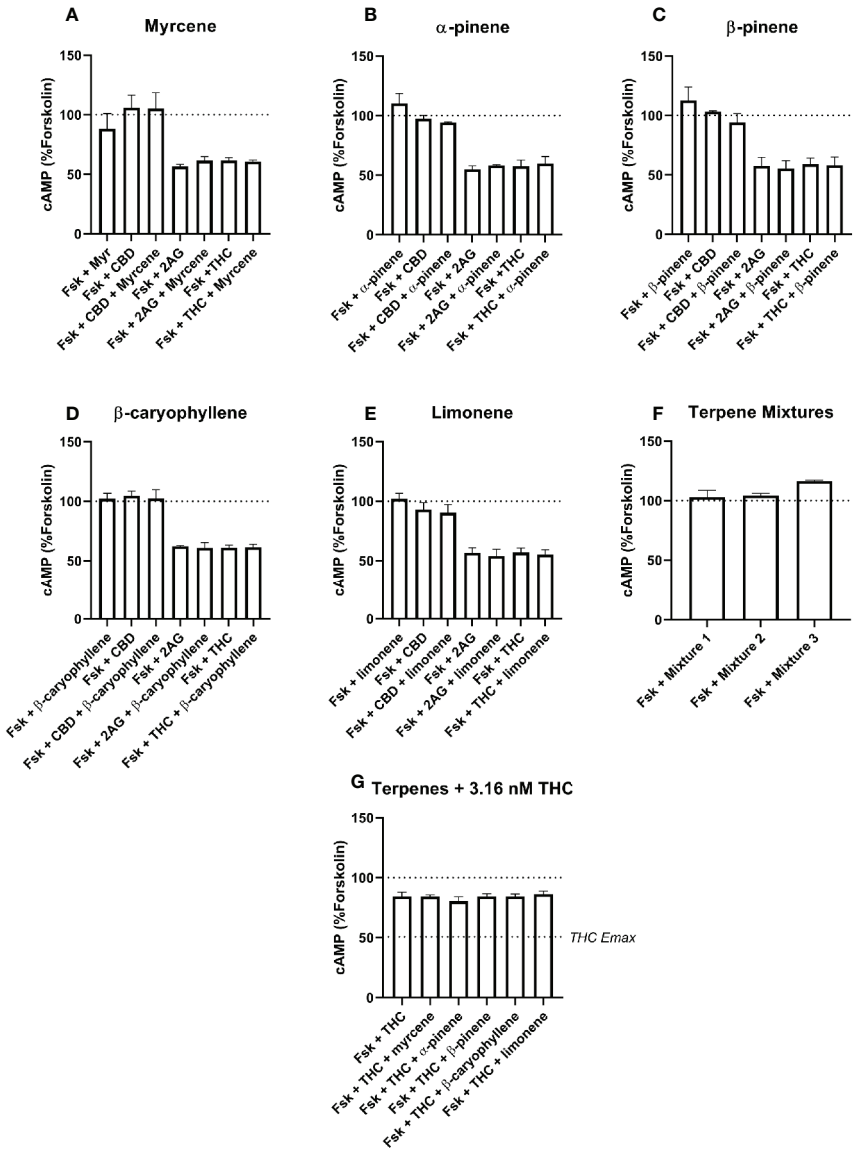


FIGURE 3 | hCB1-Mediated inhibition of cAMP production in response to forskolin (Fsk) and drug combinations with 10 μM myrcene **(A)**, α-pinene **(B)**, β-pinene **(C)**, β-caryophyllene **(D)**, limonene **(E)**, or terpene mixtures **(F)**. Figure **(G)** shows inhibition of the Fsk response by an approx. EC50 concentration of THC (3.16 nM) in combination with 10 μM of each of the five terpenes. Data are normalized to forskolin (100%) and basal (0%). cAMP responses were stimulated with forskolin (5 μM), and all cannabinoids and terpenes were at a final concentration of 10 μM. Data are means ± SEM of three independent determinations.

(reviewed in Lindsley et al., 2016); receptor functional modulation may not necessarily be predicted by altered binding and *vice versa*. However, neither CB1 or CB2 cAMP signaling was detectably modified by terpenes or terpene mixtures in this study. Terpenes failed to alter the efficacy (Emax) of Δ^9-THC or 2-AG, and also showed no ability to change Δ^9-THC potency at either CB1 or CB2. A change in potency would have been detected through the signaling assays carried out at approx. EC50 concentrations of Δ^9-THC) (**Figures 3G** and **4G**). This approach has good sensitivity to detect potency shifts either toward Emax (i.e., increasing the potency of Δ^9-THC) or away from Emax (decreasing the potency of Δ^9-THC). This negative finding for signaling modulation is particularly inconsistent for β-caryophyllene, which has previous been described as a CB2 agonist with affinity in the high nanomolar range (Gertsch et al., 2008). The reason for this lack of effect is not clear, although notably our data is consistent with the recent report by Santiago et al. (2019).

Importantly, this study cannot rule out the existence of an entourage effect for terpenoids. However, in combination with Santiago et al. (2019), there *is* likely now sufficient data to rule out *direct interactions* with either cannabinoid receptor as being the

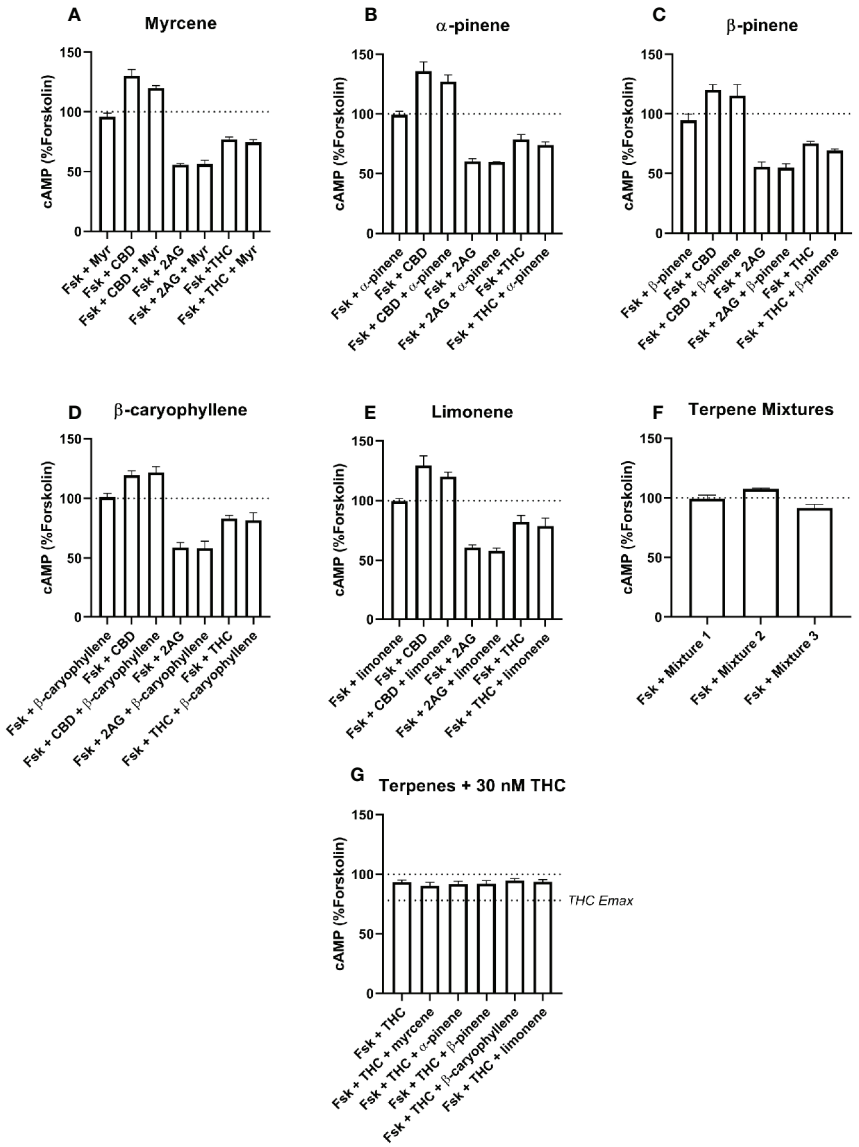


FIGURE 4 | hCB2-Mediated inhibition of cAMP production in response to forskolin (Fsk) and drug combinations with 10 μM myrcene **(A)**, α-pinene **(B)**, β-pinene **(C)**, β-caryophyllene **(D)**, limonene **(E)**, or terpene mixtures **(F)**. Figure **(G)** shows inhibition of the Fsk response by an approx. EC50 concentration of THC (30 nM) in combination with 10 μM of each of the five terpenes. Data are normalized to forskolin (100%) and basal (0%). cAMP responses were stimulated with forskolin (5 μM), and all cannabinoids and terpenes were at a final concentration of 10 μM. Data are means ± SEM of three independent determinations.

mechanism by which an entourage effect is mediated, so attention must move to other types of effect. Within the endocannabinoid system, this would mean investigating the effect of terpenoids on metabolism or synthesis of the endocannabinoids.

Some researchers suggest that an entourage-related mechanism of action may not be necessary—terpenes may merely have their own biological activity, and interact functionally with the activity of Δ^9-THC (Murataeva et al., 2016). Another mechanism which may help explain putative differences between whole cannabis and Δ^9-THC alone is that relevant compounds may synergize functionally through different receptor targets. Such a mechanism has been suggested to explain the activity of N-acyl lipids on anandamide, *via* effects mediated by TRPV1 receptors (Smart et al., 2002; Ho et al., 2008). Other non-cannabinoid targets for terpenes have also been proposed, including the suggestion that limonene may exhibit anxiolytic-like activity *via* a GABAergic mechanism (de Almeida et al., 2012; Lima et al., 2013), although these data do not necessarily reflect direct GABA receptor effects. In another example, the terpene linalool has been put forward as a candidate NMDA receptor antagonist in a study involving both molecular and *in vivo* characterization (Elisabetsky et al., 1999). In fact, the spectrum of possible effects—including both polypharmacy (functional

interactions derived from simultaneous effects of multiple drugs acting in a biological system) and polypharmacology (functional interactions derived from simultaneous effects of a drug acting at more than one target)—may help explain the entourage effect, even if this tangle of complex interactions cannot yet be unfurled by the limits of current scientific method. Finally, considering the volatility of the terpenoids (terpenoids, not cannabinoids, give cannabis its odor), it is possible that its effects may be sensory. This hypothesis also has precedent; for example, citrus terpenoids (which includes limonene, the most common naturally occurring terpenoid) have been shown to have therapeutic effects in humans, as patients who were hospitalized for depression and were exposed to citrus fragrance demonstrated improvements in Hamilton Depression Scores (Komori et al., 1995; reviewed in Russo, 2011).

It is often very difficult to distinguish between studies that support the idea of biological activity of terpenoids (including many reviewed by Russo, 2011) and studies that *specifically* address the putative entourage effect of whole cannabis, of which there are far fewer. It is worth nothing that even in human subjects, evidence is adduced against entourage—a notable instance is a study comparing the analgesic effects of pure Δ^9-THC (dronabinol) with smoked marijuana in a rigorous (randomized, placebo-controlled, double-dummy, double-blind) clinical study. Although both groups demonstrated modest improvements in pain-related endpoints, peak changes in pain sensitivity and tolerance did not differ between marijuana and dronabinol groups (Cooper et al., 2013); indeed the author of this study has stated that she "has only ever seen evidence against the entourage effect" (Chen, 2017).

As the use of cannabis and cannabis extracts is becoming more prevalent, it remains important to investigate the potential pharmacological properties of terpenoids used in conjunction with cannabinoids. As some commentators note that "There really isn't the science out there to support (the entourage hypothesis for whole cannabis)" (Worth, 2019), the research community must be reminded to view common opinion with some skepticism if it is not based on robust science.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

AUTHOR CONTRIBUTIONS

DF performed experiments, contributed to data analysis, and wrote the paper. KS and MN performed experiments and contributed to data analysis. CJ contributed to the writing of the manuscript. MG gave oversight to the project, analyzed the data, and wrote the paper.

FUNDING

All research costs were paid by Soma Group.

REFERENCES

Adams, R., Hunt, M., and Clark, J. H. (1940a). Structure of Cannabidiol, a Product Isolated from the Marihuana Extract of Minnesota Wild Hemp. I. *J. Am. Chem. Soc.* 62 (1), 196–200. doi: 10.1021/ja01858a058

Adams, R., Pease, D. C., Cain, C. K., Baker, B. R., Clark, J. H., Wolff, H., et al. (1940b). Conversion of cannabidiol to a product with marihuana activity. A type reaction for synthesis of analogous substances. Conversion of cannabidiol to cannabinol. *J. Am. Chem. Soc.* 62 (8), 2245–2246. doi: 10.1021/ja01865a508

Ahn, K. H., Mahmoud, M. M., and Kendall, D. A. (2012). Allosteric modulator ORG27569 induces CB1 cannabinoid receptor high affinity agonist binding state, receptor internalization, and Gi protein-independent ERK1/2 kinase activation. *J. Biol. Chem.* 287 (15), 12070–12082. doi: 10.1074/jbc.M111.316463

Aizpurua-Olaizola, O., Soydaner, U., Öztürk, E., Schibano, D., Simsir, Y., Navarro, P., et al. (2016). Evolution of the Cannabinoid and Terpene Content during the Growth of Cannabis sativa Plants from Different Chemotypes. *J. Nat. Prod.* 79 (2), 324–331. doi: 10.1021/acs.jnatprod.5b00949

Ben-Shabat, S., Fride, E., Sheskin, T., Tamiri, T., Rhee, M. H., Vogel, Z., et al. (1998). An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur. J. Pharmacol.* 353 (1), 23–31. doi: 10.1016/s0014-2999(98)00392-6

Bih, ,. C. I., Chen, T., Nunn, A. V. W., Bazelot, M., Dallas, M., and Whalley, B. J. (2015). Molecular Targets of Cannabidiol in Neurological Disorders. *Neurotherapeutics: J. Am. Soc. Exp. Neurother.* 12 (4), 699–730. doi: 10.1007/s13311-015-0377-3

Cahn, R. S. (1933). 326. Cannabis indica resin. Part IV. The synthesis of some 2: 2-dimethyldibenzopyrans, and confirmation of the structure of cannabinol. *J. Chem. Soc. (Resumed)* 1400–1405. doi: 10.1039/jr9330001400

Cawston, E. E., Redmond, W. J., Breen, C. M., Grimsey, N. L., Connor, M., and Glass, M. (2013). Real-time characterization of cannabinoid receptor 1 (CB_1) allosteric modulators reveals novel mechanism of action. *Br. J. Pharmacol.* 170 (4), 893–907. doi: 10.1111/bph.12329

Cawston, E. E., Connor, M., Di Marzo, V., Silvestri, R., and Glass, M. (2015). Distinct Temporal Fingerprint for Cyclic Adenosine Monophosphate (cAMP) Signaling of Indole-2-carboxamides as Allosteric Modulators of the Cannabinoid Receptors. *J. Med. Chem.* 58 (15), 5979–5988. doi: 10.1021/acs.jmedchem.5b00579

Chen, A. (2017). Some of the Parts: Is Marijuana's "Entourage Effect" Scientifically Valid? Retrieved February 20, 2020, from https://www.scientificamerican.com/article/some-of-the-parts-is-marijuana-rsquo-s-ldquo-entourage-effect-rdquo-scientifically-valid/.

Cooper, Z. D., Comer, S. D., and Haney, M. (2013). Comparison of the analgesic effects of dronabinol and smoked marijuana in daily marijuana smokers. *Neuropsychopharmacology* 38 (10), 1984–1992. doi: 10.1038/npp.2013.97

de Almeida, A. A. C., Costa, J. P., de Carvalho, R. B. F., de Sousa, D. P., and de Freitas, R. M. (2012). Evaluation of acute toxicity of a natural compound (+)-limonene epoxide and its anxiolytic-like action. *Brain Res.* 1448, 56–62. doi: 10.1016/j.brainres.2012.01.070

Devinsky, O., Cross, J. H., and Wright, S. (2017). Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. *N. Engl. J. Med.* 377 (7), 699–700. doi: 10.1056/NEJMc1708349

Elisabetsky, E., Brum, L. F. S., and Souza, D. O. (1999). Anticonvulsant properties of linalool in glutamate-related seizure models. *Phytomedicine* 6 (2), 107–113. doi: 10.1016/S0944-7113(99)80044-0

ElSohly, M. A., and Slade, D. (2005). Chemical constituents of marijuana: The complex mixture of natural cannabinoids. *Life Sci.* 78 (5), 539–548. doi: 10.1016/J.LFS.2005.09.011

Finlay, D. B., Cawston, E. E., Grimsey, N. L., Hunter, M. R., Korde, A., Vemuri, V. K., et al. (2017). $G\alpha_s$ signalling of the CB_1 receptor and the influence of receptor number. *Br. J. Pharmacol.* 174 (15), 2545–2562. doi: 10.1111/bph.13866

Gaoni, Y., and Mechoulam, R. (1964). Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. *J. Am. Chem. Soc.* 86 (8), 1646–1647. doi: 10.1021/ja01062a046

Gershenzon, J., and Dudareva, N. (2007). The function of terpene natural products in the natural world. *Nat. Chem. Biol.* 3 (7), 408–414. doi: 10.1038/nchembio.2007.5

Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J.-Z., Xie, X.-Q., et al. (2008). Beta-caryophyllene is a dietary cannabinoid. *Proc. Natl. Acad. Sci.* 105 (26), 9099–9104. doi: 10.1073/pnas.0803601105

Grimsey, N. L., Goodfellow, C. E., Dragunow, M., and Glass, M. (2011). Cannabinoid receptor 2 undergoes Rab5-mediated internalization and recycles via a Rab11-dependent pathway. *Biochim. Biophys. Acta (BBA) - Mol. Cell Res.* 1813 (8), 1554–1560. doi: 10.1016/j.bbamcr.2011.05.010

Ho, W.-S. V., Barrett, D. A., and Randall, M. D. (2008). "Entourage" effects of N-palmitoylethanolamide and N-oleoylethanolamide on vasorelaxation to anandamide occur through TRPV1 receptors. *Br. J. Pharmacol.* 155 (6), 837–846. doi: 10.1038/bjp.2008.324

Ignatowska-Jankowska, B. M., Baillie, G. L., Kinsey, S., Crowe, M., Ghosh, S., Owens, R. A., et al. (2015). A Cannabinoid CB1 Receptor-Positive Allosteric Modulator Reduces Neuropathic Pain in the Mouse with No Psychoactive Effects. *Neuropsychopharmacology* 40 (13), 2948–2959. doi: 10.1038/npp.2015.148

Jiang, L. I., Collins, J., Davis, R., Lin, K.-M., DeCamp, D., Roach, T., et al. (2007). Use of a cAMP BRET Sensor to Characterize a Novel Regulation of cAMP by the Sphingosine 1-Phosphate/G $_{13}$ Pathway. *J. Biol. Chem.* 282 (14), 10576–10584. doi: 10.1074/jbc.M609695200

Komori, T., Fujiwara, R., Tanida, M., Nomura, J., and Yokoyama, M. M. (1995). Effects of citrus fragrance on immune function and depressive states. *Neuro. Immuno. Modulation* 2 (3), 174–180. doi: 10.1159/000096889

Laprairie, R. B., Bagher, A. M., Kelly, M. E. M., and Denovan-Wright, E. M. (2015). Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. *Br. J. Pharmacol.* 172 (20), 4790–4805. doi: 10.1111/bph.13250

Laux, L. C., Bebin, E. M., Checketts, D., Chez, M., Flamini, R., Marsh, E. D., et al. (2019). Long-term safety and efficacy of cannabidiol in children and adults with treatment resistant Lennox-Gastaut syndrome or Dravet syndrome: Expanded access program results. *Epilepsy Res.* 154, 13–20. doi: 10.1016/j.eplepsyres.2019.03.015

Lima, N. G. P. B., De Sousa, D. P., Pimenta, F. C. F., Alves, M. F., De Souza, F. S., MacEdo, R. O., et al. (2013). Anxiolytic-like activity and GC-MS analysis of (R)-(+)-limonene fragrance, a natural compound found in foods and plants. *Pharmacol. Biochem. Behav.* 103 (3), 450–454. doi: 10.1016/j.pbb.2012.09.005

Lindsley, C. W., Emmitte, K. A., Hopkins, C. R., Bridges, T. M., Gregory, K. J., Niswender, C. M., et al. (2016). Practical Strategies and Concepts in GPCR Allosteric Modulator Discovery: Recent Advances with Metabotropic Glutamate Receptors. *Chem. Rev.* 116 (11), 6707–6741. doi: 10.1021/acs.chemrev.5b00656

Loewe, S. (1946). Studies on the pharmacology and acute toxicity of compounds with marihuana activity. *J. Pharmacol. Exp. Ther.* 88 (2), 154–161.

McPartland, J. M., Glass, M., and Pertwee, R. G. (2007). Meta-analysis of cannabinoid ligand binding affinity and receptor distribution: interspecies differences. *Br. J. Pharmacol.* 152 (5), 583–593. doi: 10.1038/sj.bjp.0707399

Murataeva, N., Dhopeshwarkar, A., Yin, D., Mitjavila, J., Bradshaw, H., Straiker, A., et al. (2016). Where's my entourage? The curious case of 2-oleoylglycerol, 2-linolenoylglycerol, and 2-palmitoylglycerol. *Pharmacol. Res.* 110, 173–180. doi: 10.1016/j.phrs.2016.04.015

Pertwee, R. G. (2008a). Ligands that target cannabinoid receptors in the brain: from THC to anandamide and beyond. *Addict. Biol.* 13 (2), 147–159. doi: 10.1111/j.1369-1600.2008.00108.x

Pertwee, R. G. (2008b). The diverse CB 1 and CB 2 receptor pharmacology of three plant cannabinoids: Δ 9 -tetrahydrocannabinol, cannabidiol and Δ 9 -tetrahydrocannabivarin. *Br. J. Pharmacol.* 153 (2), 199–215. doi: 10.1038/sj.bjp.0707442

Price, M. R., Baillie, G. L., Thomas, A., Stevenson, L. A., Easson, M., Goodwin, R., et al. (2005). Allosteric modulation of the Cannabinoid CB1 receptor. *Mol. Pharmacol.* 68 (5), 1484–1495. doi: 10.1124/mol.105.016162

Russo, E. B. (2011). Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *Br. J. Pharmacol.* 163 (7), 1344–1364. doi: 10.1111/j.1476-5381.2011.01238.x

Santiago, M., Sachdev, S., Arnold, J. C., McGregor, I. S., and Connor, M. (2019). Absence of Entourage: Terpenoids Commonly Found in Cannabis sativa Do Not Modulate the Functional Activity of Δ9-THC at Human CB1 and CB2 Receptors. *Cannabis Cannabinoid Res.* 4 (3), 165–176. doi: 10.1089/can.2019.0016

Scherma, M., Masia, P., Deidda, M., Fratta, W., Tanda, G., and Fadda, P. (2018). New Perspectives on the Use of Cannabis in the Treatment of Psychiatric Disorders. *Medicines (Basel Switzerland)* 5 (4). doi: 10.3390/medicines5040107

Smart, D., Jonsson, K.-O., Vandevoorde, S., Lambert, D. M., and Fowler, C. J. (2002). "Entourage" effects of N-acyl ethanolamines at human vanilloid receptors. Comparison of effects upon anandamide-induced vanilloid receptor activation and upon anandamide metabolism. *Br. J. Pharmacol.* 136 (3), 452–458. doi: 10.1038/sj.bjp.0704732

Thomas, A., Baillie, G. L., Phillips, A. M., Razdan, R. K., Ross, R. A., and Pertwee, R. G. (2007). Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *Br. J. Pharmacol.* 150 (5), 613–623. doi: 10.1038/sj.bjp.0707133

Turcotte, C., Blanchet, M.-R., Laviolette, M., and Flamand, N. (2016). The CB2 receptor and its role as a regulator of inflammation. *Cell. Mol. Life Sci.* 73 (23), 4449–4470. doi: 10.1007/s00018-016-2300-4

Turner, S. E., Williams, C. M., Iversen, L., and Whalley, B. J. (2017). Molecular Pharmacology of Phytocannabinoids. *Prog. Chem. Org. Nat. Prod.* 103, 61–101. doi: 10.1007/978-3-319-45541-9_3

Wollner, H. J., Matchett, J. R., Levine, J., and Loewe, S. (1942). Isolation of a Physiologically Active Tetrahydrocannabinol from Cannabis Sativa Resin. *J. Am. Chem. Soc.* 64 (1), 26–29. doi: 10.1021/ja01253a008

Worth, T. (2019). Cannabis's chemical synergies. *Nature* 572 (7771), S12–S13. doi: 10.1038/d41586-019-02528-1

Conflict of Interest: CJ was employed by Soma Group.

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.

The research was funded by Soma Group. Soma is a New Zealand based science-led medicinal cannabis company. While they conceived the initial concept for the study they were not involved in the final experimental design, collection, or analysis of the data or interpretation of the results.

frontiers in Pharmacology

REVIEW
published: 29 November 2021
doi: 10.3389/fphar.2021.777804

Minor Cannabinoids: Biosynthesis, Molecular Pharmacology and Potential Therapeutic Uses

Kenneth B. Walsh[1]*, *Amanda E. McKinney*[2,3] and *Andrea E. Holmes*[3,4]

[1]Department of Pharmacology, Physiology and Neuroscience, School of Medicine, University of South Carolina, Columbia, SC, United States, [2]Institute for Human and Planetary Health, Crete, NE, United States, [3]School of Integrative Learning, Doane University, Crete, NE, United States, [4]Precision Plant Molecules, Denver, CO, United States

The medicinal use of *Cannabis sativa L.* can be traced back thousands of years to ancient China and Egypt. While marijuana has recently shown promise in managing chronic pain and nausea, scientific investigation of cannabis has been restricted due its classification as a schedule 1 controlled substance. A major breakthrough in understanding the pharmacology of cannabis came with the isolation and characterization of the phytocannabinoids *trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD). This was followed by the cloning of the cannabinoid CB1 and CB2 receptors in the 1990s and the subsequent discovery of the endocannabinoid system. In addition to the major phytocannabinoids, Δ^9-THC and CBD, cannabis produces over 120 other cannabinoids that are referred to as minor and/or rare cannabinoids. These cannabinoids are produced in smaller amounts in the plant and are derived along with Δ^9-THC and CBD from the parent cannabinoid cannabigerolic acid (CBGA). While our current knowledge of minor cannabinoid pharmacology is incomplete, studies demonstrate that they act as agonists and antagonists at multiple targets including CB1 and CB2 receptors, transient receptor potential (TRP) channels, peroxisome proliferator-activated receptors (PPARs), serotonin 5-HT_{1a} receptors and others. The resulting activation of multiple cell signaling pathways, combined with their putative synergistic activity, provides a mechanistic basis for their therapeutic actions. Initial clinical reports suggest that these cannabinoids may have potential benefits in the treatment of neuropathic pain, neurodegenerative diseases, epilepsy, cancer and skin disorders. This review focuses on the molecular pharmacology of the minor cannabinoids and highlights some important therapeutic uses of the compounds.

Keywords: *Cannabis sativa*, minor cannabinoids, TRP channel, endocannabinoids, therapeutics, CB1–CB2 cannabinoid receptors

OPEN ACCESS

Edited by:
Kathia Honorio,
Universidade de São Paulo, Brazil

Reviewed by:
Carl R. Lupica,
National Institute on Drug Abuse (NIDA), United States
Jenny Wiley,
RTI International, United States

***Correspondence:**
Kenneth B. Walsh
walsh@uscmed.sc.edu

Specialty section:
This article was submitted to Experimental Pharmacology and Drug Discovery, a section of the journal Frontiers in Pharmacology

Received: *15 September 2021*
Accepted: *08 November 2021*
Published: *29 November 2021*

Citation:
Walsh KB, McKinney AE and Holmes AE (2021) Minor Cannabinoids: Biosynthesis, Molecular Pharmacology and Potential Therapeutic Uses. Front. Pharmacol. 12:777804. doi: 10.3389/fphar.2021.777804

INTRODUCTION

The marijuana plant has been grown and cultivated for medical, industrial and recreational uses throughout recorded history. Based on the physical characteristics of the plant, two main species of cannabis were originally described; *Cannabis indica* (short plant with broad leaves) and *Cannabis sativa* (tall plant with thin leaves) (Schultes, et al., 1974). However, numerous cannabis strains have been selected through breeding programs whose chemotaxonomic properties do not correlate with a

Cannabis indica or *Cannabis sativa* lineage (Hillig and Mahlberg, 2004). More recently, the existence of only one species (*Cannabis sativa L.*), has been proposed (Small, 2015) with the strains categorized according to the content of the cannabinoids *trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD). Δ^9-THC dominant strains with low CBD content induce intoxicating, psychotropic effects including euphoria, enhancement of sensory perception and impairment in memory. In contrast, CBD dominant strains with low Δ^9-THC content are considered to be nonpsychotropic.

Some of the earliest recorded medicinal uses of cannabis trace back to China and the pharmacopoeia of the Emperor Shen Nung (approximately 2500 BC), where the plant was indicated for the treatment of rheumatic pain, constipation, malaria and gynecological disorders (Russo, 2007; Pisanti and Bifulco, 2019). Along with China, cannabis medicine developed in India and then spread to Egypt, Greece and Rome. It was in Egypt that preparations of cannabis were first used in the treatment of glaucoma. By the 1800s extracts and tinctures of cannabis were recognized in the Western world for their relief of migraine headaches and their anti-emetic effects. In response to the perceived abuse of marijuana in the United States in the early 1900s, the Marijuana Tax Act was introduced which banned the sale and use of cannabis. This was followed in the 1970s by the classification of marijuana as a schedule 1 narcotic under the U.S. Controlled Substance Act. In Europe, the majority of countries have legalized the medical use of marijuana and decriminalized possession of small amounts of cannabis. However, the laws governing the use of cannabis can vary from one country to another with some countries having legalized only derivatives of the plant.

An important advancement in understanding the pharmacology of cannabis came with the isolation and structural determination of the phytocannabinoids CBD (Adams, et al., 1940a; Mechoulam and Shvo, 1963) and Δ^9-THC (Gaoni and Mechoulam, 1971). Δ^9-THC is the most abundant phytocannabinoid found in drug-type cannabis strains and the main psychotropic compound in the plant. In contrast, fiber-type strains have a higher content of CBD compared with Δ^9-THC. CBD lacks psychotropic activity, but is reported to reduce the adverse effects (anxiety, psychosis, etc.) of Δ^9-THC (Pennypacker and Romero-Sandoval, 2020). In addition to Δ^9-THC and CBD, *Cannabis sativa L.* produces over 120 other phytocannabinoids as well as an abundance of related compounds including flavonoids, non-cannabinoid phenols, phenylpropanoids, fatty acids and terpenoids (Hanus, et al., 2016; Gülck and Möller, 2020). Phytocannabinoids are meroterpenoids (21- and 22-carbon terpenophenolic compounds with an alkyl side chain) produced in the plant's glandular trichomes (Hanus, et al., 2016; Gülck and Möller, 2020). Cannabigerolic acid (CBGA), synthesized in cannabis from geranyl pyrophosphate and olivetolic acid, represents the parent cannabinoid from which the acidic and neutral minor cannabinoids are derived. In general, Δ^9-THC and CBD are considered the major phytocannabinoids, while other phytocannabinoids, present in smaller amounts in the plant, are referred to as minor (or rare) cannabinoids.

More than 230 million people worldwide consume marijuana making it the most commonly used illicit substance (World Health Organization, 2016). In recent years, cannabis has become more accessible in the United States and Europe due to its legalization for medicinal and recreational purposes. While administered for a large number of medical conditions including nausea, anorexia, glaucoma, and muscle spasms, observational studies and user surveys indicate that pain management is the most common indication for the use of cannabis (Romero-Sandoval, et al., 2018). For this purpose, medical cannabis can be smoked or vaporized (using the floral buds of the plant), applied *via* oromucosal spray preparations [cannabis extract in Nabiximols (Sativex®)] or swallowed in capsule form as Nabilone (Cesamet®, synthetic cannabinoid) and Dronabinol (Marinol®, synthetic Δ^9-THC). Anecdotal evidence indicates that the combination of the phytocannabinoids, terpenoids and other phytochemicals present in the whole cannabis plant provides a greater efficacy (called the "entourage effect") in treating chronic pain when compared to oral cannabinoid formulations (Russo, 2011). However, definitive experimental data supporting this synergistic effect are currently lacking (Santiago, et al., 2019; Finlay, et al., 2020). Finally, in addition to its own direct anti-nociceptive effects, medical cannabis may have opioid drug-sparing actions: thus allowing lower doses of opioids to be used for pain relief (Khan, et al., 2019).

This review provides a brief description of the biosynthesis of the phytocannabinoids and an overview of the endocannabinoid system. This is followed by a discussion of the molecular pharmacology and potential therapeutic uses of the minor cannabinoids. Readers desiring information on Δ^9-THC, CBD or synthetic cannabinoids are directed to these recent reviews (Banister, et al., 2019; de Almeida and Devi, 2020; Alves, et al., 2020; Walsh and Andersen, 2020).

BIOSYNTHESIS OF PHYTOCANNABINOIDS

Phytocannabinoids are meroterpenoids consisting of 21 or 22 carbon atoms that usually contain a propyl or pentyl side chain (Hanus, et al., 2016; Gülck and Möller, 2020). In *Cannabis sativa L.* the phytocannabinoids and terpenes are synthesized and stored in the glandular trichomes that are found in highest density in the female flowers of the plant (Hanus, et al., 2016; Gülck and Möller, 2020). The synthesis of the cannabinoids involves two pathways located in two separate sites within the glandular trichomes. In the first pathway olivetolic acid (OA) is produced in the cytosol of the gland cells from hexanoic acid. In the second, geranyl diphosphate (GPP) is generated in the plastidial organelles *via* the mevalonate-dependent isoprenoid (MEP) pathway. CBGA, the precursor of phytocannabinoids containing a pentyl side chain, is then synthesized from the GPP prenylation of olivetolic acid; a reaction catalyzed by olivetolate geranyltransferase (GOT) (**Figure 1**). Synthesis of tetrahydrocannabinolic acid (THCA), cannabidioloic acid (CBDA), and cannabichromenic acid (CBCA) proceeds through the appropriate oxidocyclases, THCA synthase, CBDA synthase and CBCA synthase, respectively (**Figure 1**). As

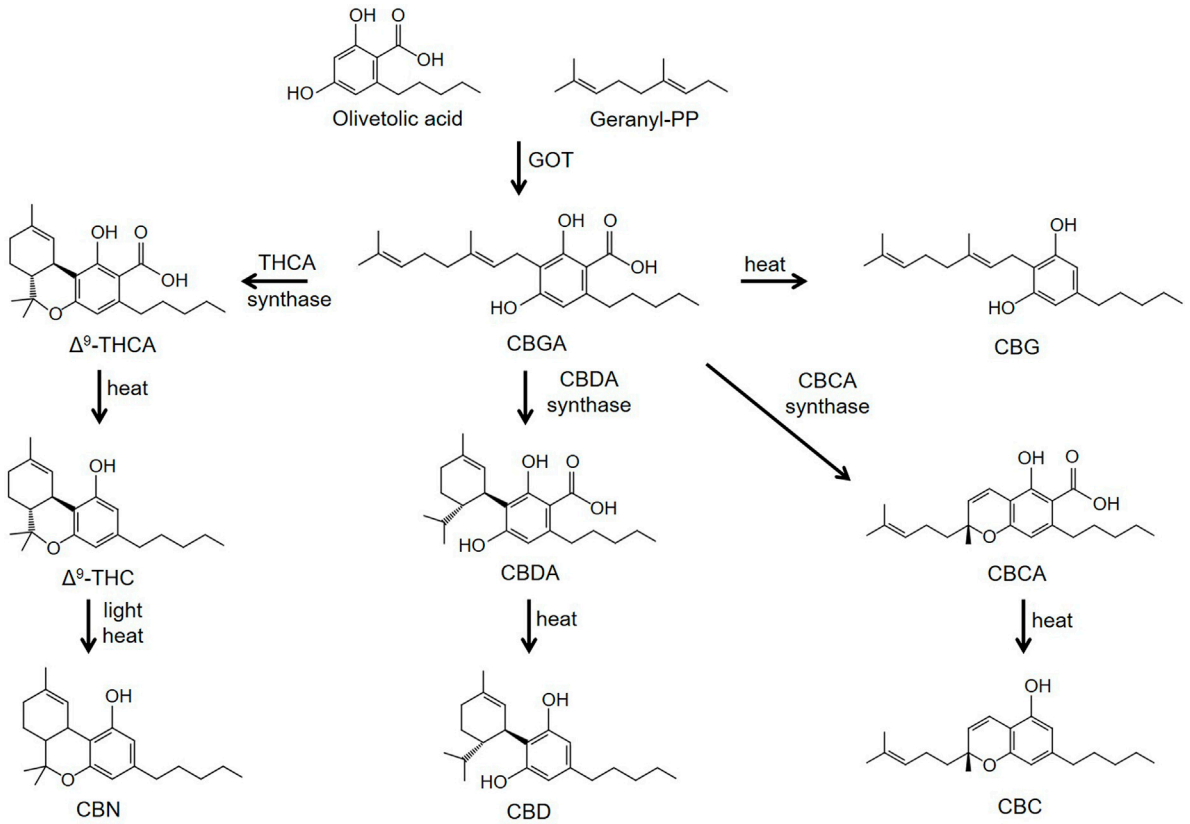


FIGURE 1 | Biosynthesis pathways of phytocannabinoids. Abbreviations: CBN, cannabinol; CBC, cannabichromene; CBD, cannabidiol; CBG, cannabigerol; CBDA, cannabidioloic acid; CBGA, cannabigerolic acid; CBCA, cannabichromenic acid; GOT, olivetolate geranyltransferase; Δ^9-THCA, Δ^9-tetrahydrocannabinolic acid; Δ^9-THC, Δ^9-tetrahydrocannabinol.

described below, the neutral phytocannabinoids are then derived from the acidic forms through non-enzymatic decarboxylation during exposure to heat or light. Cannabigerovarin acid (CBGVA), the precursor to the variant cannabinoids (propyl side chain), is synthesized from divarinolic acid through geranyltransferase (Hanus, et al., 2016).

Various methodologies including extraction/isolation, semi-synthesis or full synthesis and microbial engineering (*E. coli*, algae, yeast etc.,) are being utilized to obtain minor cannabinoids. Selective cannabis crossbreeding to enhance or decrease certain cannabinoids or terpenes is a common practice. For example, selective breeding has been used to yield cannabis varieties rich in CBG, CBC, THCV and CBDV (de Meijer and Hammond, 2005; de Meijer, et al., 2009). In a major advancement in the field, Luo et al. (2019) successfully introduced the MEP, GPP and hexanoic pathways along with THCA and CBDA synthases in yeast (*Saccharomyces cerevisiae*); establishing a platform for the large-scale fermentation of natural minor cannabinoids. Interestingly, unnatural cannabinoids with tailored alkyl side chains were produced by feeding different fatty acid precursors to the yeast (Luo, et al., 2019). Since the length and chemistry of the alkyl side chain modulates the affinity of the cannabinoids for the CB1 and CB2 receptors (Martin, et al., 1999), this platform could provide a novel method for discovering new and novel cannabinoid receptor specific agonists and antagonists.

THE ENDOCANNABINOID SYSTEM

Cannabinoid CB1 and CB2 Receptors

Cannabinoid investigators initially hypothesized that Δ^9-THC might act by disturbing cell membranes due to its lipophilic properties. However, binding assays obtained using the radio-labeled synthetic cannabinoid CP-55,940 [^{3}(H)-CP-55,940], identified selective, high affinity binding sites for the compound in rat brain preparations (Devane, et al., 1988). This finding led to the cloning of the cannabinoid type 1 (CB1) (Matsuda, et al., 1990) and type 2 (CB2) (Munro, et al., 1993) receptors in the early 1990s. Both the CB1 and CB2 receptors are members of the G protein-coupled receptor (GPCR) super-family of proteins. CB1 receptors are primarily localized to presynaptic nerve terminals in the central and peripheral nervous system. Tissues expressing high levels of the CB1 receptor include the amygdala, hippocampus, cerebral cortex, cerebellum and spinal column (Herkenham, et al., 1990; Tsou, et al., 1998). In contrast, CB2 receptors are found in the

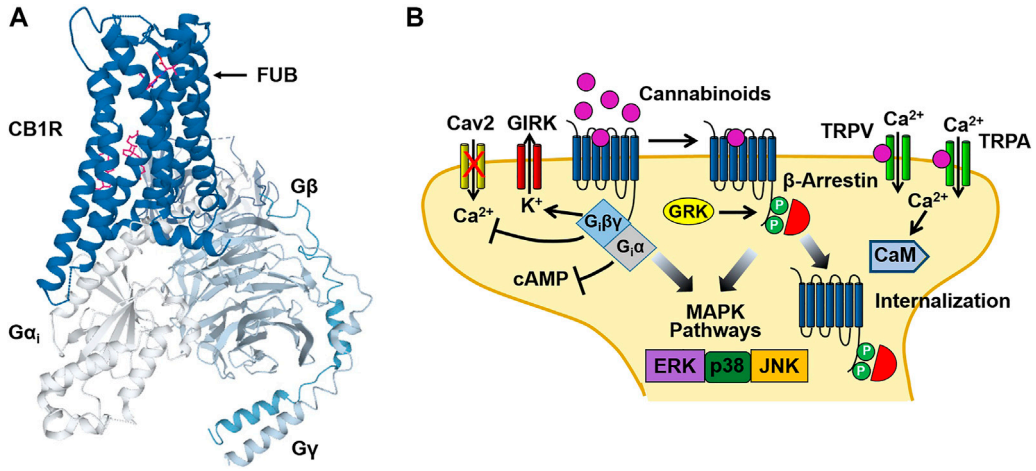


FIGURE 2 | Cannabinoid CB1 receptor structure and signaling. **(A)** Structural model of the CB1 receptor (CB1R)-G_i protein complex obtained from cryoelectron microscopy. The binding site for the synthetic cannabinoid MDMB-FUBINACA (FUB) is indicated by the magenta structure. The CB1-G_i receptor complex structure was obtained from the Protein Data Bank (code 6N4B). **(B)** Binding of cannabinoids to the neuronal CB1 receptor stimulates both neuronal G_i/G_o and β-arrestin signaling pathways leading to an inhibition of adenylyl cyclase, activation of G protein-gated inward rectifier K^+ (GIRK) channels and receptor internalization. In addition, activation of transient receptor potential vanilloid (TRPV) and ankyrin (TRPA) channels by cannabinoids causes Ca^{2+} influx that activates Ca^{2+}-sensitive enzymes such as Ca^{2+}/calmodulin-dependent protein kinase (CaM). **Figure 2B** was adapted from Walsh and Andersen (Walsh and Andersen, 2020).

cells of the immune system and in astrocytes and microglia of the CNS (Munro, et al., 1993; Galiégue, et al., 1995; Stella, 2010). As is the case with other Class A GPCRs, cannabinoid receptors contain seven transmembrane domains (TM1-7) with intracellular (ICLs) and extracellular loops (ECLs), an N-terminal ECL and an intracellular domain that interacts with pertussis toxin-sensitive G proteins (G_i/G_o) (**Figure 2**). Using computational modeling with the CB1 receptor crystal structure, Hua et al. (2017) predicted that Δ^9-THC interacts with the ECL2 and TM3, TM6 and TM7. Binding of cannabinoids is postulated to activate a toggle switch in the CB1 receptor (consisting of residues F200 and W356 in the TM3/TM6 binding pocket) that results in G_i/G_o protein interaction (Hua, et al., 2017). The strong interaction of the indazole ring of the synthetic cannabinoid MDMB-FUBINACA (FUB) with the toggle switch stabilizes the active conformation of the receptor and brings about the high efficacy of this ligand (Kumar et al., 2019) (**Figure 2**). In contrast, (Kumar et al., 2019) suggested that the lack of toggle switch interaction by Δ^9-THC may account for its partial agonist activity. Although cannabinoid receptors primarily couple to G_i/G_o, they can also stimulate G_s and G_q proteins under certain conditions (Glass and Northup, 1999; Lauckner, et al., 2005).

Binding of Δ^9-THC and synthetic cannabinoids (WIN 55,212-2, CP 55,940, etc.,) to the CB1 and CB2 receptors causes the dissociation of the βγ subunits of the G protein heterotrimer from the α subunit ($G_i\alpha$) (**Figure 2**). $G_i\alpha$ inhibits adenylyl cyclase resulting in a decrease in intracellular levels of cAMP (Howlett, et al., 1986). In contrast, $G_i\beta\gamma$ inhibits the opening of voltage-gated Ca^{2+} channels (N and P/Q type) while activating G protein-gated inward rectifier K^+ (GIRK) channels (Mackie, et al., 1995; Guo and Ikeda, 2004). In addition to GIRK channels, cannabinoid binding also couples to other K^+ channels including M-type and A-type channels in cultured neurons (Schlicker and Kathmann, 2001). Together, these cannabinoid actions bring about an acute inhibition of synaptic neurotransmitter release and dampens neuronal excitability (Shen, et al., 1996; Vaughan, et al., 2000). These signaling effects are followed by receptor phosphorylation [by G protein receptor kinase (GRK)] that recruits β-arrestin1 (βarr1) and β-arrestin2 (βarr2) to the receptor and results in CB1 receptor desensitization and internalization (**Figure 2**) (Jin, et al., 1999; Ahn, et al., 2013). Both G_i and β-arrestin can also stimulate mitogen-activated protein kinases (MAPKs), including the extracellular signal-regulated kinases (ERK1/2), bringing about additional cellular effects (Bouaboula, et al., 1995; Galve-Roperh, et al., 2002; Derkinderen, et al., 2003).

Endocannabinoids

Following the discovery of the CB1 and CB2 receptors, the endogenous cannabinoids (or endocannabinoids) anandamide [N-arachidonoylethanolamine (AEA)] and 2-arachidonoylglycerol (2-AG) were isolated (Devane, et al., 1992; Mechoulam, et al., 1995; Sugiura, et al., 1995). These endocannabinoids are synthesized from the cell membrane lipids N-arachidonoyl phosphatidyl ethanol (NAPE) (for AEA) and phosphatidyl inositol bis-phosphate (PIP2) (for 2-AG). Unlike the continuous cellular synthesis and storage of neurotransmitters and neuropeptides, AEA and 2-AG are produced through "on demand" cleavage of NAPE and PIP2. This provides for a temporal- and localization-dependent release of the endocannabinoids (Lu and Mackie, 2016). The actions of AEA and 2-AG are terminated following their cellular uptake and degradation by intracellular hydroxylase [fatty acid amide hydrolase (FAAH)] (for AEA) and lipase enzymes (monoacylglycerol lipase) (for 2-AG). Therefore, drugs that

inhibit the cellular uptake of AEA and 2-AG or prevent their enzymatic degradation should result in a potentiation of endocannabinoid action. In addition to AEA and 2-AG, other putative endocannabinoids include N-arachidonoyldopamine (NADA), 2-arachidonoylglycerylether (noladin ether), N-oleoylethanolamine (OEA) and palmitoylethanolamide (PEA) (Tan, et al., 2006).

Endocannabinoids bring about their pharmacological effects through a number of mechanisms. Early studies demonstrated that while 2-AG acts as a full agonist at the CB1 and CB2 receptors, both AEA and Δ^9-THC function as partial agonists when compared with the full cannabinoid agonist WIN 55,212-2 (Gonsiorek, et al., 2000; Luk, et al., 2004). Endocannabinoids can also act at receptor sites ("off targets") other than the CB1 and CB2 receptors. Transient receptor potential (TRP) channels are a superfamily of inotropic channels that are activated by thermal, physical and electrochemical stimuli. The TRP channels are subdivided into several families including the vanilloid (TRPV), ankyrin (TRPA) and melastatin (TRPM) channels. Both AEA and 2-AG bind to and activate TRPV1 channels causing cell membrane potential depolarization and Ca^{2+} influx (Zygmunt, et al., 1999; Smart, et al., 2000) (**Figure 2**). AEA can also modulate the activity of TRPA1 and TRPM8 channels (De Petrocellis, et al., 2007; De Petrocellis, et al., 2012). In addition to the TRP channels, a number of other "off targets" for endocannabinoids have been identified. This includes the de-orphanized G protein-coupled receptors 18 (GPR18) and 55 (GPR55), as well as Peroxisome Proliferator-activated Receptors (PPARs). GPR18 and GPR55 are proposed to regulate acute and chronic pain pathways and are activated by the endogenous ligands N-arachidonoylglycine (NAgly) and lysophophatidylinositol (LPI), respectively (Kohno, et al., 2006; Henstidge, et al., 2009). Binding of endocannabinoids to these receptors stimulates cell signaling events through $G\alpha_i$ (GPR18), $G\alpha_{12}$ and $G\alpha_{12/13}$ (GPR55). PPARs are members of the nuclear hormone receptor family of proteins that function as ligand-inducible transcription factors. A number of endocannabinoids including AEA and 2-AG, as well as the phytocannabinoids Δ^9-THC and CBD, are agonists at the PPARs (O'Sullivan, 2007).

MINOR CANNABINOID PHARMACOLOGY AND THERAPEUTICS

Minor cannabinoids are divided into neutral, acidic and varinic phytocannabinoids. Minor cannabinoids include cannabinol (CBN), cannabichromene (CBC), cannabigerol (CBG), cannabidioloic acid (CBDA), cannabigerolic acid (CBGA), tetrahydrocannabinolic acid (THCA), cannabinolic acid (CBNA), cannabidivarin (CBDV), tetrahydrocannabivarin (THCV), cannabigerovarin (CBGV), cannabichromevarin (CBCV), and others (Hanus, et al., 2016; Gülck and Möller, 2020). Cannabinoids appear naturally in the cannabis plant in their acidic forms and are thought to confer antioxidant and defense mechanisms (insecticidal, antimicrobial, etc.,) to the plant. Acidic cannabinoids undergo decarboxylation during heating and are converted to the corresponding neutral cannabinoids (**Figure 1**). For example, THCA is converted to Δ^9-THC when cannabis is smoked or vaporized. Some decarboxylation also occurs with passage of time at room temperature and during exposure to light. Cannabis products intended to contain the acidic forms of cannabinoids nearly universally also contain low levels of cannabinoids in their neutral forms. The varinic cannabinoids are considered rare but are now emerging as new targets of selective breeding. Varin compounds such as CBDV and THCV contain two fewer carbon atoms than their non-varin counterparts (CBD and Δ^9-THC) endowing these cannabinoids with unique pharmacological properties (see below).

As described for the endocannabinoids, the overall pharmacological action of the minor cannabinoids often results from binding at both cannabinoid and "off target" receptors. This combination of receptor-mediated actions makes them well suited as multi-target therapeutic agents. While a number of minor cannabinoids including CBN and THCV bind to the CB1 receptor, they have significantly less binding activity when compared with Δ^9-THC (Rhee, et al., 1997; Zagzoog, et al., 2020). To date, none of the minor cannabinoids have been clinically demonstrated to act as psychotropic drugs. The reported potencies of the minor cannabinoids at the CB1 and CB2 receptors and TRP channels are summarized in **Table 1** and discussed in the sections below.

Neutral Cannabinoids

Cannabinol

CBN was originally isolated from Indian hemp in 1896 making it the first phytocannabinoid identified in cannabis (Wood, et al., 1886). The structural determination and total synthesis of CBN was carried out by Adams and colleagues in the 1940s (Adams, et al., 1940b). CBN is not synthesized in the cannabis plant, but is derived during the degradation of Δ^9-THC. Even under ideal storage conditions, exposure to UV light and/or heat over time results in the conversion of Δ^9-THC to CBN. Using ^{3}(H)-labelled synthetic cannabinoid (e.g., HU-243, CP-55,490) displacement assays it was determined that CBN has low binding affinities for the CB1 and CB2 receptors when compared with Δ^9-THC (Rhee, et al., 1997; Mahadevan, et al., 2000; Rosenthaler, et al., 2014). In addition, CBN is less potent than Δ^9-THC in CB1 receptor-mediated inhibition of adenylyl cyclase, but displays equal potency in CB2 receptor-mediated inhibition (Rhee, et al., 1997). CBN is an agonist at TRPV1, TRPV2, TRPV3 and TRPV4 channels stimulating cell Ca^{2+} influx with the activation of Ca^{2+}-dependent pathways (De Petrocellis, et al., 2011). It is also a potent and efficacious agonist of the TRPA1 channel. In addition, CBN acts as a potent antagonist of icilin activation of the TRPM8 channel (De Petrocellis, et al., 2011).

CBN has been identified as a potential analgesic and anti-inflammatory agent. CBN isolate has been reported to relieve chronic muscle pain disorders such as temporomandibular disorders and fibromyalgia in a rat model of myofascial pain (Wong and Cairns, 2019). For example, CBN (1 mg/ml) reduces mechanical sensitivity induced by intramuscular injection of nerve growth factor in the masseter muscle (Wong and Cairns, 2019). While CBN is not as widely recognized as CBD

TABLE 1 | Pharmacology of the minor cannabinoids.

Receptor/Cell	EC_{50}/IC_{50} (μM)[a]								References
	Assay	CBN	C-BC	CBG	CBDA	CBGA	THCA	CBDV	THCV	
CB1/COS-7[b]	cAMP inhibition	0.12								Rhee, et al. (1997)
CB1/HEK293[c]	cAMP inhibition						>10			McPartland, et al. (2017)
CB1/HEK293[c]	cAMP inhibition[k]				1	1		1		Navarro, et al. (2020)
CB1/CHO[d]	cAMP -inhibition[l]		0.19	0.12	0.03		>10	>10	0.26	Zagzoog, et al. (2020)
CB1/cerebellum[e]	GTPγS binding								0.03	Dennis, et al. (2008)
CB1/HEK293[c]	GTPγS binding	0.31		>10		0.18	>10	>10	>10	Husni, et al. (2014)
CB1/vas deferens[f]	EECs								>10	Thomas, et al. (2005)
CB2/COS-7[b]	cAMP inhibition	0.290								Rhee, et al. (1997)
CB2/HEK293[c]	cAMP inhibition				0.1			>1		Navarro, et al. (2020)
CB2/CHO[d]	cAMP inhibition		0.007	0.13	0.14		1.8	0.005	0.28	Zagzoog, et al. (2020)
CB2/CHO[d]	cAMP inhibition								0.038	Bolognini, et al. (2010)
CB2/HEK293[c]	GTPγS binding	0.29		1.21			>10	0.003	>10	Husni, et al. (2014)
CB2/AtT20[g]	membrane potential		>3							Udoh, et al. (2019)
TRPV1/HEK293[h]	Ca^{2+} signal	6.2	24.2	1.3	19.7	21.0		3.6	1.5	De Petrocellis, et al. (2011)
TRPV2/HEK29[h]	Ca^{2+} signal	19.0		1.7			18.4	7.3	4.1	De Petrocellis, et al. (2011)
TRPV3/HEK29[h]	Ca^{2+} signal					12.6			3.8	De Petrocellis, et al. (2012)
TRPV4/HEK29[h]	Ca^{2+} signal	16.1		5.1		28.8		0.9	6.4	De Petrocellis, et al. (2012)
TRPA1/HEK293[i]	Ca^{2+} signal	0.18	0.09	0.7	5.3	8.4	2.7	0.42	1.5	De Petrocellis, et al. (2011)
TRPM8/HEK293[j]	Ca^{2+} signal	0.21	40.7	0.14	0.9	1.31	0.14	0.9	0.87	De Petrocellis, et al. (2008)

[a]*Cannabinoid potencies as agonists (EC_{50}) and antagonists (IC_{50}).*
[b]*African green monkey kidney (COS-7) cells expressing either the rat CB1 or CB2 receptor.*
[c]*HEK293 cells expressing either the human CB1 or CB2 receptor.*
[d]*Chinese hamster ovary (CHO) cells expressing either the human CB1 or CB2 receptor.*
[e]*Antagonism of WIN-55,212-2 stimulation of (^{35}S)GTPγS binding in rodent brain cerebellum.*
[f]*Inhibition of electrically-evoked contractions (EECs) of mouse vas deferens (IC_{50}).*
[g]*Membrane potential measured using a fluorescent dye in pituitary AtT20 cells expressing the CB2 receptor.*
[h]*Ca^{2+} fluorescence measured in HEK293 cells expressing the rat TRPV1-TRPV4 channels.*
[i]*Ca^{2+} fluorescence measured in HEK293 cells expressing the rat TRPA1 channel.*
[j]*Ca^{2+} fluorescence measured in HEK293 cells expressing the rat TRPV1-TRPV4 channels (IC_{50} against icilin).*
[k]*EC_{50} values are not given by Navarro et al., 2020. Listed values were estimated from the displayed concentration versus response curves.*
[l]*CHO cells treated for 90 min with cannabinoids.*

and Δ^9-THC for its anti-inflammatory properties, it may have therapeutic benefits in treating allergic airway diseases. CBN attenuates the production of interleukins 2, 4, 5, 13 and decreases allergen mucus production in OVA-sensitized and challenged A/J mice (Jan, et al., 2003). CBN and Δ^9-THC (but not CBD) can also be used to treat glaucoma since they prevent inflammation that causes elevated intraocular pressure (ElSohly, et al., 1981). In addition, preliminary data indicate that CBN decreases cell damage and acts as an antioxidant in a cell culture model of Huntington's disease (Aiken, et al., 2004).

CBN also shows promise as an antibacterial agent and an appetite stimulant. As with other cannabinoids (e.g., CBC and CBG), CBN has been found to be highly efficacious against multiple antibiotic-resistant bacteria, including methicillin-resistant Staphylococcus aureus (MRSA), making it a potentially viable treatment for staph infections (Appendino, et al., 2008). CBN also stimulates hyperphagia and increases food consumption and feeding time in rats (Farrimond et al., 2012a; Farrimond, et al., 2012b). Although CBN is not as potent an appetite stimulant as Δ^9-THC, CBN administration is not associated with the psychotropic effects of Δ^9-THC. Thus, CBN represents a non-intoxicating alternative to Δ^9-THC as an appetite stimulant.

CBN-rich products are advertised for promoting sleep or relaxation without the impairment caused by Δ^9-THC. Since CBN is a degradation product of Δ^9-THC it is found in greater quantities in aged cannabis preparations. For this reason it is marketed as "the sleepy cannabinoid in old weed." However, laboratory results obtained from sleep studies with CBN have been equivocal. In mice, CBN was reported to increase barbiturate-induced sleep time in one study (Yoshida, et al., 1995), while having no effects on sleep in another (Chesner, et al., 1974). When administered along with Δ^9-THC in rats, CBN produces greater sedation compared with either cannabinoid alone (Fernandes, et al., 1974; Takahashi and Karniol, 1975). In one clinical study involving a small number of participants, the combination of Δ^9-THC and CBN caused greater drowsiness than with Δ^9-THC used alone (Karniol, et al., 1975). However, in a recent review of the CBN literature, Corroon (2021) found little evidence supporting a sleep promoting effect of CBN. Therefore, controlled studies are warranted to substantiate sleep-related claims of CBN containing products.

Cannabichromene

CBC is one of the most abundant minor cannabinoids found in cannabis. The structure of CBC was first determined using NMR spectroscopy by Gaoni and Mechoulam (Gaoni and Mechoulam, 1966). Although cannabinoid receptor studies using CBC are limited, the cannabinoid was initially identified as partial CB2 receptor agonist (Rosenthaler, et al., 2014). This was supported by

experiments using a "hyperpolarization assay" with pituitary AtT20 cells in which CBC was found to be selective in stimulating the CB2 receptor over the CB1 receptor (Udoh, et al., 2019). In this same study, CBC was more potent and efficacious than Δ^9-THC in causing cell hyperpolarization *via* the CB2 receptor. In contrast to these results, CBC was shown in a recent paper to display similar affinities for the CB1 and CB2 receptors and to cause both CB1 and CB2 receptor-mediated decreases in cellular cAMP levels (Zagzoog, et al., 2020). As is the case for CBN, CBC is a potent activator of the TRPA1 channel (De Petrocellis, et al., 2011). In addition, it activates TRPV3 and TRPV4 channels when applied at micromolar and submicromolar concentrations (De Petrocellis, et al., 2011). Other proposed sites of action of CBN are discussed below.

Anti-inflammatory effects of CBC were first reported in the 1980s using a rat model of edema. High doses of CBC were more efficacious than the nonsteroidal anti-inflammatory drug (NSAID) phenylbutazone in carrageenan-induced paw edema (Turner and ElSohly, 1981). CBC has been shown to reduce pain and inflammation associated with osteoarthritis in rats without the negative side effects of NSAIDs (Maione, et al., 2011). In addition, CBC attenuates lipopolysaccharide (LPS)-induced increases in nitric oxide levels in an *in vitro* model of colitis (Romano, et al., 2013) and reduces inflammation-induced GI motility (Izzo, et al., 2012). CBC also displays a modest anti-nociceptive effect in the mouse tail-withdrawal assay (Maione, et al., 2011; Zagzoog, et al., 2020). CBC regulates a number of cellular pathways involved in anti-nociception that include the stimulation of adenosine A1 receptors, CB1 receptors and TRPA1 channels (De Petrocellis, et al., 2011; Maione, et al., 2011). In addition, CBC has been proposed to inhibit AEA (anandamide) reuptake; thus potentiating the physiological effects of AEA (De Petrocellis, et al., 2011). Like other synergistic actions of cannabinoids, CBC has a greater anti-inflammatory response when combined with Δ^9-THC than when either cannabinoid is used alone (DeLong, et al., 2010).

The anti-inflammatory actions of CBC may be important in its ability to function as a neuroprotective drug. CBC increases the viability of neural stem progenitor cells (NSPCs) *in vitro* through an ERK dependent mechanism (Shinjyo and Di Marzo, 2013). In addition, CBC inhibits astroglial differentiation of the NSPCs (Shinjyo and Di Marzo, 2013). NSPCs are modulated by surrounding microglial cells, brain immune cells, and astrocytes, which produce both pro- and anti-inflammatory factors. The potential anti-inflammatory and neuroprotective effects of CBC may occur through its suppression of reactive astrocytes (Covelo, et al., 2021). CBC inhibition of NSPCs differentiation into astrocytes may therefore offer a protective effect against neuro-inflammation, Alzheimer's disease, and hepatic encephalopathy (Covelo, et al., 2021).

Δ^9-THC possesses anti-tumor properties and is used for treating several different forms of cancer (Fraguas-Sánchez and Torres-Suárez, 2018). However, the psychotropic qualities of Δ^9-THC limit its use as a chemotherapy agent. CBC may be beneficial in cancer treatment due to its ability to increase blood levels of AEA (see above). AEA has been shown to inhibit breast cancer cell proliferation and induce death of colon cancer cells (De Petrocellis, et al., 1998; Patsos, et al., 2005). CBC was also shown to inhibit cell migration and disrupt the cell cytoskeleton in an *in vitro* model of urothelial cancer (Anis, et al., 2021). In one study that examined the anti-tumor effects of several minor cannabinoids, only CBG was more potent than CBC at inhibiting the growth of several cancer cell lines (Ligresti, et al., 2006).

Cannabigerol

CBG is produced *via* decarboxylation of CBGA, the precursor molecule of the Δ^9-THC and CBD branches of the cannabis synthesis pathway (**Figure 1**). Results obtained using cAMP assays revealed that CBG displays weak partial agonist activity at the CB1 and CB2 receptors (see **Table 1**) (Husni, et al., 2014; Navarro, et al., 2020; Zagzoog, et al., 2020). In CHO cells expressing both CB1 and CB2 receptors, CBG binds to the receptors with K_is in the low micromolar range (Navarro, et al., 2018). Of special note, in this same study CBG was found to compete with ^{3}(H)-CP-55,490 for binding to the CB1 receptor, but not ^{3}(H)-WIN-55,212-2 (Navarro, et al., 2018). This suggests the CBG and CP-55,490 (but not WIN-55,212-2) bind to the same orthosteric site on the receptor. CBG activates TRPV1, TRPV2, TRPV3, TRPV4 and TRPA1 channels at low micromolar concentrations (De Petrocellis, et al., 2011). CBG has also been shown to act through other off-target sites including the 5-HT_{1a} receptor and the α_2-adrenergic receptor. For example, CBG competitively antagonizes the ability of the 5-HT_{1a} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) to stimulate (^{35}S)GTPγS binding in rat brain membranes (Cascio, et al., 2010). Furthermore, CBG inhibits electrically-induced contractions of the vas deferens and stimulates (^{35}S)GTPγS binding in rat brain membranes through agonist activity at the α_2-adrenergic receptor (Cascio, et al., 2010). CBG, along with acidic cannabinoids THCA and CBDA, also binds to and activates PPARγ (D'Aniello, et al., 2019) (see below).

As with other minor cannabinoids, CBG may reduce the severity of inflammatory diseases and peripheral pain. The anti-inflammatory properties of CBG are postulated to result from binding to the CB2 receptor, TRP channels, PPARγ and other targets (De Petrocellis, et al., 2011; Cascio, et al., 2010; Ruhaak, et al., 2011). There is anecdotal human and preclinical evidence for CBG having a benefit in cases of inflammatory bowel diseases including Crohn's and ulcerative colitis. In a mouse model of colitis, CBG was found to reduce bowel inflammation, nitric oxide production [from increased nitric oxide synthase (iNOS) expression during inflammation] and oxidative stress in intestinal cells (Borrelli, et al., 2013; Pagano, et al., 2021). Similar to CBC, CBG (3 mg/kg and 10 mg/kg i.p.) produces a weak anti-nociceptive effect in mice (Zagzoog, et al., 2020). In one study this effect of CBG was inhibited by the α_2-adrenergic receptor antagonist yohimbine, suggesting a role of α_2-adrenergic regulation in CBG analgesia (Cascio, et al., 2010).

Inflammation and oxidative stress are both contributors to neurodegeneration, which is linked to Alzheimer's and Huntington's disease as well as Multiple Sclerosis (MS). CBG may protect against both neuroinflammation and oxidative stress, helping to prevent neuronal cell loss (Gugliandolo, et al., 2018).

Carrillo-Salinas et al. (2014) examined the effect of the CBG analog VCE-003 on human T-cells and its efficacy in a mouse model of autoimmune MS. When tested *in vitro*, VCE-003 inhibited antigen-induced T-cell proliferation, cell cycle progression and the expression of surface activation markers. VCE-003 also prevented the expression of the pro-inflammatory enzyme iNOS in microglia. In animals, VCE-003 attenuated MS through activating CB2 and PPARγ receptors (Carrillo-Salinas, et al., 2014). CBG was also investigated in a mouse model of Huntington's disease induced using 3-nitropropionate (3-NP) (Valdeolivas, et al., 2015). Treatment with CBG (10 mg/kg) reduced levels of the pro-inflammatory cytokines IL-6 and tumor necrosis factor α in the 3-NP treated mice. CBG also partially improved motor deficits and preserved striatum neurons in the R2/6 transgenic model of Huntington's disease (Valdeolivas, et al., 2015).

As noted previously, CBG is effective in suppressing cancer cell growth (Ligresti, et al., 2006). In a murine colon cancer model, CBG was found to promote cancer cell death and inhibit the growth of tumors (Borrelli, et al., 2014). This inhibition was mimicked by TRPM8 channel antagonists (Borrelli, et al., 2014). Additionally, *in vitro* experiments using leukemia cell lines suggest this anti-cancer activity is enhanced when CBG is combined with other cannabinoids such as CBD (Scott, et al., 2013). Clinical studies are currently underway to determine if these results are translatable to treatment in humans. Individuals living with cancer and AIDS commonly experience anorexia and cachexia. CBG represents a non-psychoactive alternative to Δ^9-THC for treating anorexia since it stimulates appetite and increases food consumption (Brierley, et al., 2017). Interestingly, CBG as part of a whole plant cannabis extract is more potent in stimulating appetite than CBG as an isolate (Brierley, et al., 2017). Thus, these results provide additional evidence that synergism of minor cannabinoids with other components of the cannabis plant may enhance their clinical efficacy (Russo, 2011).

Cannabinoid Acids

Cannabidioloic Acid

CBDA was first isolated in 1955 and its structure elucidated in 1965 (Mechoulam and Gaoni, 1965). CBDA has a low affinity for both the CB1 and CB2 receptors based on ^{3}[H]-CP-55,490 displacement assays (Zagzoog, et al., 2020). However, it shows moderate efficacy in inhibiting adenylyl cyclase through these receptors (Navarro, et al., 2020; Zagzoog, et al., 2020). In addition, CBDA is one of several minor cannabinoids (along with THCA and THCV) that functions as allosteric regulators at 5-HT_{1a} receptors (Bolognini, et al., 2013). CBDA enhances 8-OH-DPAT-stimulated (^{35}S)GTPγS binding to 5-HT_{1a} receptors expressed in rat brain and CHO cell membranes possibly by binding to an allosteric site on the receptor (Bolognini, et al., 2013). CBDA was reported to be 1,000 times more potent than CBD in stimulating (^{35}S)GTPγS binding at the 5-HT_{1a} receptor. Based on *in silico* docking experiments, it was predicted that CBDA, along with the cannabinoids CBGA and CBG, bind to PPARs (D'Aniello, et al., 2019). *In vitro* reporter assays carried out with CHO cells confirmed that all three minor cannabinoids activate PPARα and PPARγ (D'Aniello, et al., 2019).

CBDA produces dose-dependent anti-hyperalgesia and anti-inflammatory effects in a rodent model of carrageenan-induced hind paw inflammation (Rock, et al., 2018). The anti-hyperalgesia effect of CBDA is blocked by AMG9810, an antagonist of the TRPV1 channel. In addition, when combined with Δ^9-THC, low doses of CBDA are more effective in preventing hyperalgesia and reducing inflammation. CBDA may also produce anti-inflammatory effects *via* cyclooxygenase 2 (COX-2) enzyme inhibition; the same mechanism of action as the NSAID Celecoxib (Takeda, et al., 2008; Ruhaak, et al., 2011). CBDA inhibits the COX-2 enzyme *in vitro* with an EC_{50} of 2 μM and has 9-fold greater selectivity in inhibiting the COX-2 enzyme over the COX-1 enzyme. This selective inhibition is dependent on the presence of the carboxylic acid moiety in the CBDA molecule (Takeda, et al., 2008).

CBDA has anti-nausea effects at low doses in mice that are mediated *via* agonist activity at CNS 5-HT_{1A} receptors (Pertwee, et al., 2018). CBDA was found to be 1000-fold more potent than CBD in reducing nausea-induced conditioned gaping disgust responses (Rock et al., 2020). The drug HU-580, a stable analogue of CBDA that is not metabolized to CBD, also reduces LiCl-induced conditioned gaping (Pertwee, et al., 2018). In addition to suppressing acute nausea, CBDA decreases anticipatory nausea and vomiting which occurs upon re-exposure to a contextual stimulus previously associated with acute nausea (e.g., a chemotherapy session) (Limebeer, et al., 2014). CBDA combined with ondansetron, a commonly used antiemetic drug, enhances ondansetron's effect when applied at low doses (Rock and Parker, 2013).

Anderson et al. (2019) examined the anti-seizure activity of CBDA using Scn1aRX/+ mouse model of Dravet Syndrome. The Scn1aRX/+ mice develop generalized tonic-clonic seizures in response to elevated body temperature and thus recapitulate the seizures observed in children with Dravet Syndrome. When administered using i.p. injection (10 and 30 mg/kg), CBDA raised the temperature threshold required for seizures in the mice (Anderson, et al., 2019). CBDA also displayed dose-dependent protection in rats against electroshock-induced seizures (Goerl, et al., 2021). While clinical trials have not been reported, CBDA may be more effective than CBD in reducing seizures in humans. According to a patent application by GW Pharmaceuticals, the makers of Epidiolex® (a sublingual spray containing 100 mg of CBD/100 ml of solution), CBDA displays greater bioavailability and potency in treating epilepsy (GW PHARMA LTD, 2015).

Cannabigerolic Acid

CBGA is the precursor cannabinoid to THCA, CBDA, and CBCA (see **Figure 1**). Since CBGA is decarboxylated over time to CBG, it is rarely found in significant amounts in mature cannabis flowers. Thus, harvesting hemp very early yields higher levels of CBGA compared to later in the plant's life. In addition, some cultivars have increased yields of CBGA through selective breeding to inhibit its transformation into other cannabinoids during the plant's maturation (Garfinkel, et al., 2021). Similar to CBDA, CBGA displays low affinity for both the CB1 and CB2 receptors (Navarro, et al., 2020). Nonetheless, CBGA is equally as

efficacious as Δ^9-THC in decreasing intracellular cAMP levels though the CB1 receptor (**Table 1**) (Navarro, et al., 2020). However, unlike Δ^9-THC, it is not effective in recruiting βarr2 to the CB1 receptor (Navarro, et al., 2020). CBGA has important off target effects including activating PPARs (D'Aniello, et al., 2019). In addition, fractions of *Cannabis sativa* containing high amounts of CBG/CBGA inhibit the aldose reductase enzyme (Smeriglio, et al., 2018).

While less in known about the therapeutic uses of CBGA compared with the other minor cannabinoids, it may play a role in controlling diabetes mellitus and preventing the cardiovascular complications that can accompany Type 2 diabetes (D'Aniello, et al., 2019). Through activation of PPARs, CBGA can improve lipid metabolism and reduce the accumulation of adipose tissue; thus reducing insulin resistance in the Type 2 patient (Gao, et al., 2015). Type 2 diabetes is considered a "coronary artery disease equivalent" and mortality in Type 2 diabetes primarily results from cardiovascular events including acute coronary syndrome (ACS). By inhibiting the enzyme aldose reductase, CBGA improves cardiac glucose metabolism and reduces the risk of ACS (Smeriglio, et al., 2018). Synthetic inhibitors of aldose reductase have severe side effects including elevations in blood liver enzymes from hepatotoxicity, as well as nausea and vomiting. Therefore, plant-derived CBGA offers a promising alternative to these inhibitors.

CBGA may also be beneficial in treating some types of cancer. A cannabis fraction containing high amounts of CBGA was reported to have cytotoxic activity against colon cancer cells (Nallathambi, et al., 2018). Interestingly, synergistic toxic effects were observed when CBGA was given with a cannabis fraction high in THCA. These two fractions also prevented the growth and proliferation of adenomatous colon polyps that are colon cancer precursors. When tested at micromolar concentrations, CBGA was also shown to have cytotoxic actions in human leukemia cancer cell lines (Scott, et al., 2013). In further support of cannabinoid synergism, the IC_{50} for CBGA leukemia cell toxicity was reduced when co-applied with CBD (Scott, et al., 2013; Scott, et al., 2017).

Tetrahydrocannabinolic Acid

THCA is a non-psychotropic cannabinoid that is converted to Δ^9-THC through decarboxylation by exposure to heat (**Figure 1**). Since THCA is a precursor to Δ^9-THC, and because no sample of THCA is completely free of Δ^9-THC, possession of this cannabinoid could be prosecuted under the U.S. government Federal Analogue Act. THCA displays roughly 60- and 125-fold lower affinity for the CB1 and CB2 receptors compared with Δ^9-THC (McPartland, et al., 2017). While high concentrations of THCA inhibit forskolin-stimulated increases in cAMP through the CB1 receptor, it produces no inhibition through the CB2 receptor (McPartland, et al., 2017). Nagal et al. (2017) compared the effects of several cannabinoids, including CBDA, CBGA and THCA on PPAR activity. When compared with CBDA and CBGA, THCA has the highest binding affinity for PPARγ (Nadal, et al., 2017). In addition, THCA is more potent than other minor cannabinoids in inducing PPARγ-mediated transcriptional activity.

THCA was recently shown to possess potent anti-inflammatory activity in mice fed a high fat diet (HFD) (Palomares, et al., 2020; Carmona-Hidalgo, et al., 2021). THCA treatment reduced the expression of inflammatory molecules including tumor necrosis factor alpha (TNF-α4) and cytokine interleukin 10 (IL-10) in the HFD mice. This effect was mediated *via* PPARγ stimulation (Palomares, et al., 2020). THCA also improved glucose tolerance and attenuated liver fibrosis in the HFD mice (Carmona-Hidalgo, et al., 2021). Using an *in vitro* COX-1/COX-2 assay it was determined that Δ^9-THCA inhibits both COX-1 and COX-2 enzymes with a concentration causing 50% inhibition (IC_{50}) in the high micromolar range (Ruhaak, et al., 2011). Nallathambi et al. (2017) reported that cannabis fractions containing high amounts of THCA produce anti-inflammatory effects (e.g., reduction in IL-8) in several colon epithelial cell lines and in colon tissue biopsies. Anti-inflammatory effects of THCA were inhibited by treatment with the GPR55 antagonist CID16020046, but not by the CB1 and CB2 receptor antagonist rimonabant and SR144528 (Nallathambi, et al., 2017). In addition to its anti-inflammatory properties, THCA also has anti-nausea and antiemetic properties in mice at doses much lower than Δ^9-THC (Rock, et al., 2013a). Thus, THCA may present a non-psychotropic alternative to Δ^9-THC for treating nausea and vomiting.

As discussed previously for CBC and CBG, THCA may also exhibit neuroprotective properties that could be beneficial in the treatment of neurodegenerative diseases. THCA improved neuronal viability through a PPARγ-dependent pathway in an *in vitro* model of Huntington's disease (Nadal, et al., 2017). THCA also caused an improvement in hind limb dystonia and locomotor activity in mice treated with 3-NPA. These neuroprotective actions of THCA were significantly reduced when mice were co-administered the PPARγ antagonist T0070903. In contrast, THCA had no effects on the survival of dopaminergic neurons in a 1-methyl-4-phenyl pyridinium (MPP^+) cell culture model of Parkinson's disease (Moldzio, et al., 2012). Since THCA undergoes decarboxylation to Δ^9-THC, it is possible that the reported neuroprotective effects of THCA in Huntington's disease may have resulted from contamination by Δ^9-THC (Sagredo, et al., 2011). In addition, THCA displays poor brain penetration properties when tested using two vehicles (vegetable oil and Tween 80) (Anderson, et al., 2019); a limitation that could reduce its clinical efficacy.

Anecdotal reports have long suggested that THCA acts as an anticonvulsant. Over 40 years ago Karler and Turkanis reported that THCA (200 mg/kg) reduces seizures in the mouse maximal electroshock test (Karler and Turkanis, 1979). In a more recent mouse study the anticonvulsant effects of THCA were found to vary depending on the seizure model utilized and whether Δ^9-THC was given along with the THCA (Benson, et al., 2020). When used alone, THCA (2, 30, and 100 mg/kg) was ineffective in the 6-Hz threshold (6-HzT) model of psychomotor seizures, but had anticonvulsant activity when given with Δ^9-THC. Conversely, THCA used alone or with Δ^9-THC did not reduce hyperthermia-induced seizures in the Scn1aRX/+ mice model (compared with the protective effects of CBDA described above).

More encouraging results were reported with THCA in a clinical study. The frequency and duration of seizures were reduced in four case reports of children using low doses of THCA (0.1–1 mg/kg per day) in conjunction with conventional antiepileptic drugs and full spectrum cannabis (Sulak, et al., 2017). In contrast, Epidiolex® (CBD), which is approved by the U.S. Food and Drug Administration (FDA) for treating epilepsy, is dosed from 5 to 25 mg/kg per day. Thus, THCA may be ten to hundred times more potent in reducing seizures. However, increased doses of THCA did not improve efficacy in this clinical study (Sulak, et al., 2017). Furthermore, formulations of THCA containing high levels of the terpenoid α-linalool were more efficacious than formulations containing low levels of the terpenoid. Thus, other components of the THCA formulation may have accounted for the beneficial effects. Finally, symptoms and seizure activity worsened in one patient after increasing the THCA dose (Sulak, et al., 2017).

Varinic Cannabinoids

Cannabidivarin

CBDV is found in landrace cannabis strains that have relatively high amounts of CBD and low amounts of Δ^9-THC. Prior to its isolation in 1969, it was assumed that all naturally occurring cannabinoids contained a pentyl side chain, rather than the propyl chain found in CBDV and THCV. CBDV displays low binding affinity for the CB1 and CB2 receptors (Rosenthaler, et al., 2014; Husni, et al., 2014). Consistent with this, high concentrations of CBDV are needed for CB1 receptor stimulation of (^{35}S)GTPγS binding, inhibition of cAMP synthesis and recruitment of βarr2 (Husni, et al., 2014; Navarro, et al., 2020; Zagzoog, et al., 2020). Overall, CBDV is a more potent and efficacious agonist at CB2 receptors (Navarro, et al., 2020; Zagzoog, et al., 2020). CBDV displays a similar pharmacological profile for TRP channels as CBN, CBG and THCV; activating TRPV1, TRPV2, TRPV3, TRPV4 and TRPA1 channels while inhibiting the TRPM8 channel (De Petrocellis, et al., 2008; De Petrocellis, et al., 2011). Other important off target sites for CBDV include the de-orphanized receptors GPR55 and GPR6. Binding of CBDV to the GPR55 receptor stimulates ERK1/2 phosphorylation and inhibits LPS-mediated signaling effects occurring through the GPR55 receptor (Anavi-Goffer, et al., 2012). These effects of CBDV are comparable to those of Δ^9-THC. GPR6 is a constitutively active receptor that couples to G_s to stimulate adenylyl cyclase and recruits βarr2. CBDV acts as an inverse agonist at the GPR6 receptor causing significant inhibition of βarr2 recruitment at concentrations of 1 and 10 μM (Laun, et al., 2018).

CBD (marketed as Epidiolex®) was approved by the U.S. Food and Drug Administration (FDA) in 2018 for preventing epileptic seizures in Lennox-Gastaut syndrome and Dravet syndrome in children. CBDV, a structural homolog of CBD, possesses anti-epileptic properties when tested in animals and humans. When examined *in vitro* in rat brain slices, CBDV attenuates epileptiform local field potentials induced by 4-amino pyridine (Hill, et al., 2012). *In vivo*, CBDV (200 mg/kg per day) significantly reduces PTZ-induced seizure activity in the rats (Hill, et al., 2012). However, when used alone, CBDV has no effect on pilocarpine-induced seizures, but requires the co-administration of valproate or phenobarbital to be effective. Consistent with this, Amada et al. (2013) reported that CBDV significantly decreases PTZ-induced seizure severity. In addition, CBDV suppresses the expression of several epilepsy-related genes in animals that respond to CBDV anti-epileptic treatment (Amada, et al., 2013). A human trial to assess the efficacy, safety, and tolerability of CBDV in adults with focal seizures was recently conducted by GW Pharmaceuticals, the maker of Epidiolex® (Brodie, et al., 2021). The drug GPW42006 (800 mg b.i.d.), containing CBDV as its major component, reduced the frequency of seizures by 41%. However, similar reductions in focal seizure frequency were observed in the CBDV and placebo (38%) groups. There was also no differences between CBDV and placebo groups for any specific seizure type. Therefore, higher doses and longer durations of treatment of GPW42006 will be needed in future clinical trials to better access the benefits of CBDV.

CBDV has been investigated as a treatment for symptoms associated with autism spectrum disorder (ASD) such as repetitive behaviors, cognitive challenges and issues with communication and social functioning (Mouro, et al., 2019). Mice carrying mutations in the MeCP2 gene and MeCP2 null mice develop Rett Syndrome (RTT), a neurodevelopment disease related to ASD. CBDV treatment (2 mg/kg) was found to rescue both behavioral and phenotypic changes in the RTT mice model (Vigli, et al., 2018). These CBDV effects included improvements in motor coordination, locomotion and brain weight. When administered using a life-long treatment schedule, CBDV also prolonged survival and delayed the appearance of neurological and motor deficits in MeCP2 null mice (Zamberletti, et al., 2019a). CBDV also reversed memory deficits in these mice. Similar behavioral improvements were reported using CBDV in a valproic acid-induced model of ASD (Zamberletti, et al., 2019b).

CBDV may also have utility in the treatment of Duchenne muscular dystrophy (DMD) and in preventing nausea. In a recent study, CBDV was found to improve muscle quality and locomotion and to slow muscle degeneration in male dystrophic mdx mice (Iannotti, et al., 2019). Muscle improvement by CBDV (and CBD) was accompanied by anti-inflammatory and pro-autophagic effects. Both CBDV (200 mg/kg) and THCV (20 mg/kg) are effective in reducing LiCl-induced conditioned gaping in rats (Rock, et al., 2013b). In contrast, CB1 receptor inverse agonists such as SR141716 and AM251 are known to enhance nausea. Thus, CBDV and THCV do not function *in vivo* as CB1 receptor inverse agonists. In conclusion, CBDV has Δ^9-THC-like, antiemetic effects in rodents consistent with a CB1 receptor agonist, but without the psychotropic activity of Δ^9-THC.

Tetrahydrocannabivarin

THCV is derived from cannabigerovarin acid (CBGVA), one of the two primary minor cannabinoid precursors, the other being CBGA (**Figure 1**). THCA synthase converts CBGVA to THCVA, which is then decarboxylated to the neutral compound THCV when exposed to heat or light (Hanus, et al., 2016). THCV is

typically found in very small amounts in cannabis flowers, though breeders are developing strains with higher concentrations. While THCV binds to both CB1 and CB2 receptors, its pharmacological effects remain controversial. In some studies, THCV has been reported to act as an antagonist/inverse agonist at the CB1 and CB2 receptors. In (^{35}S)GTPγS binding assays measured with rodent brain preparations, THCV acts as an antagonist to WIN-55,212-2 (Dennis, et al., 2008). In addition, THCV antagonizes CP-55,940-induced stimulation of (^{35}S)GTPγS binding in rodent brain and CHO cell membranes (Thomas, et al., 2005). In contrast, more recent studies using CHO cells have demonstrated that THCV functions as a partial agonist at the cannabinoid receptors to inhibit cAMP formation and to stimulate βarr2 recruitment (Zagzoog, et al., 2020). Computational docking experiments revealed that THCV interacts with the same residues as Δ^9-THC in the orthosteric site of the CB1 receptor (Jung, et al., 2018). However, the pentyl side chain of Δ^9-THC protrudes into a sub-pocket of the binding site. THCV containing a propyl side chain does not have this interaction (Jung, et al., 2018). This difference and the distinct binding energies of the two ligands might account for the higher affinity of Δ^9-THC for the CB1 receptor.

As with other minor cannabinoids, THCV has off target actions at TRP channels and 5-HT$_{1A}$ receptors. THCV acts as an agonist at TRPV1-4 and TRPA1 channels, while acting as an antagonist at the TRPM8 channel (De Petrocellis, et al., 2011). THCV (at 100 nM) was found to enhance (H^3)-8-OH-DPAT binding to the 5-HT$_{1A}$ receptor and to increase the potency of 8-OH-DPAT-stimulated (^{35}S)GTPγS binding to cell membranes (Cascio, et al., 2015). Thus, both THCV and CBDA appear to function as positive allosteric regulators of the 5-HT$_{1A}$ receptor.

THCV has been shown to reduce inflammation and inflammatory pain in mice. THCV attenuated signs of inflammation induced by intraplantar injection of carrageenan in mouse hind paws and reduced hyperalgesia from formalin hind paw injection (Bolognini, et al., 2010). Surprisingly, the ability of THCV to relieve formalin-induced hyperalgesia was significantly attenuated by both the CB1 receptor-selective antagonist rimonabant, and CB2 receptor-selective antagonist SR144528 (Bolognini, et al., 2010). Thus, when tested *in vivo*, THCV exhibits both CB1 and CB2 receptor agonist activity. These anti-inflammatory actions of THCV were further supported by *in vitro* experiments using peritoneal-derived macrophages (Romano, et al., 2016). THCV was found to suppress inflammatory pathways by down-regulating LPS-induced expression of iNOS, COX-2 and interleukin 1β. THCV mediated suppression of nitrite production (from nitric oxide) was prevented by pretreatment of the macrophages with SR144528.

THCV has also shown promise as an anti-epileptic agent and in the treatment of neurodegeneration in Parkinson's disease. Hill et al. (2010) used an extracellular multi-electrode array (MEA) assay to study the effects of THCV on spontaneous epileptiform bursting in rat brain slices. Pretreatment of the brain slices with THCV (20 μM) reduced burst complex incidence and the amplitude and frequency of paroxysmal depolarizing shifts (Hill, et al., 2010). In addition, in rats treated *in vivo* with pentylenetetrazole (PTZ) to induce seizures, THCV (0.25 mg/kg) reduced the incidence of seizures. Approximately 33% of animals treated with THCV exhibited a complete absence of PTZ seizures (compared with 13% of control treated rats). Garcia et al. (2011) reported that THCV reduces slow motor movements in rats with 6-hydroxydopamine (6-OHDA)-induced Parkinson's disease. In addition, 2 weeks of treatment with THCV reduced microglial activation and preserved nigrostriatal dopaminergic neurons in the 6-OHDA Parkinson's model (García, et al., 2011). Thus, THCV may be a useful treatment for Parkinson's disease by preventing neuronal degradation and alleviating associated symptoms.

THCV regulates blood glucose levels suggesting it might be useful in weight reduction and treating diabetes. In mice with dietary-induced obesity (DIO), THCV improved fasting plasma glucose and glucose tolerance in a dose-dependent manner (Wargent, et al., 2013). In addition, THCV increased insulin sensitivity in genetically (ob/ob) obese mice. While THCV increased energy expenditure in the DIO and ob/ob mice, it did not reduce food intake or overall body weight (Wargent, et al., 2013). This contrasts with a previous study where intraperitoneal administration of THCV caused hypophagia and weight loss in rodents (Riedel, et al., 2009). Importantly, a clinical trial evaluated the effects of THCV and CBD on 62 subjects with type 2 diabetes (Jadoon, et al., 2016). Although THCV had no effect on plasma HDL levels, it significantly decreased fasting plasma glucose levels and improved pancreatic β-cell function in the type 2 patients. In addition to these findings, THCV was reported to increase the response to aversive stimuli in humans in regions of the brain (amygdala, insula and mid orbitofrontal cortex) involved in food aversion (Tudge, et al., 2015). This suggests that THCV may aid in appetite suppression and weight loss without the side effects (depression, anxiety, etc.,) caused by the CB1 receptor antagonist rimonabant (Mitchell and Morris, 2007).

Various minor cannabinoids including THCV, CBC, CBG and CBDV have shown promise in the treatment of skin disorders and are being investigated for the treatment of atopic dermatitis, psoriasis, scleroderma, acne hair growth and pigmentation disorders, keratin diseases, skin tumors, and pruritus (Tubaro, et al., 2010; Oláh, et al., 2016; Tóth, et al., 2019). It is postulated that these cannabinoids produce anti-acne effects by regulating homeostatic sebaceous lipogenesis and by exerting anti-proliferative and anti-inflammatory actions. *In vitro* experiments have shown that THCV inhibits the proliferation of human SZ95 sebocytes (Oláh, et al., 2016). This anti-proliferative effect of THCV occurs through a CBD-like mechanism of action; increasing intracellular Ca^{2+} and stimulating ERK1/2 following TRPV4 channel activation. In addition, THCV exhibits powerful anti-inflammatory properties by reducing levels of arachidonic acid (AA), needed for lipogenesis (Oláh, et al., 2016). THCV also suppresses lipid synthesis in the sebaceous glands, providing relief to acne sufferers whose condition is triggered by excessive oil production (Oláh, et al., 2016; Tóth, et al., 2019). In conclusion, THCV and other minor cannabinoids will continue to be evaluated for the management of acne.

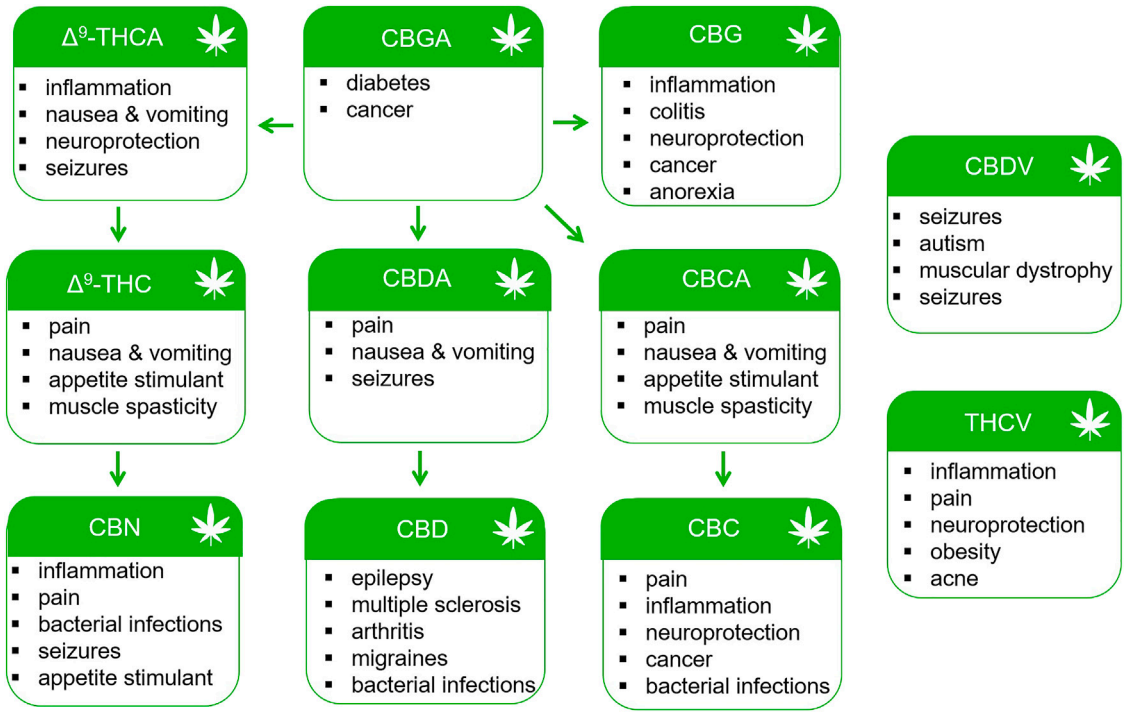


FIGURE 3 | Potential therapeutic uses of phytocannabinoids.

Other Minor Cannabinoids

Cannabitriol (CBT) and Δ^8-tetrahydrocannabinol (Δ^8-THC) are two rare cannabinoids that are gaining commercial popularity. Cannabitriol (CBT) was first isolated by Obata and Ishikawa, but its structure was not fully determined until 1977 (Obata and Ishikawa, 1966; ElSohly, et al., 1977). Although the pharmacology of CBT is largely unknown, recent virtual screening analysis of the estrogen receptor α (ER-α) indicate that CBT represents a novel estrogen antagonist that might be used for the prevention and treatment of breast cancer (Kikiowo, et al., 2021). Δ^8-THC is an isomer of Δ^9-THC that contains a double bond between carbon atoms 8 and 9. Unlike Δ^9-THC, Δ^8-THC is legally available in the U.S. through cannabis suppliers. The U.S. 2018 Farm Bill legalized cannabinoids such as CBD that are isolated from hemp. Since Δ^8-THC can be derived from CBD, it is currently considered a legal natural product. While Δ^8-THC displays roughly similar binding affinities as Δ^9-THC to the CB1 and CB2 receptors (Husni, et al., 2014), preclinical results suggest that it is less potent in producing euphoric, anti-emetic and appetite-stimulating effects (Järbe and Henriksson, 1973; Hine, et al., 1977).

CONCLUSION

Preclinical data and early clinical studies support the continued investigation of phytocannabinoids for the treatment of pain, inflammation, neurodegeneration, cancer and other disorders (**Figure 3**). Natural products have historically been valuable sources of novel compounds developed into pharmaceuticals. Such was the case with the isolation of salicin from the bark of the Willow tree and the subsequent synthesis of aspirin. Δ^9-THC (Dronabinol) is currently approved by the U.S. FDA for the treatment of nausea associated with cancer chemotherapy and as an appetite stimulant for patients with AIDS (Romero-Sandoval, et al., 2018; Fraguas-Sánchez and Torres-Suárez, 2018). Nabiximols (Sativex®) containing a mixture of Δ^9-THC and CBD from the cannabis plant is approved in Canada and Europe for the treatment of MS spasticity (Fraguas-Sánchez and Torres-Suárez, 2018). It is also indicated for the treatment of neuropathic pain in MS and for pain relief in patients with advanced cancer (Fraguas-Sánchez and Torres-Suárez, 2018). However, use of Δ^9-THC is associated with acute psychotropic effects including euphoria, sedation, anxiety, cognitive impairment, and in some patients, paranoia and hallucinations. Minor cannabinoids and their chemical homologs offer the potential medicinal benefits of Δ^9-THC without adverse effects. Recently, Δ^9-tetrahydrocannabiphorol (Δ^9-THCP) and cannabidihexol (CBDH), homologs of Δ^9-THC and CBD, were synthesized and shown to produce anti-nociceptive effects in mice at doses comparable to Δ^9-THC (Citti, et al., 2019; Linciano, et al., 2020). Future studies will need to evaluate the risk versus benefit of these and other minor cannabinoids when compared to Δ^9-THC and traditional analgesic drugs.

In addition to the CB1/CB2 receptors and "off target" binding sites described in this review, minor cannabinoids may bring about their pharmacological effects by interacting with other receptors and ion channels. Along with GPR55 and GPR18, de-orphanized receptors including GPR3, GPR6 and GPR12 are emerging as

possible targets for minor cannabinoids (Laun and Song, 2017; Brown, et al., 2017). These receptors are highly expressed in neuronal tissues and are postulated to participate in neuroprotection, anti-nociception and brain development. Although the affinity of these receptors for minor cannabinoids has not yet been examined, CBD is known to function as an inverse agonist at all three receptors. However, it is unclear whether CBD binds to an orthostatic site on the receptor or if it modifies receptor activity *via* an allosteric site. While TRP channel agonism/antagonism provides a major mechanism of action for many of the minor cannabinoids, voltage-gated ion channels, such Na^+ and Ca^{2+} channels are also regulated by cannabinoids. When tested in parathyroid cells, the synthetic cannabinoid WIN 55,212-2 and the endocannabinoid 2-AG reduce the peak Na^+ current and shift the voltage-dependence of Na^+ channel inactivation to more negative membrane potentials (Okada, et al., 2005). In addition, when applied at low micromolar concentrations, CBD inhibits the Na^+ current in heterologous cells expressing various Na^+ channel subunits ($Na_V1.1$, $Na_V1.3$ $Na_V1.6$, etc.,) (Ghovanloo, et al., 2018). CBD also inhibits T-type Ca^{2+} channels ($Ca_V3.x$) in mouse sensory neurons (Ross, et al., 2008). Whether CBD acts directly to regulate the conduction of the Na^+ and Ca^{2+} channels, or acts indirectly to alter the properties of the cell lipid membrane will require further investigation.

Advances in the bioengineering of cannabinoid synthesis enzymes in yeast and other microbial systems should expand the production of both natural and novel minor cannabinoids (Luo, et al., 2019). The ability to combine these cannabinoids with terpenes, flavonoids, polyphenols and other cannabis-based chemicals could create countless possibilities in the era of personalized healthcare. It is predicted that new cannabinoid products might be formulated to meet the therapeutic needs of different demographic groups and could be available in numerous delivery systems including topical creams, tablets, transdermal patches, vaporizers and more. Women represent one demographic group where cannabinoids could offer a variety of health care benefits. Cannabinoid receptors are ubiquitously distributed in reproductive tissues and AEA and the FAAH enzyme are found in the ovaries, oviducts and endometrium (Maia, et al., 2020). Cannabinoid-based suppositories containing Δ^9-THC and CBD are already available for relieving menstrual cramps, and as drug discovery progresses, natural and unnatural cannabinoids may prove effective for reproductive system issues, from endometriosis and fibroids to perimenopause symptoms. Of course, the effectiveness of these cannabis products must first be confirmed through large, randomized and controlled clinical trials. Much of our current knowledge of the medicinal benefits of minor cannabinoids has come from subjective and anecdotal patient reporting, rather than through rigorous clinical trials. In order to move forward, researchers, clinicians and regulatory officials will need to work together to ensure that phytocannabinoid products meet the necessary therapeutic and safety standards.

AUTHOR CONTRIBUTIONS

KW, AM, and AH have all made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

This work was supported by U.S. Public Health Service award NS-071530 and U.S. National Science Foundation award CBET-1606882 to KW.

REFERENCES

Adams, R., Hunt, M., and Clark, J. H. (1940a). Structure of Cannabidiol, a Product Isolated from the Marihuana Extract of Minnesota Wild Hemp. I. *J. Am. Chem. Soc.* 62, 196–200. doi:10.1021/ja01858a058

Adams, R., Pease, D. C., and Clark, J. H. (1940b). Isolation of Cannabinol, Cannabidiol and Quebrachitol from Red Oil of Minnesota Wild Hemp. *J. Am. Chem. Soc.* 62, 2194–2196. doi:10.1021/ja01865a080

Ahn, K. H., Mahmoud, M. M., Shim, J. Y., and Kendall, D. A. (2013). Distinct Roles of β-arrestin 1 and β-arrestin 2 in ORG27569-Induced Biased Signaling and Internalization of the Cannabinoid Receptor 1 (CB1). *J. Biol. Chem.* 288, 9790–9800. doi:10.1074/jbc.M112.438804

Aiken, C. T., Tobin, A. J., and Schweitzer, E. S. (2004). A Cell-Based Screen for Drugs to Treat Huntington's Disease. *Neurobiol. Dis.* 16, 546–555. doi:10.1016/j.nbd.2004.04.001

Alves, V. L., Gonçalves, J. L., Aguiar, J., Teixeira, H. M., and Câmara, J. S. (2020). The Synthetic Cannabinoids Phenomenon: from Structure to Toxicological Properties. A Review. *Crit. Rev. Toxicol.* 50, 359–382. doi:10.1080/10408444.2020.1762539

Amada, N., Yamasaki, Y., Williams, C. M., and Whalley, B. J. (2013). Cannabidivarin (CBDV) Suppresses Pentylenetetrazole (PTZ)-induced Increases in Epilepsy-Related Gene Expression. *PeerJ* 1, e214. doi:10.7717/peerj.214

Anavi-Goffer, S., Baillie, G., Irving, A. J., Gertsch, J., Greig, I. R., Pertwee, R. G., et al. (2012). Modulation of L-α-lysophosphatidylinositol/GPR55 Mitogen-Activated Protein Kinase (MAPK) Signaling by Cannabinoids. *J. Biol. Chem.* 287, 91–104. doi:10.1074/jbc.M111.296020

Anderson, L. L., Low, I. K., Banister, S. D., McGregor, I. S., and Arnold, J. C. (2019). Pharmacokinetics of Phytocannabinoid Acids and Anticonvulsant Effect of Cannabidiolic Acid in a Mouse Model of Dravet Syndrome. *J. Nat. Prod.* 82, 3047–3055. doi:10.1021/acs.jnatprod.9b00600

Anis, O., Vinayaka, A. C., Shalev, N., Namdar, D., Nadarajan, S., Anil, S. M., et al. (2021). Cannabis-Derived Compounds Cannabichromene and Δ9-Tetrahydrocannabinol Interact and Exhibit Cytotoxic Activity against Urothelial Cell Carcinoma Correlated with Inhibition of Cell Migration and Cytoskeleton Organization. *Molecules* 26, 465. doi:10.3390/molecules26020465

Appendino, G., Gibbons, S., Giana, A., Pagani, A., Grassi, G., Stavri, M., et al. (2008). Antibacterial Cannabinoids from Cannabis Sativa: a Structure-Activity Study. *J. Nat. Prod.* 71, 1427–1430. doi:10.1021/np8002673

Banister, S. D., Arnold, J. C., Connor, M., Glass, M., and McGregor, I. S. (2019). Dark Classics in Chemical Neuroscience: Δ9-Tetrahydrocannabinol. *ACS Chem. Neurosci.* 10, 2160–2175. doi:10.1021/acschemneuro.8b00651

Benson, M. J., Anderson, L. L., Low, I. K., Luo, J. L., Kevin, R. C., Zhou, C., et al. (2020). Evaluation of the Possible Anticonvulsant Effect of delta9-tetrahydrocannabinolic Acid in Murine Seizure Models. *Cannabis Cannabinoid Res.* online ahead of print.

Bolognini, D., Costa, B., Maione, S., Comelli, F., Marini, P., Di Marzo, V., et al. (2010). The Plant Cannabinoid delta9-tetrahydrocannabivarin Can Decrease Signs of Inflammation and Inflammatory Pain in Mice. *Br. J. Pharmacol.* 160, 677–687. doi:10.1111/j.1476-5381.2010.00756.x

Bolognini, D., Rock, E. M., Cluny, N. L., Cascio, M. G., Limebeer, C. L., Duncan, M., et al. (2013). Cannabidiolic Acid Prevents Vomiting in Suncus Murinus and Nausea-Induced Behaviour in Rats by Enhancing 5-HT1A Receptor Activation. *Br. J. Pharmacol.* 168, 1456–1470. doi:10.1111/bph.12043

Borrelli, F., Fasolino, I., Romano, B., Capasso, R., Maiello, F., Coppola, D., et al. (2013). Beneficial Effect of the Non-psychotropic Plant Cannabinoid Cannabigerol on Experimental Inflammatory Bowel Disease. *Biochem. Pharmacol.* 85, 1306–1316. doi:10.1016/j.bcp.2013.01.017

Borrelli, F., Pagano, E., Romano, B., Panzera, S., Maiello, F., Coppola, D., et al. (2014). Colon Carcinogenesis Is Inhibited by the TRPM8 Antagonist Cannabigerol, a Cannabis-Derived Non-psychotropic Cannabinoid. *Carcinogenesis* 35, 2787–2797. doi:10.1093/carcin/bgu205

Bouaboula, M., Poinot-Chazel, C., Bourrié, B., Canat, X., Calandra, B., Rinaldi-Carmona, M., et al. (1995). Activation of Mitogen-Activated Protein Kinases by Stimulation of the central Cannabinoid Receptor CB1. *Biochem. J.* 312 (Pt 2), 637–641. doi:10.1042/bj3120637

Brierley, D. I., Samuels, J., Duncan, M., Whalley, B. J., and Williams, C. M. (2017). A Cannabigerol-Rich Cannabis Sativa Extract, Devoid of Δ9-tetrahydrocannabinol, Elicits Hyperphagia in Rats. *Behav. Pharmacol.* 28, 280–284. doi:10.1097/fbp.0000000000000285

Brodie, M. J., Czapinski, P., Pazdera, L., Sander, J. W., Toledo, M., Napoles, M., et al. (2021). A Phase 2 Randomized Controlled Trial of the Efficacy and Safety of Cannabidivarin as Add-On Therapy in Participants with Inadequately Controlled Focal Seizures. *Cannabis Cannabinoid Res.* online ahead of print. doi:10.1089/can.2020.0075

Brown, K. J., Laun, A. S., and Song, Z. H. (2017). Cannabidiol, a Novel Inverse Agonist for GPR12. *Biochem. Biophys. Res. Commun.* 493, 451–454. doi:10.1016/j.bbrc.2017.09.001

Carmona-Hidalgo, B., González-Mariscal, I., García-Martín, A., Prados, M. E., Ruiz-Pino, F., Appendino, G., et al. (2021). Δ9-Tetrahydrocannabinolic Acid Markedly Alleviates Liver Fibrosis and Inflammation in Mice. *Phytomedicine* 81, 153426. doi:10.1016/j.phymed.2020.153426

Carrillo-Salinas, F. J., Navarrete, C., Mecha, M., Feliú, A., Collado, J. A., Cantarero, I., et al. (2014). A Cannabigerol Derivative Suppresses Immune Responses and Protects Mice from Experimental Autoimmune Encephalomyelitis. *PLoS ONE* 9, e94733. doi:10.1371/journal.pone.0094733

Cascio, M. G., Gauson, L. A., Stevenson, L. A., Ross, R. A., and Pertwee, R. G. (2010). Evidence that the Plant Cannabinoid Cannabigerol Is a Highly Potent Alpha2-Adrenoceptor Agonist and Moderately Potent 5HT1A Receptor Antagonist. *Br. J. Pharmacol.* 159, 129–141. doi:10.1111/j.1476-5381.2009.00515.x

Cascio, M. G., Zamberletti, E., Marini, P., Parolaro, D., and Pertwee, R. G. (2015). The Phytocannabinoid, Δ9-tetrahydrocannabivarin, Can Act through 5-HT1Areceptors to Produce Antipsychotic Effects. *Br. J. Pharmacol.* 172, 1305–1318. doi:10.1111/bph.13000

Chesner, G. B., Jackson, D. M., and Stamer, G. A. (1974). Interaction of Cannabis and General Anesthetic Agents in Mice. *Br. J Pharmacol* 50, 593–599.

Citti, C., Linciano, P., Russo, F., Luongo, L., Iannotta, M., Maione, S., et al. (2019). A Novel Phytocannabinoid Isolated from Cannabis Sativa L. With an *In Vivo* Cannabimimetic Activity Higher Than Δ9-tetrahydrocannabinol: Δ9-Tetrahydrocannabiphorol. *Sci. Rep.* 9, 20335. doi:10.1038/s41598-019-56785-1

Corroon, J. (2021). Cannabinol and Sleep: Separating Fact from Fiction. *Cannabis Cannabinoid Res6*, 366–371. doi:10.1089/can.2021.0006

Covelo, A., Eraso-Pichot, A., Fernández-Moncada, I., Serrat, R., and Marsicano, G. (2021). CB1R-dependent Regulation of Astrocyte Physiology and Astrocyte-Neuron Interactions. *Neuropharmacol* 195, 108678. doi:10.1089/can.2021.0006

D'Aniello, E., Fellous, T., Iannotti, F. A., Gentile, A., Allarà, M., Balestrieri, F., et al. (2019). Identification and Characterization of Phytocannabinoids as Novel Dual PPARα/γ Agonists by a Computational and *In Vitro* Experimental Approach. *Biochim. Biophys. Acta Gen. Subj* 1863, 586–597. doi:10.1016/j.bbagen.2019.01.002

de Almeida, D. L., and Devi, L. A. (2020). Diversity of Molecular Targets and Signaling Pathways for CBD. *Pharmacol. Res. Perspect.* 8, e00682. doi:10.1002/prp2.682

de Meijer, E. P. M., Hammond, K. M., and Micheler, M. (2009). The Inheritance of Chemical Phenotype in Cannabis Sativa L. (III): Variation in Cannabichromene Proportion. *Euphytica* 165, 293–311. doi:10.1007/s10681-008-9787-1

de Meijer, E. P. M., and Hammond, K. M. (2005). The Inheritance of Chemical Phenotype in Cannabis Sativa L. (II): Cannabigerol Predominant Plants. *Euphytica* 145, 189–198. doi:10.1007/s10681-005-1164-8

De Petrocellis, L., Ligresti, A., Moriello, A. S., Allarà, M., Bisogno, T., Petrosino, S., et al. (2011). Effects of Cannabinoids and Cannabinoid-Enriched Cannabis Extracts on TRP Channels and Endocannabinoid Metabolic Enzymes. *Br. J. Pharmacol.* 163, 1479–1494. doi:10.1111/j.1476-5381.2010.01166.x

De Petrocellis, L., Melck, D., Palmisano, A., Bisogno, T., Laezza, C., Bifulco, M., et al. (1998). The Endogenous Cannabinoid Anandamide Inhibits Human Breast Cancer Cell Proliferation. *Proc. Natl. Acad. Sci. U S A.* 95, 8375–8380. doi:10.1073/pnas.95.14.8375

De Petrocellis, L., Schiano Moriello, A., Imperatore, R., Cristino, L., Starowicz, K., and Di Marzo, V. (2012). A Re-evaluation of 9-HODE Activity at TRPV1 Channels in Comparison with Anandamide: Enantioselectivity and Effects at Other TRP Channels and in Sensory Neurons. *Br. J. Pharmacol.* 167, 1643–1651. doi:10.1111/j.1476-5381.2012.02122.x

De Petrocellis, L., Starowicz, K., Moriello, A. S., Vivese, M., Orlando, P., and Di Marzo, V. (2007). Regulation of Transient Receptor Potential Channels of Melastatin Type 8 (TRPM8): Effect of cAMP, Cannabinoid CB(1) Receptors and Endovanilloids. *Exp. Cel Res* 313, 1911–1920. doi:10.1016/j.yexcr.2007.01.008

De Petrocellis, L., Vellani, V., Schiano-Moriello, A., Marini, P., Magherini, P. C., Orlando, P., et al. (2008). Plant-derived Cannabinoids Modulate the Activity of Transient Receptor Potential Channels of Ankyrin Type-1 and Melastatin Type-8. *J. Pharmacol. Exp. Ther.* 325, 1007–1015. doi:10.1124/jpet.107.134809

DeLong, G. T., Wolf, C. E., Poklis, A., and Lichtman, A. H. (2010). Pharmacological Evaluation of the Natural Constituent of Cannabis Sativa, Cannabichromene and its Modulation by Δ(9)-tetrahydrocannabinol. *Drug Alcohol Depend* 112, 126–133. doi:10.1016/j.drugalcdep.2010.05.019

Dennis, I., Whalley, B. J., and Stephens, G. J. (2008). Effects of Delta9-tetrahydrocannabivarin on [35S]GTPgammaS Binding in Mouse Brain Cerebellum and Piriform Cortex Membranes. *Br. J. Pharmacol.* 154, 1349–1358. doi:10.1038/bjp.2008.190

Derkinderen, P., Valjent, E., Toutant, M., Corvol, J. C., Enslen, H., Ledent, C., et al. (2003). Regulation of Extracellular Signal-Regulated Kinase by Cannabinoids in hippocampus. *J. Neurosci.* 23, 2371–2382. doi:10.1523/jneurosci.23-06-02371.2003

Devane, W. A., Dysarz, F. A., Johnson, M. R., Melvin, L. S., and Howlett, A. C. (1988). Determination and Characterization of a Cannabinoid Receptor in Rat Brain. *Mol. Pharmacol.* 34, 605–613.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., et al. (1992). Isolation and Structure of a Brain Constituent that Binds to the Cannabinoid Receptor. *Science* 258, 1946–1949. doi:10.1126/science.1470919

ElSohly, M. A., El-Feraly, F. S., and Turner, C. E. (1977). Isolation and Characterization of (+)-cannabitriol and (-)-10-Ethoxy-9-Hydroxy-delta 6a [10a]-Tetrahydrocannabinol: Two New Cannabinoids from *Cannabis Sativa* L. Extract. *Lloydia* 40, 275–280.

ElSohly, M. A., Harland, E., Murphy, J. C., Wirth, P., and Waller, C. W. (1981). Cannabinoids in Glaucoma: A Primary Screening Procedure. *J. Clin. Pharmacol.* 21, 472S–478S. doi:10.1002/j.1552-4604.1981.tb02627.x

Farrimond, J. A., Whalley, B. J., and Williams, C. M. (2012a). Cannabinol and Cannabidiol Exert Opposing Effects on Rat Feeding Patterns. *Psychopharmacology (Berl)* 223, 117–129. doi:10.1007/s00213-012-2697-x

Farrimond, J. A., Whalley, B. J., and Williams, C. M. (2012b). Non-Δ^9tetrahydrocannabinol Phytocannabinoids Stimulate Feeding in Rats. *Behav. Pharmacol.* 23, 113–117. doi:10.1097/FBP.0b013e32834ed832

Fernandes, M., Schabarek, A., Coper, H., and Hill, R. (1974). Modification of delta9-THC-actions by Cannabinol and Cannabidiol in the Rat. *Psychopharmacologia* 38, 329–338. doi:10.1007/BF00429130

Finlay, D. B., Sircombe, K. J., Nimick, M., Jones, C., and Glass, M. (2020). Terpenoids from Cannabis Do Not Mediate an Entourage Effect by Acting at Cannabinoid Receptors. *Front. Pharmacol.* 11, 359. doi:10.3389/fphar.2020.00359

Fraguas-Sánchez, A. I., and Torres-Suárez, A. I. (2018). Medical Use of Cannabinoids. *Drugs* 78, 1665–1703.

Galiègue, S., Mary, S., Marchand, J., Dussossoy, D., Carrière, D., Carayon, P., et al. (1995). Expression of central and Peripheral Cannabinoid Receptors in Human

Immune Tissues and Leukocyte Subpopulations. *Eur. J. Biochem.* 232, 54–61. doi:10.1111/j.1432-1033.1995.tb20780.x

Galve-Roperh, I., Rueda, D., Gómez del Pulgar, T., Velasco, G., and Guzmán, M. (2002). Mechanism of Extracellular Signal-Regulated Kinase Activation by the CB(1) Cannabinoid Receptor. *Mol. Pharmacol.* 62, 1385–1392. doi:10.1124/mol.62.6.1385

Gao, Q., Hanh, J., Váradi, L., Cairns, R., Sjöström, H., Liao, V. W., et al. (2015). Identification of Dual PPARα/γ Agonists and Their Effects on Lipid Metabolism. *Bioorg. Med. Chem.* 23, 7676–7684. doi:10.1016/j.bmc.2015.11.013

Gaoni, Y., and Mechoulam, R. (1971). The Isolation and Structure of delta-1-tetrahydrocannabinol and Other Neutral Cannabinoids from Hashish. *J. Am. Chem. Soc.* 93, 217–224. doi:10.1021/ja00730a036

Gaoni, Y., and Mechoulam, R. (1966). Cannabichromene, a New Active Principle in Hashish. *Chem. Commun. (London)* 1, 20–21. doi:10.1039/c19660000020

García, C., Palomo-Garo, C., García-Arencibia, M., Ramos, J. A., Pertwee, R. G., and Fernández-Ruiz, J. (2011). Symptom-relieving and Neuroprotective Effects of the Phytocannabinoid delta9-THCV in Animal Models of Parkinson's Disease. *Br. J Pharmacol* 163, 1495–1506.

Garfinkel, A. R., Otten, M., and Crawford, S. (2021). SNP in Potentially Defunct Tetrahydrocannabinolic Acid Synthase Is a Marker for Cannabigerolic Acid Dominance in *Cannabis Sativa L. Genes (Basel)* 12, 228. doi:10.3390/genes12020228

Ghovanloo, M. R., Shuart, N. G., Mezeyova, J., Dean, R. A., Ruben, P. C., and Goodchild, S. J. (2018). Inhibitory Effects of Cannabidiol on Voltage-dependent Sodium Currents. *J. Biol. Chem.* 293, 16546–16558. doi:10.1074/jbc.RA118.004929

Glass, M., and Northup, J. K. (1999). Agonist Selective Regulation of G Proteins by Cannabinoid CB(1) and CB(2) Receptors. *Mol. Pharmacol.* 56, 1362–1369. doi:10.1124/mol.56.6.1362

Goerl, B., Watkins, S., Metcalf, C., Smith, M., and Beenhakker, M. (2021). Cannabidiolic Acid Exhibits Entourage-like Improvements of Anticonvulsant Activity in an Acute Rat Model of Seizures. *Epilepsy Res.* 169, 106525. doi:10.1016/j.eplepsyres.2020.106525

Gonsiorek, W., Lunn, C., Fan, X., Narula, S., Lundell, D., and Hipkin, R. W. (2000). Endocannabinoid 2-arachidonyl Glycerol Is a Full Agonist through Human Type 2 Cannabinoid Receptor: Antagonism by Anandamide. *Mol. Pharmacol.* 57, 1045–1050.

Gugliandolo, A., Pollastro, F., Grassi, G., Bramanti, P., and Mazzon, E. (2018). *In Vitro* model of Neuroinflammation: Efficacy of Cannabigerol, a Non-psychoactive Cannabinoid. *Int. J. Mol. Sci.* 19, 1992. doi:10.3390/ijms19071992

Gülck, T., and Möller, B. L. (2020). Phytocannabinoids: Origins and Biosynthesis. *Trends Plant Sci.* 25, 985–1004.

Guo, J., and Ikeda, S. R. (2004). Endocannabinoids Modulate N-type Calcium Channels and G-Protein-Coupled Inwardly Rectifying Potassium Channels via CB1 Cannabinoid Receptors Heterologously Expressed in Mammalian Neurons. *Mol. Pharmacol.* 65, 665–674. doi:10.1124/mol.65.3.665

GW PHARMA LTD (2015). Use of Cannabinoids in the Treatment of Epilepsy. *Patent*. WO2017025712A1.

Hanus, L. O., Meyer, S. M., Muñoz, E., Taglialatela-Scafati, O., and Appendino, G. (2016). Phytocannabinoids: a Unified Critical Inventory. *Nat. Prod. Rep.* 33, 1357–1392.

Henstidge, C. M., Balenga, N. A. B., Ford, L. A., Ross, R. A., Waldhoer, M., and Irving, A. J. (2009). The GPR55 Ligand L-α-Lysophosphatidylinositol Promotes RhoA-dependent Ca^{2+} Signaling and NFAT Activation. *FASEB J.* 23, 183–193.

Herkenham, M., Lynn, A. B., Little, M. D., Johnson, M. R., Melvin, L. S., de Costa, B. R., et al. (1990). Cannabinoid Receptor Localization in Brain. *Proc. Natl. Acad. Sci. U S A.* 87, 1932–1936. doi:10.1073/pnas.87.5.1932

Hill, A. J., Mercier, M. S., Hill, T. D., Glyn, S. E., Jones, N. A., Yamasaki, Y., et al. (2012). Cannabidivarin Is Anticonvulsant in Mouse and Rat. *Br. J. Pharmacol.* 167, 1629–1642. doi:10.1111/j.1476-5381.2012.02207.x

Hill, A. J., Weston, S. E., Jones, N. A., Smith, I., Bevan, S. A., Williamson, E. M., et al. (2010). Δ^9-Tetrahydrocannabivarin Suppresses *In Vitro* Epileptiform and *In Vivo* Seizure Activity in Adult Rats. *Epilepsia* 51, 1522–1532. doi:10.1111/j.1528-1167.2010.02523.x

Hillig, K. W., and Mahlberg, P. G. (2004). A Chemotaxonomic Analysis of Cannabinoid Variation in Cannabis (Cannabaceae). *Am. J. Bot.* 91, 966–975. doi:10.3732/ajb.91.6.966

Hine, B., Torreelio, M., and Gershon, S. (1977). Analgesic, Heart Rate, and Temperature Effects of delta8-THC during Acute and Chronic Administration to Conscious Rats. *Pharmacology* 15, 63–72. doi:10.1159/000136664

Howlett, A. C., Qualy, J. M., and Khachatrian, L. L. (1986). Involvement of Gi in the Inhibition of Adenylate Cyclase by Cannabimimetic Drugs. *Mol. Pharmacol.* 29, 307–313.

Hua, T., Vemuri, K., Nikas, S. P., Laprairie, R. B., Wu, Y., Qu, L., et al. (2017). Crystal Structures of Agonist-Bound Human Cannabinoid Receptor CB1. *Nature* 547, 468–471. doi:10.1038/nature23272

Husni, A. S., McCurdy, C. R., Radwan, M. M., Ahmed, S. A., Slade, D., Ross, S. A., et al. (2014). Evaluation of Phytocannabinoids from High Potency Cannabis Sativa Using *In Vitro* Bioassays to Determine Structure-Activity Relationships for Cannabinoid Receptor 1 and Cannabinoid Receptor 2. *Med. Chem. Res.* 23, 4295–4300. doi:10.1007/s00044-014-0972-6

Iannotti, F. A., Pagano, E., Moriello, A. S., Alvino, F. G., Sorrentino, N. C., D'Orsi, L., et al. (2019). Effects of Non-euphoric Plant Cannabinoids on Muscle Quality and Performance of Dystrophic Mdx Mice. *Br. J. Pharmacol.* 176, 1568–1584. doi:10.1111/bph.14460

Izzo, A. A., Capasso, R., Aviello, G., Borrelli, F., Romano, B., Piscitelli, F., et al. (2012). Inhibitory Effect of Cannabichromene, a Major Non-psychotropic Cannabinoid Extracted from Cannabis Sativa, on Inflammation-Induced Hypermotility in Mice. *Br. J. Pharmacol.* 166, 1444–1460. doi:10.1111/j.1476-5381.2012.01879.x

Jadoon, K. A., Ratcliffe, S. H., Barrett, D. A., Thomas, E. L., Stott, C., Bell, J. D., et al. (2016). Efficacy and Safety of Cannabidiol and Tetrahydrocannabivarin on Glycemic and Lipid Parameters in Patients with Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled, Parallel Group Pilot Study. *Diabetes Care* 39, 1777–1786. doi:10.2337/dc16-0650

Jan, T. R., Farraj, A. K., Harkema, J. R., and Kaminski, N. E. (2003). Attenuation of the Ovalbumin-Induced Allergic Airway Response by Cannabinoid Treatment in A/J Mice. *Toxicol. Appl. Pharmacol.* 188, 24–35. doi:10.1016/s0041-008x(03)00010-3

Järbe, T. U., and Henriksson, B. G. (1973). Effects of delta8-THC, and delta9-THC on the Acquisition of a Discriminative Positional Habit in Rats. *Psychopharmacologia* 31, 321–332.

Jin, W., Brown, S., Roche, J. P., Hsieh, C., Celver, J. P., Kovoor, A., et al. (1999). Distinct Domains of the CB1 Cannabinoid Receptor Mediate Desensitization and Internalization. *J. Neurosci.* 19, 3773–3780. doi:10.1523/jneurosci.19-10-03773.1999

Jung, S. W., Cho, A. E., and Yu, W. (2018). Exploring the Ligand Efficacy of Cannabinoid Receptor 1 (CB1) Using Molecular Dynamics Simulations. *Sci. Rep.* 8, 13787. doi:10.1038/s41598-018-31749-z

Karler, R., and Turkanis, S. A. (1979). "Cannabis and Epilepsy," in *Marihuana Biological Effects*. Editors G. G. Nahas and W. D. M. Paton (Pergamon), 619–641. doi:10.1016/b978-0-08-023759-6.50052-4

Karniol, I. G., Shirakawa, I., Takahashi, R. N., Knobel, E., and Musty, R. E. (1975). Effects of delta9-tetrahydrocannabinol and Cannabinol in Man. *Pharmacology* 13, 502–512. doi:10.1159/000136944

Khan, S. P., Pickens, T. A., and Berlau, D. J. (2019). Perspectives on Cannabis as a Substitute for Opioid Analgesics. *Pain Manag.* 9, 191–203. doi:10.2217/pmt-2018-0051

Kikiowo, B., Ogunleye, A. J., Iwaloye, O., Ijatuyi, T., Adelakun, N. S., and Alashe, W. O. (2021). Induced Fit Docking and Automated QSAR Studies Reveal the ER-α Inhibitory Activity of *Cannabis Sativa* in Breast Cancer. *Recent Pat Anticancer Drug Discov.* 16, 1–12. doi:10.2174/1574892816666210201115359

Kohno, M., Hasegawa, H., Inoue, A., Muraoka, M., Miyazaki, T., Oka, K., et al. (2006). Identification of N-Arachidonylglycine as the Endogenous Ligand for Orphan G-Protein-Coupled Receptor GPR18. *Biochem. Biophys. Res. Commun.* 347, 827–832. doi:10.1016/j.bbrc.2006.06.175

Kumar, K., Shalev-Benami, M., Robertson, M. J., Hu, H., Banister, S. D., Hollingsworth, S. A., et al. (2019). Structure of a Signaling Cannabinoid Receptor 1-G Protein Complex. *Cell* 176, 448–e12. doi:10.1016/j.cell.2018.11.040

Lauckner, J. E., Hille, B., and Mackie, K. (2005). The Cannabinoid Agonist WIN55,212-2 Increases Intracellular Calcium via CB1 Receptor Coupling to Gq/11 G Proteins. *Proc. Natl. Acad. Sci. U S A.* 102, 19144–19149. doi:10.1073/pnas.0509588102

Laun, A. S., Shrader, S. H., and Song, Z. H. (2018). Novel Inverse Agonists for the Orphan G Protein-Coupled Receptor 6. *Heliyon* 4, e00933. doi:10.1016/j.heliyon.2018.e00933

Laun, A. S., and Song, Z. H. (2017). GPR3 and GPR6, Novel Molecular Targets for Cannabidiol. *Biochem. Biophys. Res. Commun.* 490, 17–21. doi:10.1016/j.bbrc.2017.05.165

Ligresti, A., Moriello, A. S., Starowicz, K., Matias, I., Pisanti, S., De Petrocellis, L., et al. (2006). Antitumor Activity of Plant Cannabinoids with Emphasis on the Effect of Cannabidiol on Human Breast Carcinoma. *J. Pharmacol. Exp. Ther.* 318, 1375–1387. doi:10.1124/jpet.106.105247

Limebeer, C. L., Abdullah, R. A., Rock, E. M., Imhof, E., Wang, K., Lichtman, A. H., et al. (2014). Attenuation of Anticipatory Nausea in a Rat Model of Contextually Elicited Conditioned Gaping by Enhancement of the Endocannabinoid System. *Psychopharmacology (Berl)* 231, 603–612. doi:10.1007/s00213-013-3282-7

Linciano, P., Citti, C., Russo, F., Tolomeo, F., Laganà, A., Capriotti, A. L., et al. (2020). Identification of a New Cannabidiol N-Hexyl Homolog in a Medicinal Cannabis Variety with an Antinociceptive Activity in Mice: Cannabidihexol. *Sci. Rep.* 10, 22019. doi:10.1038/s41598-020-79042-2

Lu, H. C., and Mackie, K. (2016). An Introduction to the Endogenous Cannabinoid System. *Biol. Psychiatry* 79, 516–525. doi:10.1016/j.biopsych.2015.07.028

Luk, T., Jin, W., Zvonok, A., Lu, D., Lin, X. Z., Chavkin, C., et al. (2004). Identification of a Potent and Highly Efficacious, yet Slowly Desensitizing CB1 Cannabinoid Receptor Agonist. *Br. J. Pharmacol.* 142, 495–500. doi:10.1038/sj.bjp.0705792

Luo, X., Reiter, M. A., d'Espaux, L., Wong, J., Denby, C. M., Lechner, A., et al. (2019). Complete Biosynthesis of Cannabinoids and Their Unnatural Analogues in Yeast. *Nature* 567, 123–126. doi:10.1038/s41586-019-0978-9

Mackie, K., Lai, Y., Westenbroek, R., and Mitchell, R. (1995). Cannabinoids Activate an Inwardly Rectifying Potassium Conductance and Inhibit Q-type Calcium Currents in AtT20 Cells Transfected with Rat Brain Cannabinoid Receptor. *J. Neurosci.* 15, 6552–6561. doi:10.1523/jneurosci.15-10-06552.1995

Mahadevan, A., Siegel, C., Martin, B. R., Abood, M. E., Beletskaya, I., and Razdan, R. K. (2000). Novel Cannabinol Probes for CB1 and CB2 Cannabinoid Receptors. *J. Med. Chem.* 43, 3778–3785. doi:10.1021/jm0001572

Maia, J., Fonseca, B. M., Teixeira, N., and Correia-da-Silva, G. (2020). The Fundamental Role of the Endocannabinoid System in Endometrium and Placenta: Implications in Pathophysiological Aspects of Uterine and Pregnancy Disorders. *Hum. Reprod. Update* 26, 586–602. doi:10.1093/humupd/dmaa005

Maione, S., Piscitelli, F., Gatta, L., Vita, D., De Petrocellis, L., Palazzo, E., et al. (2011). Non-psychoactive Cannabinoids Modulate the Descending Pathway of Antinociception in Anaesthetized Rats through Several Mechanisms of Action. *Br. J. Pharmacol.* 162, 584–596. doi:10.1111/j.1476-5381.2010.01063.x

Martin, B. R., Jefferson, R., Winckler, R., Wiley, J. L., Huffman, J. W., Crocker, P. J., et al. (1999). Manipulation of the Tetrahydrocannabinol Side Chain Delineates Agonists, Partial Agonists, and Antagonists. *J. Pharmacol. Exp. Ther.* 290, 1065–1079.

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., and Bonner, T. I. (1990). Structure of a Cannabinoid Receptor and Functional Expression of the Cloned cDNA. *Nature* 346, 561–564. doi:10.1038/346561a0

McPartland, J. M., MacDonald, C., Young, M., Grant, P. S., Furkert, D. P., and Glass, M. (2017). Affinity and Efficacy Studies of Tetrahydrocannabinolic Acid A at Cannabinoid Receptor Types One and Two. *Cannabis Cannabinoid Res.* 2, 87–95. doi:10.1089/can.2016.0032

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., et al. (1995). Identification of an Endogenous 2-monoglyceride, Present in Canine Gut, that Binds to Cannabinoid Receptors. *Biochem. Pharmacol.* 50, 83–90. doi:10.1016/0006-2952(95)00109-d

Mechoulam, R., and Gaoni, Y. (1965). Hashish. IV. The Isolation and Structure of Cannabinolic Cannabidiolic and Cannabigerolic Acids. *Tetrahedron* 21, 1223–1229. doi:10.1016/0040-4020(65)80064-3

Mechoulam, R., and Shvo, Y. (1963). Hashish. I. The Structure of Cannabidiol. *Tetrahedron* 19, 2073–2078. doi:10.1016/0040-4020(63)85022-x

Mitchell, P. B., and Morris, M. J. (2007). Depression and Anxiety with Rimonabant. *Lancet* 370, 1671–1672. doi:10.1016/S0140-6736(07)61705-X

Moldzio, R., Pacher, T., Krewenka, C., Kranner, B., Novak, J., Duvigneau, J. C., et al. (2012). Effects of Cannabinoids Δ(9)-tetrahydrocannabinol, Δ(9)-tetrahydrocannabinolic Acid and Cannabidiol in MPP+ Affected Murine Mesencephalic Cultures. *Phytomedicine* 19, 819–824. doi:10.1016/j.phymed.2012.04.002

Mouro, F. M., Miranda-Lourenço, C., Sebastião, A. M., and Diógenes, M. J. (2019). From Cannabinoids and Neurosteroids to Statins and the Ketogenic Diet: New Therapeutic Avenues in Rett Syndrome? *Front. Neurosci.* 13, 680. doi:10.3389/fnins.2019.00680

Munro, S., Thomas, K. L., and Abu-Shaar, M. (1993). Molecular Characterization of a Peripheral Receptor for Cannabinoids. *Nature* 365, 61–65. doi:10.1038/365061a0

Nadal, X., Del Río, C., Casano, S., Palomares, B., Ferreiro-Vera, C., Navarrete, C., et al. (2017). Tetrahydrocannabinolic Acid Is a Potent PPARγ Agonist with Neuroprotective Activity. *Br. J. Pharmacol.* 174, 4263–4276. doi:10.1111/bph.14019

Nallathambi, R., Mazuz, M., Ion, A., Selvaraj, G., Weininger, S., Fridlender, M., et al. (2017). Anti-Inflammatory Activity in Colon Models Is Derived from Δ9-Tetrahydrocannabinolic Acid that Interacts with Additional Compounds in Cannabis Extracts. *Cannabis Cannabinoid Res.* 2, 167–182. doi:10.1089/can.2017.0027

Nallathambi, R., Mazuz, M., Namdar, D., Shik, M., Namintzer, D., Vinayaka, A. C., et al. (2018). Identification of Synergistic Interaction between Cannabis-Derived Compounds for Cytotoxic Activity in Colorectal Cancer Cell Lines and colon Polyps that Induces Apoptosis-Related Cell Death and Distinct Gene Expression. *Cannabis Cannabinoid Res.* 3, 120–135. doi:10.1089/can.2018.0010

Navarro, G., Varani, K., Lillo, A., Vincenzi, F., Rivas-Santisteban, R., Raïch, I., et al. (2020). Pharmacological Data of Cannabidiol- and Cannabigerol-type Phytocannabinoids Acting on Cannabinoid CB1, CB2 and CB1/CB2 Heteromer Receptors. *Pharmacol. Res.* 159, 104940. doi:10.1016/j.phrs.2020.104940

Navarro, G., Varani, K., Reyes-Resina, I., Sánchez de Medina, V., Rivas-Santisteban, R., Sánchez-Carnerero Callado, C., et al. (2018). Cannabigerol Action at Cannabinoid CB1 and CB2 Receptors and at CB1-CB2 Heteroreceptor Complexes. *Front. Pharmacol.* 9, 632. doi:10.3389/fphar.2018.00632

O'Sullivan, S. E. (2007). Cannabinoids Go Nuclear: Evidence for Activation of Peroxisome Proliferator-Activated Receptors. *Br. J. Pharmacol.* 152, 576–582. doi:10.1038/sj.bjp.0707423

Obata, Y., and Ishikawa, Y. (1966). Studies on the Constituents of Hemp Plant (Cannabis sativaL). *Agric. Biol. Chem.* 30, 619–620. doi:10.1080/00021369.1966.10858651

Okada, Y., Imendra, K. G., Miyazaki, T., Hotokezaka, H., Fujiyama, R., Zeredo, J. L., et al. (2005). Biophysical Properties of Voltage-Gated Na+ Channels in Frog Parathyroid Cells and Their Modulation by Cannabinoids. *J. Exp. Biol.* 208, 4747–4756. doi:10.1242/jeb.01967

Oláh, A., Markovics, A., Szabó-Papp, J., Szabó, P. T., Stott, C., Zouboulis, C. C., et al. (2016). Differential Effectiveness of Selected Non-psychotropic Phytocannabinoids on Human Sebocyte Functions Implicates Their Introduction in Dry/seborrhoeic Skin and Acne Treatment. *Exp. Dermatol.* 25, 701–707.

Pagano, E., Iannotti, F. A., Piscitelli, F., Romano, B., Lucariello, G., Venneri, T., et al. (2021). Efficacy of Combined Therapy with Fish Oil and Phytocannabinoids in Murine Intestinal Inflammation. *Phytother Res.* 35, 517–529. doi:10.1002/ptr.6831

Palomares, B., Ruiz-Pino, F., Garrido-Rodriguez, M., Eugenia Prados, M., Sánchez-Garrido, M. A., Velasco, I., et al. (2020). Tetrahydrocannabinolic Acid A (THCA-A) Reduces Adiposity and Prevents Metabolic Disease Caused by Diet-Induced Obesity. *Biochem. Pharmacol.* 171, 113693. doi:10.1016/j.bcp.2019.113693

Patsos, H. A., Hicks, D. J., Dobson, R. R., Greenhough, A., Woodman, N., Lane, J. D., et al. (2005). The Endogenous Cannabinoid, Anandamide, Induces Cell Death in Colorectal Carcinoma Cells: a Possible Role for Cyclooxygenase 2. *Gut* 54, 1741–1750. doi:10.1136/gut.2005.073403

Pennypacker, S. D., and Romero-Sandoval, E. A. (2020). CBD and THC: Do They Complement Each Other like Yin and Yang? *Pharmacotherapy* 40, 1152–1165. doi:10.1002/phar.2469

Pertwee, R. G., Rock, E. M., Guenther, K., Limebeer, C. L., Stevenson, L. A., Haj, C., et al. (2018). Cannabidiolic Acid Methyl Ester, a Stable Synthetic Analogue of Cannabidiolic Acid, Can Produce 5-HT1A Receptor-Mediated Suppression of

Nausea and Anxiety in Rats. *Br. J. Pharmacol.* 175, 100–112. doi:10.1111/bph.14073

Pisanti, S., and Bifulco, M. (2019). Medical Cannabis: A Plurimillennial History of an evergreen. *J. Cel Physiol* 234, 8342–8351. doi:10.1002/jcp.27725

Rhee, M. H., Vogel, Z., Barg, J., Bayewitch, M., Levy, R., Hanus, L., et al. (1997). Cannabinol Derivatives: Binding to Cannabinoid Receptors and Inhibition of Adenylylcyclase. *J. Med. Chem.* 40, 3228–3233. doi:10.1021/jm970126f

Riedel, G., Fadda, P., McKillop-Smith, S., Pertwee, R. G., Platt, B., and Robinson, L. (2009). Synthetic and Plant-Derived Cannabinoid Receptor Antagonists Show Hypophagic Properties in Fasted and Non-fasted Mice. *Br. J. Pharmacol.* 156, 1154–1166. doi:10.1111/j.1476-5381.2008.00107.x

Rock, E. M., Kopstick, R. L., Limebeer, C. L., and Parker, L. A. (2013a). Tetrahydrocannabinolic Acid Reduces Nausea-Induced Conditioned Gaping in Rats and Vomiting in *Suncus Murinus*. *Br. J. Pharmacol.* 170, 641–648. doi:10.1111/bph.12316

Rock, E. M., Limebeer, C. L., and Parker, L. A. (2018). Effect of Cannabidiolic Acid and Δ9-tetrahydrocannabinol on Carrageenan-Induced Hyperalgesia and Edema in a Rodent Model of Inflammatory Pain. *Psychopharmacology (Berl)* 235, 3259–3271. doi:10.1007/s00213-018-5034-1

Rock, E. M., and Parker, L. A. (2013). Suppression of Lithium Chloride-Induced Conditioned Gaping (A Model of Nausea-Induced Behaviour) in Rats (Using the Taste Reactivity Test) with Metoclopramide Is Enhanced by Cannabidiolic Acid. *Pharmacol. Biochem. Behav.* 111, 84–89. doi:10.1016/j.pbb.2013.08.012

Rock, E. M., Sticht, M. A., Duncan, M., Stott, C., and Parker, L. A. (2013b). Evaluation of the Potential of the Phytocannabinoids, Cannabidivarin (CBDV) and Δ(9) -tetrahydrocannabivarin (THCV), to Produce CB1 Receptor Inverse Agonism Symptoms of Nausea in Rats. *Br. J. Pharmacol.* 170, 671–678. doi:10.1111/bph.12322

Rock, E. M., Sullivan, M. T., Collins, S. A., Goodman, H., Limebeer, C. L., Mechoulam, R., et al. (2020). Evaluation of Repeated or Acute Treatment with Cannabidiol (CBD), Cannabidiolic Acid (CBDA) or CBDA Methyl Ester (HU-580) on Nausea And/or Vomiting in Rats and Shrews. *Psychopharmacology (Berl)* 237, 2621–2631. doi:10.1007/s00213-020-05559-z

Romano, B., Borrelli, F., Fasolino, I., Capasso, R., Piscitelli, F., Cascio, M., et al. (2013). The Cannabinoid TRPA1 Agonist Cannabichromene Inhibits Nitric Oxide Production in Macrophages and Ameliorates Murine Colitis. *Br. J. Pharmacol.* 169, 213–229. doi:10.1111/bph.12120

Romano, B., Pagano, E., Orlando, P., Capasso, R., Cascio, M. G., Pertwee, R., et al. (2016). Pure Δ9-tetrahydrocannabivarin and a Cannabis Sativa Extract with High Content in Δ9-tetrahydrocannabivarin Inhibit Nitrite Production in Murine Peritoneal Macrophages. *Pharmacol. Res.* 113, 199–208. doi:10.1016/j.phrs.2016.07.045

Romero-Sandoval, E. A., Fincham, J. E., Kolano, A. L., Sharpe, B. N., and Alvarado-Vázquez, P. A. (2018). Cannabis for Chronic Pain: Challenges and Considerations. *Pharmacotherapy* 38, 651–662. doi:10.1002/phar.2115

Rosenthaler, S., Pöhn, B., Kolmanz, C., Huu, C. N., Krewenka, C., Huber, A., et al. (2014). Differences in Receptor Binding Affinity of Several Phytocannabinoids Do Not Explain Their Effects on Neural Cell Cultures. *Neurotoxicol Teratol* 46, 49–56. doi:10.1016/j.ntt.2014.09.003

Ross, H. R., Napier, I., and Connor, M. (2008). Inhibition of Recombinant Human T-type Calcium Channels by delta9-tetrahydrocannabinol and Cannabidiol. *J. Biol. Chem.* 283, 16124–16134. doi:10.1074/jbc.M707104200

Ruhaak, L. R., Felth, J., Karlsson, P. C., Rafter, J. J., Verpoorte, R., and Bohlin, L. (2011). Evaluation of the Cyclooxygenase Inhibiting Effects of Six Major Cannabinoids Isolated from *Cannabis Sativa*. *Biol. Pharm. Bull.* 34, 774–778. doi:10.1248/bpb.34.774

Russo, E. B. (2007). History of Cannabis and its Preparations in Saga, Science, and Sobriquet. *Chem. Biodivers* 4, 1614–1648. doi:10.1002/cbdv.200790144

Russo, E. B. (2011). Taming THC: Potential Cannabis Synergy and Phytocannabinoid-Terpenoid Entourage Effects. *Br. J. Pharmacol.* 163, 1344–1364. doi:10.1111/j.1476-5381.2011.01238.x

Sagredo, O., Pazos, M. R., Satta, V., Ramos, J. A., Pertwee, R. G., and Fernández-Ruiz, J. (2011). Neuroprotective Effects of Phytocannabinoid-Based Medicines in Experimental Models of Huntington's Disease. *J. Neurosci. Res.* 89, 1509–1518. doi:10.1002/jnr.22682

Santiago, M., Sachdev, S., Arnold, J. C., McGregor, I. S., and Connor, M. (2019). Absence of Entourage: Terpenoids Commonly Found in Cannabis Sativa Do Not Modulate the Functional Activity of Δ9-THC at Human CB1 and CB2 Receptors. *Cannabis Cannabinoid Res.* 4, 165–176. doi:10.1089/can.2019.0016

Schlicker, E., and Kathmann, M. (2001). Modulation of Transmitter Release via Presynaptic Cannabinoid Receptors. *Trends Pharmacol. Sci.* 22, 565–572. doi:10.1016/s0165-6147(00)01805-8

Schultes, R. E., Klein, W. M., Plowman, T., and Lockwood, T. E. (1974). Cannabis: an Example of Taxonomic Neglect. *Harv. Univ. Bot. Mus. Leaflets* 23, 337–367. doi:10.5962/p.168565

Scott, K. A., Dalgleish, A. G., and Liu, W. M. (2017). Anticancer Effects of Phytocannabinoids Used with Chemotherapy in Leukaemia Cells Can Be Improved by Altering the Sequence of Their Administration. *Int. J. Oncol.* 51, 369–377. doi:10.3892/ijo.2017.4022

Scott, K. A., Shah, S., Dalgleish, A. G., and Liu, W. M. (2013). Enhancing the Activity of Cannabidiol and Other Cannabinoids *In Vitro* through Modifications to Drug Combinations and Treatment Schedules. *Anticancer Res.* 33, 4373–4380.

Shen, M., Piser, T. M., Seybold, V. S., and Thayer, S. A. (1996). Cannabinoid Receptor Agonists Inhibit Glutamatergic Synaptic Transmission in Rat Hippocampal Cultures. *J. Neurosci.* 16, 4322–4334. doi:10.1523/jneurosci.16-14-04322.1996

Shinjyo, N., and Di Marzo, V. (2013). The Effect of Cannabichromene on Adult Neural Stem/progenitor Cells. *Neurochem. Int.* 63, 432–437. doi:10.1016/j.neuint.2013.08.002

Small, E. (2015). Evolution and Classification of Cannabis Sativa (Marijuana, Hemp) in Relation to Human Utilization. *Bot. Rev.* 81, 189–294. doi:10.1007/s12229-015-9157-3

Smart, D., Gunthorpe, M. J., Jerman, J. C., Nasir, S., Gray, J., Muir, A. I., et al. (2000). The Endogenous Lipid Anandamide Is a Full Agonist at the Human Vanilloid Receptor (hVR1). *Br. J. Pharmacol.* 129, 227–230. doi:10.1038/sj.bjp.0703050

Smeriglio, A., Giofrè, S. V., Galati, E. M., Monforte, M. T., Cicero, N., D'Angelo, V., et al. (2018). Inhibition of Aldose Reductase Activity by Cannabis Sativa Chemotypes Extracts with High Content of Cannabidiol or Cannabigerol. *Fitoterapia* 127, 101–108. doi:10.1016/j.fitote.2018.02.002

Stella, N. (2010). Cannabinoid and Cannabinoid-like Receptors in Microglia, Astrocytes, and Astrocytomas. *Glia* 58, 1017–1030. doi:10.1002/glia.20983

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., et al. (1995). 2-Arachidonoylglycerol: a Possible Endogenous Cannabinoid Receptor Ligand in Brain. *Biochem. Biophys. Res. Commun.* 215, 89–97. doi:10.1006/bbrc.1995.2437

Sulak, D., Saneto, R., and Goldstein, B. (2017). The Current Status of Artisanal Cannabis for the Treatment of Epilepsy in the United States. *Epilepsy Behav.* 70, 328–333. doi:10.1016/j.yebeh.2016.12.032

Takahashi, R. N., and Karniol, I. G. (1975). Pharmacologic Interaction between Cannabinol and delta9-tetrahydrocannabinol. *Psychopharmacologia* 41, 277–284. doi:10.1007/BF00428937

Takeda, S., Misawa, K., Yamamoto, I., and Watanabe, K. (2008). Cannabidiolic Acid as a Selective Cyclooxygenase-2 Inhibitory Component in Cannabis. *Drug Metab. Dispos* 36, 1917–1921. doi:10.1124/dmd.108.020909

Tan, B., Bradshaw, H. B., Rimmerman, N., Srinivasan, H., Yu, Y. W., Krey, J. F., et al. (2006). Targeted Lipidomics: Discovery of New Fatty Acyl Amides. *AAPS J.* 8, E461–E465. doi:10.1208/aapsj080354

Thomas, A., Stevenson, L. A., Wease, K. N., Price, M. R., Baillie, G., Ross, R. A., et al. (2005). Evidence that the Plant Cannabinoid delta9-tetrahydrocannabivarin Is a Cannabinoid CB1 and CB2 Receptor Antagonist. *Br. J. Pharmacol.* 146, 917–926. doi:10.1038/sj.bjp.0706414

Tóth, K. F., Adám, D., Bíró, T., and Oláh, A. (2019). Cannabinoid Signaling in the Skin: Therapeutic Potential of the "c(ut)annabinoid System. *Molecules* 24, 918.

Tsou, K., Brown, S., Sañudo-Peña, M. C., Mackie, K., and Walker, J. M. (1998). Immunohistochemical Distribution of Cannabinoid CB1 Receptors in the Rat central Nervous System. *Neuroscience* 83, 393–411. doi:10.1016/s0306-4522(97)00436-3

Tubaro, A., Giangaspero, A., Sosa, S., Negri, R., Grassi, G., Casano, S., et al. (2010). Comparative Topical Anti-inflammatory Activity of Cannabinoids and Cannabivarins. *Fitoterapia* 81, 816–819. doi:10.1016/j.fitote.2010.04.009

Tudge, L., Williams, C., Cowen, P. J., and McCabe, C. (2015). Neural Effects of Cannabinoid CB1 Neutral Antagonist Tetrahydrocannabivarin on Food

Reward and Aversion in Healthy Volunteers. *Int. J. Neuropsychopharmacol.* 18, pyu094. doi:10.1093/ijnp/pyu094

Turner, C. E., and ElSohly, M. A. (1981). Biological Activity of Cannabichromene, its Homologs and Isomers. *J. Clin. Pharmacol.* 21, 283S–291S. doi:10.1002/j.1552-4604.1981.tb02606.x

Udoh, M., Santiago, M., Devenish, S., McGregor, I. S., and Connor, M. (2019). Cannabichromene Is a Cannabinoid CB2 Receptor Agonist. *Br. J. Pharmacol.* 176, 4537–4547. doi:10.1111/bph.14815

Valdeolivas, S., Navarrete, C., Cantarero, I., Bellido, M. L., Muñoz, E., and Sagredo, O. (2015). Neuroprotective Properties of Cannabigerol in Huntington's Disease: Studies in R6/2 Mice and 3-Nitropropionate-Lesioned Mice. *Neurotherapeutics* 12, 185–199. doi:10.1007/s13311-014-0304-z

Vaughan, C. W., Connor, M., Bagley, E. E., and Christie, M. J. (2000). Actions of Cannabinoids on Membrane Properties and Synaptic Transmission in Rat Periaqueductal gray Neurons *In Vitro*. *Mol. Pharmacol.* 57, 288–295.

Vigli, D., Cosentino, L., Raggi, C., Laviola, G., Woolley-Roberts, M., and De Filippis, B. (2018). Chronic Treatment with the Phytocannabinoid Cannabidivarin (CBDV) Rescues Behavioural Alterations and Brain Atrophy in a Mouse Model of Rett Syndrome. *Neuropharmacology* 140, 121–129. doi:10.1016/j.neuropharm.2018.07.029

Walsh, K. B., and Andersen, H. K. (2020). Molecular Pharmacology of Synthetic Cannabinoids: Delineating CB1 Receptor-Mediated Cell Signaling. *Ijms* 21, 6115. doi:10.3390/ijms21176115

Wargent, E. T., Zaibi, M. S., Silvestri, C., Hislop, D. C., Stocker, C. J., Stott, C. G., et al. (2013). The Cannabinoid Δ(9)-tetrahydrocannabivarin (THCV) Ameliorates Insulin Sensitivity in Two Mouse Models of Obesity. *Nutr. Diabetes* 3, e68. doi:10.1038/nutd.2013.9

Wong, H., and Cairns, B. E. (2019). Cannabidiol, Cannabinol and Their Combinations Act as Peripheral Analgesics in a Rat Model of Myofascial Pain. *Arch. Oral Biol.* 104, 33–39. doi:10.1016/j.archoralbio.2019.05.028

Wood, T. B., Spivey, W. T. N., and Easterfield, T. H. (1886). Charas, the Resin of Indian Hemp. *J. Chem. Soc.* 69, 539–546.

World Health Organization (2016). The Health and Social Effects of Nonmedical Cannabis Use. Available at: http://www.who.int/substance_abuse/publications/cannabis/en/.

Yoshida, H., Usami, N., Ohishi, Y., Watanabe, K., Yamamoto, I., and Yoshimura, H. (1995). Synthesis and Pharmacological Effects in Mice of Halogenated Cannabinol Derivatives. *Chem. Pharm. Bull. (Tokyo)* 43, 335–337. doi:10.1248/cpb.43.335

Zagzoog, A., Mohamed, K. A., Kim, H. J. J., Kim, E. D., Frank, C. S., Black, T., et al. (2020). *In Vitro* and *In Vivo* Pharmacological Activity of Minor Cannabinoids Isolated from Cannabis Sativa. *Sci. Rep.* 10, 20405. doi:10.1038/s41598-020-77175-y

Zamberletti, E., Gabaglio, M., Piscitelli, F., Brodie, J. S., Woolley-Roberts, M., Barbiero, I., et al. (2019a). Cannabidivarin Completely Rescues Cognitive Deficits and Delays Neurological and Motor Defects in Male Mecp2 Mutant Mice. *J. Psychopharmacol.* 33, 894–907. doi:10.1177/0269881119844184

Zamberletti, E., Gabaglio, M., Woolley-Roberts, M., Bingham, S., Rubino, T., and Parolaro, D. (2019b). Cannabidivarin Treatment Ameliorates Autism-like Behaviors and Restores Hippocampal Endocannabinoid System and Glia Alterations Induced by Prenatal Valproic Acid Exposure in Rats. *Front Cel Neurosci* 13, 367. doi:10.3389/fncel.2019.00367

Zygmunt, P. M., Petersson, J., Andersson, D. A., Chuang, H., Sørgård, M., Di Marzo, V., et al. (1999). Vanilloid Receptors on Sensory Nerves Mediate the Vasodilator Action of Anandamide. *Nature* 400, 452–457. doi:10.1038/22761

Conflict of Interest: AH is employed by the company Precision Plant Molecules.

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Pharmacology

MINI REVIEW
published: 19 January 2022
doi: 10.3389/fphar.2021.793750

Components of the Endocannabinoid System and Effects of Cannabinoids Against Bone Diseases: A Mini-Review

Yuqi Xin†, *Anqun Tang*†, *Shuting Pan and Jie Zhang**

Department of Oral and Maxillofacial Surgery, The First Affiliated Hospital of Nanchang University, Nanchang, China

Background: The endocannabinoid system (ECS) is involved in multiple physiological processes, including appetite regulation, pain perception, motor function development, and immune response regulation. Cannabinoids have been approved for the clinical treatment of nausea and vomiting caused by cytostatic therapy or cancer chemotherapy, loss of appetite in HIV/AIDS-associated cachexia, refractory spasms induced by multiple sclerosis, chronic pain, and urinary incontinence.

Methods: Check out the research on ECS and bone diseases in the past 20 years.

Results: Many studies have demonstrated that endocannabinoids (eCBs) and cannabinoid receptors (CBRs) are expressed in bone and synovial tissues, playing important roles in bone metabolism. Preclinical studies using cannabis-based therapies in animal models have shown that cannabinoids (CBs) can alleviate the development of osteoarthritis (OA), prevent osteoporosis (OP), reduce cancer-induced osteolytic destruction, and improve fracture healing, highlighting the therapeutic potential of CBs for human bone diseases.

Conclusions: The present review summarizes various components of the ECS in bone diseases and their potential as a therapeutic target.

Keywords: cannabinoids, bone loss, osteoporosis, osteoarthritis, bone tumor, bone fractures

OPEN ACCESS

Edited by:
Cristoforo Pomara, University of Catania, Italy

Reviewed by:
Paolo Tucci, University of Foggia, Italy
Chawon Yun, Northwestern University, United States

***Correspondence:**
Jie Zhang
ndyfy02134@ncu.edu.cn
zhjprs@163.com

†*These authors have contributed equally to this work and share first authorship*

Specialty section:
This article was submitted to Experimental Pharmacology and Drug Discovery, a section of the journal Frontiers in Pharmacology

Received: *13 October 2021*
Accepted: *23 December 2021*
Published: *19 January 2022*

Citation:
Xin Y, Tang A, Pan S and Zhang J (2022) Components of the Endocannabinoid System and Effects of Cannabinoids Against Bone Diseases: A Mini-Review. Front. Pharmacol. 12:793750. doi: 10.3389/fphar.2021.793750

INTRODUCTION

Cannabis Sativa has been used medicinally and recreationally for thousands of years. As early as in 2600 B.C., cannabis was already used in treating malaria, constipation, pain and dysmenorrhoea in China (Mechoulam, 1986; Grinspoon, 1993). In the late 19th century, European people began using cannabis to treat pain, muscle spasms, asthma, insomnia, depression and anorexia. However, it was not until 1964 that its major active chemical component delta-9-tetrahydrocannabinol (THC), also known as dronabinol, was discovered (Gaoni and Mechoulam, 1964). Nearly 30 years later, a specific cannabinoid receptor (CBR) was identified as the target of THC (Devane et al., 1988; Munro et al.,

Abbreviations: ECS, The endocannabinoid system; eCB, endocannabinoid; CBR, cannabinoid receptor; CB, cannabinoid; OA, osteoarthritis; OP, osteoporosis; THC, delta-9-tetrahydrocannabinol; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; OP, osteoporosis; GPR, G-protein coupled receptors; TRPV, transient receptor potential vanilloids; PPAR, peroxisome proliferator-activated receptor; OCs, osteoclasts; OB, osteoblasts; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; CBD, cannabisol; TNF, tumor necrosis factor; NF-B, nuclear factor-B; LPS, lipopolysaccharide; MMPs, matrix metalloproteinases; RA, rheumatoid arthritis.

1993). In 1992, Devane et al. discovered the first endocannabinoid (eCB) N-arachidonoylethanolamine or anandamide (AEA) as the endogenous ligand of CBR in the pig brain (Devane et al., 1992). Subsequently in 1995, the second eCB 2-arachidonoylglycerol (2-AG) was also discovered (Mechoulam et al., 1995). With the discovery of endocannabinoids, a great number of studies have investigated the physiological functions of the endocannabinoid system (ECS).

The ECS is recognized to play a significant role in regulating a variety of physiological processes, including appetite control, pain perception, and immune regulation (Idris and Ralston, 2010; Robson, 2014). Marinol (dronabinol), a cannabinoid receptor 1(CB1) agonist, has been approved in the United States of America (USA) for the clinical treatment of nausea and vomiting, and anorexia caused by cytostasis or AIDS. Nabilone has also been approved in the United Kingdom (UK) for the treatment of chemotherapy-induced adverse effects in cancer patients. A new CB drug (sativex) has also be approved in Germany for the treatment of intractable muscle spasm caused by multiple sclerosis (Grotenhermen and Müller-Vahl, 2012).

Several research groups have reported ECS expression in bone and synovial tissues and its important role in bone metabolism (Carnovali et al., 2016; Ehrenkranz and Levine, 2019; Dou et al., 2020). Preclinical studies in animal models demonstrated that CBs could alleviate the development of arthritis, prevent osteoporosis (OP), inhibit bone tumor cell proliferation, reduce bone cancer pain and improve fracture healing (Smoum et al., 2015; Carnovali et al., 2016; Frei et al., 2016; Marino et al., 2019; Yan et al., 2019). In this regard, recent progress in the application of CBs in bone diseases has been reviewed, with the expectation to provide a new direction for the clinical treatment of bone diseases. In this article, we will discuss the potential therapeutic effects of CBs for the treatment of patients with bone diseases.

CB EXPRESSION IN BONE AND JOINT TISSUES

The ECS consist of endogenous cannabinoid ligands (endocannabinoids, eCBs), their receptors, and the gene enzymes involved in their synthesis and degradation (McPartland et al., 2006; Pertwee et al., 2010). Arachidonoylethanolamine (AEA) and 2-Arachidonoylglycerol (2-AG) are two firstly identified and most studied eCBs, which are believed to be involved in a wide range of physiological processes including appetite stimulation, pain modulation and energy expenditure (Di Marzo and Petrosino, 2007). Cannabinoid receptor 1 (CB1) and 2 (CB2) are the characterised cannabinoid receptors, to which AEA and 2-AG bind in the nanomolar range. 2-AG is considered a full agonist, and AEA is considered a partial agonist (Pertwee and Ross, 2002). Other receptors known for eCBs include G-protein coupled receptors (GPR55 and GPR119), transient receptor potential vanilloids (TRPV1 and TRPV4), peroxisome proliferator-activated receptors (PPARα and PPARγ), and various ion channels (Pertwee, 2010).

The ECS exists in most mammalian organs and tissues, playing primarily important roles in the nervous and immune systems (Joshi and Onaivi, 2019; Silver Robert, 2019). ECBs and their receptors are also expressed in the bone (Rossi et al., 2009; Whyte et al., 2009). CB1, CB2 and TRPV1 have been identified in human osteoclasts (OCs) and GPR55, and found to be expressed in both human osteoblasts (OBs) and OCs (Rossi et al., 2009; Whyte et al., 2009). Mouse OBs and OCs express CB1, CB2, GPR55 and TRPV1 (Idris et al., 2005; Ofek et al., 2006; Idris et al., 2009; Whyte et al., 2009; Idris et al., 2010). Studies of the innervation of the mouse bone have shown that CB1 and TRPV1 are expressed in sympathetic nerve fibers (Ghilardi et al., 2005; Tam et al., 2006). AEA and 2-AG are responsible for most of the pharmacological effects associated with CBRs in mammalian cells (Pertwee, 2005). Jiang S et al. discovered AEA and 2-AG are produced in bone marrow (Bab et al., 2008; Tam et al., 2008; Jiang et al., 2010). AEA and 2-AG are detectable in human OC- and OB-like cells *in vitro* (Rossi et al., 2009; Whyte et al., 2012). Rossi et al. (2009) reported that cultured human OCs produced 2-AG and a certain amount of AEA *in vitro*, and the level of eCBs increased when the culture was treated with URB597, a fatty acid amide hydrolase (FAAH) inhibitor. Whyte et al. (2012) reported that differentiation of human OCs was related to the increased AEA level and decreased 2-AG level. These observations suggest that AEA and 2-AG may be produced by bone cells and within the cultured bone (Tam et al., 2008; Maccarrone et al., 2015). The enzyme diacylglycerol lipases *α* and *ß* and NAPE-phospholipase D, which are required for 2-AG and AEA synthesis respectively, are also expressed in OBs and OCs (Tam et al., 2008; Rossi et al., 2009). Similarly, the degradation enzymes FAAH and monoacylglycerol lipase (MAGL) have been found in human OCs and mouse OBs (Hutchins et al., 2011; Rossi et al., 2011). The role of cannabinoid receptor ligands in regulating osteoclasts, osteoblasts and adipocytes *in vitro* and *in vivo* are shown in **Table 1**.

The ECS is also expressed in synovial tissues of joints. Richardson et al. (2008) reported that CB1 and CB2 receptors were expressed in synovial biopsies of human osteoarthritis (OA) and rheumatoid arthritis (RA) by Western Blot detection, and played a role in regulating physiological functions. Further evidence showed that AEA and 2-AG could be detected in synovial fluid from OA and RA patients but not in synovial fluid from normal subjects. Dunn et al. (2016) reported that a wide range of CBRs including CB1, CB2, GPR55, PPARαand PPARγ were expressed in chondrocytes of OA joints, and even in degenerate tissues.

CANNABINOIDS AFFECT BONE DISEASES

Osteoporosis

OBs are known to synthesize bone cells to produce AEA and 2-AG, and express CB1 receptors on their surfaces (Tam et al., 2008; Maccarrone et al., 2015). Activation of CB1 in OBs inhibits the release of norepinephrine, which to some extent suppresses the process of bone formation, i.e., CB1 activation inhibits bone

TABLE 1 | The role of cannabinoid receptor ligands in regulating osteoclasts, osteoblasts, and adipocytes *in vitro* and *in vivo*.

	Ligand	Receptor	Bone metabolism			
			Oc	Oc	Ob	Ac
			Number	Activity	Number	Number
Agonists	AEA	CB1/CB2/GPR55/TRPV1	↑	↑	↑	-
	2-AG	CB1/CB2/GPR55	↑	↑	↑	-
	Δ9-THC	CB1/CB2	-	-	-	-
	CP55,940	CB1/CB2	↑↓	↑	↑	↓
	WIN55,212	CB1	-	-	↑	-
	HU308	CB2	↑↓↓	-	↑↑	↓
	JWH133	CB2	↑	↑	↑	↓
	JWH139	CB2	-	-	-	-
	JWH015	CB2	-	↑	↑	↓
	AM1241	CB2	-	-	-	-
	Lysophosphatidyl inositol	GPR55	↓	↑	-	-
	O-1602	GPR55	↓	↑	↑	-
Antagonists	AM630	CB2>CB1/GPR55	↓↓↑	↓	↓	-
	SR144528	CB2>CB1	↓	↓	↓	-
	AM251	CB1>CB2/GPR55	↓↓↑	↓	↓	↑
	SR141716A	CB1>CB2	↓↓	↓	↓	-
	Cannabidiol	GPR55	↑	↓↓	-	-

Abbreviations: CB1, cannabinoid type 1 receptor; CB2, cannabinoid type 2 receptor; GPR55, G protein-coupled receptor 55; TRPV1, transient receptor potential vanilloid type 1. Oc., osteoclast; Ob., osteolblast; Ac., adipocyte. ↑, increase; ↓, decrease; –, non tested. Black and red arrows denote in vitro *and* in vivo *data, respectively.*

production (Tam et al., 2006). In addition, OCs also express AEA and 2-AG, but with CB2 receptors instead of CB1 (Whyte et al., 2012). CB2 activation in OCs suppresses osteolysis activity, thereby preserving the bone tissue (Whyte et al., 2012). This effect proves highly beneficial to balancing the relationship between hyperactive OCs and inactive OBs in OP, leading to increased bone resorption without compensatory bone formation. These findings support that the ECS is the main regulatory system of the bone. Although norepinephrine is directly responsible for the activities of OCs and OBs, the level of norepinephrine is mainly mediated both by the ECS expressed in the sympathetic nervous system and that expressed in the bone tissue itself.

CBs have been shown to regulate bone formation, bone loss and bone turnover. The ECS system is an important regulator of bone mass. CBR agonists promote differentiation of mouse mesenchymal stem cells (MSCs) into OBs (Zhang et al., 2019). Idris et al. (Hutchins et al., 2011) reported that CB1 receptor inactivation increased bone mass and prevented bone loss due to ovariectomy, an *in vivo* model of OP in 2005. In addition, Rossi et al. reported that CB2 receptors had an anti-osteoporosis function (Rossi et al., 2019). CB2 receptor agonists increased bone mass by enhancing the number and activity of OBs, inhibiting the proliferation of OCs, and stimulating fibroblastic colony formation by myeloid cells (Ofek et al., 2006). Furthermore, CB2 receptor regulates bone loss also involving the regulation of osteoclast function (Sophocleous et al., 2017). Therefore, CB2 provides a molecular target for the diagnosis and treatment of OP.

GPR55 was expressed in human and mouse OCs and OBs. In contrast to the bone turnover function of CB1 and CB2 receptors, GPR55 inhibited OC formation but stimulates OB function. Histomorphometric and microcomputed tomography analysis of the long bones in male GPR55 (−/−) mice revealed that the number of OCs was increased, but the volume and thickness of the trabecular bone was increased significantly with no cartilage resorption observed, a possibility is that osteoclast numbers were increased, but osteoclast function was impaired (Whyte et al., 2009). Therefore, GPR55 receptor agonists promote bone loss. A recent study of OCs from patients with OP suggested that GPR55 desensitization by FAAs or its enhanced transport, and TRPV1 agonist-induced overexpression of CB2 receptor might be critical to reduce calciumentry into OCs, which could lead to over-activation of cells and increase bone resorption and bone loss. TRPV1 agonistsCB together with CB2 agonists were reported useful for the treatment of OP (Rossi et al., 2011). These results indicate that CBRs agonists could be used for the prevention and treatment of OP.

Osteoarthritis

OA is characterized by degeneration of the articular cartilage, which is mediated by complex interactions of proinflammatory cytokines including IL-1, inflammatory mediators and proteases. CBs have been shown to prevent IL-1-induced matrix breakdown of collagen and proteoglycan, suggesting that they may play an important role in cartilage protection (Mbvundula et al., 2006). CBs exert their effects through several CBRs, and therefore it is important to identify the key CBs and CBRs involved in cartilage protection. CBRs are expressed in synovial tissues and osteoarthritic articular chondrocytes and produce important physiological effects such as reducing arthritis inflammation and alleviating arthritis-associated pain symptoms (Mbvundula et al., 2006; Guidetti et al., 2014). Malfait et al. (2000) described the effect of a high potency dimeric cannabinoid, named cannabisol (CBD), in a mouse arthritis model and found that CBD had immunosuppressive and anti-inflammatory activities

and could improve the symptoms of arthritis in a murine collagen-induced arthritis model by both intraperitoneal and oral administration methods. They also found that CBD could reduce joint damage. The *in vitro* effects of CBD included dose-dependent inhibition of lymphocyte proliferation, both mitogen-stimulated and antigen-specific, and reactive oxygen burst triggered by peritoneal granulocytes blocking zymosan. It was also found that CBD administration could block the increase of serum tumor necrosis factor (TNF) induced by lipopolysaccharide in C57/BL mice. Sumariwalla et al. (2004) used a synthetic CB (HU-320) in a similar experiment and found that this novel synthetic CB HU-320 could be used to treat arthritis in mice due to its strong anti-inflammatory and immunosuppressive properties without showing psychoactive effects. HU-320 inhibited the production of tumor necrosis factor (TNF) and reactive oxygen intermediates (ROIs) from mouse macrophages and RAW 264.7 cells respectively, as well as the increased serum TNF level following endotoxin attack.

Oversecretion of proinflammatory cytokines from OBs plays an essential role in the development of OA (Liu et al., 2014; Sun et al., 2014), and high levels of pro-inflammatory factors in bones and joints induce pain, cartilage loss, and even joint dysfunction (Yang et al., 2013; Karsdal et al., 2014). Therefore, reducing the release of pro-inflammatory cytokines from OBs is an effective therapy for OA. Yang et al. (2015) have reported that THC inhibited the release of pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, and IL-8, decreased nuclear factor-B (NF-B) expression, and inhibited the upregulation of cofilin-1 protein (a cytoskeleton protein involved in inflammation of OA of lipopolysaccharide (LPS)-stimulated MG-63 cells. The administration of the CB2 receptor antagonist or the CB2-siRNA partially abolished the above-mentioned THC-induced anti-inflammatory effect. In addition, overexpression of cofilin-1 significantly reversed the THC-induced anti-inflammatory effect in MG-63 cells. These results indicate that CB2 is involved in the anti-inflammation induced by THC in LPS-stimulated MG-63 cells, and suggest that the anti-inflammatory effect may be mediated by cofilin-1 (Yang et al., 2015).

Cannabinoids exert chondroprotective effects and are useful for OA treatment (Dunn et al., 2012). Dunn et al. (Dunn et al., 2016) designed CBs to bind to their receptors and found that they inhibited the catabolic and pain pathways within the arthritic joint without causing psychoactive effects, suggesting a therapeutic potential for arthritis. Sophocleous et al. (2015) reported that CB2-selective agonist HU308 reduced the severity of total knee joint OA following surgical destabilization of the medial meniscus (DMM) in wide type mice. When compared with wild-type chondrocytes, cultured articular chondrocytes from CB2 deletion ($CB2^{-/-}$) mice produced less proteoglycans *in vitro*, indicated that the CB2 pathway played a role in the pathophysiology of murine OA, and that the pharmacological activity of CB2 had a protective effect against OA.

There is increasing evidence that the ECS, especially CB2, also plays an important role in the pathophysiology of rheumatoid arthritis (RA) (Lowin et al., 2019). Gui et al. (2014) reported that many members of the ECS inhibit synovial inflammation, hyperplasia, and cartilage destruction in RA. In particular, activation of CB2 may relieve RA by inhibiting the production of autoantibodies, proinflammatory cytokines, and matrix metalloproteinases (MMPs), as well as bone erosion, T cells mediated immune response, and the proliferation of FLSs (Gui et al., 2014).

CBs can also reduce the loss of thealveolar bone (Ossola et al., 2016). Ossola et al. have demonstrated that CB-2 receptor was expressed in OBs and OCs to promote bone metabolism. And the results of their studies in rat models showed that alveolar bone loss was greatly attenuated by the use of CB-2 receptor agonist HU-308 in LPS-induced periodontitis and as such demonstrated anti-inflammatroy, osteoprotective, prohomeostatic effects (Ossola et al., 2016).

CBs can not only reduce the inflammation of arthritis but relieve the pain symptoms of arthritis. The termination of endocannabinoid activity is achieved by cellular uptake, followed by intracellular hydrolysis by fatty acid amide hydrolase (FAAH) (Schuelert et al., 2011). Schuelert et al. (2011) indicated local injection of FAAH inhibitor URB597 into the OA knee joints reduced mechanical nociception and pain in two OA rodent models, and this response was eliminated by CB1 receptor antagonists, indicating that CB1 receptor could be used as an arthritic pain treatment target.

Bone Tumors

Several studies have demonstrated the positive effects of the ECS in the treatment of cancer. CBRs can inhibit tumor cell proliferation, reduce tumor cell invasion, cause tumor regression and prevent tumor metastasis. For example, Qamri et al. (2009) reported that synthetic CBR agonists inhibited tumor growth and metastasis of breast cancer. Preet et al. (2008) indicated that delta9-Tetrahydrocannabinol inhibited lung cancer cell migration *in vitro* as well as its growth and metastasis *in vivo*. Gustafsson et al. (2008) showed that therapeutic options using ABR ligands had the efficacy of reducing tumor burden in malignant lymphoma overexpressing CB1 and CB2. However, the role of CBs in bone metastasis remains to be studied.

There have been many studies concerning the use of the ECS in the treatment of primary bone tumors. For tumor cells, ECS can affect their growth, movement, invasion, spread, and colonization of distant organs. It was found that CB2 was modulated in the genetic and phrenological processes, thus affecting bone cell activity in remodeling in both healthy individuals and patients (Di Marzo, 2008; Marino and Idris, 2017). Furthermore, Niu et al. (2015) have demonstrated that the synthetic cannabinoid CB receptor agonist WIN-55,212-2 has therapeutic effect on the MG-63 human osteosarcoma cell line. WIN-55,212-2 inhibit migration, invasion and angiogenic activity of this cell line. The mechanism of this inhibition is associated with the downregulation of the Notch-1 and MMP-2 signaling pathways, which are known as important pathways associated with cell proliferation and apotosis as well as degradation of extra-cellular matrix, the key to tumor invasion. High level of MMP-2 is considered a key indicator

TABLE 2 | The role of cannabinoid receptor ligands in regulating tumor cells and bone diseases *in vitro* and *in vivo*.

	Ligand	Receptor	Bone tumor		
			Tm.Growth	Osteolysis	Pain
Agonists	AEA	CB1/CB2/GPR55/TRPV1	↓↓	-	↓
	2-AG	CB1/CB2/GPR55	↓	↓	-
	Δ9-THC	CB1/CB2	↓↓↑↑	-	↓
	CP55,940	CB1/CB2	↓	-	
	WIN55,212	CB1	↓↓	-	
	HU308	CB2	↑↓	↑	-
	JWH133	CB2	↑↓↓	↓↓↑	
	JWH139	CB2	↓	-	
	JWH015	CB2	↓↓	↓	↓
	AM1241	CB2	↓	↓	
	Lysophosphatidyl inositol	GPR55	-	-	-
	O-1602	GPR55	-	-	
Antagonists	AM630	CB2>CB1/GPR55	-	↓	
	SR144528	CB2>CB1	-	-	
	AM251	CB1>CB2/GPR55	-	-	
	SR141716A	CB1>CB2	-	-	
	Cannabidiol	GPR55	↓↓	-	↓

Abbreviations: CB1, cannabinoid type 1 receptor; CB2, cannabinoid type 2 receptor; GPR55, G protein-coupled receptor 55; TRPV1, transient receptor potential vanilloid type 1. Tm., tumor cell. ↑, increase; ↓, decrease; –, non tested. Black and red arrows denote in vitro *and* in vivo *data, respectively.*

of cancer metastasis (Berx et al., 1998; Jezierska and Motyl, 2009; Niu et al., 2015).

Lozano-Ondoua et al. noted that the CB2 receptor agonist reduced the degree of tumor burden within the intramedullary cavity of the femoris and produced anti-progressive effects of the tumor *in vivo* (Lozano-Ondoua et al., 2013).

Lozano-Ondoua et al. (2010) showed that CB2 receptor agonists could attenuate sarcoma-induced pain, reduce cancer-induced osteolytic destruction, and prevent the occurrence of pathological bone fracture. CB1 and CB2 were found to be associated with mediating ligands and molecular mechanisms associated with synthesis, transport and metabolism with potential effects of reducing complications of primary bone tumors. Especially, CB1 and CB2 agonists reduced bone cancer pain in animal models (Curto-Reyes et al., 2010; Kawamata et al., 2010). Therefore, this approach may be applied as analgesic treatment in patients with Bone tumors. The role of cannabinoid receptor ligands in regulating tumor cells and bone diseases *in vitro* and *in vivo* are shown in **Table 2**.

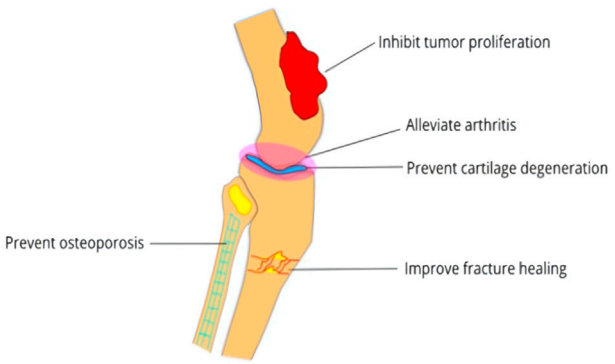


FIGURE 1 | The potential therapeutic effects of cannabinoids in bone diseases.

Bone Fractures

Bone fractures are highly prevalent, involving prolonged immobilization and discomfort. Some researchers have found that CBRs trigger bone formation and strengthen the bridge that connects broken bones (Kogan et al., 2015). Kogan et al. (2015) reported that the major non-psychoactive cannabis constituent CBD enhanced the biomechanical properties of rat mid-femoral fracture healing. Micro–computed tomography (μCT) showed that the fracture callus size was transiently reduced by either CBD or THC 4 weeks after fracture but reached control level after 6 and 8 weeks. The callus material density was unaffected by CBD and/or THC. In contrast, CBD stimulated mRNA expression of Plod1 in primary OB cultures to encode an enzyme that catalyzes lysine hydroxylation, which in turn was involved in collagen cross-linking and stabilization. These data show that CBD can improve fracture healing and plays a critical mechanical role of collagen cross-linked enzymes. The bones of rats treated with CBD alone not only healed faster but the previous fracture was less likely to break in the future because of a strengthened fracture callus. Therefore, CBD provides research directions for the treatment and prognosis of fractures.

Bab et al. (2011) showed that fatty acid amides (FAAs) assisted in the process of bone metabolism by interacting with CBRs. FAAs are important because they are broken down by a particular enzyme (FAAH) that is blocked by CBD. For quite some time, CBD was known to inhibit FAAH, knowing that it could prevent the enzyme from breaking down bone forming compounds. An effective function of bone anabolic-antiresorptive is shared by many skeletal FAAs. Inhibition of the FAA degrading enzyme (FAAH) may prove to be an effective therapeutic strategy for the treatment of bone fractures.

In summary, CBs could alleviate the development of arthritis, prevent osteoporosis, inhibit bone tumor cell proliferation, reduce bone cancer pain and improve fracture healing (**Figure 1**).

CONCLUSION

Bones provide structural support and physical protection to our soft tissues and allow us to walk, eat, breed and carry out the life activities. The ECS has been shown to regulate bone metabolism. An eCB deficiency may affect the skeletal system. No CB compound has been approved for the treatment of bone diseases at present. However, the important skeletal actions of the ECS have prompted both preclinical researchers and industrial companies to explore the clinical therapeutic potential based on CBs in the treatment of various bone diseases.

Evidence indicates the potential role of the ECS in the treatment of bone diseases because of the multiple targets involved in the pathologic process of various bone conditions including OP, OA, bone tumors and bone fractures. However, there is currently limited research on the use of CBs in the treatment of bone diseases, and the evaluation of medicinal cannabis in humans remains in its infancy. The following work still need to be further explored.

Firstly, the effect of CBR regulation on bone tissue metabolism needs further investigation in details. As it is necessary to further study the regulatory mechanisms of eCBs on osteogenesis, bone loss, synovial inflammatory response and arthritis pain, comprehensive evaluations of *in vitro* and *in vivo* mechanisms and pharmacologicals should be performed on each member of the eCB family. Secondly, to ensure clinical applicability of CBs, it is necessary to explore new ways to improve the therapeutic effects of CBs and reduce their neurological adverse effects, such as synthesizing new CB drugs and using CB hydrolase inhibitors to increase endogenous levels of CBs. Finally, further investigations on the function of the ECS and its role in bone diseases are required to provide a solid foundation for the evolution and refinement of cannabis-based medicines. Comprehensive evaluations through high-quality randomized controlled trials (RCTs) are also required to identify the true clinical efficacy and long-term risks associated with CB therapy.

The ECS plays a role in maintaining the bones strength and combating bone diseases, and holds promise as a novel drug for bone disease treatment.

AUTHOR CONTRIBUTIONS

YX designed the study and AT prepared the first draft of the paper. SP was responsible for figure and table. JZ modified the study. He is guarantor. All authors revised the paper critically for intellectual content and approved the final version. All authors agree to be accountable for the work and to ensure that any questions relating to the accuracy and integrity of the paper are investigated and properly resolved.

FUNDING

This work was supported by the National Natural Science Foundation of China (Grant No. 81960200 and 82160184).

REFERENCES

Bab, I., Ofek, O., Tam, J., Rehnelt, J., and Zimmer, A. (2008). Endocannabinoids and the Regulation of Bone Metabolism. *J. Neuroendocrinol* 20, 69–74. doi:10.1111/j.1365-2826.2008.01675.x

Bab, I., Smoum, R., Bradshaw, H., and Mechoulam, R. (2011). Skeletal Lipidomics: Regulation of Bone Metabolism by Fatty Acid Amide Family. *Br. J. Pharmacol.* 163 (7), 1441–1446. doi:10.1111/j.1476-5381.2011.01474.x

Berx, G., Nollet, F., and van Roy, F. (1998). Dysregulation of the E-Cadherin/catenin Complex by Irreversible Mutations in Human Carcinomas. *Cell Adhes. Commun.* 6 (null), 171–184. doi:10.3109/15419069809004474

Carnovali, M., Ottria, R., Pasqualetti, S., Banfi, G., Ciuffreda, P., and Mariotti, M. (2016). Effects of Bioactive Fatty Acid Amide Derivatives in Zebrafish Scale Model of Bone Metabolism and Disease. *Pharmacol. Res.* 104 (undefined), 1–8. doi:10.1016/j.phrs.2015.12.009

Curto-Reyes, V., Llames, S., Hidalgo, A., Menéndez, L., and Baamonde, A. (2010). Spinal and Peripheral Analgesic Effects of the CB2 Cannabinoid Receptor Agonist AM1241 in Two Models of Bone Cancer-Induced Pain. *Br. J. Pharmacol.* 160 (3), 561–573. doi:10.1111/j.1476-5381.2009.00629.x

Devane, W. A., Dysarz, F. A., Johnson, M. R., Melvin, L. S., and Howlett, A. C. (1988). Determination and Characterization of a Cannabinoid Receptor in Rat Brain. *Mol. Pharmacol.* 34 (5), 605–613.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., et al. (1992). Isolation and Structure of a Brain Constituent that Binds to the Cannabinoid Receptor. *Science* 258 (5090), 1946–1949. doi:10.1126/science.1470919

Di Marzo, V., and Petrosino, S. (2007). Endocannabinoids and the Regulation of Their Levels in Health and Disease. *Curr. Opin. Lipidol.* 18 (2), 129–140. doi:10.1097/MOL.0b013e32803dbdec

Di Marzo, V. (2008). Targeting the Endocannabinoid System: to Enhance or Reduce? *Nat. Rev. Drug Discov.* 7, 438–455. doi:10.1038/nrd2553

Dou, Z.-j., Gao, X.-L., Jia, Y.-l., Chen, J., Yang, J.-J., Chen, Y., et al. (2020). CB1 Receptor Antagonist Rimonabant Protects against Chronic Intermittent Hypoxia-Induced Bone Metabolism Disorder and Destruction in Rats. *Sleep Breath* 24, 1441–1449. doi:10.1007/s11325-019-02009-9

Dunn, S. L., Wilkinson, J. M., Crawford, A., Bunning, R. A. D., and Le Maitre, C. L. (2016). Expression of Cannabinoid Receptors in Human Osteoarthritic Cartilage: Implications for Future Therapies. *Cannabis Cannabinoid Res.* 1 (1), 3–15. doi:10.1089/can.2015.0001

Dunn, S. L., Wilkinson, J. M., Crawford, A., Le Maitre, C. L., and Bunning, R. A. (2012). Cannabinoids: Novel Therapies for Arthritis? *Future Med. Chem.* 4 (6), 713–725. doi:10.4155/fmc.12.20

Ehrenkranz, J., and Levine, M. A. (2019). Bones and Joints: The Effects of Cannabinoids on the Skeleton. *J. Clin. Endocrinol. Metab.* 104 (10), 4683–4694. doi:10.1210/jc.2019-00665

Frei, R. B., Luschnig, P., Parzmair, G. P., Peinhaupt, M., Schranz, S., Fauland, A., et al. (2016). Cannabinoid Receptor 2 Augments Eosinophil Responsiveness and Aggravates Allergen-Induced Pulmonary Inflammation in Mice. *Allergy* 71 (7), 944–956. doi:10.1111/all.12858

Gaoni, Y., and Mechoulam, R. (1964). Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. *J. Am. Chem. Soc.* 86, 1646–1647. doi:10.1021/ja01062a046

Ghilardi, J. R., Röhrich, H., Lindsay, T. H., Sevcik, M. A., Schwei, M. J., Kubota, K., et al. (2005). Selective Blockade of the Capsaicin Receptor TRPV1 Attenuates Bone Cancer Pain. *J. Neurosci.* 25 (12), 3126–3131. doi:10.1523/JNEUROSCI.3815-04.2005

Grinspoon, J. (1993). "Cannabis as a Medicine," in *Marijuana: The Forbidden Medicine* (New Haven: Yale University Press), 67–81.

Grotenhermen, F., and Müller-Vahl, K. (2012). The Therapeutic Potential of Cannabis and Cannabinoids. *Dtsch Arztebl Int.* 109 (null), 495–501. doi:10.3238/arztebl.2012.0495

Gui, H., Liu, X., Wang, Z. W., He, D. Y., Su, D. F., and Dai, S. M. (2014). Expression of Cannabinoid Receptor 2 and its Inhibitory Effects on Synovial Fibroblasts in Rheumatoid Arthritis. *Rheumatology (Oxford)* 53 (5), 802–809. doi:10.1093/rheumatology/ket447

Guidetti, R., Astles, P. C., Sanderson, A. J., Hollinshead, S. P., Johnson, M. P., and Chambers, M. G. (2014). The SAR Development of Substituted Purine Derivatives as Selective CB2 Agonists for the Treatment of Chronic Pain. *Bioorg. Med. Chem. Lett.* 24 (24), 5572–5575. doi:10.1016/j.bmcl.2014.11.006

Gustafsson, K., Wang, X., Severa, D., Eriksson, M., Kimby, E., Christensson, B., et al. (2008). Expression of Cannabinoid Receptors Type 1 and Type 2 in Non-hodgkin Lymphoma: Growth Inhibition by Receptor Activation. *Int. J. Cancer* 123 (5), 1025–1033. doi:10.1002/ijc.23584

Hutchins, H. L., Li, Y., Hannon, K., and Watkins, B. A. (2011). Eicosapentaenoic Acid Decreases Expression of Anandamide Synthesis Enzyme and Cannabinoid Receptor 2 in Osteoblast-like Cells. *J. Nutr. Biochem.* 22 (2), 195–200. doi:10.1016/j.jnutbio.2010.06.001

Idris, A. I., Landao-Bassonga, E., and Ralston, S. H. (2010). The TRPV1 Ion Channel Antagonist Capsazepine Inhibits Osteoclast and Osteoblast Differentiation *In Vitro* and Ovariectomy Induced Bone Loss *In Vivo*. *Bone* 46 (4), 1089–1099. doi:10.1016/j.bone.2010.01.368

Idris, A. I., and Ralston, S. H. (2010). Cannabinoids and Bone: Friend or Foe? *Calcif. Tissue Int.* 87 (4), 285–297. doi:10.1007/s00223-010-9378-8

Idris, A. I., Sophocleous, A., Landao-Bassonga, E., Canals, M., Milligan, G., Baker, D., et al. (2009). Cannabinoid Receptor Type 1 Protects against Age-Related Osteoporosis by Regulating Osteoblast and Adipocyte Differentiation in Marrow Stromal Cells. *Cell Metab* 10 (2), 139–147. doi:10.1016/j.cmet.2009.07.006

Idris, A. I., van 't Hof, R. J., Greig, I. R., Ridge, S. A., Baker, D., Ross, R. A., et al. (2005). Regulation of Bone Mass, Bone Loss and Osteoclast Activity by Cannabinoid Receptors. *Nat. Med.* 11 (7), 774–779. doi:10.1038/nm1255

Jezierska, A., and Motyl, T. (2009). Matrix Metalloproteinase-2 Involvement in Breast Cancer Progression: a Mini-Review. *Med. Sci. Monit.* 15 (2), RA32–40. doi:10.1016/j.mehy.2008.08.012

Jiang, S., Zagozdzon, R., Jorda, M. A., Parmar, K., Fu, Y., Williams, J. S., et al. (2010). Endocannabinoids Are Expressed in Bone Marrow Stromal Niches and Play a Role in Interactions of Hematopoietic Stem and Progenitor Cells with the Bone Marrow Microenvironment. *J. Biol. Chem.* 285 (46), 35471–35478. doi:10.1074/jbc.M110.144758

Joshi, N., and Onaivi, E. S. (2019). Endocannabinoid System Components: Overview and Tissue Distribution. *Adv. Exp. Med. Biol.* 1162 (undefined), 1–12. doi:10.1007/978-3-030-21737-2_1

Karsdal, M. A., Bay-Jensen, A. C., Lories, R. J., Abramson, S., Spector, T., Pastoureau, P., et al. (2014). The Coupling of Bone and Cartilage Turnover in Osteoarthritis: Opportunities for Bone Antiresorptives and Anabolics as Potential Treatments? *Ann. Rheum. Dis.* 73 (2), 336–348. doi:10.1136/annrheumdis-2013-204111

Kawamata, T., Niiyama, Y., Yamamoto, J., and Furuse, S. (2010). Reduction of Bone Cancer Pain by CB1 Activation and TRPV1 Inhibition. *J. Anesth.* 24 (2), 328–332. doi:10.1007/s00540-010-0919-0

Kogan, N. M., Melamed, E., Wasserman, E., Raphael, B., Breuer, A., Stok, K. S., et al. (2015). Cannabidiol, a Major Non-psychotropic Cannabis Constituent Enhances Fracture Healing and Stimulates Lysyl Hydroxylase Activity in Osteoblasts. *J. Bone Miner Res.* 30, 1905–1913. doi:10.1002/jbmr.2513

Liu, X., Xu, Y., Chen, S., Tan, Z., Xiong, K., Ye, Y., et al. (2014). Rescue of Proinflammatory Cytokine-Inhibited Chondrogenesis by the Antiarthritic Effect of Melatonin in Synovium Mesenchymal Stem Cells via Suppression of Reactive Oxygen Species and Matrix Metalloproteinases. *Free Radic. Biol. Med.* 68 (undefined), 234–246. doi:10.1016/j.freeradbiomed.2013.12.012

Lowin, T., Schneider, M., and Pongratz, G. (2019). Joints for Joints: Cannabinoids in the Treatment of Rheumatoid Arthritis. *Curr. Opin. Rheumatol.* 31 (3), 271–278. doi:10.1097/BOR.0000000000000590

Lozano-Ondoua, A. N., Hanlon, K. E., Symons-Liguori, A. M., Largent-Milnes, T. M., Havelin, J. J., Ferland, H. L., et al. (2013). Disease Modification of Breast Cancer-Induced Bone Remodeling by Cannabinoid 2 Receptor Agonists. *J. Bone Miner Res.* 28 (1), 92–107. doi:10.1002/jbmr.1732

Lozano-Ondoua, A. N., Wright, C., Vardanyan, A., King, T., Largent-Milnes, T. M., Jimenez-Andrade, J. M., et al. (2010). A Cannabinoid 2 Receptor Agonist Attenuates Bone Cancer-Induced Pain and Bone Loss. *Life Sci.* 86 (null), 646–653. doi:10.1016/j.lfs.2010.02.014

Maccarrone, M., Bab, I., Bíró, T., Cabral, G. A., Dey, S. K., Di Marzo, V., et al. (2015). Endocannabinoid Signaling at the Periphery: 50 Years after THC. *Trends Pharmacol. Sci.* 36 (5), 277–296. doi:10.1016/j.tips.2015.02.008

Malfait, A. M., Gallily, R., Sumariwalla, P. F., Malik, A. S., Andreakos, E., Mechoulam, R., et al. (2000). The Nonpsychoactive Cannabis Constituent Cannabidiol Is an Oral Anti-arthritic Therapeutic in Murine Collagen-Induced Arthritis. *Proc. Natl. Acad. Sci. U S A.* 97 (17), 9561–9566. doi:10.1073/pnas.160105897

Marino, S., de Ridder, D., Bishop, R. T., Renema, N., Ponzetti, M., Sophocleous, A., et al. (2019). Paradoxical Effects of JZL184, an Inhibitor of Monoacylglycerol Lipase, on Bone Remodelling in Healthy and Cancer-Bearing Mice. *EBioMedicine* 44, 452–466. doi:10.1016/j.ebiom.2019.05.048

Marino, S., and Idris, A. I. (2017). Emerging Therapeutic Targets in Cancer Induced Bone Disease: A Focus on the Peripheral Type 2 Cannabinoid Receptor. *Pharmacol. Res.* 119 (undefined), 391–403. doi:10.1016/j.phrs.2017.02.023

Mbvundula, E. C., Bunning, R. A., and Rainsford, K. D. (2006). Arthritis and Cannabinoids: HU-210 and Win-55,212-2 Prevent IL-1alpha-induced Matrix Degradation in Bovine Articular Chondrocytes *In-Vitro*. *J. Pharm. Pharmacol.* 58 (3), 351–358. doi:10.1211/jpp.58.3.0009

McPartland, J. M., Matias, I., Di Marzo, V., and Glass, M. (2006). Evolutionary Origins of the Endocannabinoid System. *Gene* 370 (undefined), 64–74. doi:10.1016/j.gene.2005.11.004

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., et al. (1995). Identification of an Endogenous 2-monoglyceride, Present in Canine Gut, that Binds to Cannabinoid Receptors. *Biochem. Pharmacol.* 50 (1), 83–90. doi:10.1016/0006-2952(95)00109-d

Mechoulam, R. (1986). "The Pharmacohistory of Cannabis Sativa," in *Cannabinoids as Therapeutic Agents* (Roca Raton: CRC Press), 1–19.

Munro, S., Thomas, K. L., and Abu-Shaar, M. (1993). Molecular Characterization of a Peripheral Receptor for Cannabinoids. *Nature* 365, 61–65. doi:10.1038/365061a0

Niu, F., Zhao, S., Xu, C. Y., Sha, H., Bi, G. B., Ye, L., et al. (2015). Potentiation of the Antitumor Activity of Adriamycin against Osteosarcoma by Cannabinoid WIN-55,212-2. *Oncol. Lett.* 10 (4), 2415–2421. doi:10.3892/ol.2015.3525

Ofek, O., Karsak, M., Leclerc, N., Fogel, M., Frenkel, B., Wright, K., et al. (2006). Peripheral Cannabinoid Receptor, CB2, Regulates Bone Mass. *Proc. Natl. Acad. Sci. U S A.* 103 (3), 696–701. doi:10.1073/pnas.0504187103

Ossola, C. A., Surkin, P. N., Mohn, C. E., Elverdin, J. C., and Fernández-Solari, J. (2016). Anti-Inflammatory and Osteoprotective Effects of Cannabinoid-2 Receptor Agonist HU-308 in a Rat Model of Lipopolysaccharide-Induced Periodontitis. *J. Periodontol.* 87, 725–734. doi:10.1902/jop.2016.150612

Pertwee, R. G. (2005). Pharmacological Actions of Cannabinoids," in *Handb Exp. Pharmacol.*, Chap. 1, 1–51. doi:10.1007/3-540-26573-2_1

Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P., Di Marzo, V., Elphick, M. R., et al. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid Receptors and Their Ligands: beyond CB_1 and CB_2. *Pharmacol. Rev.* 62 (4), 588–631. doi:10.1124/pr.110.003004

Pertwee, R. G. (2010). Receptors and Channels Targeted by Synthetic Cannabinoid Receptor Agonists and Antagonists. *Curr. Med. Chem.* 17 (14), 1360–1381. doi:10.2174/092986710790980050

Pertwee, R. G., and Ross, R. A. (2002). Cannabinoid Receptors and Their Ligands. *Prostaglandins Leukot. Essent. Fatty Acids* 66, 101–121. doi:10.1054/plef.2001.0341

Preet, A., Ganju, R. K., and Groopman, J. E. (2008). Delta9-Tetrahydrocannabinol Inhibits Epithelial Growth Factor-Induced Lung Cancer Cell Migration *In Vitro* as Well as its Growth and Metastasis *In Vivo*. *Oncogene* 27 (3), 339–346. doi:10.1038/sj.onc.1210641

Qamri, Z., Preet, A., Nasser, M. W., Bass, C. E., Leone, G., Barsky, S. H., et al. (2009). Synthetic Cannabinoid Receptor Agonists Inhibit Tumor Growth and Metastasis of Breast Cancer. *Mol. Cancer Ther.* 8 (11), 3117–3129. doi:10.1158/1535-7163.MCT-09-0448

Richardson, D., Pearson, R. G., Kurian, N., Latif, M. L., Garle, M. J., Barrett, D. A., et al. (2008). Characterisation of the Cannabinoid Receptor System in Synovial

Tissue and Fluid in Patients with Osteoarthritis and Rheumatoid Arthritis. *Arthritis Res. Ther.* 10 (2), R43. doi:10.1186/ar2401

Robson, P. J. (2014). Therapeutic Potential of Cannabinoid Medicines. *Drug Test. Anal.* 6 (null), 24–30. doi:10.1002/dta.1529

Rossi, F., Bellini, G., Luongo, L., Torella, M., Mancusi, S., De Petrocellis, L., et al. (2011). The Endovanilloid/endocannabinoid System: a New Potential Target for Osteoporosis Therapy. *Bone* 48 (5), 997–1007. doi:10.1016/j.bone.2011.01.001

Rossi, F., Siniscalco, D., Luongo, L., De Petrocellis, L., Bellini, G., Petrosino, S., et al. (2009). The Endovanilloid/endocannabinoid System in Human Osteoclasts: Possible Involvement in Bone Formation and Resorption. *Bone* 44 (3), 476–484. doi:10.1016/j.bone.2008.10.056

Rossi, F., Tortora, C., Punzo, F., Bellini, G., Argenziano, M., Di Paola, A., et al. (2019). The Endocannabinoid/Endovanilloid System in Bone: From Osteoporosis to Osteosarcoma. *Int. J. Mol. Sci.* 20 (8). doi:10.3390/ijms20081919

Schuelert, N., Johnson, M. P., Oskins, J. L., Jassal, K., Chambers, M. G., and McDougall, J. J. (2011). Local Application of the Endocannabinoid Hydrolysis Inhibitor URB597 Reduces Nociception in Spontaneous and Chemically Induced Models of Osteoarthritis. *Pain* 152 (5), 975–981. doi:10.1016/j.pain.2010.11.025

Silver Robert, J. (2019). The Endocannabinoid System of Animals. *Animals (Basel)* 9, 686. doi:10.3390/ani9090686

Smoum, R., Baraghithy, S., Chourasia, M., Breuer, A., Mussai, N., Kogan, N. M., et al. (2015). CB2 Cannabinoid Receptor Agonist Enantiomers HU-433 and HU-308: An Inverse Relationship between Binding Affinity and Biological Potency. *Proc. Natl. Acad. Sci. U S A.* 112 (28), 8774–8779. doi:10.1073/pnas.1503395112

Sophocleous, A., Börjesson, A. E., Salter, D. M., and Ralston, S. H. (2015). The Type 2 Cannabinoid Receptor Regulates Susceptibility to Osteoarthritis in Mice. *Osteoarthritis Cartilage* 23 (9), 1586–1594. doi:10.1016/j.joca.2015.04.020

Sophocleous, A., Marino, S., Kabir, D., Ralston, S. H., and Idris, A. I. (2017). Combined Deficiency of the Cnr1 and Cnr2 Receptors Protects against Age-Related Bone Loss by Osteoclast Inhibition. *Aging Cell* 16 (5), 1051–1061. doi:10.1111/acel.12638

Sumariwalla, P. F., Gallily, R., Tchilibon, S., Fride, E., Mechoulam, R., and Feldmann, M. (2004). A Novel Synthetic, Nonpsychoactive Cannabinoid Acid (HU-320) with Antiinflammatory Properties in Murine Collagen-Induced Arthritis. *Arthritis Rheum.* 50 (3), 985–998. doi:10.1002/art.20050

Sun, R., Zhang, B., Chen, L., and Sun, J. (2014). Role of Nuclear Factor of Activated T Cells 1 in the Pathogenesis of Osteoarthritis. *Exp. Ther. Med.* 7 (1), 195–198. doi:10.3892/etm.2013.1390

Tam, J., Ofek, O., Fride, E., Ledent, C., Gabet, Y., Zimmer, A., et al. (2006). Involvement of Neuronal Cannabinoid Receptor CB1 in Regulation of Bone Mass and Bone Remodeling. *Mol. Pharmacol.* 70 (3), 786–792. doi:10.1124/mol.106.026435

Tam, J., Trembovler, V., Di Marzo, V., Petrosino, S., Leo, G., Alexandrovich, A., et al. (2008). The Cannabinoid CB1 Receptor Regulates Bone Formation by Modulating Adrenergic Signaling. *FASEB J.* 22 (1), 285–294. doi:10.1096/fj.06-7957com

Whyte, L. S., Ford, L., Ridge, S. A., Cameron, G. A., Rogers, M. J., and Ross, R. A. (2012). Cannabinoids and Bone: Endocannabinoids Modulate Human Osteoclast Function *In Vitro*. *Br. J. Pharmacol.* 165 (8), 2584–2597. doi:10.1111/j.1476-5381.2011.01519.x

Whyte, L. S., Ryberg, E., Sims, N. A., Ridge, S. A., Mackie, K., Greasley, P. J., et al. (2009). The Putative Cannabinoid Receptor GPR55 Affects Osteoclast Function *In Vitro* and Bone Mass *In Vivo*. *Proc. Natl. Acad. Sci. U S A.* 106 (38), 16511–16516. doi:10.1073/pnas.0902743106

Yan, W., Cao, Y., Yang, H., Han, N., Zhu, X., Fan, Z., et al. (2019). CB1 Enhanced the Osteo/dentinogenic Differentiation Ability of Periodontal Ligament Stem Cells via P38 MAPK and JNK in an Inflammatory Environment. *Cell Prolif* 52, e12691. doi:10.1111/cpr.12691

Yang, L., Li, F. F., Han, Y. C., Jia, B., and Ding, Y. (2015). Cannabinoid Receptor CB2 Is Involved in Tetrahydrocannabinol-Induced Anti-inflammation against Lipopolysaccharide in MG-63 Cells. *Mediators Inflamm.* 2015 (undefined), 362126. doi:10.1155/2015/362126

Yang, S., Dubé, C. E., Eaton, C. B., McAlindon, T. E., and Lapane, K. L. (2013). Longitudinal Use of Complementary and Alternative Medicine Among Older Adults with Radiographic Knee Osteoarthritis. *Clin. Ther.* 35 (11), 1690–1702. doi:10.1016/j.clinthera.2013.09.022

Zhang, M., Shi, X., Wu, J., Wang, Y., Lin, J., Zhao, Y., et al. (2019). CoCl2 Induced Hypoxia Enhances Osteogenesis of Rat Bone Marrow Mesenchymal Stem Cells through Cannabinoid Receptor 2. *Arch. Oral Biol.* 108, 104525. doi:10.1016/j.archoralbio.2019.104525

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 25 January 2022
doi: 10.3389/fphar.2022.831052

Metabolomic Fingerprint of Behavioral Changes in Response to Full-Spectrum Cannabis Extracts

*Zaid H. Maayah[1], Pamela J. F. Raposo[2], Heidi Silver[1], Rupasri Mandal[3,4], Lee Ellis[5], Abrar S. Alam[1], Shingo Takahara[1], Mourad Ferdaoussi[1], Kyle E. Mathewson[6,7], Dean T. Eurich[8], Karim Fouad[2,6], David S. Wishart[3,4] and Jason R. B. Dyck[1]**

[1]Cardiovascular Research Centre, Department of Pediatrics, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada, [2]Faculty of Rehabilitation Medicine - Physical Therapy, University of Alberta, Edmonton, AB, Canada, [3]The Metabolomics Innovation Centre (TMIC), University of Alberta, Edmonton, AB, Canada, [4]Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada, [5]National Research Council of Canada, Halifax, NS, Canada, [6]Neuroscience and Mental Health Institute, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada, [7]Department of Psychology, Faculty of Science, University of Alberta, Edmonton, AB, Canada, [8]School of Public Health, University of Alberta, Edmonton, AB, Canada

OPEN ACCESS

Edited by:
Francesca Baratta, University of Turin, Italy

Reviewed by:
Carmela Maria Montone, Sapienza University of Rome, Italy
Michihito Deguchi, Penn State Harrisburg, United States

***Correspondence:**
Jason R. B. Dyck
jason.dyck@ualberta.ca

Specialty section:
This article was submitted to Experimental Pharmacology and Drug Discovery, a section of the journal Frontiers in Pharmacology

Received: *07 December 2021*
Accepted: *03 January 2022*
Published: *25 January 2022*

Citation:
Maayah ZH, Raposo PJ, Silver H, Mandal R, Ellis L, Alam AS, Takahara S, Ferdaoussi M, Mathewson KE, Eurich DT, Fouad K, Wishart DS and Dyck JRB (2022) Metabolomic Fingerprint of Behavioral Changes in Response to Full-Spectrum Cannabis Extracts. Front. Pharmacol. 13:831052. doi: 10.3389/fphar.2022.831052

Numerous existing full-spectrum cannabis extract products have been used in clinical trials for the treatment of various diseases. Despite their efficacy, the clinical use of some of these full-spectrum cannabis extracts is limited by behavioral side effects such as cognitive dysfunction and impaired motor skills. To better understand what constitutes cannabis-induced behavioral effects, our objective was to identify a novel panel of blood-based metabolites that are predictive, diagnostic, and/or prognostic of behavioral effects.

At 8 weeks of age, male rats were randomly assigned to groups and were gavage fed with full-spectrum cannabis extract (tetrahydrocannabinol/cannabidiol (THC/CBD) along with all other cannabis compounds, 15 mg/kg), broad-spectrum cannabis extract (CBD along with all other cannabis compounds, 15 mg/kg), or vehicle oil. Four hours after being gavage fed, behavioral assessments were determined using the open field test and the elevated plus maze. Following these assessments, serum was collected from all rats and the serum metabolites were identified and quantified by LC–MS/MS and 1H NMR spectroscopy.

We found that only rats treated with full-spectrum cannabis extract exhibited behavioral changes. Compared to vehicle-treated and broad-spectrum extract–treated rats, full-spectrum extract–treated rats demonstrated higher serum concentrations of the amino acid phenylalanine and long-chain acylcarnitines, as well as lower serum concentrations of butyric acid and lysophosphatidylcholines. This unique metabolomic fingerprint in response to cannabis extract administration is linked to behavioral effects and may represent a biomarker profile of cannabis-induced behavioral changes. If validated, this work may allow a metabolomics-based decision tree that would aid in the rapid diagnosis of cannabis-induced behavioral changes including cognitive impairment.

Keywords: cannabis, metabolomics, behavior, THC - tetrahydrocannabinol, CBD - cannabidiol

INTRODUCTION

Cannabis has been used for centuries due to its medicinal benefits (Maayah et al., 2020b). Numerous existing full-spectrum cannabis extract products, that is, tetrahydrocannabinol/cannabidiol (THC/CBD), along with many other compounds such as terpenes and cannabinoids, have been used for the treatment of various conditions such as neuropathic pain and inflammation (Izzo et al., 2009; Maayah et al., 2020a; Maayah and Dyck, 2020). However, despite its efficacy in small patient studies (Maayah et al., 2020a; Maayah and Dyck, 2020), the clinical use of full-spectrum cannabis extract is limited due to some of the side effects such as behavioral changes (Misner and Sullivan, 1999; Maayah et al., 2020a; Maayah and Dyck, 2020). Behavioral side effects of full-spectrum cannabis extract involve impaired short-term memory and concentration (Misner and Sullivan, 1999; Maayah and Dyck, 2020) as well as delayed reaction time (Misner and Sullivan, 1999; Maayah and Dyck, 2020), that is, cognitive dysfunction (Misner and Sullivan, 1999; Maayah and Dyck, 2020). In addition, cannabis-related behavioral effects often involve reduced motor skills and coordination, which can eventually affect an individual's overall quality of life (Misner and Sullivan, 1999; Maayah and Dyck, 2020) even for tasks like impairing the ability to drive (Hartman and Huestis, 2013; Lee et al., 2021). However, there is no evidence that the simple presence of cannabis impairs cognitive function or affects motor skills and coordination (Brubacher et al., 2019). Also, given that many cannabinoids are present in cannabis and a number of these can induce behavioral changes (Montone et al., 2020), a test designed to measure THC levels in biological fluid samples may be misleading as these levels may not be truly indicative of cognitive dysfunction or impairment in motor skills (Brubacher et al., 2019). Thus, there remains a crucial need to identify tests that can help in the detection of full-spectrum cannabis–related behavioral effects and then devise a real-time decision tree that would have an impact on different aspects of a user's day-to-day activities like the ability to operate a motor vehicle.

To better understand what constitutes cannabis-induced behavioral effects, we have used quantitative metabolomics to identify a novel panel of blood-based metabolites that are predictive, diagnostic, and/or prognostic of cannabis-induced behavioral effects. If validated, this work may allow a simple and reliable decision tree that would aid in the rapid diagnosis of cannabis-induced behavioral effects including cognitive impairment.

METHODS

Experimental Design and Treatment Protocol

All protocols involving rodents were approved by the University of Alberta Institutional Animal Care and Use Committee (Health Sciences) and conform to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (eighth edition; revised 2011). The University of Alberta adheres to the principles for biomedical research involving animals developed by the Council for International Organizations of Medical Sciences and complies with the Canadian Council on Animal Care guidelines.

Male Sprague–Dawley rats were purchased from Charles River Laboratories. All rats were housed under standard conditions (25°C, 12:12-h light/ dark cycle) with ad libitum access to food and water. At 8 weeks of age, rats were randomly assigned into groups and were gavage-fed with broad-spectrum cannabis extract (i.e., CBD extract along with all other compounds and cannabinoids, except THC) (15 mg/kg), full-spectrum cannabis extract products (i.e., THC/CBD extract along with all other compounds and cannabinoids) (15 mg/kg), or vehicle oil (**Supplementary Table S1**). Four hours after being gavage-fed, behavioral observation assessments and blood collection were performed. Blood samples were collected in evacuated blood collection tubes and then the samples were allowed to clot by leaving them undisturbed at room temperature for 15 min. Subsequently, the serum was separated by centrifugation of blood samples at 2,000 × g for 10 min at 4°C. Following centrifugation, the serum was transferred into clean polypropylene tubes and stored at -80°C.

Behavioral Testing

Tests were performed during the light cycle by an experimenter blind to the group conditions. All testing apparatuses were cleaned with unscented soap and water and dried between each animal. The trainer was blinded to treatment groups and performed behavioral analysis also post session.

Open Field Test

To assess general locomotor performance and exploratory activity, rats were placed in the center of an open-field arena (100 × 80 × 30 cm) for 5 min and video recorded from above (Walsh and Cummins, 1976). Offline video analysis of the distance traveled was performed using customized tracking software (https://github.com/cdoolin/rat-apps) (Walsh and Cummins, 1976). The total distance traveled, percentage of time spent, and distance traveled in the inner 45% of the arena were measured, as described previously (Walsh and Cummins, 1976).

Elevated Plus Maze

To assess anxiety-like behavior, rats were placed in the junction of two open arms and two closed arms (each arm is 50 cm long and 10 cm wide) elevated 65 cm above the ground while being video recorded from above for 10 min (Itoh et al., 1990; Sharma and Kulkarni, 1992). Offline video analysis was performed using customized motion tracking software (https://github.com/cdoolin/rat-apps) to analyze the percentage of time spent in the open arms and total distance traveled (Itoh et al., 1990; Sharma and Kulkarni, 1992). Entries into the open and closed arms of the elevated plus maze (EPM) were counted when all 4 paws were located in the arm, as described previously (Itoh et al., 1990; Sharma and Kulkarni, 1992).

Combined Direct Flow Injection and Liquid Chromatography–Tandem Mass Spectrometry Compound Identification and Quantification

We applied a targeted quantitative metabolomics approach to analyze the samples using a combination of direct injection mass spectrometry with a reverse-phase LC–MS/MS custom assay. This custom assay, in combination with an AB Sciex 4000 QTRAP (Applied Biosystems/MDS Sciex) mass spectrometer, can be used for the targeted identification and quantification of up to 143 different endogenous metabolites including amino acids, acylcarnitines, biogenic amines and derivatives, uremic toxins, glycerophospholipids, sphingolipids, and sugars (Sung et al., 2017;Foroutan et al., 2019; Foroutan et al., 2020). The method combines the derivatization and extraction of analytes and the selective mass spectrometric detection using multiple reaction monitoring (MRM) pairs. Isotope-labeled internal standards and other internal standards are used for metabolite quantification. The custom assay contains a 96-well deep well plate with a filter plate attached with sealing tape, and reagents and solvents used to prepare the plate assay. The first 14 wells were used for one blank, three zero samples, seven standards, and three quality control samples. For all metabolites, except organic acid, samples were thawed on ice and were vortexed and centrifuged at 13,000 ×g. Then 10 µl of each sample was loaded onto the center of the filter on the upper 96-well plate and dried in a stream of nitrogen. Subsequently, phenyl isothiocyanate was added for derivatization. After incubation, the filter spots were dried again using an evaporator. Extraction of the metabolites were then achieved by adding 300 µl of extraction solvent. The extracts were obtained by centrifugation into the lower 96-well deep well plate, followed by a dilution step with MS running solvent.

For organic acid analysis, 150 µl of ice-cold methanol and 10 µl of isotope-labeled internal standard mixture were added to 50 µl of the serum sample for overnight protein precipitation. Then it was centrifuged at 13,000× g for 20 min. After that, 50 µl of supernatant was loaded at the center of the wells of a 96-well deep well plate, followed by the addition of 3-nitrophenylhydrazine (NPH) reagent. After incubation for 2 h, a BHT stabilizer and water were added before LC–MS injection.

Mass spectrometric analysis was performed on an AB Sciex 4000 QTRAP® tandem mass spectrometry instrument (Applied Biosystems/MDS Analytical Technologies, Foster City, CA) equipped with an Agilent 1260 series UHPLC system (Agilent Technologies, Palo Alto, CA). The samples were delivered to the mass spectrometer by an LC method, followed by a direct injection (DI) method. Data analysis was performed using Analyst 1.6.2.

Nuclear Magnetic Resonance Spectroscopy

Serum samples contain a significant concentration of large–molecular weight proteins and lipoproteins, which affect the identification of the small–molecular weight metabolites by NMR spectroscopy. Therefore, a deproteinization step, involving ultrafiltration, as previously described (Psychogios et al., 2011), was therefore introduced in the protocol to remove serum proteins. Prior to filtration, 3-KDa cutoff centrifugal filter units (Amicon Microcon YM-3) were rinsed five times each with 0.5 ml of H_2O and centrifuged (10,000 rpm for 10 min) to remove residual glycerol bound to the filter membranes. Aliquots of each serum sample were then transferred into the centrifuge filter devices and spun (10,000 rpm for 20 min) to remove macromolecules (primarily protein and lipoproteins) from the sample. The filtrates were checked visually for any evidence that the membrane was compromised, and for these samples, the filtration process was repeated with a different filter and the filtrate inspected again. The subsequent filtrates were collected, and the volumes were recorded. If the total volume of the sample was under 250 µl, an appropriate amount from a 150 mM KH_2PO_4 buffer (pH 7) was added until the total volume of the sample was 173.5 µ. Any sample that had to have buffer added to bring the solution volume to 173.5 µl was annotated with the dilution factor, and metabolite concentrations were corrected in the subsequent analysis. Subsequently, 46.5 µl of a standard buffer solution (54% D_2O:46% 1.75 mM KH_2PO_4 pH 7.0 v/v containing 5.84 mM DSS (2,2-dimethyl-2-silcepentane-5-sulphonate), 5.84 mM 2-chloropyrimidine-5 carboxylate, and 0.1% NaN_3 in H_2O) was added to the sample.

The serum sample (250 µl) was then transferred to a 3-mm SampleJet NMR tube for subsequent spectral analysis. All 1H NMR spectra were collected on a 700 MHz Avance III (Bruker) spectrometer equipped with a 5-mm HCN Z-gradient pulsed-field gradient (PFG) cryoprobe. 1H NMR spectra were acquired at 25°C using the first transient of the NOESY pre-saturation pulse sequence (noesy1dpr), chosen for its high degree of quantitative accuracy (Saude et al., 2006). All FIDs (free induction decays) were zero-filled to 250K data points. The singlet produced by the DSS methyl groups was used as an internal standard for chemical shift referencing (set to 0 ppm), and for quantification, all 1H NMR spectra were processed and analyzed using an in-house version of the MAGMET automated analysis software package using a custom metabolite library. MAGMET allows for qualitative and quantitative analysis of an NMR spectrum by automatically fitting spectral signatures from an internal database to the spectrum. Each spectrum was further inspected by an NMR spectroscopist to minimize compound misidentification and misquantification. Typically, all visible peaks were assigned. Most of the visible peaks were annotated with a compound name. It has been previously shown that this fitting procedure provides absolute concentration accuracy of 90% or better (Zordoky et al., 2015; Ravanbakhsh et al., 2015).

Cannabinoid Analysis Method

For cannabinoid analysis, 150 µl of ice-cold methanol and 10 µl of isotope-labeled internal standard mixture were added to 50 µl of each individual sample (PBS as blank sample, calibration standards, QC standards, and serum samples) for a 1-h protein precipitation. All the samples were then centrifuged at 13,000 × g for 20 min. For each sample, 180 µl of the supernatant

was loaded into the center of corresponding wells of a 96-well deep well plate, followed by the addition of 90 µl of water to each well. The plate was then shaken at 600 rpm for 15 min before LC–MS/MS analysis. Mass spectrometric analysis was performed on an AB Sciex 5500 QTRAP® tandem mass spectrometry instrument (Applied Biosystems/MDS Analytical Technologies, Foster City, CA) equipped with an Agilent 1290 series UHPLC system (Agilent Technologies, Palo Alto, CA). Data analysis was done using Analyst 1.6.3.

Statistical Analysis

Results are shown as means ± SEM. Statistical analysis was carried out using GraphPad Prism software (version 7.04) (GraphPad Software, Inc., La Jolla, CA). The Shapiro–Wilk test was used to assess the normality of distribution of each parameter. One-way analysis of variance (ANOVA), followed by the Tukey–Kramer post hoc multiple comparison test, unpaired two-tailed *t*-test for normally distributed data, or Kruskal–Wallis test for non-normally distributed data were carried out to assess which treatment group(s) showed a significant difference.

For metabolomic data analysis, log-transformation was applied to all quantified metabolites to normalize the concentration distributions. Heat maps were generated with the concentrations of potential candidate metabolites, which were extracted with univariate analysis. It was generated without hierarchical cluster analysis unlike usual structures of heat maps and simply arranged by grouping similar metabolites together for use in pathway analysis through intuitive pattern discovery. The heat map displays an increase in each metabolite in relative concentration as red color and a decrease in a metabolite as blue color. The metabolites are listed at the left side of each row, and the subjects are shown at the bottom of each column. Partial least squares discriminant analysis (PLS-DA) score plots were used to compare serum metabolite data across and between study groups; 100-fold permutation tests were used to minimize the possibility that the observed separation of the PLS-DA was due to chance. Coefficient scores and least absolute shrinkage and selection operator (LASSO) algorithm were used to identify the most discriminating metabolites for group comparisons. A receiver operating characteristic (ROC) curve was determined. The ROC calculations included bootstrap 95% confidence intervals for the desired model specificity and other measures including accuracy and false discovery rates (FDR). Metabolite data analyses were done using MetaboAnalyst (Lopez-Hernandez et al., 2021).

RESULTS

Full-Spectrum Cannabis Extract Induces Behavioral Changes in Rats

Considering the fact that rats are commonly used animals in behavioral research (Feyissa et al., 2017), we sought to test the effect of full-spectrum and broad-spectrum cannabis extracts on behavioral changes in our rat model using a clinically relevant dose of cannabis (Huestis, 2007). Given that there is no evidence that the simple presence of cannabis (i.e., low concentration of THC) impairs behavior (Brubacher et al., 2019), the dosage of full-spectrum cannabis extract (15 mg/kg, per oral) we used in our rat model was selected from the literature from studies in rodents to provide the highest achievable concentration of THC in humans (Huestis, 2007). To do this, rats were treated with full-spectrum cannabis extract, broad-spectrum cannabis extract, or vehicle (**Figures 1A–C**). Interestingly, while broad-spectrum cannabis extract-treated rats did not demonstrate significant behavioral changes, rats treated with full-spectrum cannabis extract traveled significantly less distance and made significantly fewer open arm entries than broad-spectrum cannabis extract–treated and vehicle-treated rats in the elevated plus maze (**Figures 1D–F**). Taken together, these data suggest that rats administered with full-spectrum cannabis extract displayed a significant behavioral change.

To further assess general locomotor performance and exploratory activity, we performed open field tests in our rat model (**Figure 1H**). Our results show that full-spectrum cannabis extract–treated rats displayed a significant reduction in the total distance traveled in the open field (**Figure 1I**). In addition, full-spectrum cannabis extract–treated rats demonstrated a significant increase in the percentage of inner time compared to broad-spectrum cannabis extract–treated and vehicle-treated rats (**Figure 1J**). However, there was no difference between all experimental groups in the % time open arms in the elevated plus maze as well as the inner distance in the open field test (**Figures 1G,K**). Overall, our data indicate that full-spectrum cannabis extract induces clear behavioral effects in our rats.

Serum Metabolite Profile Differences Between Full-Spectrum Cannabis Extract and Controls

Serum metabolomic analysis of a total of 181 analyzed metabolites from DI-MS (148 metabolites) and NMR (33 metabolites) revealed that the serum concentrations of some medium-chain and long-chain acylcarnitines, kynurenine, phosphatidylcholines (PC) such as PC ae C40:6, some lysophosphatidylcholines (LysoPC), sphingomyelins (SM) such as SM(OH) C22:2, and several amino acids such as phenylalanine were higher in full-spectrum cannabis extract–treated group than controls (**Figure 2A**, **Supplementary Table S1**). Conversely, the serum concentrations of some short-chain acylcarnitines such as C4, carnitine, LysoPC such as LysoPC a C18:2, LysoPC a C20:3 and LysoPC a C20:4, butyric acid, glutamic acid, methylmalonic acid, lactic acid, hippuric acid, homovanillic acid, alpha-ketoglutaric acid, uric acid, methionine sulfoxide, and several amino acids such as tyrosine were lower in full-spectrum cannabis extract–treated group than controls (**Figure 2A**, **Supplementary Table S2**).

To identify potential metabolite biomarkers of cannabis-related behavioral effects, PLS-DA was performed to find the most parsimonious model to discriminate full-spectrum cannabis extract–treated group from the controls. A small number of metabolites including phenylalanine, tyrosine, butyric acid, LysoPC a C18:2, C0, alanine, C4, C16, C18:1, proline, *trans*-hydroxyproline, and glutamic acid was able to discriminate full-

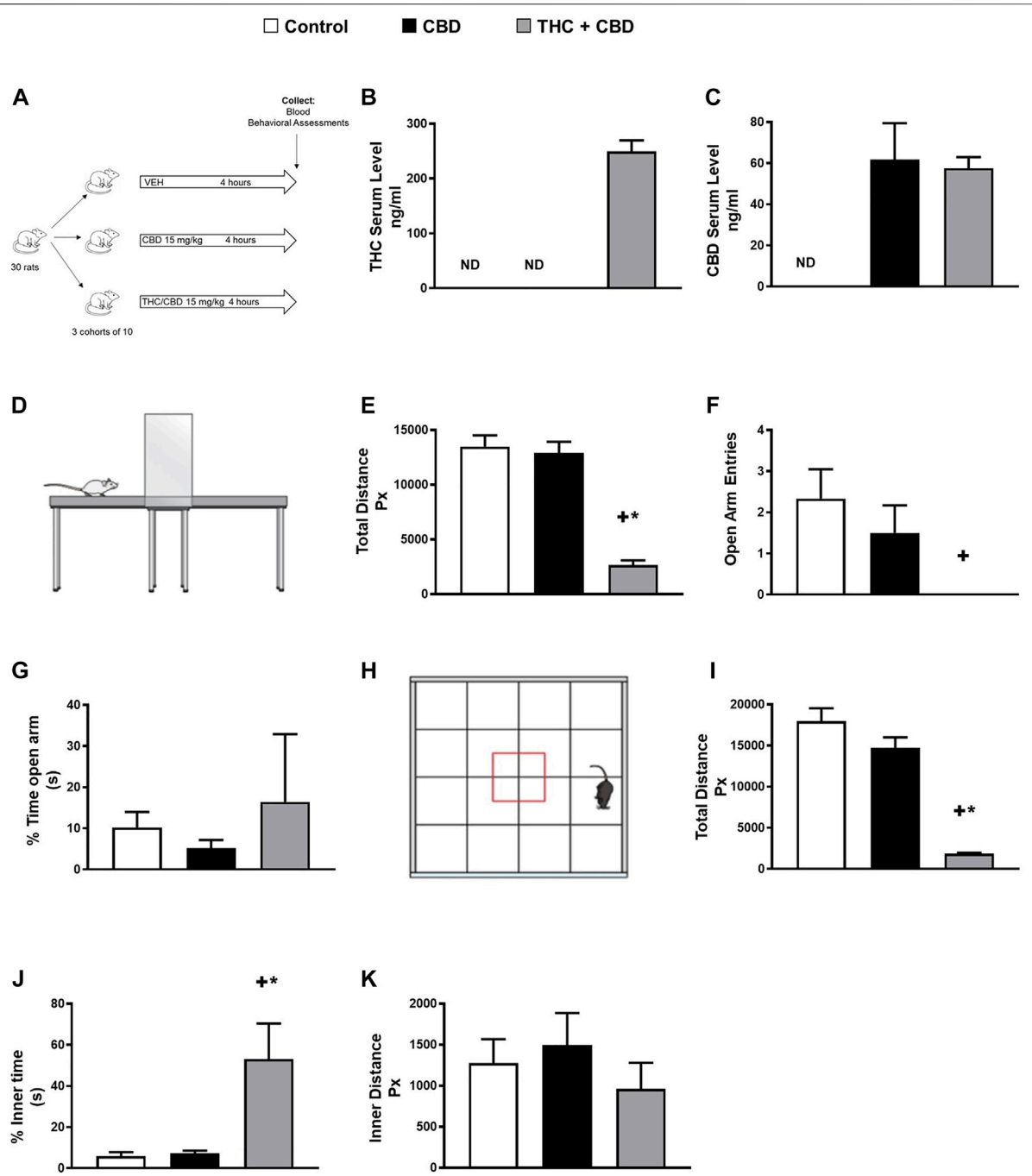


FIGURE 1 | Full-spectrum cannabis extract induces behavioral changes in rats. **(A)** Scheme of study design for identifying novel metabolite biomarkers of behavioral changes in response to cannabis extracts. **(B)** THC serum concentration and **(C)** CBD serum concentration that was determined in vehicle-treated, broad-spectrum cannabis extract–treated, and full-spectrum cannabis extract–treated rats. **(D)** Image showing a rat in the elevated plus maze apparatus. **(E)** Total distance travelled, **(F)** open arm entries, and **(G)** % time open arm in vehicle-treated, broad-spectrum cannabis extract–treated and full-spectrum cannabis extract–treated rats (n = 6). **(H)** Image showing a rat in the open field apparatus, **(I)** total distance travelled, **(J)** % inner time, and **(K)** inner distance in vehicle-treated, broad-spectrum cannabis extract–treated, and full-spectrum cannabis extract–treated rats (n = 6). Results are shown as means ± SEM. Comparisons between three groups were made by one-way ANOVA with a Tukey–Kramer post hoc multiple comparison test or Kruskal–Wallis test. $+p < 0.05$ vs. vehicle control group. $*p < 0.05$ vs. broad-spectrum cannabis extract group. THC, tetrahydrocannabinol; CBD, cannabidiol.

spectrum cannabis extract–treated group from the controls. Receiver operating characteristic (ROC) curve analysis using only these selected metabolites produced an area under the curve (AUC) of 0.997 (**Figure 2B**). The permutation test's result (p-value < 0.04) for model validation with an average accuracy of 0.948 indicated that the model was significant

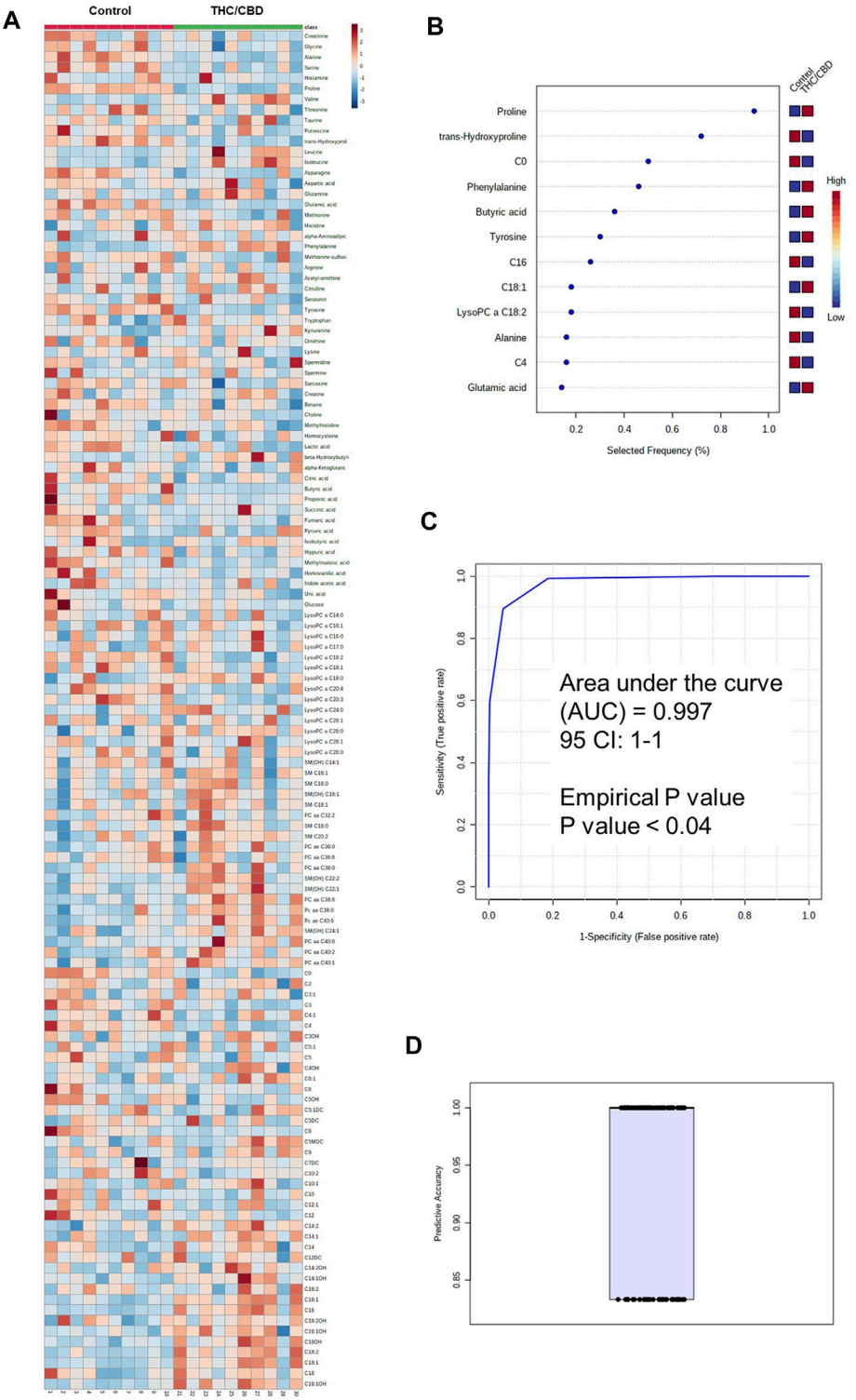


FIGURE 2 | Metabolomic differences between full-spectrum cannabis extract and controls. **(A)** Heat map of metabolomic differences between full-spectrum cannabis extract and controls. Heat maps were generated with the concentrations of potential candidate metabolites with univariate analysis. Similar metabolites were arranged together for use in pathway analysis through intuitive pattern discovery. The heat map displays an increase in each metabolite in relative concentration as red color and a decrease in a metabolite as blue color. The metabolites are listed at the left side of each row, and the subjects are shown at the bottom of each column. **(B)** Rank of the different metabolites (the top 12) identified by the PLS-DA according to the selected frequency on the *x*-axis. The most discriminating metabolites are shown in descending order of their scores. The color boxes indicate whether metabolite concentration has increased (red) or decreased (blue) in vehicle-treated and full-spectrum cannabis extract–treated rats. **(C)** ROC curve of the metabolite model. **(D)** Predictive accuracy of the metabolite model in vehicle-treated and full-spectrum cannabis extract–treated rats. The figures were drawn via MetaboAnalyst software v 4.0 (https://www.metaboanalyst.ca).

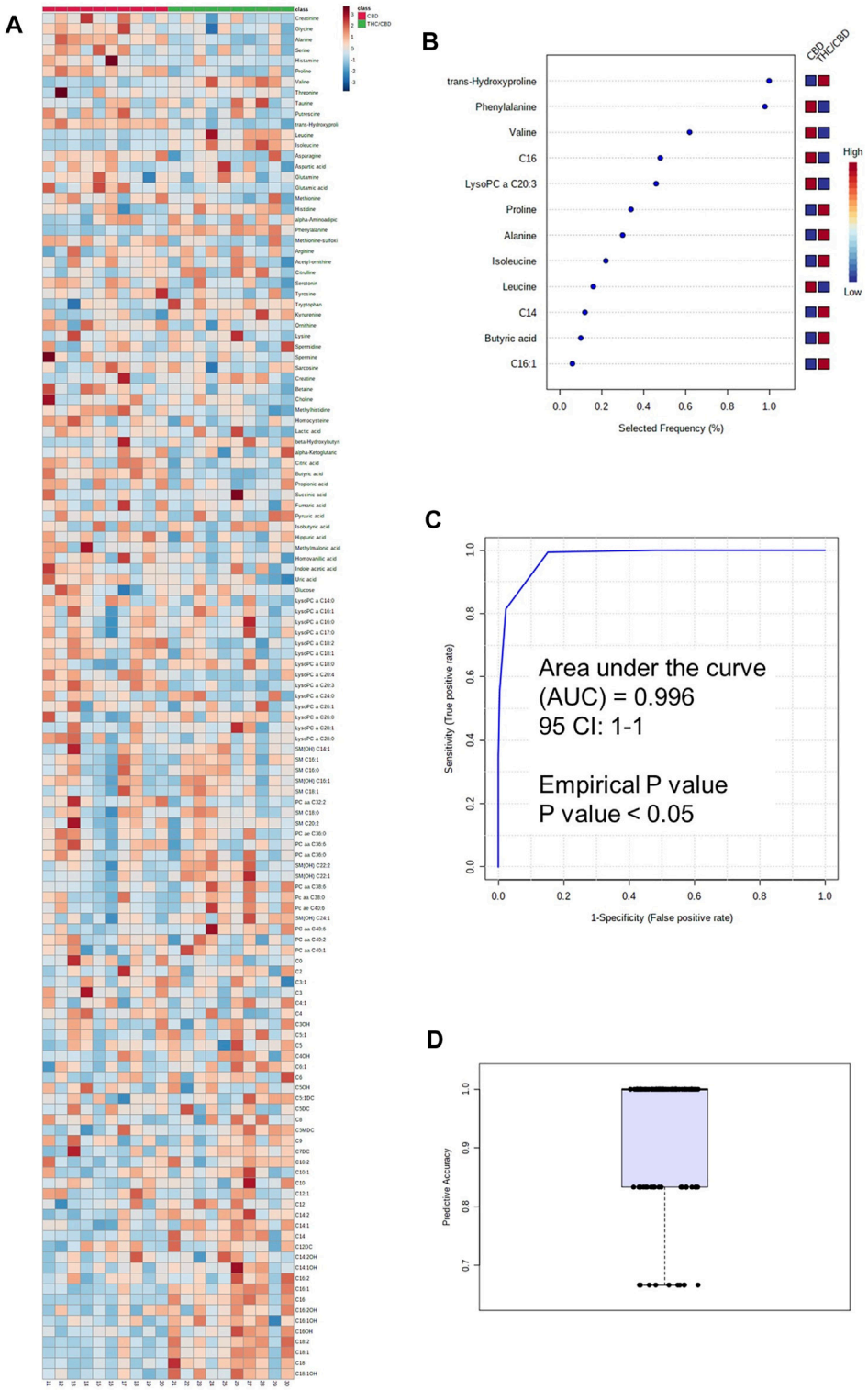


FIGURE 3 | Metabolomic differences between full-spectrum cannabis extract and broad-spectrum cannabis extract. **(A)** Heat map of metabolomic differences between full-spectrum cannabis extract and broad-spectrum cannabis extract groups. Heat maps were generated with the concentrations of potential candidate metabolites with univariate analysis. Similar metabolites were arranged together for use in pathway analysis through intuitive pattern discovery. The heat map displays an increase in each metabolite in relative concentration as red color and a decrease in a metabolite as blue color. The metabolites are listed at the left side of each row, and the subjects are shown at the bottom of each column. **(B)** Rank of the different metabolites (the top 12) identified by the PLS-DA according to the selected frequency

(*Continued*)

FIGURE 3 | on the *x*-axis. The most discriminating metabolites are shown in descending order of their scores. The color boxes indicate whether metabolite concentration has increased (red) or decreased (blue) in broad-spectrum cannabis extract–treated and full-spectrum cannabis extract–treated rats. **(C)** ROC curve of the metabolite model. **(D)** Predictive accuracy of the metabolite model in broad-spectrum cannabis extract–treated and full-spectrum cannabis extract–treated rats. The figures were drawn via MetaboAnalyst software v 4.0 (https://www.metaboanalyst.ca).

(**Figures 2B,C**). Intriguingly, since the aforementioned metabolite biomarkers are also detected in human and rodents with cognitive impairment (Ravaglia et al., 2004; Mapstone et al., 2014; Ashe et al., 2019; Heyck and Ibarra, 2019), our findings are highly suggestive that full-spectrum cannabis extract induces cognitive dysfunction in our rat model.

Metabolomic Differences Between Full-Spectrum Cannabis Extract and Broad-Spectrum Cannabis Extract

The metabolomic analysis showed that the serum concentrations of some medium- and long-chain acylcarnitines, some amino acids such as phenylalanine, SM(OH) C24:1, SM(OH) C22:2, PC aa C40:6, tryptophan, and kynurenine were higher in the full-spectrum cannabis extract–treated group than the broad-spectrum cannabis extract–treated group, whereas the serum concentrations of C4, LysoPC a C20:3, LysoPC a C18:2, LysoPC a C20:4, LysoPC a C14:0, butyric acid, glutamic acid, hippuric acid, uric acid, methionine sulfoxide, methylhistidine, asparagine, histamine, creatinine, glycine, serotonin, and several amino acids were found to be lower in the full-spectrum cannabis extract–treated group than the broad-spectrum cannabis extract–treated group (**Figure 3**, **Supplementary Table S3**).

For the identification of a potential biomarker panel of metabolites, we performed a similar PLS-DA of metabolites from the full-spectrum cannabis extract–treated group and the broad-spectrum cannabis extract–treated group. Another small number of metabolites including phenylalanine, butyric acid, alanine, LysoPC a C20:3, C16:1, C14, C16, proline, *trans*-hydroxyproline, valine, isoleucine, and leucine discriminated full-spectrum cannabis extract–treated group from the broad-spectrum cannabis extract–treated rats. ROC curve analysis produced an AUC of 0.996 (**Figure 3B**). The permutation test's result (p-value < 0.05) for model validation with an average accuracy of 0.932 indicated that the model was significant (**Figures 3B,C**). Based on our findings, it is clear that phenylalanine, butyric acid, alanine, C16, proline, and *trans*-hydroxyproline discriminated the full-spectrum cannabis extract–treated group from the both control and broad-spectrum cannabis extract–treated rats (**Figure 2B**, **3B**).

Full-Spectrum Cannabis Extract Significantly Reduces Phenylalanine Hydroxylase Enzyme Activity

Given that 1) phenylalanine discriminated full-spectrum cannabis extract group from both control and broad-spectrum cannabis extract-treated rats (**Figure 2B**, **3B**), and 2) a reduction in the activity of phenylalanine hydroxylase enzyme is known to cause behavioral problems and cognitive dysfunction (Ravaglia et al., 2004; Ashe et al., 2019), we sought to test the effect of full-spectrum cannabis extract on phenylalanine hydroxylase enzyme activity by measuring the ratio of tyrosine/phenylalanine. Interestingly, we found that the full-spectrum cannabis extract–treated group displayed a significant reduction in the ratio of tyrosine/phenylalanine compared to both control and broad-spectrum cannabis extract–treated groups, suggesting that the behavioral effect induced by the full-spectrum cannabis extract might be attributed, at least in part, to a reduction in the activity of phenylalanine hydroxylase enzyme (**Figure 4**).

DISCUSSION

Herein, we tested the effect of cannabis extracts on behavioral changes in our rat model using a clinically relevant dose of cannabis (Huestis, 2007). Given that there is no evidence that the simple presence of cannabis (i.e., low concentration of THC) impairs behavior (Brubacher et al., 2019), the dosage of the full-spectrum cannabis extract we used in our rat model was selected from studies in rodents to provide the highest achievable concentration of THC in humans (Huestis, 2007). Interestingly, while the broad-spectrum cannabis extract administered in this study did not result in any signs of behavioral changes, the full-spectrum cannabis extract displayed significant behavioral changes in our rat model. Thus, we conclude that consistent with other studies (Misner and Sullivan, 1999; Maayah and Dyck, 2020), THC is the primary component of cannabis that is responsible for behavioral changes (Misner and Sullivan, 1999). Notably, our observations are also in agreement with studies showing that only high concentration of THC induces behavioral changes (Brubacher et al., 2019; Lee et al., 2021), suggesting that the behavioral effect of the full-spectrum cannabis extract is dose-dependent and supports the notion that the consumption of low dose of the full-spectrum cannabis extract is not associated with significant behavioral changes (Brubacher et al., 2019).

To better understand what constitutes the cannabis-induced behavioral effect, we used quantitative metabolomics to identify a novel panel of blood-based metabolites that are predictive, diagnostic, and/or prognostic of cannabis behavioral effect. If validated, this could be used as a test to help in the reliable detection of full-spectrum cannabis extract–related behavioral changes. For instance, in the present study, we show that phenylalanine hydroxylase is implicated in the behavioral changes of the full-spectrum cannabis extract. Our findings support the hypothesis that an impaired conversion of the reduced amino acid phenylalanine to tyrosine as a consequence of the phenylalanine hydroxylase activity may contribute to the behavioral changes induced by the full-spectrum cannabis extract (Ashe et al., 2019). In agreement

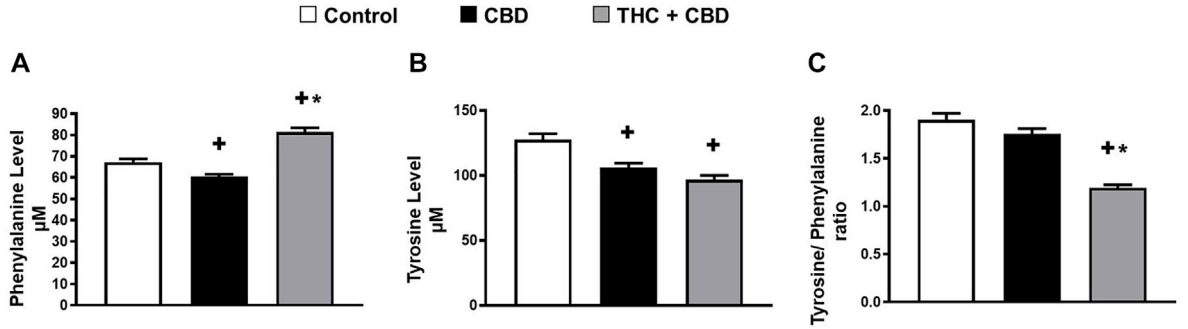


FIGURE 4 | Full-spectrum cannabis extract may significantly reduce phenylalanine hydroxylase enzyme activity. **(A)** Serum level of phenylalanine; **(B)** serum level of tyrosine, and **(C)** the ratio of tyrosine to phenylalanine (phenylalanine hydroxylase) in vehicle-treated, broad-spectrum cannabis extract–treated, and full-spectrum cannabis extract–treated rats (n = 10). Results are shown as mean ± SEM. Comparisons between three groups were made by one-way ANOVA with a Tukey–Kramer post hoc multiple comparison test. $+p < 0.05$ vs. vehicle group. $^{*}p < 0.05$ vs. full-spectrum cannabis extract group.

with this hypothesis, elevated phenylalanine concentrations in blood were found in patients with mild cognitive impairment and are known to cause severe intellectual disability and cognitive impairment (Ravaglia et al., 2004; Ashe et al., 2019). Our observations are also in agreement with several reports showing that impairment in phenylalanine hydroxylase may be linked to the change in the production of other catecholamine neurotransmitters such as dopamine, epinephrine, and norepinephrine (Romani et al., 2017; Winn et al., 2018). Thus, it is likely that phenylalanine hydroxylase activity and/or phenylalanine concentrations in the blood could be used as a diagnostic and prognostic tool of cannabis-induced behavioral effect including cognitive dysfunction.

The present study also sheds light on the potential contribution of microbial by-products, in particular butyric acid, to full-spectrum cannabis extract–induced behavioral changes. This finding is congruent with the concept that the gut microbiome plays a vital role in mental health and neurological conditions such as motor impairment and cognitive dysfunction (Tooley, 2020). Given the fact that 1) butyric acid is known to improve cognitive function and motor skills (Cantu-Jungles et al., 2019; Heyck and Ibarra, 2019), and 2) we found that the full-spectrum cannabis extract dramatically reduces the serum concentration of butyric acid, we speculate that the behavioral changes in our rat model is also attributed to, at least in part, the disturbances in gut microbiome composition and a subsequent reduction in butyric acid. Based on this, in addition to the utilization of the serum level of butyric acid as a diagnostic tool of the cannabis behavioral effect, we suggest that butyrogenic prebiotic fibers or sodium butyrate could be attempted in preclinical and clinical studies to lessen cannabis-induced behavioral changes including cognitive dysfunction. Thus, butyrogenic prebiotic fibers or sodium butyrate may hold promise as a repurposed therapy to reduce cannabis-related behavioral effects.

Numerous other metabolic perturbations were detected in our full-spectrum cannabis extract rat model that warrants discussion. For instance, the serum concentrations of long-chain acyl carnitine are significantly higher in full-spectrum cannabis extract–treated rats than broad-spectrum cannabis extract and controls. Given the fact that carnitine and its acyl derivatives play a vital role in mitochondrial metabolism and fatty acid uptake, the higher long-chain acyl carnitine concentration in full-spectrum cannabis extract–treated rats may suggest inefficient whole-body β-oxidation (Adams et al., 2009). In addition to inefficient β-oxidation and the disruption in mitochondrial energy production (Wajner and Amaral, 2015), long-chain acyl carnitine derivatives are themselves pro-inflammatory, neurotoxic, and involved in the pathophysiology of the cerebral damage (Rutkowsky et al., 2014; Wajner and Amaral, 2015). Thus, we assume that long-chain acyl carnitine derivatives may contribute, at least in part, to the full-spectrum cannabis extract–induced behavioral effect in our rat model. In contrast to long-chain acyl carnitine, serum concentrations of neuroprotective LysoPCs, in particular LysoPC a C18:2, were lower in full-spectrum cannabis extract–treated rats than in broad-spectrum cannabis extract–treated and control rats (Semba, 2020). Interestingly, our observations are in agreement with those of an important recent study on patients with mild cognitive impairment and Alzheimer's disease where LysoPC a C18:2 was found to be lower in patients with mild cognitive impairment (Mapstone et al., 2014). Thus, it is likely that LysoPC a C18:2 concentrations in blood could be used as a diagnostic and prognostic tool of cannabis-induced cognitive impairment.

Since we have detected distinct metabolomic fingerprints of the full-spectrum cannabis extract–treated rat model, we sought to use these fingerprints to discover novel panels of biomarkers that are predictive, diagnostic, and/or prognostic of the cannabis-behavioral effect, which can be used later as a test to help in the rapid detection of full-spectrum cannabis–related behavioral changes including cognitive dysfunction and impaired motor skills. Using phenylalanine, butyric acid, alanine, C16, proline, and *trans*-hydroxyproline, we discovered a novel panel of biomarkers that reliably distinguishes the full-spectrum cannabis extract from the broad-spectrum cannabis extract and control in rats. Thus, our newly identified panel of metabolites may have potential to be used clinically in the diagnosis of a cannabis-related behavioral effect and then utilized to devise a real-time decision tree that allows people

to work and operate a motor vehicle. To the best of our knowledge, this is the first report to identify a set of biomarkers that can be used as novel metabolite biomarkers of the behavioral effect in response to the consumption of cannabis extracts.

In summary, our results suggest that decreased phenylalanine hydroxylase activity, low serum level of butyric acid and LysoPC a C18:2, and increased long-chain acyl carnitine may play a crucial role in the full-spectrum cannabis extract–induced behavioral effects in our rat model. We also used an unbiased and systematic approach to identify novel metabolite biomarkers of behavioral effect in response to the full-spectrum cannabis extract. Thus, once our findings are clinically validated and refined, our work may allow a simple decision tree that would aid in the rapid diagnosis of cannabis-related behavioral effects. This would have potential applicability in different aspects of a users' day-to-day activities such as operating a motor vehicle or heavy machinery.

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 reviewed and approved by the University of Alberta Institutional Animal Care and Use Committee (Health Sciences) and conform to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (eighth edition; revised 2011).

AUTHOR CONTRIBUTIONS

All authors have read and approved the manuscript. Participated in research design: ZHM, KEM, KD, DSW, DTE, and JRBD. Conducted experiments: ZHM, PJFR, HS, RM, AA, ST, and MF. Performed data analysis: ZHM, PJFR, RM, KF, DSW, and JRBD. Wrote or contributed to the writing of the manuscript: ZHM, LE, ST, KEM, KF, DTE, and JRBD.

FUNDING

This work was supported by a grant from Mitacs Accelerate, Alberta Innovates mCannabis Program, and Canadian Institutes of Health Research (CIHR) to JD. JD is a Canada Research Chair in Molecular Medicine. KF is a Canada Research Chair in Spinal Cord Injury. ZM is the recipient of the Alberta Innovates and the CIHR postdoctoral fellowship awards.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.831052/full#supplementary-material

REFERENCES

Adams, S. H., Hoppel, C. L., Lok, K. H., Zhao, L., Wong, S. W., Minkler, P. E., et al. (2009). Plasma Acylcarnitine Profiles Suggest Incomplete Long-Chain Fatty Acid Beta-Oxidation and Altered Tricarboxylic Acid Cycle Activity in Type 2 Diabetic African-American Women. *J. Nutr.* 139 (6), 1073–1081. doi:10.3945/jn.108.103754

Ashe, K., Kelso, W., Farrand, S., Panetta, J., Fazio, T., De Jong, G., et al. (2019). Psychiatric and Cognitive Aspects of Phenylketonuria: The Limitations of Diet and Promise of New Treatments. *Front. Psychiatry* 10, 561. doi:10.3389/fpsyt.2019.00561

Brubacher, J. R., Chan, H., Erdelyi, S., Macdonald, S., Asbridge, M., Mann, R. E., et al. (2019). Cannabis Use as a Risk Factor for Causing Motor Vehicle Crashes: a Prospective Study. *Addiction* 114 (9), 1616–1626. doi:10.1111/add.14663

Cantu-Jungles, T. M., Rasmussen, H. E., and Hamaker, B. R. (2019). Potential of Prebiotic Butyrogenic Fibers in Parkinson's Disease. *Front. Neurol.* 10, 663. doi:10.3389/fneur.2019.00663

Feyissa, D. D., Aher, Y. D., Engidawork, E., Höger, H., Lubec, G., and Korz, V. (2017). Individual Differences in Male Rats in a Behavioral Test Battery: A Multivariate Statistical Approach. *Front. Behav. Neurosci.* 11, 26. doi:10.3389/fnbeh.2017.00026

Foroutan, A., Fitzsimmons, C., Mandal, R., Piri-Moghadam, H., Zheng, J., Guo, A., et al. (2020). The Bovine Metabolome. *Metabolites* 10 (6). doi:10.3390/metabo10060233

Foroutan, A., Guo, A. C., Vazquez-Fresno, R., Lipfert, M., Zhang, L., Zheng, J., et al. (2019). Chemical Composition of Commercial Cow's Milk. *J. Agric. Food Chem.* 67 (17), 4897–4914. doi:10.1021/acs.jafc.9b00204

Hartman, R. L., and Huestis, M. A. (2013). Cannabis Effects on Driving Skills. *Clin. Chem.* 59 (3), 478–492. doi:10.1373/clinchem.2012.194381

Heyck, M., and Ibarra, A. (2019). Microbiota and Memory: A Symbiotic Therapy to Counter Cognitive Decline. *Brain Circ.* 5 (3), 124–129. doi:10.4103/bc.bc_34_19

Huestis, M. A. (2007). Human Cannabinoid Pharmacokinetics. *Chem. Biodivers* 4 (8), 1770–1804. doi:10.1002/cbdv.200790152

Itoh, J., Nabeshima, T., and Kameyama, T. (1990). Utility of an Elevated Plus-Maze for the Evaluation of Memory in Mice: Effects of Nootropics, Scopolamine and Electroconvulsive Shock. *Psychopharmacology (Berl)* 101 (1), 27–33. doi:10.1007/BF02253713

Izzo, A. A., Borrelli, F., Capasso, R., Di Marzo, V., and Mechoulam, R. (2009). Non-psychotropic Plant Cannabinoids: New Therapeutic Opportunities from an Ancient Herb. *Trends Pharmacol. Sci.* 30 (10), 515–527. doi:10.1016/j.tips.2009.07.006

Lee, C., Voaklander, D., Minhas-Sandhu, J. K., Hanlon, J. G., Hyshka, E., Dyck, J. R. B., et al. (2021). Cohort Study of Medical Cannabis Authorization and Motor Vehicle Crash-Related Healthcare Visits in 2014-2017 in Ontario, Canada. *Inj. Epidemiol.* 8 (1), 33. doi:10.1186/s40621-021-00321-1

López-Hernández, Y., Monárrez-Espino, J., Oostdam, A. H., Delgado, J. E. C., Zhang, L., Zheng, J., et al. (2021). Targeted Metabolomics Identifies High Performing Diagnostic and Prognostic Biomarkers for COVID-19. *Sci. Rep.* 11 (1), 14732. doi:10.1038/s41598-021-94171-y

Maayah, Z. H., and Dyck, J. R. B. (2020). The Pharmacological Effects of Inhaled Cannabis on Pain in Patients with Multiple Sclerosis: Risks versus Rewards. *Inflamm. Res.* 69 (11), 1073–1076. doi:10.1007/s00011-020-01396-0

Maayah, Z. H., Takahara, S., Ferdaoussi, M., and Dyck, J. R. B. (2020a). The Anti-inflammatory and Analgesic Effects of Formulated Full-Spectrum Cannabis Extract in the Treatment of Neuropathic Pain Associated with Multiple Sclerosis. *Inflamm. Res.* 69 (6), 549–558. doi:10.1007/s00011-020-01341-1

Maayah, Z. H., Takahara, S., Ferdaoussi, M., and Dyck, J. R. B. (2020b). The Molecular Mechanisms that Underpin the Biological Benefits of Full-Spectrum

Cannabis Extract in the Treatment of Neuropathic Pain and Inflammation. *Biochim. Biophys. Acta Mol. Basis Dis.* 1866 (7), 165771. doi:10.1016/j.bbadis.2020.165771

Mapstone, M., Cheema, A. K., Fiandaca, M. S., Zhong, X., Mhyre, T. R., MacArthur, L. H., et al. (2014). Plasma Phospholipids Identify Antecedent Memory Impairment in Older Adults. *Nat. Med.* 20 (4), 415–418. doi:10.1038/nm.3466

Misner, D. L., and Sullivan, J. M. (1999). Mechanism of Cannabinoid Effects on Long-Term Potentiation and Depression in Hippocampal CA1 Neurons. *J. Neurosci.* 19 (16), 6795–6805. doi:10.1523/jneurosci.19-16-06795.1999

Montone, C. M., Cerrato, A., Botta, B., Cannazza, G., Capriotti, A. L., Cavaliere, C., et al. (2020). Improved Identification of Phytocannabinoids Using a Dedicated Structure-Based Workflow. *Talanta* 219, 121310. doi:10.1016/j.talanta.2020.121310

Psychogios, N., Hau, D. D., Peng, J., Guo, A. C., Mandal, R., Bouatra, S., et al. (2011). The Human Serum Metabolome. *PLoS One* 6 (2), e16957. doi:10.1371/journal.pone.0016957

Ravaglia, G., Forti, P., Maioli, F., Bianchi, G., Martelli, M., Talerico, T., et al. (2004). Plasma Amino Acid Concentrations in Patients with Amnestic Mild Cognitive Impairment or Alzheimer Disease. *Am. J. Clin. Nutr.* 80 (2), 483–488. doi:10.1093/ajcn/80.2.483

Ravanbakhsh, S., Liu, P., Bjorndahl, T. C., Bjordahl, T. C., Mandal, R., Grant, J. R., et al. (2015). Accurate, Fully-Automated NMR Spectral Profiling for Metabolomics. *PLoS One* 10 (5), e0124219. doi:10.1371/journal.pone.0124219

Romani, C., Palermo, L., MacDonald, A., Limback, E., Hall, S. K., and Geberhiwot, T. (2017). The Impact of Phenylalanine Levels on Cognitive Outcomes in Adults with Phenylketonuria: Effects across Tasks and Developmental Stages. *Neuropsychology* 31 (3), 242–254. doi:10.1037/neu0000336

Rutkowsky, J. M., Knotts, T. A., Ono-Moore, K. D., McCoin, C. S., Huang, S., Schneider, D., et al. (2014). Acylcarnitines Activate Proinflammatory Signaling Pathways. *Am. J. Physiol. Endocrinol. Metab.* 306 (12), E1378–E1387. doi:10.1152/ajpendo.00656.2013

Saude, E. J., Slupsky, C. M., and Sykes, B. D. (2006). Optimization of NMR Analysis of Biological Fluids for Quantitative Accuracy. *Metabolomics* 2 (3), 113–123. doi:10.1007/s11306-006-0023-5

Semba, R. D. (2020). Perspective: The Potential Role of Circulating Lysophosphatidylcholine in Neuroprotection against Alzheimer Disease. *Adv. Nutr.* 11 (4), 760–772. doi:10.1093/advances/nmaa024

Sharma, A. C., and Kulkarni, S. K. (1992). Evaluation of Learning and Memory Mechanisms Employing Elevated Plus-Maze in Rats and Mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 16 (1), 117–125. doi:10.1016/0278-5846(92)90014-6

Sung, M. M., Kim, T. T., Denou, E., Soltys, C. M., Hamza, S. M., Byrne, N. J., et al. (2017). Improved Glucose Homeostasis in Obese Mice Treated With Resveratrol Is Associated With Alterations in the Gut Microbiome. *Diabetes* 66 (2), 418–425. doi:10.2337/db16-0680

Tooley, K. L. (2020). Effects of the Human Gut Microbiota on Cognitive Performance, Brain Structure and Function: A Narrative Review. *Nutrients* 12 (10). doi:10.3390/nu12103009

Wajner, M., and Amaral, A. U. (2015). Mitochondrial Dysfunction in Fatty Acid Oxidation Disorders: Insights from Human and Animal Studies. *Biosci. Rep.* 36 (1), e00281. doi:10.1042/BSR20150240

Walsh, R. N., and Cummins, R. A. (1976). The Open-Field Test: a Critical Review. *Psychol. Bull.* 83 (3), 482–504. doi:10.1037/0033-2909.83.3.482

Winn, S. R., Scherer, T., Thöny, B., Ying, M., Martinez, A., Weber, S., et al. (2018). Blood Phenylalanine Reduction Corrects CNS Dopamine and Serotonin Deficiencies and Partially Improves Behavioral Performance in Adult Phenylketonuric Mice. *Mol. Genet. Metab.* 123 (1), 6–20. doi:10.1016/j.ymgme.2017.10.009

Zordoky, B. N., Sung, M. M., Ezekowitz, J., Mandal, R., Han, B., Bjorndahl, T. C., et al. (2015). Metabolomic Fingerprint of Heart Failure with Preserved Ejection Fraction. *PLoS One* 10 (5), e0124844. doi:10.1371/journal.pone.0124844

Conflict of Interest: JD is a shareholder, board Member, and chief scientific officer of Australis Capital, which is a cannabis company operating out of the United States.

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Pharmacology

REVIEW
published: 21 April 2022
doi: 10.3389/fphar.2022.881810

Medicinal Cannabis and Central Nervous System Disorders

Yuma T. Ortiz[1], *Lance R. McMahon*[2] *and Jenny L. Wilkerson*[2]*

[1]Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL, United States, [2]Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, TX, United States

Cannabinoids, including those found in cannabis, have shown promise as potential therapeutics for numerous health issues, including pathological pain and diseases that produce an impact on neurological processing and function. Thus, cannabis use for medicinal purposes has become accepted by a growing majority. However, clinical trials yielding satisfactory endpoints and unequivocal proof that medicinal cannabis should be considered a frontline therapeutic for most examined central nervous system indications remains largely elusive. Although cannabis contains over 100 + compounds, most preclinical and clinical research with well-controlled dosing and delivery methods utilize the various formulations of Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), the two most abundant compounds in cannabis. These controlled dosing and delivery methods are in stark contrast to most clinical studies using whole plant cannabis products, as few clinical studies using whole plant cannabis profile the exact composition, including percentages of all compounds present within the studied product. This review will examine both preclinical and clinical evidence that supports or refutes the therapeutic utility of medicinal cannabis for the treatment of pathological pain, neurodegeneration, substance use disorders, as well as anxiety-related disorders. We will predominately focus on purified THC and CBD, as well as other compounds isolated from cannabis for the aforementioned reasons but will also include discussion over those studies where whole plant cannabis has been used. In this review we also consider the current challenges associated with the advancement of medicinal cannabis and its derived potential therapeutics into clinical applications.

Keywords: cannabinoid 1 receptor, cannabinoid 2 receptor, serotonin 1a receptor, clinical research, addiction, pain, neurodegeneration, anxiety

OPEN ACCESS

Edited by:
Shimon Ben -Shabat, Ben-Gurion University of the Negev, Israel

Reviewed by:
Ethan Budd Russo, CReDO Science, United States
Maria Scherma, University of Cagliari, Italy

***Correspondence:**
Jenny L. Wilkerson
jenny.wilkerson@ttuhsc.edu

Specialty section:
This article was submitted to Experimental Pharmacology and Drug Discovery, a section of the journal Frontiers in Pharmacology

Received: *23 February 2022*
Accepted: *05 April 2022*
Published: *21 April 2022*

Citation:
Ortiz YT, McMahon LR and Wilkerson JL (2022) Medicinal Cannabis and Central Nervous System Disorders. Front. Pharmacol. 13:881810. doi: 10.3389/fphar.2022.881810

1 INTRODUCTION

Interest in cannabinoids has continued to grow as they steadily show increased potential as therapeutics for treating a diverse range of diseases and illnesses. Therapeutic actions of these cannabinoids are in part the result of two identified cannabinoid receptors, both of which are G protein-coupled receptors. Cannabinoid 1 receptors (CB_1R) appear in high densities among

Abbreviations: AD, alzheimer's disease; ALS, amyotrophic lateral sclerosis; CBD, cannabidiol; CB1R, cannabinoid 1 receptor; CB2R, cannabinoid 2 receptor; CNS, central nervous system; GAD, generalized anxiety disorder; HD, Huntington's disease; MS, multiple sclerosis; OCD, obsessive-compulsive disorder; PD, Parkinson's disease; PTSD, post-traumatic stress disorder; 5-HT1A, serotonin 1a; SEDDS, Self-emulsifying drug delivery system; SSRIs, selective serotonin reuptake inhibitors; SAD, social anxiety disorder; THC, Δ9-tetrahydrocannabinol.

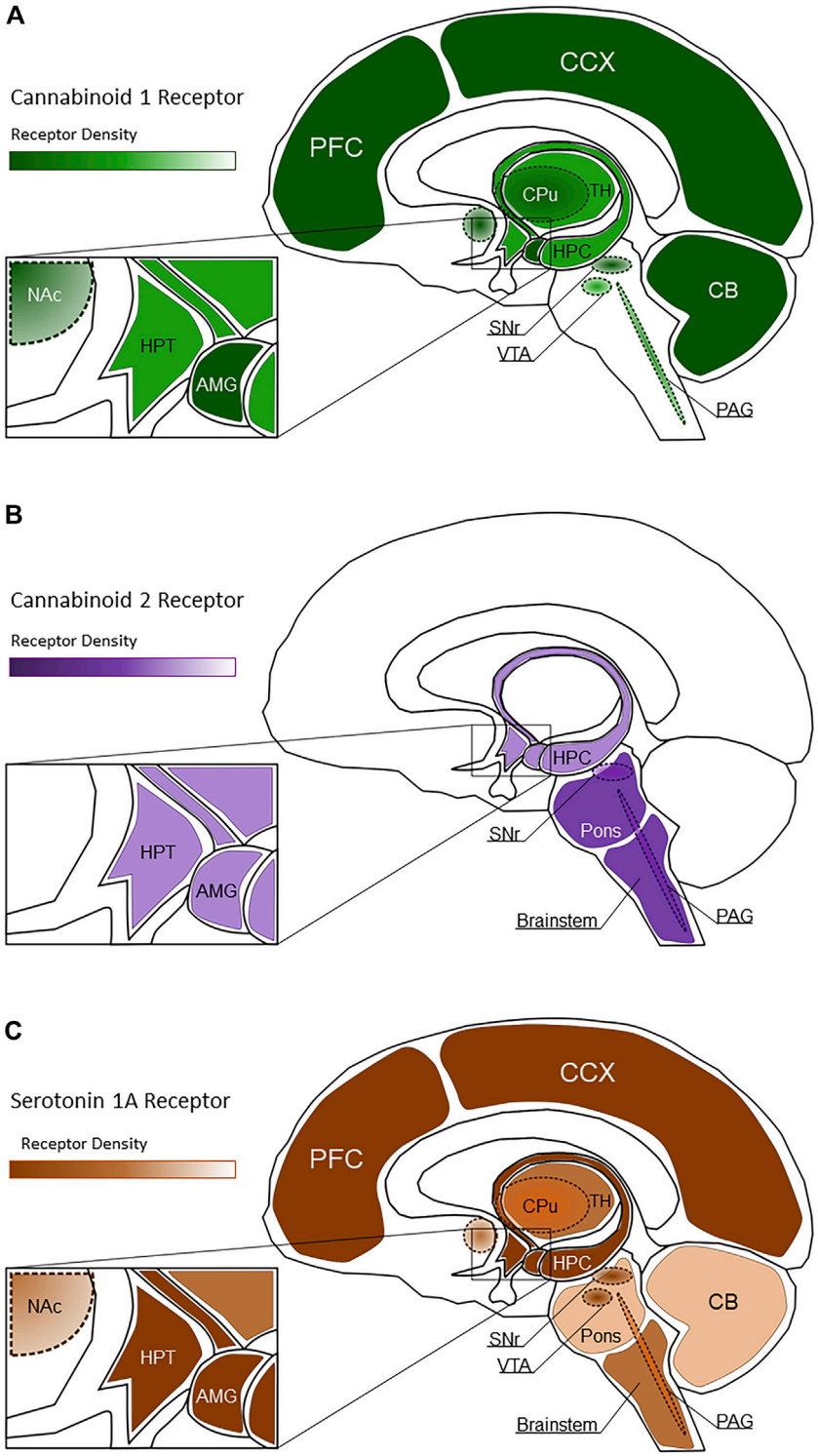


FIGURE 1 | Spatial distribution of CB_1R [**(A)**, green shading], CB_2R [**(B)**, purple shading], and 5-HT1A receptors [**(C)**, brown shading] within healthy brain regions. Lighter shaded regions represent low receptor density while darker shaded regions represent high receptor density. In these images, PFC, Prefrontal Cortex; CCX, Cerebral Cortex; CB, Cerebellum; CPu, Caudate Putamen; HPC, Hippocampus; TH, Thalamus; HPT, Hypothalamus; Nac, Nucleus Accumbens; SNr, Substantia Nigra pars compacta; VTA, Ventral Tegmental Area; PAG, Periaqueductal gray; AMG, Amygdala.

presynaptic neurons within the central nervous system (CNS), particularly among GABAergic interneurons, and peripheral neurons as well as on astrocytes and oligodendrocytes (Huang et al., 2001; Katona et al., 2001; Ohno-Shosaku et al., 2001; Szabo et al., 2005). The behavioral effects of cannabinoid consumption, termed as "cannabimimetic" behavioral effects, are mediated by neuronal CB_1R. Such cannabimimetic effects include acute antinociception, decreased locomotion, catalepsy, and hypothermia (Little et al., 1988; Ledent et al., 1999; Grim et al., 2016). CB_1R are also associated with anti-inflammatory mechanisms, contributing to therapeutic prospects of CB_1R agonists (Richardson et al., 1998; Kraus et al., 2009; Newton et al., 2009). CB2 receptors (CB_2R) are expressed by immune cells including microglia, astrocytes, oligodendrocytes (Munro et al., 1993;Galiegue et al., 1995; Schatz et al., 1997; Carayon et al., 1998), and discrete neuronal populations (Onaivi et al., 2008a) within the brainstem (Van Sickle et al., 2005), and the hippocampus (Stempel et al., 2016). Unlike CB_1R agonism, CB_2R agonism does not result in the cannabimimetic effects observed with CB_1R agonists whilst still producing anti-inflammatory signaling cascades (Rahn et al., 2011). Despite the current, vast library of synthetic cannabinoid ligands generated, clinical research has extensively utilized variations of Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) due to their well-controlled dosing and delivery methods. Within this review, we report on the use of THC (a non-selective CB_1R/CB_2R agonist) and its clinically approved synthetic formulations, dronabinol and nabilone, THC/CBD formulations as nabiximols and medicinal cannabis, and CBD (limited agonism at CB_1R/CB_2R) and its clinically approved formulation, Epidiolex. Not discussed at length within this review, it should be noted that medicinal cannabis contains over 100 different compounds, including acid phytocannabinoids, cannabigerol, as well as cannabis-related terpenoids (Russo, 2018). Each of these cannabis compounds has its own pharmacology which can include activity at receptors not discussed within this review, and these compounds may modify resultant THC, CBD activity as well as overall medicinal cannabis therapeutic potency.

The therapeutic actions of CBD are generally attributed to non-CB_1R/ CB_2R activity, including partial agonist activity at the 5-HT1A receptor, although potential endocannabinoid modulatory effects of CBD cannot be eliminated (De Gregorio et al., 2019; King et al., 2017). Serotonin 5-HT1A receptors are G protein-coupled receptors located on presynaptic serotonergic neurons and postsynaptic non-serotonergic neurons, astrocytes, oligodendrocytes, and microglia, with a high density of distribution within limbic brain areas (Barnes & Sharp, 1999; Riad et al., 1991, 2000). Additionally, 5-HT1A receptors are also expressed within primary afferent neurons and their peripheral terminals (Björk et al., 1992; Granados-Soto et al., 2010; Laporte et al., 1995; Perrin et al., 2011). **Figure 1**shows the spatial distribution of CB_1R, CB_2R, and 5-HT1A receptors within the brain. Agonism of 5-HT1A receptors has been shown to inhibit nociception (Gjerstad et al., 1996; Bardin et al., 2003; Haleem & Nawaz, 2017), exhibit neuroprotective effects (Miyazaki et al., 2013; Isooka et al., 2020; Kikuoka et al., 2020), and alleviate the severity of several anxiety disorders (Sussman and Joffe, 1998; Campos and Guimarães, 2008).

The diverse physiological activity resultant of these cannabinoids indicates a wide breadth of potential therapeutic indications. As medicinal cannabis related clinical research has predominately focused on CNS-related diseases, such as neurodegeneration and neurological disorders, pain, substance use disorders, and anxiety disorders, this review will first examine evidence that supports or refutes the therapeutic utility of cannabinoids for the treatment of neurodegenerative disease, pain, mood disorders, and substance use disorders. **Table 1** summarizes these clinical studies. Not discussed at length within this review, medical cannabis has also shown promise in treating other CNS-related diseases, such as brain tumors and gliomas (Russo, 2018). Despite continued growing interest and an increasing trove of preclinical research that exemplifies the therapeutic potential of cannabinoids, the development of viable, approved therapeutics remains elusive due to various challenges in formulation and bioavailability, efficacy, and tolerability. In this review we will address some of the challenges and considerations within the cannabinoid field that may be important in advancing such therapeutics into the clinic while presenting recent findings that provide a more up to date understanding of where the field currently lies regarding the therapeutic viability of cannabinoids.

2 CANNABINOID INVOLVEMENT IN CENTRAL NERVOUS SYSTEM DISEASES AND DISORDERS

2.1 Neurodegenerative and Neurological Diseases

Neurodegenerative and neurological diseases that commonly afflict those in mid to late life have steadily become a common cause of mortality worldwide as elderly populations have continued to grow. Epidemiological reviews of these neurodegenerative diseases show that associated deaths have increased within the last 25 years, having increased worldwide by more than 35% (Group GNDC, 2017).

2.1.1 Alzheimer's Disease

Alzheimer's disease (AD) is the most common neurodegenerative disease that contributes to approximately 60–80% of all dementia cases globally (Erkkinen et al., 2018) and is characterized by the formation of β-amyloid plaques, phosphorylated tau proteins, formation of neurofibrillary tangles, glial activation, and neuronal death (Scheltens et al., 2021). Structural imaging of AD brains observed atrophy of the hippocampus and in later stages, the frontal cortex, areas with high density distribution of CB_1R (Glass et al., 1997; Biegon & Kerman, 2001; Erkkinen et al., 2018). However, such atrophy is not necessarily correlative of observed deficits in declarative memory and recall (Nelson et al., 2009; Iqbal et al., 2010; Aschenbrenner et al., 2018).

Alterations in CB_1R expression resultant of AD maintains itself as a point of contention. Studies have reported considerable decreases in CB_1R expression within post-mortem AD patient

TABLE 1 | Cannabinoids and the clinical work done investigating them as novel therapeutics.

Compound	Safety	Clinical outcomes	References
Nabilone	No major adverse effects: minor side effects include fatigue, dizziness, anxiety, dry mouth	- Improvements among both motor and non-motor symptoms of PD. - Improved motor function in MS patients. No improvement in cognitive function in MS patients. Self-reported improvements in pain measures among MS patients - Minimal effect on symptoms of OCD, significant therapeutic effect observed when paired with behavioral therapy - Nabilone exhibits anti-inflammatory effects in instances of AD. - Failed to minimize post operative nausea and vomiting - Reduced cannabis use among cannabis dependent patients, not discernable from placebo - No reduction on maximal pain levels experienced by women undergoing medical abortion	Colwill et al., 2020; Hill et al., 2017; Kayser et al., 2020a; Levin et al., 2017; Peball et al., 2020; Ruthirakuhan et al., 2020
Dronabinol	No major adverse effects: side effects include euphoria, dry mouth	- Inconsistent acute analgesia observed with hydromorphone coadministration in healthy patients. No effect on chronic pain - Reduced pain intensity, though no different from placebo in alleviating neuropathic pain - Reduced pain perception in patients with noncardiac chest pains - Dronabinol did not enhance analgesia produced by oxycodone and increased abuse-related subjective effects	Babalonis et al., 2019; Dunn et al., 2021; Malik et al., 2017; Schimrigk et al., 2017; University of California, Davis, 2018
Nabiximols	No major adverse effects: side effects include sedation, dizziness, nausea	- Slight improvements in self-reported pain evaluations in advanced cancer. Improved quality of life for secondary symptoms associated with advanced cancer - Reduced the amount of cannabis consumed by cannabis dependent patients and reduced the number of cravings - Reduced spasticity in patients with motor neuron disease and MS.	Collin et al., 2007; Fallon et al., 2017; Johnson et al., 2010; Kavia et al., 2010; Lichtman et al., 2018; Lintzeris et al., 2019; Markovà et al., 2019; Notcutt et al., 2004; Nurmikko et al., 2007; Portenoy et al., 2012; Riva et al., 2019; Rog et al., 2005; Trigo et al., 2018; Wade et al., 2004; Wade et al., 2006
Cannabidiol	No major adverse effects: side effects include fatigue, diarrhea	- Reduction of tremors, improved sleep, and improved emotional control in PD patients - Chronic pain patients reduced or eliminated use of prescribed opioids when CBD is added to regimens - Symptomatic relief of peripheral neuropathy of the lower extremities	Capano et al., 2020; Leehey et al., 2020; Xu et al., 2020
Whole Cannabis	No major adverse effects: side effects include sedation, anxiety in high THC concentrations	- Reduced reported intensity of chronic pain among patients with general improvements to anxiety and depression - Decrease in maximum strength among those with MS and consuming medicinal cannabis. Relief of muscle stiffness observed after 12 weeks of consumption - No difference in anxiolytic effects observed compared to placebo in PTSD patients - Minimal acute effect on OCD associated anxiety compared to placebo - Reduced consumption of prescribed opioids among patients with chronic pain. Instances of opioid cessation	Bonn-Miller et al., 2021; Haroutounian et al., 2016; Kayser et al., 2020b; O'Connell et al., 2019; Rudroff, 2020; Vigil et al., 2017; Ware et al., 2010, 2015; Zajicek et al., 2012

brain tissues (Ramírez et al., 2005), particularly those compared to age-matched controls (Solas et al., 2013). Others found no change regarding distribution or expression of CB_1R within the hippocampus and cortex (Benito et al., 2003; Lee et al., 2010; Mulder et al., 2011; Ahmad et al., 2014). CB_2R expression has been found to be significantly increased with the accumulation of b-amyloid plaques (Benito et al., 2003; Ramirez et al., 2005; Solas et al., 2013), even in instances where plaque accumulation did not

induce cognitive impairment (Solas et al., 2013). Increasing evidence supports a potential contributory role of the serotonergic system in AD. Serotonin 5-HT1A receptors are expressed in the hippocampus and are involved with memory processing and learning (Ögren et al., 2008; Muzerelle et al., 2016; Solís-Guillén et al., 2021).

Preclinical studies have shown marked decreases in 5-HT1A receptor expression across various regions sampled from human AD brains (Bowen et al., 1983; Cross et al., 1984; Lai et al., 2003). 5-HT1A receptors as a target for alleviating cognitive dysfunction has shown promise. Continued research with 5-HT1A receptor antagonists, such as lecozotan, enhanced cognitive function in a rat model of scopolamine induced amnesia (Skirzewski et al., 2010), as well as enhanced cognitive performance in aged rhesus monkeys and reversed cognitive deficits associated with cholinergic lesions in marmosets (Schechter et al., 2005). As partial agonists buspirone and tandospirone both improved AD patient mood and behavior, further research into 5-HT1A modulation, *via* CBD or other cannabinoids with serotonergic activity, may provide promising therapeutics able to alleviate AD associated cognitive dysfunction, behavioral decline and memory impairment (Salzman, 2001; Sato et al., 2007). Indeed, in accordance with CBD's serotonergic activity profile, CBD has shown utility in animal AD models. Utilizing intracerebroventricular administration of beta-amyloid in mice to simulate cognitive impairment associated with AD, it was shown that intraperitoneal administration of CBD was able to modulate beta-amyloid activation of microglia and restore cognitive function as indicated by decreased latencies in the Morris water maze compared to vehicle controls (Martín-Moreno et al., 2011). Furthermore, THC exhibits neuroprotective effects when administered within a transgenic, beta-amyloid expressing AD mouse model with observed reductions in neuronal loss and reduced accumulation of b-amyloid compared to vehicle controls (Franke et al., 2019).

Recent clinical studies have demonstrated potential therapeutic benefit with nabilone for the treatment of neurodegenerative and neuroinflammatory diseases. In a double-blind, randomized cross-over AD study, markers for oxidative stress and neuroinflammation, such as tumor necrosis factor-α, were decreased following nabilone administration (1–2 mg), indicating a correlative association with reductions in agitation and decreased inflammation markers (Ruthirakuhan et al., 2020).

2.1.2 Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disease after AD, with an estimated prevalence of 572 per 100,000 among those aged 45 years and older (Marras et al., 2018), and doubling of such instances within the next 2 decades is expected (Dorsey et al., 2018). Characterized by the loss of dopaminergic neurons within the substantia nigra pars compacta, the resulting loss of dopaminergic striatal input nigra leads to the hallmark observable changes of PD. These changes include reductions in motor function such as resting tremor, bradykinesia, postural instability, and rigidity (Davie, 2008) as well as cognitive impairment, mood disorders, and pain sensory disturbances (Erkkinen et al., 2018). While instances of PD are thought to be sporadic, genetic mutations are heavily linked to PD onset, including missense mutations with genes PARK1, PARK2, and PARK7 (Blauwendraat et al., 2020). Proinflammatory signaling is thought to play a role in disease progression as well (for reviews see Wang et al., 2015; Klein and Westenberger, 2012), providing therapeutic potential to cannabinoids and their anti-inflammatory nature.

Research has shown that CB_1R mRNA expression is decreased within preclinical rat models of toxin induced PD (Silverdale et al., 2001; Walsh et al., 2010) and genetic mouse models of PD (García-Arencibia et al., 2009). However, decreases in mRNA expression observed in genetic mouse models were shown to be reversed in later disease stages with increased CB_1R mRNA expression (García-Arencibia et al., 2009). Significant reductions in CB_1R expression within the ventral mesencephalic region are observed in early-stage PD patients compared to healthy controls (Van Laere et al., 2012). Similarly, CB_1R mRNA was found to be reduced in the caudate nucleus, anterior dorsal putamen, and the external globus pallidus in human post-mortem brain tissues taken from PD patients (Hurley et al., 2003; Van Laere et al., 2012). However, up regulation of both CB_1R and CB_2R expression has been observed within the substantia nigra in human post-mortem striatal brain tissues taken from medicated PD patients (Navarrete et al., 2018). Serotonergic systems are affected alongside the dopaminergic denervation associated with PD with observed decreases in serotonin and dopamine concentration in cerebrospinal fluid and human striatum (Tohgi et al., 1992; Kish et al., 2008; Politis & Niccolini, 2015). Using positron emission tomography, it was found that 5-HT1A receptor binding was reduced significantly in PD patients compared to healthy controls, with a significant correlation between reduction in binding and tremor severity (Doder et al., 2003).

Blocking serotonergic signaling with the 5-HT1A agonist buspirone was found to reduce the development of l-DOPA-induced dyskinesia in a 6-hydroxydopamine (6-OHDA) lesion model of PD in rats (Eskow et al., 2007). Using the same 6-OHDA lesion model to induce dopamine depletion associated with PD in rats, it was found that CBD was able to recover dopamine levels when given immediately after lesion induction (García-Arencibia et al., 2009). However, the same study had found that administration of CBD 1 week after the lesion did not affect dopamine levels. Preclinical research studying the effects of THC in PD models have reported potential neuroprotective effects. Utilizing an *in vitro* model of PD with SH-SY5Y cells and PD relevant toxins, THC was shown to have an active neuroprotective effect that mitigated cell death following exposure to toxins that generate free radicals and inhibit mitochondrial function (Carroll et al., 2012). THC treatment within a marmoset PD model was also shown to improve locomotor activity associated with spontaneous exploratory behavior and complex tasks requiring hand-eye coordination (van Vliet et al., 2008). Neuroprotective effects were observed following daily intraperitoneal administration of THC over the course of 2 weeks within a preclinical rat model of toxin induced

PD, with THC having reduced dopaminergic loss (Lastres-Becker et al., 2005).

Clinical studies utilizing CBD administration in PD patients have observed a reduction in the occurrence of psychotic symptoms that include sleep disturbances, hallucinations, and delusions (Zuardi et al., 2009), reduced tremor amplitude (de Faria et al., 2020), and an overall observed improvement in patient well-being and motor function (Chagas et al., 2014). A recent clinical trial utilizing CBD (Epidiolex) found similar improvements in PD associated symptoms with patients experiencing good tolerability with no major adverse effects with the 5–25 mg/kg/day dosing schedule (Leehey et al., 2020). A phase II, randomized, placebo-controlled, double-blind study to examine the effectiveness of nabilone to impact non-motor adverse effects related to PD has recently concluded and had found that PD patients given nabilone responded positively to doses up to 1 mg with good tolerability and with no major adverse effects reported (Peball et al., 2020). Clinical assessment surveys and self-scoring methods from this study indicated that patients receiving nabilone experienced improvements to non-motor adverse effects as opposed to the placebo arm, which reported increased disturbances resultant of non-motor adverse effects.

2.1.3 Huntington's Disease

Huntington's disease (HD) is a rare genetic neurodegenerative disorder resultant of excessive extension of CAG repeats within the huntingtin gene. Symptoms of HD include alterations in movement, mood, and cognition (see McColgan and Tabrizi, 2018) with an estimated prevalence of approximately 3 cases per 100,000 (Erkkinen et al., 2018). Cardinal features of HD include neuronal death and neuroinflammation in the striatum, globus pallidus, substantia nigra, and cerebral cortex (Hickey and Chesselet, 2003), with advanced stages of HD exhibiting widespread neuronal death among the cerebellum, hippocampus, and brain stem (Erkkinen et al., 2018).

Preclinical studies utilizing genetic mouse models of HD observed decreases in CB_1R mRNA within the striatum, cortex, and hippocampus in initial phases of HD (Denovan-Wright and Robertson, 2000; McCaw et al., 2004; Dowie et al., 2009). In rat preclinical studies utilizing a pharmacological model of HD, both CB_1R mRNA and protein were decreased in the caudate putamen, basal ganglia, globus pallidus, and substantia nigra (Lastres-Becker et al., 2001, 2002), though administration of substances that increased endocannabinoid activity was found to have activated the decreased population of CB_1R and improve subject motor function (Lastres-Becker et al., 2002). Utilizing quantitative autoradiography in post-mortem brain tissue sections from HD patients, a significant loss in CB_1R protein was observed within the globus pallidus, and substantia nigra (Glass et al., 1993; Richfield & Herkenham, 1994). Conversely, reductions in CB_1R expression have been accompanied by increased expression of CB_2R among astrocytes and microglia in preclinical rat pharmacological models of HD (Fernández-Ruiz et al., 2007; Basavarajappa et al., 2017). Post-mortem brain tissues from HD patients and transgenic mice models also exhibit increased CB_2R expression within the caudate putamen as well as in striatal microglia (Palazuelos et al., 2008). Disruption of the serotonergic system has been observed in striatal samples from HD patient brains where serotonin transporter protein was found in increased concentrations compared to age matched healthy controls and early-stage HD brain samples (He et al., 2019). In brains taken from a transgenic HD mouse, binding analyses of 5-HT1A receptors found reduced binding of 5-HT1A receptor agonists among hippocampal and striatal regions of the brain, indicating further disruption of the serotonergic system within HD (Yohrling IV et al., 2002).

Within a rat pharmacological model of HD, THC produced neuroprotective effects, which further suggests that THC may have therapeutic potential, as well as lends additional credence that cannabinoid receptor dysfunction may be involved in HD etiology (Lastres-Becker et al., 2004). In a preclinical study utilizing a rat model of striatal atrophy, CBD administration was able to reverse neurodegeneration following a 5 mg/kg/day dosing schedule over 5 days, and the authors found that these effects were likely the result of the intrinsic antioxidant potential held by CBD (Sagredo et al., 2007).

One small scale pilot study has indicated potential therapeutic capacity of nabilone in HD, having observed improvements to motor skills and participant cognition (Curtis et al., 2009). A case report also observed that medicinal cannabis and nabilone were able to improve patient motor function and cognitive behavior, though no measured responses were taken and reports were anecdotal (Curtis & Rickards, 2006) In contrast, clinical trials have observed either no significant difference in motor function and cognition with nabiximols compared to placebo controls (Lopez-Sendon Moreno et al., 2016), failure to provide symptomatic protection with CBD (Consroe et al., 1991), or significant increases in involuntary movements with nabilone (Müller-Vahl et al., 1999).

2.1.4 Multiple Sclerosis

Multiple sclerosis (MS) is a debilitating neurodegenerative disease largely affecting individuals in early adult life with an increasing prevalence of 1 per 3,000 individuals, or approximately 2.8 million people worldwide (Walton et al., 2020). Characterized pathologically by hallmarks that include inflammation, axonal and neuronal loss, demyelination, and astrocytic gliosis within the brain stem and spinal cord (Goldenberg, 2012; Thompson et al., 2018), MS is physiologically characterized by episodes of sensory and motor impairments driven largely by neurodegeneration.

Preclinical studies utilizing experimental autoimmune encephalomyelitis MS models have observed reduced expression of CB_1R among the striatum and cortex of rats (Berrendero et al., 2001), and mice deficient in CB_1R used in experimental autoimmune encephalomyelitis MS models exhibit enhanced neurodegeneration compared to control subjects (Pryce et al., 2003). Similarly, studies utilizing human post-mortem CNS tissue samples have observed increased expression of CB_1R among cortical neurons, oligodendrocytes, oligodendrocyte precursor cells and macrophages near plaques associated with MS (Benito et al., 2007; Palazuelos et al., 2008). Likewise, CB_2R receptor expression and density were found to have been increased in MS, particularly in T-lymphocytes,

astrocytes, microglia, and macrophages near active plaques (Yiangou et al., 2006; Benito et al., 2007). Utilizing photon emission tomography, patients with MS were found to have lower availability of serotonin transporters throughout the limbic system, a factor that may contribute to the psychiatric symptoms associated with MS, as well as disturbed modulation of the immune system (Hesse et al., 2014). CBD has been shown to provide therapeutic benefits for the treatment of MS, though further research is needed to understand the mechanisms driving such activity. Preclinical studies utilizing experimental autoimmune encephalomyelitis in mice have found that CBD administration ameliorated the severity of MS symptoms when given during disease onset (Kozela et al., 2011), inhibited production of pro-inflammatory cytokines (Giacoppo et al., 2017), and attenuated the infiltration of $CD4^+$ T cells and macrophages into the central nervous system (Constantinescu et al., 2011; Giacoppo et al., 2017).

Although nabiximols administration has not been found to improve cognitive function of patients (Rekand, 2014; Lopez-Sendon Moreno et al., 2016; Riva et al., 2019), the THC:CBD spray combination has not been associated with cognitive decline in long-term use (Rekand, 2014), a salient concern, given that long-term THC use has been linked to poor cognitive health (Crean et al., 2011). Compared to placebo controls, early clinical trials demonstrated that nabiximols displayed efficacy in the alleviation of MS-associated spasticity and reduced both spasm number instances and severity during treatment (Collin et al., 2007; Novotna et al., 2011; Wade et al., 2004, 2006). A more recent clinical trial utilizing nabiximols for the treatment of symptoms associated with MS observed superior improvement of MS induced spasticity compared to adjustments in first-line anti-spasticity medication alone (Markova et al., 2019). Similar improvements have been reported with clinical trials utilizing 10 mg dronabinol, where reductions in spasticity and improved ambulation (University of California, Davis, 2018) and modest improvements in pain assessments (Svendsen et al., 2004) were observed. While these studies report good tolerability with no major adverse effects, dronabinol was found to have no improvements on cognitive function and indications of worsening cognitive function with time (University of California, Davis, 2018). Whole cannabis extract was also found to have relieved muscle stiffness experienced by MS patients (Zajicek et al., 2012). Finally, medicinal cannabis was found to have slightly reduced fatigue among MS patients compared to age/sex matched controls with no record of cannabis use (Rudroff, 2020). Though this comparative observational study reports good tolerability as well, there is no mention of total cannabis consumption among patients. These findings are summarized in **Table 1**.

2.1.5 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with a prevalence between 4.1 and 8.4 per 100,000 persons, characterized by gradual loss of muscle control resultant of increasing muscle weakness and wasting (Longinetti & Fang, 2019). This results in the progressive loss of the ability to chew, swallow, talk and breathe, ultimately leading to death (Zarei et al., 2015). Portions of those with ALS will also experience frontotemporal dementia and changes in behavior and cognition as the disease progresses (Masrori & Damme, 2020). While 90–95% of all ALS cases are sporadic with unknown etiology (Zarei et al., 2015) and pathogenesis is not completely understood, it is thought that mechanisms associated with excitotoxicity, oxidative stress, and neuroinflammation are implicated with ALS onset (Rao & Weiss, 2004; Liu & Wang, 2017; Batra et al., 2019).

Preclinical research utilizing a genetic mouse model of ALS presents conflicting observations regarding CB_1R. Compared to healthy controls, reductions in spinal cord motor neuron CB_1R expression has been observed in the early, pre-symptomatic stage in a mouse ALS model, with an elevation of expression observed in the symptomatic stage (Zhao et al., 2008). The authors suggest that this may be indicative of a neuroprotective action compensating for initial losses in CB_1R, though ultimately, expression of CB_1R was reduced in end-stage ALS mouse models, suggesting continued declination in neuronal health (Zhao et al., 2008). Another preclinical study utilizing genetic ablation of CB_1R in a genetic mouse model of ALS observed an extension of life span compared to wild type subjects (Bilsland et al., 2006). However, ablation also resulted in significant motor neuron death and decreased survival of remaining motor neurons. An immunocytochemistry analysis of post-mortem spinal cord tissue taken from ALS patients observed increased CB_2R immunoreactivity within areas exhibiting neuronal degeneration (Yiangou et al., 2006). Further analyses will be needed to understand the role of cannabinoid receptors in ALS experienced by humans. Motor neurons preferentially affected in ALS are densely innervated by 5-HT expressing neurons; their degeneration may provide the pathological link to the spasticity commonly seen with ALS (Sandyk, 2006; Dentel et al., 2013). In ALS patient brainstem samples, it was found that there was severe degeneration of serotonergic neurons compared to healthy controls (Dentel et al., 2013).

Studies utilizing a transgenic mouse model of ALS have observed that mice treated with THC (Raman et al., 2004; Weydt et al., 2005), experienced delayed disease progression and prolonged survival. An *in vitro* component to one such study found that THC effectively reduced oxidative stress and minimized excitotoxicity within mouse spinal cord cultures (Raman et al., 2004), suggesting that THC possesses potential neuroprotective effects which may be beneficial in treating ALS.

Current clinical research into the use of cannabinoids in ALS is limited, though previously mentioned clinical trials in other neurodegenerative diseases suggest therapeutic potential in ALS. Observations gathered from patient surveys suggest that medicinal cannabis provides therapeutic relief of symptoms of ALS such as pain, spasticity, and excessive drooling, however these observations are limited by the comparatively small number (10% of those surveyed) of those having used cannabis recently at the time of survey (Amtmann et al., 2004). A randomized placebo-controlled clinical study utilizing nabiximols in patients with ALS report reductions in spasticity symptoms with no report of major adverse effects (Riva et al., 2019). Finally, a clinical study investigating the efficacy of THC in mitigating cramping associated with ALS observed no

subjective improvement of cramp intensity among ALS patients, though THC was well tolerated with no major adverse effects reported with 10 mg daily oral administrations (Weber et al., 2010). These findings are summarized in **Table 1**.

2.1.6 Epilepsy

Seizures are the result of abnormal synchronous neuronal excitation within the brain with etiologies including genetic predisposition, injury, brain tumors, and neurodegenerative diseases (Falco-Walter, 2020). The prevalence of epilepsy is between 50.4 to 81.7 per 100,000 persons annually and continues to rise as advances in healthcare lead to increased survivability of traumatic head injuries, stroke, and increased lifespans (GBD 2016 Epilepsy Collaborators, 2019).

As discussed above cannabinoid compounds have exhibited antispastic capacity in a range of neurodegenerative disease states that gives further support to the use of cannabinoids for the treatment of epileptic convulsions. THC-related anticonvulsant activity is likely the result of CB_1R stimulation. Preclinical evidence suggests that the endogenous cannabinoid system contributes to the regulation of seizure frequency. Mice lacking functional CB_1R or mice that have genetic alterations in endogenous cannabinoid system activity which lead to decreased CB_1R tone are characterized to be seizure prone (Clement et al., 2003; Marsicano et al., 2003). THC completely abolished spontaneous seizures within a rat model of epilepsy, while CB_1R antagonism with SR141716A increased seizure duration and frequency (Wallace et al., 2003). The same study also revealed that CB_1R expression was significantly increased within epileptic hippocampi (Wallace et al., 2003). CBD-related anticonvulsant activity is likely due to its 5-HT agonist properties, actions at voltage-gated sodium ion channels, as well as its ability to modulate intracellular calcium storage. *In vitro* studies, CBD selectively inhibited aberrant sodium currents in mutated sodium ion channel expressing cells and had no effect on normal sodium channel activity (Patel et al., 2016). In a mouse *ex vivo* epilepsy model CBD pre-exposure blocked aberrant hippocampal nerve firing, and these protective effects were inhibited by either a reduction in serotonin tone, or pharmacologically, by a calcium store antagonist (Maggio et al., 2018).

Clinical research has yielded promising results with the use of cannabinoids for treating epilepsy. However, current interest has been focused largely on CBD due to good tolerability and lack of psychoactive effects (Jones et al., 2010). CBD utilization within clinical trials of treatment-resistant epilepsy (Devinsky et al., 2016; Gaston et al., 2019) and Dravet syndrome (Devinsky et al., 2018, 2019) with clinically approved Epidiolex was found have significantly reduced the occurrence and duration of epileptic seizures. Long term safety and quality of life studies with Epidiolex also indicated that CBD provides effective long-term treatment with good tolerability and improves patient quality of life (Gaston et al., 2019; Laux et al., 2019).

2.1.7 Increasing Prevalence Requires Further Research

The prevalence of neurodegenerative and neurological disease continues to rise globally as improvements in healthcare result in improved survivability of many previously fatal diseases and longer life spans. However, with increases by more than 35% in death rates among those with neurodegenerative diseases within the past 25 years, therapeutics are needed urgently. Many of the pathologies discussed above lack any current clinically approved cures or treatments, with the current extent of our therapies only providing symptomatic relief. With endocannabinoid targets such as CB_1R and CB_2R and serotonergic involvement with 5-HT1A, development of cannabinoid-based therapeutics shows promise and further research and development is critical considering our aging population.

2.2 Pain

Pathological pain is a substantial component of many chronic illnesses and diseases and can be divided into pain that arises from inflammatory insults, known as inflammatory pain, and pain that is the result of nerve injury, known as neuropathic pain. Both inflammatory and neuropathic pain alter neuronal processing and immune cell function. These alterations ultimately lead to perceived pain with a prevalence of 6.9–10% for neuropathic pain, with much higher estimates for inflammatory pain as it is ubiquitous in many disease states (van Hecke et al., 2014). Diseases with exhibitions of neuropathic pain include diabetes, neurodegeneration, human immunodeficiency virus, and chemotherapy induced peripheral neuropathy. Cancer itself has both inflammatory and neuropathic components that contribute to pain perception, with common cancers such as breast, prostate, kidney, and lung cancers resulting in metastasis to bone that further drive and contribute to pathological pain in cancer (Mantyh, 2014).

2.2.1 Endocannabinoid Targets in Pain

There is continued interest in the endocannabinoid system and its involvement in pain modulation (see Donvito et al., 2018). This is due to the extensive expression of CB_1R throughout the CNS in pain relevant regions such as afferent nerve fibers (Hohmann et al., 1999; Morisset and Urban, 2001), spinal cord interneurons (Jennings et al., 2001), trigeminal sensory neurons (Price et al., 2003), and neurons within the periaqueductal grey (Mailleux and Vanderhaeghen, 1992). As for the periphery, CB_1R is observed on peripheral nociceptors (Richardson et al., 1998) and the dorsal root ganglia (Hohmann and Herkenham, 1999). As for CB_2R, these cannabinoid receptors are expressed in peripheral macrophages and lymphocytes (Munro et al., 1993; Bouaboula et al., 1993; Galiegue et al., 1995) as well as astrocytes, oligodendrocytes, and microglia within the central nervous system (Zhang et al., 2003; Maresz et al., 2005; Beltramo et al., 2006; Racz et al., 2008), suggesting potential mediation of inflammatory pain with cannabinoids. The spatial distribution of CB_1R and CB_2R within the CNS are shown in **Figure 1**. CB_2R may also be implicated with neuropathic pain, as CB_2R has observed expression among sensory neurons of the dorsal horn after sciatic nerve section or spinal nerve ligation in rats (Wotherspoon et al., 2005). 5-HT1A receptors are expressed in areas relevant to pain signaling and transmission such as primary afferent neurons, peripheral terminals, astrocytes,

oligodendrocytes, and microglia (Björk et al., 1992; Laporte et al., 1995; Granados-Soto et al., 2010; Perrin et al., 2011). The spatial distribution of 5-HT1A receptors within the CNS is shown in **Figure 1**.

2.2.2 Preclinical Studies in Pain

Preclinical studies assessing CBD for pain relief have painted a promising picture for the cannabinoid. Though as discussed in the next section, expectations should be tempered as the translational efficacy of cannabinoids into humans is unclear. CBD has been found to exert analgesic effects in animal models of neuropathic pain, such as surgically induced nerve injury and chemotherapy induced neuropathy (Harris et al., 2016; Ward et al., 2011, Ward et al., 2014), though effects are dependent on dose and route of administration (Costa et al., 2007; Abraham et al., 2020). While the therapeutic window of THC is limited by its psychoactive side effects, various studies utilizing combinations of THC and CBD have found improved efficacy in low dose administrations to treat pain. In models of either surgically induced nerve injury (Casey et al., 2017; Linher-Melville et al., 2020) or chemotherapy induced neuropathy (King et al., 2017), 1:1 THC + CBD combinations exhibited greater efficacy at low doses that were ineffective with either THC or CBD alone.

2.2.3 Clinical Trials in Pain

Despite these preclinical studies suggesting therapeutic potential of cannabinoids as analgesics, review of recent clinical trials in various pain pathologies suggests an inconclusive viability of cannabinoids as a therapeutic for pain. Within a double-blind placebo-controlled study in MS patients experiencing neuropathic pain, THC (dronabinol), taken orally up to a maximum dose of 15.9 mg over 16 weeks, decreases patient reported pain measurements, though no significant difference from placebo was ever observed (Schimrigk et al., 2017). Studies in safety also found that whole cannabis was able to alleviate chronic pain whilst improving patient quality of life with both acute and long-term administrations (Ware et al., 2010, 2015). An open label, long-term efficacy and safety portion of this clinical trial also observed maintained decreases in patient reported pain intensities with low occurrences of serious adverse effects being reported (Schimrigk et al., 2017). A clinical trial utilizing THC (dronabinol) for pain relief in patients with noncardiac chest pains observed that 10 mg oral administrations improved patient pain thresholds significantly compared to placebo with good tolerability and with no major adverse effects being reported (Malik et al., 2017). Clinical trials utilizing nabiximols have observed pain relief in patients experiencing pain resultant of a range of pathologies including MS (Notcutt et al., 2004; Rog et al., 2005; Kavia et al., 2010), peripheral neuropathy (Nurmikko et al., 2007), and cancer (Johnson et al., 2010; Portenoy et al., 2012; Fallon et al., 2017; Lichtman et al., 2018). A clinical trial with nabiximols in patients experiencing chronic pain associated with late-stage cancer observed a 15.5% improvement among patient reported perceptions of pain (Lichtman et al., 2018). An identical companion study to this clinical trial once again observed similar improvements with nabiximols (Fallon et al., 2017). It should be noted that patient pools utilized cohorts from both the United States and Eastern Europe, with significant improvements in pain relief compared to placebo among American patients and general improvements among Eastern Europeans, though these effects were not significant compared to placebo in Eastern European cohorts (Fallon et al., 2017; Lichtman et al., 2018). It should be noted in these studies the Eastern European cohort was sicker than the American cohort, suggesting that patient selection criteria may have contributed to the divergent study findings. Additionally, nabiximols administration did meet several secondary endpoints associated with quality of life, which, as suggested by the authors, may indicate therapeutic utility in cancer pain as an adjuvant therapeutic with a low opioid dose (Lichtman et al., 2018). In a small double-blinded, placebo-controlled, crossover design clinical study, oral CBD had no effect on muscle damage markers or muscle soreness in exercised untrained men (Cochrane-Snyman et al., 2021). A recent double-blind, placebo controlled clinical study in an emergency room setting found that orally administered CBD was equal to placebo and did not adequately control acute non-traumatic low back pain (Bebee et al., 2021). Although these above clinical studies suggest CBD may not be a frontline analgesic, further clinical studies examining various route of administration and dosing strategies are needed. Additionally, it is unknown if the other compounds in medicinal cannabis may yield enhanced utility of these cannabinoids in clinical pain management settings.

2.2.4 Opioids and Cannabinoids for Pain

The ability of medical cannabis to augment the analgesic potency of opioids without additional enhancement of opioid associated side effects is an area of growing research interest with recent clinical trials yielding mixed results. Clinical pain research suggests that medicinal cannabis or cannabinoids for chronic pain may yield opioid sparing effects, a major consideration given the interest in minimizing opioid use for severe pain to avoid opioid tolerance, dependance risk, and side effects such as somnolence and respiratory depression. Studies utilizing smoked or oral medicinal cannabis among habitual opioid using, chronic pain patient cohorts observed improvements to quality of life, pain, and opioid prescription cessation (Haroutounian et al., 2016; Capano et al., 2020). Indeed, following 6 months of opioid/cannabis cotreatments, prescribed morphine use was found to have dropped significantly compared to baseline usage among patients with chronic pains, with reductions observed earlier at 3 months opioid/cannabis cotreatment (O'Connell et al., 2019). Similar reductions in prescribed, opioid use were also observed over a 21-month period with 83.8% of patients (N = 37) reporting reduced prescribed daily opioid dosage and 40.5% of patients ceasing opioid prescriptions altogether (Vigil et al., 2017). A clinical trial utilizing healthy participants with no prior indication of pathological pain had observed that orally administered THC (dronabinol, 5 mg) was not able enhance the analgesic effects of oxycodone in coadministrations and reported an increase in both abuse and impairment related effects associated with opioid use (Babalonis et al., 2019). A similar study in healthy subjects also

reported that THC (dronabinol, max 10 mg) had no consistent dose-effect relationship with the opioid agonist hydromorphone in measures of both acute and chronic pain, though significant analgesia in acute pain with hydropmorphone and 2.5 mg dronabinol compared to placebo was observed (Dunn et al., 2021). Additionally, a clinical trial utilizing healthy cannabis smokers found that the combination of oxycodone (2.5 mg) and cannabis (cigarettes, 5.6% THC) was not able to provide analgesia in measures of acute pain and increased abuse-related subjective effects but did increase pain thresholds and tolerance (Cooper et al., 2018). This discrepancy may be due to the contributing pharmacological effects of the other compounds found in cannabis rather than THC alone, though more work is needed to explore the complex pharmacology between cannabinoids and opioids.

2.2.5 Disconnect Between Preclinical and Clinical Research Findings

Pain is a substantial component of many chronic illnesses and diseases that range from cancer to diabetes. With extensive expression of CB_1R and CB_2R and 5-HT receptors, cannabinoid compounds have great potential as novel pain therapeutics for pathologies such as chemotherapy induced peripheral neuropathy, cancer, and neurodegenerative diseases. Current preclinical literature shows promise with cannabinoids being able to effectively alleviate pain across different animal pain models. However, clinical research suggests that more work is needed to examine dose, pain indication, and route of administration questions, given that many studies observe general, but not significant, improvements in pain when compared to proven analgesics such as oxycodone and other opioids. Despite this, these studies and others still report improvements in patient reported assessments of pain and quality of life compared to placebo controls. As such, further clinical research is warranted to determine whether cannabinoids can provide effective pain relief alone or as an adjunctive therapeutic in human pain pathologies.

2.3 Addiction and Substance Use Disorders

From alcohol to opioids, addiction can occur with a variety of psychoactive drugs and consists of use disorders characterized by heavy consumption, loss of intake control, and withdrawal experiences (Zou et al., 2017). Most of these addictive substances can result in elevations of extracellular dopamine that, with time, downregulate the expression of dopamine receptors and negatively affect dopaminergic neurons, like those found in the mesocorticolimbic pathway (Wise & Robble, 2020).

2.3.1 Endocannabinoid Targets in Addiction and Substance Use Disorders

In addition to dopaminergic receptors, CB_1R are expressed in abundance throughout this pathway in brain regions involved in reward signaling such as the ventral tegmental area, nucleus accumbens, amygdala, pre-frontal cortex, and hippocampus (Oleson et al., 2021). Preclinical studies utilizing either CB_1R antagonists or deletion of the receptor have observed reduced motivation for the consumption and self-administration of ethanol in rat and mouse alcohol dependence models (Gallate et al., 2004; Thanos et al., 2005; Femenía et al., 2010). Rimonabant, the CB_1R inverse agonist/functional CB_1R antagonist, was shown to reduce conditioned place preference and self-administration of alcohol (Arnone et al., 1997), heroin (De Vries et al., 2003), and nicotine (Cohen et al., 2005; Robinson et al., 2018). While serious psychiatric side effects such as anxiety, depression, and suicide ideation have prevented rimonabant from passing clinical trials (Manzanares et al., 2018), it supports the notion that CB_1R antagonism may allow for the attenuation of substance use disorders. CB_2R in substance use disorders may also provide a potential target with cannabinoid therapeutics as research suggests their potential role in modulating behaviors associated with addiction (Onaivi et al., 2008b; Agudelo et al., 2013; Galaj et al., 2020). Similarly, given the role that the serotonergic system plays in both motivational and reinforcement processes, serotonergic modulation may provide a solution to alleviating substance use disorders (Müller & Homberg, 2015; Yagishita, 2020). Research shows that extracellular serotonin is acutely increased following administration of morphine (Tao & Auerbach, 1994; Fadda et al., 2005) and alcohol (Yoshimoto et al., 1992; Bare et al., 1998; Thielen et al., 2002). Additionally, chronic administration of psychoactive substances such as morphine, ethanol, and cocaine have been found to reduce the basal levels of extracellular serotonin within the brain, potentially resulting in increased sensitivity (Pelloux et al., 2012; Müller & Homberg, 2015). Preclinical research focused on 5-HT1A receptor modulation in the context of drug reward and addictive behaviors found CBD decreased morphine-induced reward facilitation in an operant behavioral paradigm within rats that was mediated through 5-HT1A receptor activation in the dorsal raphe nucleus (Katsidoni et al., 2013). Though further studies are required, current research has provided proof of concept regarding the treatment of drug dependency and use disorders with cannabinoids, suggesting their use as an alternative or co-adjuvant therapeutic.

2.3.2 Alcohol Use Disorder

US Food and Drug Administration approval for drugs in the treatment of alcohol use disorder has not occurred since 2004 with the approval of acamprosate. Preclinical research has shown that CBD may hold particular promise as an alcohol use disorder therapeutic. Activity at CB_2R may provide for an initial target in therapeutic development with cannabinoids. An early study utilizing the CB_2R agonist, JWH 015 in stressed mice observed enhanced alcohol preference compared to controls (Onaivi et al., 2008a). Upregulation of CB_2R was also observed in dendritic cells from patients with alcohol abuse disorders (Agudelo et al., 2013). Preclinical studies utilizing mice in the two-bottle choice paradigm and oral ethanol self-administration demonstrated that systemic CBD administration significantly reduced both ethanol consumption and preference, suggesting that CBD can reduce the motivational properties of ethanol (Viudez-Martínez et al., 2018). The same study found that CBD administration prevented relapse in oral ethanol self-administration (Viudez-

Martínez et al., 2018). Contrary to the potential benefits observed with CBD, THC has been found to reinstate alcohol seeking behavior in abstinent rats (McGregor et al., 2005). Utilizing a beer (4.5% ethanol v/v) self-administration paradigm, the study observed that intraperitoneal THC administration significantly reinstated responding previously reinforced with beer. However, both sucrose trained subjects and beer trained subjects had self-administration responses reinstated with THC administration (McGregor et al., 2005).

2.3.3 Opioid Use Disorder

Preclinical studies with cannabinoids as a therapeutic for opioid use disorders are largely motivated by the neurobiological interactions between the cannabinoid and opioid systems (Rodríguez et al., 2001; Schoffelmeer et al., 2006). A preclinical study in rats utilizing a self-administration, drug-seeking behavior model found that CBD inhibited reinstatement of cue-induced heroin seeking behavior, though such effects were not observed with drug seeking behavior initiated by a priming dose of heroin (Ren et al., 2009). A similar study utilizing a conditioned place preference paradigm in mice with morphine treatment found that CBD decreased the establishment of opioid reward as indicated by an attenuation of morphine place preference (Markos et al., 2018). In contrast, some research suggests that THC may not be a viable therapeutic for treating opioid use disorders, though use in treating withdrawal symptoms show promise. It has been observed that subjects pre-exposed in adolescence shown marked opiate sensitivity with higher consumption of heroin and upward shifts in self-administration acquisition (Ellgren et al., 2007). Similar studies observing the effects of systemic THC administration in cannabinoid-opioid system interactions have reported similar results with enhanced opioid intake in operant behavioral studies (Vela et al., 1998; Solinas et al., 2004). THC may not be without therapeutic opioid use disorder utility. Current research, though limited, demonstrates that systemic THC administration inhibits symptoms (jumping, rearing, wet shakes, diarrhea) associated with naloxone-induced opioid withdrawal (Hine et al., 1975; Bhargava, 1976).

2.3.4 Tobacco Use Disorder

While currently limited, there is increasing evidence that cannabinoid compounds are able to modulate tobacco use disorder. However, some studies are seemingly contradictory, and have left the exact therapeutic utility of cannabinoids for the treatment of tobacco use disorder unclear. A small-scale pilot study in treatment-seeking smokers had found that use of a CBD inhaler resulted in reduced self-reported smoking compared to placebo treatment over a 7-day period, although cravings for cigarettes remained unchanged (Morgan et al., 2013). Acute administration of THC has been found to attenuate the somatic and motivational manifestations of nicotine withdrawal in mice, though it is unlikely a result of the compensatory changes on CB_1R density following chronic nicotine exposure (Balerio et al., 2004). A similar study assessing nicotine and THC coadministration in mice found enhancement of both the expression of nicotine withdrawal symptoms and nicotine induced conditioned place preference (Valjent et al., 2002). Further research will be required to elucidate any potential therapeutics for nicotine use disorders.

2.3.5 Cocaine Use Disorder

With no currently approved therapeutics for psychostimulant addiction, the use of cannabinoids as a treatment for cocaine addiction has garnered interest despite a small body of literature. Recent preclinical work with CBD administration in mice observed reduced CB_1R expression within the nucleus accumbens with simultaneous increases in CB_2R expression (Calpe-López et al., 2019). These results are intriguing and raise the possibility that although CBD may not act directly *via* CB_1R or CB_2R based upon binding affinity, it may alter cannabinoid receptor tone. An earlier study utilizing JWH133, a CB_2R agonist, found that CB_2R agonism was able to dose dependently inhibit cocaine-enhanced locomotion and cocaine self-administration in mice (Xi et al., 2011). Such effects were not observed in CB_2R knockout mice and were blocked with AM630, a CB_2R antagonist, suggesting a role for CB_2R in modulating cocaine-induced rewarding and locomotor enhancing effects (Xi et al., 2011). A similar study conducted last year in mice also reported similar benefits, as CBD prevented behavioral alterations associated with cocaine addiction that included locomotor stimulation and memory deficits related to cocaine withdrawal (Ledesma et al., 2021). Finally, in a rat cocaine self-administration model, it was observed that CBD reduced cocaine self-administration, and these effects are blocked following CB_2R and 5-HT1A antagonist administration (Galaj et al., 2020).

2.3.6 Considerations of Cannabis Dependence and Therapeutic Capacity

As mentioned earlier, the two primary constituents of cannabis are THC and CBD, with THC having psychoactive properties and marked effects on dopamine release like other drugs of addiction. Indeed, clinical research shows that acute administration of THC does elicit dopamine release within the striatum (Bossong et al., 2015; Bloomfield et al., 2016), with such effects being dose dependent. Chronic cannabis use is also associated with increased risk of substance use disorder development and development of withdrawal behaviors that include irritability, anxiety, depression, fever, and tremors (Katz et al., 2014; Volkow et al., 2014). Furthermore, utilization of THC in anxiolytic therapies is limited due to psychoactive sequelae, risk of abuse, and anxiogenic effects (Kayser et al., 2020b; García-Gutiérrez et al., 2020). While the therapeutic window of THC is limited by its psychoactive side effects, various studies utilizing combinations of THC and CBD have found improved efficacy in low dose administrations to treat pain, anxiety, and depression. Although the negative attributes of cannabis are largely attributed to THC, as described above, CBD continues to draw attention as an anxiolytic and analgesic. The minor components of cannabis may also prove to be beneficial in either the selective development of whole cannabis therapeutics or as isolated cannabinoid compound mixtures. This rationale is due to the discovery that cannabis terpenoids and minor phytocannabinoids exhibit therapeutic capacity in a variety of pathologies, including

epilepsy, neurodegenerative disease, and traumatic brain injuries (Russo & Marcu, 2017; Russo, 2018).

2.4 Anxiety Disorders

Early epidemiological studies observing the prevalence of mood disorders had found that anxiety disorders are highly prevalent within the United States (Weissman, 1988; Stein et al., 2017). Anxiety disorders to date are maintained as the most common mood-related disorders (Vos et al., 2015; Penninx et al., 2021) both within the United States and worldwide. Psychological symptoms of common anxiety disorders include frequent and prolonged states of amplified fear and/or anxiety (Giacobbe & Flint, 2018).

2.4.1 Endocannabinoid Targets in Anxiety

Brain regions relevant in feelings of anxiety and fear include the prefrontal cortex, hippocampus, amygdala, hypothalamic nuclei, and the bed nucleus of the stria terminalis (Lafenetre et al., 2007), regions with notable expression of neuronal CB_1R. Additionally, CB_2R present within the periphery and the CNS, have been implicated in both anxiety disorders and anxiety regulation (Garcia-Gutierrez and Manzanares, 2011; Liu et al., 2017; Patel et al., 2017). Like CB_1R, 5-HT1A receptors are present in high densities throughout the CNS in areas associated with emotional control and anxiety, including regions such as the hippocampus, amygdala, and cerebral cortex (Mestikawy et al., 1991; Wang et al., 2009; Marcinkiewcz et al., 2016). While their direct role in anxiety onset is unclear, the contribution of the serotonergic system is evident as 5-HT1A receptor knockout mice exhibit increased anxiety-like behavior in assays such as the elevated-plus maze and open-field test, both of which provide face and predictive validity in human models of anxiety (Hirshfeld et al., 1992; Graeff et al., 1998; Lesch, 2005).

2.4.2 Cannabinoid Consideration for Anxiety

Primary first line pharmacotherapeutics for the treatment of anxiety are serotonergic, which include selective serotonin reuptake inhibitors (SSRIs) and azapirones like buspirone. These therapeutics are generally well tolerated with short-term adverse effects that include nausea, diarrhea, and constipation. However, more problematic adverse effects include sexual dysfunction (Jing & Straw-Wilson, 2016), suicide ideation in pediatric patients (Hammell et al., 2016), and serotonin syndrome (Volpi-Abadie et al., 2013) with SSRIs and the development of buspirone induced movement disorders (Rissardo & Caprara, 2020). While the utilization of THC in anxiolytic therapies is limited due to psychoactive sequelae, risk of abuse, and anxiogenic effects (Kayser et al., 2020b; García-Gutiérrez et al., 2020), CBD continues to draw increasing attention in its use as an anxiolytic as work continues in developing therapeutics that can mimic the beneficial effects of current first line anxiety therapeutics while having improved side effect profiles over SSRIs and buspirone. CBD has been indicated as a potential treatment of a range of anxiety disorders that include both generalized anxiety disorder (GAD) and social anxiety disorder (SAD) as well as the excessive anxiety associated with post-traumatic stress disorder (PTSD) and obsessive-compulsive disorder (OCD) (Micale et al., 2013; Blessing et al., 2015).

2.4.3 Preclinical Studies in Anxiety

Preclinical literature regarding CBD in rodent models of generalized anxiety suggest CBD's efficacy in minimizing anxiety associated behaviors relevant in GAD, SAD, PTSD, and OCD. Studies utilizing CBD in elevated plus and elevated T mazes with rodents have observed anxiolytic effects following both acute systemic administration (Campos et al., 2012; Campos et al., 2013a; Campos et al., 2013b) and acute local administrations in areas such as the amygdala central nucleus (Hsiao et al., 2012), bed nucleus of the stria terminalis (Gomes et al., 2011), and the intra-dorsal periaqueductal gray (Soares et al., 2010). Anxiolytic effects of CBD in these models are presented as a bell-shaped dose-response curve, with anxiolytic effects generally observed at moderate doses; 2.5–10.0 mg/kg in rats (Guimarães et al., 1990), 1 and 10 mg/kg in mice (Onaivi et al., 1990). Chronic administrations of CBD have also been found to produce anxiolytic effects in mice with the open-field test (Long et al., 2010), though contrasting results from a later study show that chronic CBD had no such effect in the elevated plus maze (Schiavon et al., 2016). Despite these mixed results and considering current preclinical evidence, use of CBD as an anxiolytic appears favorable with an improved side effect profile and no risk of anxiogenic effects (Garakani et al., 2020).

2.4.4 Clinical Trials in Anxiety

Secondary outcomes of clinical trials utilizing dronabinol (Malik et al., 2017), nabilone (John Redmond et al., 2008), and oral titrations of THC (Attal et al., 2004) all have reported general improvements to patient anxiety alongside their primary outcomes on pain relief. Studies assessing cannabinoid/opioid cotreatments also observed improvements to patient quality of life with secondary outcomes looking at measures of anxiety (Haroutounian et al., 2016; Capano et al., 2020). Direct assessments of patient anxiety provide clinical evidence that suggests that CBD has potential as a treatment for anxiety disorders, though such studies have generally focused on acute administrations utilizing small subject sizes often in healthy patients (Blessing et al., 2015; García-Gutiérrez et al., 2020). Clinical studies utilizing the simulation public speaking test had found that acute oral administration of CBD capsules reduced subjective (visual analog mood scale) and physiological (blood pressure, heart rate) measures of stress in healthy patients (Zuardi et al., 2009; Bergamaschi et al., 2011). Of these studies, a treatment naïve patient group with SAD was given CBD and had also exhibited indications of reduced anxiety, both subjective and physiological (Bergamaschi et al., 2011). Clinical studies assessing the anxiolytic properties of cannabinoids in PTSD and chronic pain pathologies have also observed general improvements in patients having consumed whole cannabis products (Greer et al., 2014; Bonn-Miller et al., 2021) or CBD alone (Elms et al., 2019) as indicated by lowered scores in clinician administered posttraumatic stress scales and PTSD checklists which assess emotional response and cognitive

function. Instances of self-reported anxiety associated with OCD were found to have been no different than placebo after administration of cannabis high in either CBD (0.4% THC/10.4% CBD) or THC (7.0% THC/0.18% CBD) (Kayser et al., 2020a). Another retrospective clinical study had found that general anxiety experienced by patients was reduced following continued CBD administration using the Hamilton anxiety rating scale, though it should be noted that this study utilized open-label treatment for patients without a comparison group (Shannon et al., 2019). These studies support the potential for CBD as a treatment for anxiety disorders, especially when paired with preclinical findings. However, larger clinical trials assessing both acute and chronic dosing in additional anxiety disorders are needed.

2.4.5 CBD, But Not THC for Anxiolytic Development

Anxiety disorders are highly prevalent within the United States and is maintained as the most common mood-related disorder worldwide. First line therapeutics for the treatment of anxiety include SSRIs and buspirone and while generally tolerated, these therapeutics are associated with problematic adverse effects that include sexual dysfunction and suicide ideation in pediatric patients. Therefore, development of cannabinoid-based anxiolytics would provide a potentially safer alternative to current therapies. It should be noted though that the psychoactive components of cannabis, such as THC, are generally anxiogenic at higher doses and while clinical research has indicated anxiolytic effects at low doses, THC alone seems to have fallen out of favor in anxiolytic development. With indications from both preclinical and clinical research, CBD may prove to be an effective cannabinoid in relieving anxiety in patients and further development of cannabinoid-based anxiolytics is warranted.

3 CONSIDERATIONS FOR CANNABINOID ADMINISTRATION AND FORMULATION

Cannabinoids exhibit particular characteristics that must be considered for both compound formulation and routes of administration as the pharmacokinetics and effects observed are heavily dependent on these (Lucas et al., 2018). Cannabinoids such as THC exhibit high lipophilicity, low aqueous solubility, and susceptibility to degradation *via* light, heat, and auto-oxidation (Grotenhermen, 2003). Interest in cannabinoid formulation, delivery strategies, and utilization of optimal routes of administration continues to grow in parallel with interests in the use of cannabinoids for potential therapeutic applications. Formulation strategies have been developed to overcome challenges brought upon by characteristics such as high lipophilicity in other compounds, though these strategies require testing in cannabinoids to determine if they would provide favorable pharmacokinetic improvements in items such as distribution and bioavailability (Kumari et al., 2010; Allen and Cullis, 2013; Dengler et al., 2013).

3.1 Oral Administration

Current clinically approved cannabinoids such as nabilone, dronabinol, and cannabidiol (Epidiolex) utilize oral administration and is the most prevalent route of administration for therapeutic applications. In the case of dronabinol, an exciting opportunity presents itself where direct comparisons can be made between the capsule and liquid formulation of the cannabinoid regarding efficacy. Companion clinical trials aiming to assess potential differences between these capsule and liquid formulations had observed a large, though insignificant, difference in dronabinol absorption times with 4.25 mg liquid formulations being superior to 5 mg capsules (Parikh et al., 2016; Oh et al., 2017). However, peak serum concentration was higher for both dronabinol and its metabolite, 11-OH-Δ9-THC, with the capsule formulation (Parikh et al., 2016; Oh et al., 2017). This disparity among formulations may likely be the result of hydrophobic drugs being less bioavailable when delivered in oil-based formulations (MacGregor et al., 1997). Indeed, both THC and CBD oral administrations in sesame oil exhibit poor bioavailability, as low as 6% in humans (Agurell et al., 1981; Huestis, 2005), likely resulting from variable absorption and extensive first pass metabolism (Lucas et al., 2018). Utilization of a self-emulsifying drug delivery system (SEDDS) could provide a more desirable endpoint for compound bioavailability as the mixing oils, surfactants, solvents, and other excipients can improve the oral bioavailability of lipophilic compounds. SEDDS may provide a viable solution to the challenges brought upon by the inherent lipophilicity of cannabinoids as patents filed by Murty Pharmaceuticals show a growing body of research that supports this drug delivery system (Murty and Murty, 2012). A recent clinical study utilizing a SEDDS-CBD oral administration (standardized to 25 mg) in healthy volunteers has observed significant improvements across pharmacokinetic parameters, including increased CBD plasma values, enhanced bioavailability, and fast absorption with no safety concerns being noted (Knaub et al., 2019). Ultimately, these oral formulations could provide symptomatic relief over prolonged periods (Lucas et al., 2018), making them suitable for continued administrations for the chronic symptomatic relief.

3.2 Nasal and Oral Mucosal Administrations

Alternative routes of administration can provide methods of circumventing variable absorption and extensive first pass metabolism. Both the oral mucosa and nasal cavity provide attractive targets for alternative routes of administration due to thin layering coupled with extensive vascularization. An assessment report conducted by the Australian Department of Health's Therapeutic Goods Administration for nabiximols surmised that oromucosal formulations of nabiximols are rapidly absorbed, resulting in higher plasma concentrations of THC and CBD compared to oral formulations (Therapeutic Goods Administration, 2013). Though administration *via* the nasal mucosal membrane provides favorable absorption rates, current formulations are not as attractive given patient reluctance, formulation safety concerns, and nasal spray particle size (Therapeutic Goods Administration, 2013). It is

likely that these issues associated with nasal administration has resulted in few recent developments regarding intranasal formulations (Bryson & Sharma, 2017; Bruni et al., 2018). Current oromucosal formulations of cannabinoids are therefore preferable in providing rapid, potentially therapeutic effects in a manner that is comfortable to patients and is more likely to be self-administered.

3.3 Pulmonary Administration

Among the possible routes of administration utilized for cannabinoids, pulmonary administration of cannabis is likely the most well-known route of administration among the general population. Like nasal and oral mucosa, pulmonary administration is highly effective given the high bioavailability, rapid onset, and avoidance of first-pass metabolism this route provides (Grotenhermen, 2003). However, critical issues associated with both intrapatient and interpatient variability arise given the inherent variability with pulmonary administration without the use of standardized methods. These include variations in inhalation depth, irritation or discomfort, technique and experience, and pharmacokinetic parameters such as maximum plasma concentration (Ohlsson et al., 1982; Hunault et al., 2010; Solowij et al., 2014; Lucas et al., 2018). Factors such as these could ultimately affect the efficacy of inhaled cannabis or cannabinoids and should be considered with concerns of dosing frequency and of side effects such as intoxication and cognitive function with psychoactive components like THC. Much interest surrounds the development of a standardized system or device that can deliver a metered dose of inhaled cannabinoid as a result. Comparatively, cannabinoid vaporization has grown in popularity due to ease of use and relative safety compared traditional combustion methods, such as with cannabis cigarettes (Gieringer et al., 2004). However, a standardized methodology has yet to be developed to account for sources of variability such as inhalation depth, though some have presented method proposals (Solowij et al., 2014; Lanz et al., 2016) and metered inhalation device patents (Davidson et al., 2018). A clinical study utilizing this metered inhaler observed that the product was able to administer consistent doses (15.1 ± 0.1 mg) of cannabis (19.9% THC, 0.1% CBD, 0.2% cannabinol) that provided effective neuropathic pain relief in patients (Eisenberg et al., 2014).

3.4 Topical Applications

Like mucosal and pulmonary administration, topical administration of cannabinoids provides an avoidance of first-pass metabolism, steady administration over time, and consistent dosing. However, due to the hydrophobicity of cannabinoids, diffusion across the skin is limited and such topical formulations require enhancements to permeation (Challapalli & Stinchcomb, 2002; Lodzki et al., 2003). Current applications for topical cannabinoids, though almost exclusively CBD, range from treating inflammatory dermatological disorders to localized pain relief among instances of arthritis and joint pain, though continued research suggests potential benefit in neuropathic pains (Baswan et al., 2020; D'Andre et al., 2021; Hammell et al., 2016; Xu et al., 2020). Similar to other aspects of cannabinoids as potential therapeutics, advances in formulation and optimization of administration drives further interest research into cannabinoids, though more work is needed.

3.5 Standardized Oral Administrations Over Non Standardized Pulmonary Administrations

Pharmacokinetic characteristics of cannabinoids is dependent on route of administration. While clinically approved cannabinoids such as dronabinol utilize oral administrations, general consumption of cannabis is primarily pulmonary with inhalation of cannabis smoke. However, unlike these oral administrations with a standardized formulation and administration method, pulmonary administrations of cannabis introduce numerous variables that could introduce interpatient and interpatient variability. These same factors can also directly affect the kinetics of the cannabinoid. While standardization and development of inhalation devices are being developed, current oral administrations, either through ingestion or through the oral mucosa, provide ease of use among patients and standardization to ensure consistency in dosing.

4 DISCUSSION

The field of cannabinoid research continues to experience advancements as interest in therapeutic applications continuously grows, whether that be from urgent needs in replacement therapeutics or the development of a therapeutic the first of its kind. This review of current and past studies finds that preclinical research indicates therapeutic potential for cannabis, THC, and CBD mediated through either CB_1R, CB_2R, 5-HT1A, or a variable combination of these receptors. Clinical research utilizing cannabinoids within instances of neurodegenerative disease, pain, addiction, and anxiety suggest both tolerability and therapeutic potential either alone or in combination with current therapeutics. However, preclinical literature dominates, and additional clinical studies are required to clarify these therapeutic indications before definitive declarations can be made. Further advancement of cannabinoids to the clinical setting is dependent on these clinical trials. There still exists a wide gap between the purported and anecdotal medicinal cannabis uses and specific therapeutic indications irrefutably supported by strong scientific evidence. One possible explanation for this disparity may lie in the complex pharmacological nature of cannabis. Although this review focuses on THC and CBD, there are over 100 different compounds in cannabis including minor cannabinoids, cannabis terpenoids, and phytocannabinoids which have additional pharmacological and biological activity. Additional work in the field of medicinal cannabis to identify the exact composition of studied strains, including minor cannabinoid and terpenoid profiles and concentrations, which can vary dramatically between different cannabis strains, is sparse. This information is desperately needed within the field to study interactive effects between minor cannabinoids, terpenoids, as well as THC and CBD. Indeed, it may be that the interactive pharmacological profiles of minor cannabinoids and terpenoids may underlie at

least some of the purported medicinal cannabis benefits that have so far been elusive to definitively confirm.

AUTHOR CONTRIBUTIONS

Wrote or contributed to the writing of the manuscript: YTO, LRM and JLW.

FUNDING

This work was supported by the National Institute on Drug Abuse DA25267 and DA48353 (LRM). JLW received funding from the 2021 Research Grants Program of the Consortium for Medical Marijuana Clinical Outcomes Research, which is funded through State of Florida appropriations.

REFERENCES

Abraham, A. D., Leung, E. J. Y., Wong, B. A., Rivera, Z. M. G., Kruse, L. C., Clark, J. J., et al. (2020). Orally Consumed Cannabinoids Provide Long-Lasting Relief of Allodynia in a Mouse Model of Chronic Neuropathic Pain. *Neuropsychopharmacology* 45 (7), 1105–1114. doi:10.1038/s41386-019-0585-3

Agudelo, M., Yndart, A., Morrison, M., Figueroa, G., Muñoz, K., Samikkannu, T., et al. (2013). Differential Expression and Functional Role of Cannabinoid Genes in Alcohol Users. *Drug Alcohol Depend* 133 (2), 789–793. doi:10.1016/j.drugalcdep.2013.08.023

Agurell, S., Carlsson, S., Lindgren, J. E., Ohlsson, A., Gillespie, H., and Hollister, L. (1981). Interactions of delta 1-tetrahydrocannabinol with Cannabinol and Cannabidiol Following Oral Administration in Man. Assay of Cannabinol and Cannabidiol by Mass Fragmentography. *Experientia* 37 (10), 1090–1092. doi:10.1007/BF02085029

Ahmad, R., Goffin, K., Van den Stock, J., De Winter, F. L., Cleeren, E., Bormans, G., et al. (2014). *In Vivo* type 1 Cannabinoid Receptor Availability in Alzheimer's Disease. *Eur. Neuropsychopharmacol.* 24 (2), 242–250. doi:10.1016/j.euroneuro.2013.10.002

Allen, T. M., and Cullis, P. R. (2013). Liposomal Drug Delivery Systems: From Concept to Clinical Applications. *Adv. Drug Deliv. Rev.* 65 (1), 36–48. doi:10.1016/j.addr.2012.09.037

Amtmann, D., Weydt, P., Johnson, K. L., Jensen, M. P., and Carter, G. T. (2004). Survey of Cannabis Use in Patients with Amyotrophic Lateral Sclerosis. *Am. J. Hosp. Palliat. Care* 21 (2), 95–104. doi:10.1177/104990910402100206

Arnone, M., Maruani, J., Chaperon, F., Thiébot, M. H., Poncelet, M., Soubrié, P., et al. (1997). Selective Inhibition of Sucrose and Ethanol Intake by SR 141716, an Antagonist of central Cannabinoid (CB1) Receptors. *Psychopharmacology (Berl)* 132 (1), 104–106. doi:10.1007/s002130050326

Aschenbrenner, A. J., Gordon, B. A., Benzinger, T. L. S., Morris, J. C., and Hassenstab, J. J. (2018). Influence of Tau PET, Amyloid PET, and Hippocampal Volume on Cognition in Alzheimer Disease. *Neurology* 91 (9), e859. doi:10.1212/WNL.0000000000006075

Attal, N., Brasseur, L., Guirimand, D., Clermond-Gnamien, S., Atlami, S., and Bouhassira, D. (2004). Are Oral Cannabinoids Safe and Effective in Refractory Neuropathic Pain? *Eur. J. Pain* 8 (2), 173–177. doi:10.1016/S1090-3801(03)00084-3

Babalonis, S., Lofwall, M. R., Sloan, P. A., Nuzzo, P. A., Fanucchi, L. C., and Walsh, S. L. (2019). Cannabinoid Modulation of Opioid Analgesia and Subjective Drug Effects in Healthy Humans. *Psychopharmacology (Berl)* 236 (11), 3341–3352. doi:10.1007/s00213-019-05293-1

Balerio, G. N., Aso, E., Berrendero, F., Murtra, P., and Maldonado, R. (2004). Delta9-tetrahydrocannabinol Decreases Somatic and Motivational Manifestations of Nicotine Withdrawal in Mice. *Eur. J. Neurosci.* 20 (10), 2737–2748. doi:10.1111/j.1460-9568.2004.03714.x

Bardin, L., Tarayre, J. P., Malfetes, N., Koek, W., and Colpaert, F. C. (2003). Profound, Non-opioid Analgesia Produced by the High-Efficacy 5-HT(1A) Agonist F 13640 in the Formalin Model of Tonic Nociceptive Pain. *Pharmacology* 67 (4), 182–194. doi:10.1159/000068404

Bare, D. J., McKinzie, J. H., and McBride, W. J. (1998). Development of Rapid Tolerance to Ethanol-Stimulated Serotonin Release in the Ventral Hippocampus. *Alcohol. Clin. Exp. Res.* 22 (6), 1272–1276. doi:10.1111/j.1530-0277.1998.tb03908.x

Barnes, N. M., and Sharp, T. (1999). A Review of central 5-HT Receptors and Their Function. *Neuropharmacology* 38 (8), 1083–1152. doi:10.1016/S0028-3908(99)00010-6

Basavarajappa, B. S., Shivakumar, M., Joshi, V., and Subbanna, S. (2017). Endocannabinoid System in Neurodegenerative Disorders. *J. Neurochem.* 142 (5), 624–648. doi:10.1111/jnc.14098

Baswan, S. M., Klosner, A. E., Glynn, K., Rajgopal, A., Malik, K., Yim, S., et al. (2020). Therapeutic Potential of Cannabidiol (CBD) for Skin Health and Disorders. *Ccid* 13, 927–942. doi:10.2147/CCID.S286411

Batra, G., Jain, M., Singh, R. S., Sharma, A. R., Singh, A., Prakash, A., et al. (2019). Novel Therapeutic Targets for Amyotrophic Lateral Sclerosis. *Indian J. Pharmacol.* 51 (6), 418–425. doi:10.4103/ijp.IJP_823_19

Bebee, B., Taylor, D. M., Bourke, E., Pollack, K., Foster, L., Ching, M., et al. (2021). The CANBACK Trial: a Randomised, Controlled Clinical Trial of Oral Cannabidiol for People Presenting to the Emergency Department with Acute Low Back Pain. *Med. J. Aust.* 214 (8), 370–375. doi:10.5694/mja2.51014

Beltramo, M., Bernardini, N., Bertorelli, R., Campanella, M., Nicolussi, E., Fredduzzi, S., et al. (2006). CB2 Receptor-Mediated Antihyperalgesia: Possible Direct Involvement of Neural Mechanisms. *Eur. J. Neurosci.* 23 (6), 1530–1538. doi:10.1111/j.1460-9568.2006.04684.x

Benito, C., Núñez, E., Tolón, R. M., Carrier, E. J., Rábano, A., Hillard, C. J., et al. (2003). Cannabinoid CB2 Receptors and Fatty Acid Amide Hydrolase Are Selectively Overexpressed in Neuritic Plaque-Associated Glia in Alzheimer's Disease Brains. *J. Neurosci.* 23 (35), 11136–11141. doi:10.1523/JNEUROSCI.23-35-11136.2003

Benito, C., Romero, J. P., Tolón, R. M., Clemente, D., Docagne, F., Hillard, C. J., et al. (2007). Cannabinoid CB1 and CB2 Receptors and Fatty Acid Amide Hydrolase Are Specific Markers of Plaque Cell Subtypes in Human Multiple Sclerosis. *J. Neurosci.* 27 (9), 2396–2402. doi:10.1523/JNEUROSCI.4814-06.2007

Bergamaschi, M. M., Queiroz, R. H., Chagas, M. H., de Oliveira, D. C., De Martinis, B. S., Kapczinski, F., et al. (2011). Cannabidiol Reduces the Anxiety Induced by Simulated Public Speaking in Treatment-Naïve Social Phobia Patients. *Neuropsychopharmacology* 36 (6), 1219–1226. doi:10.1038/npp.2011.6

Berrendero, F., Sánchez, A., Cabranes, A., Puerta, C., Ramos, J. A., García-Merino, A., et al. (2001). Changes in Cannabinoid CB(1) Receptors in Striatal and Cortical Regions of Rats with Experimental Allergic Encephalomyelitis, an Animal Model of Multiple Sclerosis. *Synapse* 41 (3), 195–202. doi:10.1002/syn.1075

Bhargava, H. N. (1976). Inhibition of Naloxone-Induced Withdrawal in Morphine Dependent Mice by 1-trans-delta9-tetrahydrocannabinol. *Eur. J. Pharmacol.* 36 (1), 259–262. doi:10.1016/0014-2999(76)90283-1

Biegon, A., and Kerman, I. A. (2001). Autoradiographic Study of Pre- and Postnatal Distribution of Cannabinoid Receptors in Human Brain. *NeuroImage* 14 (6), 1463–1468. doi:10.1006/nimg.2001.0939

Bilsland, L. G., Dick, J. R., Pryce, G., Petrosino, S., Di Marzo, V., Baker, D., et al. (2006). Increasing Cannabinoid Levels by Pharmacological and Genetic Manipulation Delay Disease Progression in SOD1 Mice. *FASEB J.* 20 (7), 1003–1005. doi:10.1096/fj.05-4743fje

Björk, L., Fredriksson, A., Hacksell, U., and Lewander, T. (1992). Effects of (R)-8-OH-DPAT and the Enantiomers of UH-301 on Motor Activities in the Rat: Antagonism of (R)-8-OH-DPAT-induced Effects. *Eur. Neuropsychopharmacol.* 2 (2), 141–147. doi:10.1016/0924-977X(92)90024-3

Blauwendraat, C., Nalls, M. A., and Singleton, A. B. (2020). The Genetic Architecture of Parkinson's Disease. *Lancet Neurol.* 19 (2), 170–178. doi:10.1016/S1474-4422(19)30287-X

Blessing, E. M., Steenkamp, M. M., Manzanares, J., and Marmar, C. R. (2015). Cannabidiol as a Potential Treatment for Anxiety Disorders. *Neurotherapeutics* 12 (4), 825–836. doi:10.1007/s13311-015-0387-1

Bloomfield, M. A., Ashok, A. H., Volkow, N. D., and Howes, O. D. (2016). The Effects of Δ9-tetrahydrocannabinol on the Dopamine System. *Nature* 539 (7629), 369–377. doi:10.1038/nature20153

Bonn-Miller, M. O., Sisley, S., Riggs, P., Yazar-Klosinski, B., Wang, J. B., Loflin, M. J. E., et al. (2021). The Short-Term Impact of 3 Smoked Cannabis Preparations versus Placebo on PTSD Symptoms: A Randomized Cross-Over Clinical Trial. *PLoS One* 16 (3), e0246990. doi:10.1371/journal.pone.0246990

Bossong, M. G., Mehta, M. A., van Berckel, B. N., Howes, O. D., Kahn, R. S., and Stokes, P. R. (2015). Further Human Evidence for Striatal Dopamine Release Induced by Administration of Δ9-tetrahydrocannabinol (THC): Selectivity to Limbic Striatum. *Psychopharmacology (Berl)* 232 (15), 2723–2729. doi:10.1007/s00213-015-3915-0

Bouaboula, M., Rinaldi, M., Carayon, P., Carillon, C., Delpech, B., Shire, D., et al. (1993). Cannabinoid-receptor Expression in Human Leukocytes. *Eur. J. Biochem.* 214 (1), 173–180. doi:10.1111/j.1432-1033.1993.tb17910.x

Bowen, D. M., Allen, S. J., Benton, J. S., Goodhardt, M. J., Haan, E. A., Palmer, A. M., et al. (1983). Biochemical Assessment of Serotonergic and Cholinergic Dysfunction and Cerebral Atrophy in Alzheimer's Disease. *J. Neurochem.* 41 (1), 266–272. doi:10.1111/j.1471-4159.1983.tb11838.x

Bruni, N., Della Pepa, C., Oliaro-Bosso, S., Pessione, E., Gastaldi, D., and Dosio, F. (2018). Cannabinoid Delivery Systems for Pain and Inflammation Treatment. *Molecules* 23 (10), 2478. doi:10.3390/molecules23102478

Bryson, N., and Sharma, A. C. (2017). *Nasal Cannabidiol Compositions (World Intellectual Property Organization Patent No. WO2017208072A2)*. Availableat: https://patents.google.com/patent/WO2017208072A2/en.

Calpe-López, C., García-Pardo, M. P., and Aguilar, M. A. (2019). Cannabidiol Treatment Might Promote Resilience to Cocaine and Methamphetamine Use Disorders: A Review of Possible Mechanisms. *Molecules* 24 (14), 2583. doi:10.3390/molecules24142583

Campos, A. C., de Paula Soares, V., Carvalho, M. C., Ferreira, F. R., Vicente, M. A., Brandão, M. L., et al. (2013a). Involvement of Serotonin-Mediated Neurotransmission in the Dorsal Periaqueductal gray Matter on Cannabidiol Chronic Effects in Panic-like Responses in Rats. *Psychopharmacology (Berl)* 226 (1), 13–24. doi:10.1007/s00213-012-2878-7

Campos, A. C., Ferreira, F. R., and Guimarães, F. S. (2012). Cannabidiol Blocks Long-Lasting Behavioral Consequences of Predator Threat Stress: Possible Involvement of 5HT1A Receptors. *J. Psychiatr. Res.* 46 (11), 1501–1510. doi:10.1016/j.jpsychires.2012.08.012

Campos, A. C., and Guimarães, F. S. (2008). Involvement of 5HT1A Receptors in the Anxiolytic-like Effects of Cannabidiol Injected into the Dorsolateral Periaqueductal gray of Rats. *Psychopharmacology (Berl)* 199 (2), 223–230. doi:10.1007/s00213-008-1168-x

Campos, A. C., Ortega, Z., Palazuelos, J., Fogaça, M. V., Aguiar, D. C., Díaz-Alonso, J., et al. (2013b). The Anxiolytic Effect of Cannabidiol on Chronically Stressed Mice Depends on Hippocampal Neurogenesis: Involvement of the Endocannabinoid System. *Int. J. Neuropsychopharmacol.* 16 (6), 1407–1419. doi:10.1017/S1461145712001502

Capano, A., Weaver, R., and Burkman, E. (2020). Evaluation of the Effects of CBD Hemp Extract on Opioid Use and Quality of Life Indicators in Chronic Pain Patients: A Prospective Cohort Study. *Postgrad. Med.* 132 (1), 56–61. doi:10.1080/00325481.2019.1685298

Carayon, P., Marchand, J., Dussossoy, D., Derocq, J. M., Jbilo, O., Bord, A., et al. (1998). Modulation and Functional Involvement of CB2 Peripheral Cannabinoid Receptors during B-Cell Differentiation. *Blood* 92 (10), 3605–3615. doi:10.1182/blood.V92.10.3605

Carroll, C. B., Zeissler, M.-L., Hanemann, C. O., and Zajicek, J. P. (2012). Δ9-tetrahydrocannabinol (Δ9-THC) Exerts a Direct Neuroprotective Effect in a Human Cell Culture Model of Parkinson's Disease. *Neuropathol. Appl. Neurobiol.* 38 (6), 535–547. doi:10.1111/j.1365-2990.2011.01248.x

Casey, S. L., Atwal, N., and Vaughan, C. W. (2017). Cannabis Constituent Synergy in a Mouse Neuropathic Pain Model. *Pain* 158 (12), 2452–2460. doi:10.1097/j.pain.0000000000001051

Chagas, M. H., Zuardi, A. W., Tumas, V., Pena-Pereira, M. A., Sobreira, E. T., Bergamaschi, M. M., et al. (2014). Effects of Cannabidiol in the Treatment of Patients with Parkinson's Disease: an Exploratory Double-Blind Trial. *J. Psychopharmacol.* 28 (11), 1088–1098. doi:10.1177/0269881114550355

Challapalli, P. V., and Stinchcomb, A. L. (2002). *In Vitro* experiment Optimization for Measuring Tetrahydrocannabinol Skin Permeation. *Int. J. Pharm.* 241 (2), 329–339. doi:10.1016/s0378-5173(02)00262-4

Clement, A. B., Hawkins, E. G., Lichtman, A. H., and Cravatt, B. F. (2003). Increased Seizure Susceptibility and Proconvulsant Activity of Anandamide in Mice Lacking Fatty Acid Amide Hydrolase. *J. Neurosci.* 23 (9), 3916–3923. doi:10.1523/jneurosci.23-09-03916.2003

Cochrane-Snyman, K. C., Cruz, C., Morales, J., and Coles, M. (2021). The Effects of Cannabidiol Oil on Noninvasive Measures of Muscle Damage in Men. *Med. Sci. Sports Exerc.* 53 (7), 1460–1472. doi:10.1249/MSS.0000000000002606

Cohen, C., Perrault, G., Griebel, G., and Soubrié, P. (2005). Nicotine-associated Cues Maintain Nicotine-Seeking Behavior in Rats Several Weeks after Nicotine Withdrawal: Reversal by the Cannabinoid (CB1) Receptor Antagonist, Rimonabant (SR141716). *Neuropsychopharmacology* 30 (1), 145–155. doi:10.1038/sj.npp.1300541

Collin, C., Davies, P., Mutiboko, I. K., and Ratcliffe, S.Sativex Spasticity in MS Study Group (2007). Randomized Controlled Trial of Cannabis-Based Medicine in Spasticity Caused by Multiple Sclerosis. *Eur. J. Neurol.* 14 (3), 290–296. doi:10.1111/j.1468-1331.2006.01639.x

Colwill, A. C., Alton, K., Bednarek, P. H., Bayer, L. L., Jensen, J. T., Garg, B., et al. (2020). Cannabinoids for Pain Control During Medical Abortion: A Randomized Controlled Trial. *Obstet Gynecol* 135, 1289–1295.

Consroe, P., Laguna, J., Allender, J., Snider, S., Stern, L., Sandyk, R., et al. (1991). Controlled Clinical Trial of Cannabidiol in Huntington's Disease. *Pharmacol. Biochem. Behav.* 40 (3), 701–708. doi:10.1016/0091-3057(91)90386-g

Constantinescu, C. S., Farooqi, N., O'Brien, K., and Gran, B. (2011). Experimental Autoimmune Encephalomyelitis (EAE) as a Model for Multiple Sclerosis (MS). *Br. J. Pharmacol.* 164 (4), 1079–1106. doi:10.1111/j.1476-5381.2011.01302.x

Cooper, Z. D., Bedi, G., Ramesh, D., Balter, R., Comer, S. D., and Haney, M. (2018). Impact of Co-administration of Oxycodone and Smoked Cannabis on Analgesia and Abuse Liability. *Neuropsychopharmacology* 43 (10), 2046–2055. doi:10.1038/s41386-018-0011-2

Costa, B., Trovato, A. E., Comelli, F., Giagnoni, G., and Colleoni, M. (2007). The Non-psychoactive Cannabis Constituent Cannabidiol Is an Orally Effective Therapeutic Agent in Rat Chronic Inflammatory and Neuropathic Pain. *Eur. J. Pharmacol.* 556 (1–3), 75–83. doi:10.1016/j.ejphar.2006.11.006

Crean, R. D., Crane, N. A., and Mason, B. J. (2011). An Evidence Based Review of Acute and Long-Term Effects of Cannabis Use on Executive Cognitive Functions. *J. Addict. Med.* 5 (1), 1–8. doi:10.1097/ADM.0b013e31820c23fa

Cross, A. J., Crow, T. J., Ferrier, I. N., Johnson, J. A., Bloom, S. R., and Corsellis, J. A. (1984). Serotonin Receptor Changes in Dementia of the Alzheimer Type. *J. Neurochem.* 43 (6), 1574–1581. doi:10.1111/j.1471-4159.1984.tb06081.x

Curtis, A., Mitchell, I., Patel, S., Ives, N., and Rickards, H. (2009). A Pilot Study Using Nabilone for Symptomatic Treatment in Huntington's Disease. *Mov Disord.* 24 (15), 2254–2259. doi:10.1002/mds.22809

Curtis, A., and Rickards, H. (2006). Nabilone Could Treat Chorea and Irritability in Huntington's Disease. *J. Neuropsychiatry Clin. Neurosci.* 18 (4), 553–554. doi:10.1176/jnp.2006.18.4.553

D'Andre, S., McAllister, S., Nagi, J., Giridhar, K. V., Ruiz-Macias, E., and Loprinzi, C. (2021). Topical Cannabinoids for Treating Chemotherapy-Induced Neuropathy: A Case Series. *Integr. Cancer Ther.* 20, 15347354211061739. doi:10.1177/15347354211061739

Davidson, P., Almog, S., and Kindler, S. (2018). *Methods, Devices and Systems for Pulmonary Delivery of Active Agents (United States Patent No. US10118006B2)*. Availableat: https://patents.google.com/patent/US10118006B2/en.

Davie, C. A. (2008). A Review of Parkinson's Disease. *Br. Med. Bull.* 86, 109–127. doi:10.1093/bmb/ldn013

de Faria, S. M., de Morais Fabrício, D., Tumas, V., Castro, P. C., Ponti, M. A., Hallak, J. E., et al. (2020). Effects of Acute Cannabidiol Administration on Anxiety and Tremors Induced by a Simulated Public Speaking Test in Patients with Parkinson's Disease. *J. Psychopharmacol.* 34 (2), 189–196. doi:10.1177/0269881119895536

De Gregorio, D., McLaughlin, R. J., Posa, L., Ochoa-Sanchez, R., Enns, J., Lopez-Canul, M., et al. (2019). Cannabidiol Modulates Serotonergic Transmission and Reverses Both Allodynia and Anxiety-like Behavior in a Model of Neuropathic Pain. *Pain* 160 (1), 136–150. doi:10.1097/j.pain.0000000000001386

De Vries, T. J., Homberg, J. R., Binnekade, R., Raasø, H., and Schoffelmeer, A. N. M. (2003). Cannabinoid Modulation of the Reinforcing and Motivational Properties of Heroin and Heroin-Associated Cues in Rats. *Psychopharmacology (Berl)* 168 (1–2), 164–169. doi:10.1007/s00213-003-1422-1

Dengler, E. C., Liu, J., Kerwin, A., Torres, S., Olcott, C. M., Bowman, B. N., et al. (2013). Mesoporous Silica-Supported Lipid Bilayers (Protocells) for DNA Cargo Delivery to the Spinal Cord. *J. Control. Release* 168 (2), 209–224. doi:10.1016/j.jconrel.2013.03.009

Denovan-Wright, E. M., and Robertson, H. A. (2000). Cannabinoid Receptor Messenger RNA Levels Decrease in a Subset of Neurons of the Lateral Striatum, Cortex and hippocampus of Transgenic Huntington's Disease Mice. *Neuroscience* 98 (4), 705–713. doi:10.1016/S0306-4522(00)00157-3

Dentel, C., Palamiuc, L., Henriques, A., Lannes, B., Spreux-Varoquaux, O., Gutknecht, L., et al. (2013). Degeneration of Serotonergic Neurons in Amyotrophic Lateral Sclerosis: A Link to Spasticity. *Brain* 136 (Pt 2), 483–493. doi:10.1093/brain/aws274

Devinsky, O., Marsh, E., Friedman, D., Thiele, E., Laux, L., Sullivan, J., et al. (2016). Cannabidiol in Patients with Treatment-Resistant Epilepsy: An Open-Label Interventional Trial. *Lancet Neurol.* 15 (3), 270–278. doi:10.1016/S1474-4422(15)00379-8

Devinsky, O., Nabbout, R., Miller, I., Laux, L., Zolnowska, M., Wright, S., et al. (2019). Long-term Cannabidiol Treatment in Patients with Dravet Syndrome: An Open-Label Extension Trial. *Epilepsia* 60 (2), 294–302. doi:10.1111/epi.14628

Devinsky, O., Patel, A. D., Thiele, E. A., Wong, M. H., Appleton, R., Harden, C. L., et al.GWPCARE1 Part A Study Group (2018). Randomized, Dose-Ranging Safety Trial of Cannabidiol in Dravet Syndrome. *Neurology* 90 (14), e1204–e1211. doi:10.1212/WNL.0000000000005254

Doder, M., Rabiner, E. A., Turjanski, N., Lees, A. J., and Brooks, D. J. (2003). Tremor in Parkinson's Disease and Serotonergic Dysfunction: an 11C-WAY 100635 PET Study. *Neurology* 60 (4), 601–605. doi:10.1212/01.WNL.0000031424.51127.2B

Donvito, G., Nass, S. R., Wilkerson, J. L., Curry, Z. A., Schurman, L. D., Kinsey, S. G., et al. (2018). The Endogenous Cannabinoid System: A Budding Source of Targets for Treating Inflammatory and Neuropathic Pain. *Neuropsychopharmacology* 43 (1), 52–79. doi:10.1038/npp.2017.204

Dorsey, E. R., Sherer, T., Okun, M. S., and Bloem, B. R. (2018). The Emerging Evidence of the Parkinson Pandemic. *J. Parkinsons Dis.* 8 (Suppl. 1), S3. doi:10.3233/JPD-181474

Dowie, M. J., Bradshaw, H. B., Howard, M. L., Nicholson, L. F., Faull, R. L., Hannan, A. J., et al. (2009). Altered CB1 Receptor and Endocannabinoid Levels Precede Motor Symptom Onset in a Transgenic Mouse Model of Huntington's Disease. *Neuroscience* 163 (1), 456–465. doi:10.1016/j.neuroscience.2009.06.014

Dunn, K. E., Bergeria, C. L., Huhn, A. S., Speed, T. J., Mun, C. J., Vandrey, R., et al. (2021). Within-subject, Double-Blinded, Randomized, and Placebo-Controlled Evaluation of the Combined Effects of the Cannabinoid Dronabinol and the Opioid Hydromorphone in a Human Laboratory Pain Model. *Neuropsychopharmacology* 46 (8), 1451–1459. doi:10.1038/s41386-021-01007-4

Eisenberg, E., Ogintz, M., and Almog, S. (2014). The Pharmacokinetics, Efficacy, Safety, and Ease of Use of a Novel Portable Metered-Dose Cannabis Inhaler in Patients with Chronic Neuropathic Pain: A Phase 1a Study. *J. Pain Palliat. Care Pharmacother.* 28 (3), 216–225. doi:10.3109/15360288.2014.941130

el Mestikawy, S., Fargin, A., Raymond, J. R., Gozlan, H., and Hnatowich, M. (1991). The 5-HT1A Receptor: An Overview of Recent Advances. *Neurochem. Res.* 16 (1), 1–10. doi:10.1007/BF00965820

Ellgren, M., Spano, S. M., and Hurd, Y. L. (2007). Adolescent Cannabis Exposure Alters Opiate Intake and Opioid Limbic Neuronal Populations in Adult Rats. *Neuropsychopharmacology* 32 (3), 607–615. doi:10.1038/sj.npp.1301127

Elms, L., Shannon, S., Hughes, S., and Lewis, N. (2019). Cannabidiol in the Treatment of Post-Traumatic Stress Disorder: A Case Series. *J. Altern. Complement. Med.* 25 (4), 392–397. doi:10.1089/acm.2018.0437

Erkkinen, M. G., Kim, M. O., and Geschwind, M. D. (2018). Clinical Neurology and Epidemiology of the Major Neurodegenerative Diseases. *Cold Spring Harb Perspect. Biol.* 10 (4). doi:10.1101/cshperspect.a033118

Eskow, K. L., Gupta, V., Alam, S., Park, J. Y., and Bishop, C. (2007). The Partial 5-HT(1A) Agonist Buspirone Reduces the Expression and Development of L-DOPA-Induced Dyskinesia in Rats and Improves L-DOPA Efficacy. *Pharmacol. Biochem. Behav.* 87 (3), 306–314. doi:10.1016/j.pbb.2007.05.002

Fadda, P., Scherma, M., Fresu, A., Collu, M., and Fratta, W. (2005). Dopamine and Serotonin Release in Dorsal Striatum and Nucleus Accumbens Is Differentially Modulated by Morphine in DBA/2J and C57BL/6J Mice. *Synapse* 56 (1), 29–38. doi:10.1002/syn.20122

Falco-Walter, J. (2020). Epilepsy-Definition, Classification, Pathophysiology, and Epidemiology. *Semin. Neurol.* 40 (6), 617–623. doi:10.1055/s-0040-1718719

Fallon, M. T., Albert Lux, E., McQuade, R., Rossetti, S., Sanchez, R., Sun, W., et al. (2017). Sativex Oromucosal spray as Adjunctive Therapy in Advanced Cancer Patients with Chronic Pain Unalleviated by Optimized Opioid Therapy: Two Double-Blind, Randomized, Placebo-Controlled Phase 3 Studies. *Br. J. Pain* 11 (3), 119–133. doi:10.1177/2049463717710042

FemenÃa, T., GarcÃa-GutiÃ©rrez, M. a. S., and Manzanares, J. (2010). CB1 Receptor Blockade Decreases Ethanol Intake and Associated Neurochemical Changes in Fawn-Hooded Rats. *Alcohol. Clin. Exp. Res.* 34 (1), 131–141. doi:10.1111/j.1530-0277.2009.01074.x

Fernández-Ruiz, J., Romero, J., Velasco, G., Tolón, R. M., Ramos, J. A., and Guzmán, M. (2007). Cannabinoid CB2 Receptor: A New Target for Controlling Neural Cell Survival? *Trends Pharmacol. Sci.* 28 (1), 39–45. doi:10.1016/j.tips.2006.11.001

Franke, T., Irwin, C., Beindorff, N., Bouter, Y., and Bouter, C. (2019). Effects of Tetrahydrocannabinol Treatment on Brain Metabolism and Neuron Loss in a Mouse Model of Sporadic Alzheimer's Disease. *Nuklearmedizin - NuclearMedicine* 58 (2), P94. doi:10.1055/s-0039-1683689

Galaj, E., Bi, G. H., Yang, H. J., and Xi, Z. X. (2020). Cannabidiol Attenuates the Rewarding Effects of Cocaine in Rats by CB2, 5-HT1A and TRPV1 Receptor Mechanisms. *Neuropharmacology* 167, 107740. doi:10.1016/j.neuropharm.2019.107740

Galiègue, S., Mary, S., Marchand, J., Dussossoy, D., Carrière, D., Carayon, P., et al. (1995). Expression of Central and Peripheral Cannabinoid Receptors in Human Immune Tissues and Leukocyte Subpopulations. *Eur. J. Biochem.* 232 (1), 54–61. doi:10.1111/j.1432-1033.1995.tb20780.x

Gallate, J. E., Mallet, P. E., and McGregor, I. S. (2004). Combined Low Dose Treatment with Opioid and Cannabinoid Receptor Antagonists Synergistically Reduces the Motivation to Consume Alcohol in Rats. *Psychopharmacology (Berl)* 173 (1), 210–216. doi:10.1007/s00213-003-1694-5

Garakani, A., Murrough, J. W., Freire, R. C., Thom, R. P., Larkin, K., Buono, F. D., et al. (2020). Pharmacotherapy of Anxiety Disorders: Current and Emerging Treatment Options. *Front. Psychiatry* 11, 595584. doi:10.3389/fpsyt.2020.595584

García-Arencibia, M., García, C., Kurz, A., Rodríguez-Navarro, J. A., Gispert-Sánchez, S., Mena, M. A., et al. (2009). "Cannabinoid CB1 Receptors Are Early DownRegulated Followed by a Further UpRegulation in the Basal Ganglia of Mice with Deletion of Specific Park Genes," in *Birth, Life and Death of Dopaminergic Neurons in the Substantia Nigra*. Editors G. Giovanni, V. Di Matteo, and E. Esposito (Springer), 269–275. doi:10.1007/978-3-211-92660-4_22

García-Gutiérrez, M. S., Navarrete, F., Gasparyan, A., Austrich-Olivares, A., Sala, F., and Manzanares, J. (2020). Cannabidiol: A Potential New Alternative for the Treatment of Anxiety, Depression, and Psychotic Disorders. *Biomolecules* 10 (11), 1575. doi:10.3390/biom10111575

García-Gutiérrez, M. S., and Manzanares, J. (2011). Overexpression of CB2 Cannabinoid Receptors Decreased Vulnerability to Anxiety and Impaired Anxiolytic Action of Alprazolam in Mice. *J. Psychopharmacol.* 25 (1), 111–120. doi:10.1177/0269881110379507

Gaston, T. E., Szaflarski, M., Hansen, B., Bebin, E. M., and Szaflarski, J. P.UAB CBD Program (2019). Quality of Life in Adults Enrolled in an Open-Label Study of Cannabidiol (CBD) for Treatment-Resistant Epilepsy. *Epilepsy Behav.* 95, 10–17. doi:10.1016/j.yebeh.2019.03.035

GBD 2016 Epilepsy Collaborators (2019). Global, Regional, and National burden of Epilepsy, 1990-2016: a Systematic Analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 18 (4), 357–375. doi:10.1016/S1474-4422(18)30454-X

Giacobbe, P., and Flint, A. (2018). Diagnosis and Management of Anxiety Disorders. *Continuum (Minneap Minn)* 24 (3), 893–919. doi:10.1212/CON.0000000000000607

Giacoppo, S., Pollastro, F., Grassi, G., Bramanti, P., and Mazzon, E. (2017). Target Regulation of PI3K/Akt/mTOR Pathway by Cannabidiol in Treatment of Experimental Multiple Sclerosis. *Fitoterapia* 116, 77–84. doi:10.1016/j.fitote.2016.11.010

Gieringer, D., St. Laurent, J., and Goodrich, S. (2004). Cannabis Vaporizer Combines Efficient Delivery of THC with Effective Suppression of Pyrolytic Compounds. *J. Cannabis Ther.* 4 (1), 7–27. doi:10.1300/J175v04n01_02

Gjerstad, J., Tjolsen, A., and Hole, K. (1996). The Effect of 5-HT1A Receptor Stimulation on Nociceptive Dorsal Horn Neurones in Rats. *Eur. J. Pharmacol.* 318 (2–3), 315–321. doi:10.1016/s0014-2999(96)00819-9

Glass, M., Dragunow, M., and Faull, R. L. (1997). Cannabinoid Receptors in the Human Brain: A Detailed Anatomical and Quantitative Autoradiographic Study in the Fetal, Neonatal and Adult Human Brain. *Neuroscience* 77 (2), 299–318. doi:10.1016/S0306-4522(96)00428-9

Glass, M., Faull, R. L., and Dragunow, M. (1993). Loss of Cannabinoid Receptors in the Substantia Nigra in Huntington's Disease. *Neuroscience* 56 (3), 523–527. doi:10.1016/0306-4522(93)90352-g

Goldenberg, M. M. (2012). Multiple Sclerosis Review. *P T* 37 (3), 175–184.

Gomes, F. V., Resstel, L. B., and Guimarães, F. S. (2011). The Anxiolytic-like Effects of Cannabidiol Injected into the Bed Nucleus of the Stria Terminalis Are Mediated by 5-HT1A Receptors. *Psychopharmacology (Berl)* 213 (2–3), 465–473. doi:10.1007/s00213-010-2036-z

Graeff, F. G., Netto, C. F., and Zangrossi, H., Jr (1998). The Elevated T-Maze as an Experimental Model of Anxiety. *Neurosci. Biobehav Rev.* 23 (2), 237–246. doi:10.1016/S0149-7634(98)00024-4

Granados-Soto, V., Argüelles, C. F., Rocha-González, H. I., Godínez-Chaparro, B., Flores-Murrieta, F. J., and Villalón, C. M. (2010). The Role of Peripheral 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E and 5-HT1F Serotonergic Receptors in the Reduction of Nociception in Rats. *Neuroscience* 165 (2), 561–568. doi:10.1016/j.neuroscience.2009.10.020

Greer, G. R., Grob, C. S., and Halberstadt, A. L. (2014). PTSD Symptom Reports of Patients Evaluated for the New Mexico Medical Cannabis Program. *J. Psychoactive Drugs* 46 (1), 73–77. doi:10.1080/02791072.2013.873843

Grim, T. W., Morales, A. J., Gonek, M. M., Wiley, J. L., Thomas, B. F., Endres, G. W., et al. (2016). Stratification of Cannabinoid 1 Receptor (CB1R) Agonist Efficacy: Manipulation of CB1R Density through Use of Transgenic Mice Reveals Congruence between *In Vivo* and *In Vitro* Assays. *J. Pharmacol. Exp. Ther.* 359 (2), 329–339. doi:10.1124/jpet.116.233163

Grotenhermen, F. (2003). Pharmacokinetics and Pharmacodynamics of Cannabinoids. *Clin. Pharmacokinet.* 42 (4), 327–360. doi:10.2165/00003088-200342040-00003

Guimarães, F. S., Chiaretti, T. M., Graeff, F. G., and Zuardi, A. W. (1990). Antianxiety Effect of Cannabidiol in the Elevated Plus-Maze. *Psychopharmacology (Berl)* 100 (4), 558–559. doi:10.1007/BF02244012

Haleem, D. J., and Nawaz, S. (2017). Inhibition of Reinforcing, Hyperalgesic, and Motor Effects of Morphine by Buspirone in Rats. *J. Pain* 18 (1), 19–28. doi:10.1016/j.jpain.2016.10.001

Hammell, D. C., Zhang, L. P., Ma, F., Abshire, S. M., McIlwrath, S. L., Stinchcomb, A. L., et al. (2016). Transdermal Cannabidiol Reduces Inflammation and Pain-Related Behaviours in a Rat Model of Arthritis. *Eur. J. Pain* 20 (6), 936–948. doi:10.1002/ejp.818

Haroutounian, S., Ratz, Y., Ginosar, Y., Furmanov, K., Saifi, F., Meidan, R., et al. (2016). The Effect of Medicinal Cannabis on Pain and Quality-Of-Life Outcomes in Chronic Pain: A Prospective Open-Label Study. *Clin. J. Pain* 32 (12), 1036–1043. doi:10.1097/AJP.0000000000000364

Harris, H. M., Sufka, K. J., Gul, W., and ElSohly, M. A. (2016). Effects of Delta-9-Tetrahydrocannabinol and Cannabidiol on Cisplatin-Induced Neuropathy in Mice. *Planta Med.* 82 (13), 1169–1172. doi:10.1055/s-0042-106303

He, Y., Suofu, Y., Yablonska, S., Wang, X., Larkin, T. M., Kim, J., et al. (2019). Increased Serotonin Transporter Expression in Huntington's Disease Patients Is Not Consistently Replicated in Murine Models. *J. Huntingtons Dis.* 8 (4), 449–457. doi:10.3233/JHD-180318

Hesse, S., Moeller, F., Petroff, D., Lobsien, D., Luthardt, J., Regenthal, R., et al. (2014). Altered Serotonin Transporter Availability in Patients with Multiple Sclerosis. *Eur. J. Nucl. Med. Mol. Imaging* 41 (5), 827–835. doi:10.1007/s00259-013-2636-z

Hickey, M. A., and Chesselet, M. F. (2003). Apoptosis in Huntington's Disease. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 27 (2), 255–265. doi:10.1016/S0278-5846(03)00021-6

Hill, M. N., Campolongo, P., Yehuda, R., and Patel, S. (2017). Integrating Endocannabinoid Signaling and Cannabinoids into the Biology and Treatment of Posttraumatic Stress Disorder. *Neuropsychopharmacol.* 43, 80–102.

Hine, B., Friedman, E., Torrelio, M., and Gershon, S. (1975). Morphine-Dependent Rats: Blockade of Precipitated Abstinence by Tetrahydrocannabinol. *Science* 187, 443–445. doi:10.1126/science.1167428

Hirshfeld, D. R., Rosenbaum, J. F., Biederman, J., Bolduc, E. A., Faraone, S. V., Snidman, N., et al. (1992). Stable Behavioral Inhibition and its Association with Anxiety Disorder. *J. Am. Acad. Child. Adolesc. Psychiatry* 31 (1), 103–111. doi:10.1097/00004583-199201000-00016

Hohmann, A. G., Briley, E. M., and Herkenham, M. (1999). Pre- and Postsynaptic Distribution of Cannabinoid and Mu Opioid Receptors in Rat Spinal Cord. *Brain Res.* 822 (1), 17–25. doi:10.1016/S0006-8993(98)01321-3

Hohmann, A. G., and Herkenham, M. (1999). Cannabinoid Receptors Undergo Axonal Flow in Sensory Nerves. *Neuroscience* 92 (4), 1171–1175. doi:10.1016/S0306-4522(99)00220-1

Hsiao, Y. T., Yi, P. L., Li, C. L., and Chang, F. C. (2012). Effect of Cannabidiol on Sleep Disruption Induced by the Repeated Combination Tests Consisting of Open Field and Elevated Plus-Maze in Rats. *Neuropharmacology* 62 (1), 373–384. doi:10.1016/j.neuropharm.2011.08.013

Huang, S. M., Bisogno, T., Petros, T. J., Chang, S. Y., Zavitsanos, P. A., Zipkin, R. E., et al. (2001). Identification of a New Class of Molecules, the Arachidonyl Amino Acids, and Characterization of One Member that Inhibits Pain. *J. Biol. Chem.* 276 (46), 42639–42644. doi:10.1074/jbc.M107351200

Huestis, M. A. (2005). "Pharmacokinetics and Metabolism of the Plant Cannabinoids, Δ9-Tetrahydrocannibinol, Cannabidiol and Cannabinol," in *Cannabinoids*. Editor R. G. Pertwee (Springer), 657–690. doi:10.1007/3-540-26573-2_23

Hunault, C. C., van Eijkeren, J. C., Mensinga, T. T., de Vries, I., Leenders, M. E., and Meulenbelt, J. (2010). Disposition of Smoked Cannabis with High Δ(9)-tetrahydrocannabinol Content: a Kinetic Model. *Toxicol. Appl. Pharmacol.* 246 (3), 148–153. doi:10.1016/j.taap.2010.04.019

Hurley, M. J., Mash, D. C., and Jenner, P. (2003). Expression of Cannabinoid CB1 Receptor mRNA in Basal Ganglia of normal and Parkinsonian Human Brain. *J. Neural Transm. (Vienna)* 110 (11), 1279–1288. doi:10.1007/s00702-003-0033-7

Iqbal, K., Liu, F., Gong, C. X., and Grundke-Iqbal, I. (2010). Tau in Alzheimer Disease and Related Tauopathies. *Curr. Alzheimer Res.* 7 (8), 656–664. doi:10.2174/156720510793611592

Isooka, N., Miyazaki, I., Kikuoka, R., Wada, K., Nakayama, E., Shin, K., et al. (2020). Dopaminergic Neuroprotective Effects of Rotigotine via 5-HT1A Receptors: Possibly Involvement of Metallothionein Expression in Astrocytes. *Neurochem. Int.* 132, 104608. doi:10.1016/j.neuint.2019.104608

Jennings, E. A., Vaughan, C. W., and Christie, M. J. (2001). Cannabinoid Actions on Rat Superficial Medullary Dorsal Horn Neurons *In Vitro*. *J. Physiol.* 534 (3), 805–812. doi:10.1111/j.1469-7793.2001.00805.x

Jing, E., and Straw-Wilson, K. (2016). Sexual Dysfunction in Selective Serotonin Reuptake Inhibitors (SSRIs) and Potential Solutions: A Narrative Literature Review. *Ment. Health Clin.* 6 (4), 191–196. doi:10.9740/mhc.2016.07.191

Johnson, J. R., Burnell-Nugent, M., Lossignol, D., Ganae-Motan, E. D., Potts, R., and Fallon, M. T. (2010). Multicenter, Double-Blind, Randomized, Placebo-Controlled, Parallel-Group Study of the Efficacy, Safety, and Tolerability of THC:CBD Extract and THC Extract in Patients with Intractable Cancer-Related Pain. *J. Pain Symptom Manage.* 39 (2), 167–179. doi:10.1016/j.jpainsymman.2009.06.008

Jones, N. A., Hill, A. J., Smith, I., Bevan, S. A., Williams, C. M., Whalley, B. J., et al. (2010). Cannabidiol Displays Antiepileptiform and Antiseizure Properties *In Vitro* and *In Vivo*. *J. Pharmacol. Exp. Ther.* 332 (2), 569–577. doi:10.1124/jpet.109.159145

Katona, I., Rancz, E. A., Acsady, L., Ledent, C., Mackie, K., Hajos, N., et al. (2001). Distribution of CB1 Cannabinoid Receptors in the Amygdala and Their Role in the Control of GABAergic Transmission. *J. Neurosci.* 21 (23), 9506–9518. doi:10.1523/JNEUROSCI.21-23-09506.2001

Katsidoni, V., Anagnostou, I., and Panagis, G. (2013). Cannabidiol Inhibits the Reward-Facilitating Effect of Morphine: Involvement of 5-HT1A Receptors in the Dorsal Raphe Nucleus. *Addict. Biol.* 18 (2), 286–296. doi:10.1111/j.1369-1600.2012.00483.x

Katz, G., Lobel, T., Tetelbaum, A., and Raskin, S. (2014). Cannabis Withdrawal - A New Diagnostic Category in DSM-5. *Isr. J. Psychiatry Relat. Sci.* 51 (4), 270–275.

Kavia, R. B., De Ridder, D., Constantinescu, C. S., Stott, C. G., and Fowler, C. J. (2010). Randomized Controlled Trial of Sativex to Treat Detrusor Overactivity in Multiple Sclerosis. *Mult. Scler.* 16 (11), 1349–1359. doi:10.1177/1352458510378020

Kayser, R. R., Haney, M., Raskin, M., Arout, C., and Simpson, H. B. (2020a). Acute Effects of Cannabinoids on Symptoms of Obsessive-Compulsive Disorder: A Human Laboratory Study. *Depress. Anxiety* 37 (8), 801–811. doi:10.1002/da.23032

Kayser, R. R., Raskin, M., Snorrason, I., Hezel, D. M., Haney, M., and Simpson, H. B. (2020b). Cannabinoid Augmentation of Exposure-Based Psychotherapy for Obsessive-Compulsive Disorder. *J. Clin. Psychopharmacol.* 40 (2), 207–210. doi:10.1097/JCP.0000000000001179

Kikuoka, R., Miyazaki, I., Kubota, N., Maeda, M., Kagawa, D., Moriyama, M., et al. (2020). Mirtazapine Exerts Astrocyte-Mediated Dopaminergic Neuroprotection. *Sci. Rep.* 10 (1), 20698. doi:10.1038/s41598-020-77652-4

King, K. M., Myers, A. M., Soroka-Monzo, A. J., Tuma, R. F., Tallarida, R. J., Walker, E. A., et al. (2017). Single and Combined Effects of Δ9 -tetrahydrocannabinol and Cannabidiol in a Mouse Model of Chemotherapy-Induced Neuropathic Pain. *Br. J. Pharmacol.* 174 (17), 2832–2841. doi:10.1111/bph.13887

Kish, S. J., Tong, J., Hornykiewicz, O., Rajput, A., Chang, L. J., Guttman, M., et al. (2008). Preferential Loss of Serotonin Markers in Caudate versus Putamen in Parkinson's Disease. *Brain* 131 (Pt 1), 120–131. doi:10.1093/brain/awm239

Klein, C., and Westenberger, A. (2012). Genetics of Parkinson's Disease. *Cold Spring Harb Perspect. Med.* 2 (1), a008888. doi:10.1101/cshperspect.a008888

Knaub, K., Sartorius, T., Dharsono, T., Wacker, R., Wilhelm, M., and Schön, C. (2019). A Novel Self-Emulsifying Drug Delivery System (SEDDS) Based on VESIsorb® Formulation Technology Improving the Oral Bioavailability of Cannabidiol in Healthy Subjects. *Molecules* 24 (16), 2967. doi:10.3390/molecules24162967

Kozela, E., Lev, N., Kaushansky, N., Eilam, R., Rimmerman, N., Levy, R., et al. (2011). Cannabidiol Inhibits Pathogenic T Cells, Decreases Spinal Microglial Activation and Ameliorates Multiple Sclerosis-like Disease in C57BL/6 Mice. *Br. J. Pharmacol.* 163 (7), 1507–1519. doi:10.1111/j.1476-5381.2011.01379.x

Kraus, J., Börner, C., and Höllt, V. (2009). 22. Cannabinoid and Opioid-Mediated Increase in Cyclic AMP Inhibits T Cell Receptor-Triggered Signaling. *Brain Behav. Immun.* 23 (23), S14. doi:10.1016/j.bbi.2009.05.025

Kumari, A., Yadav, S. K., and Yadav, S. C. (2010). Biodegradable Polymeric Nanoparticles Based Drug Delivery Systems. *Colloids Surf. B Biointerfaces* 75 (1), 1–18. doi:10.1016/j.colsurfb.2009.09.001

Lafenêtre, P., Chaouloff, F., and Marsicano, G. (2007). The Endocannabinoid System in the Processing of Anxiety and Fear and How CB1 Receptors May Modulate Fear Extinction. *Pharmacol. Res.* 56 (5), 367–381. doi:10.1016/j.phrs.2007.09.006

Lai, M. K., Tsang, S. W., Francis, P. T., Esiri, M. M., Keene, J., Hope, T., et al. (2003). Reduced Serotonin 5-HT1A Receptor Binding in the Temporal Cortex Correlates with Aggressive Behavior in Alzheimer Disease. *Brain Res.* 974 (1), 82–87. doi:10.1016/S0006-8993(03)02554-X

Lanz, C., Mattsson, J., Soydaner, U., and Brenneisen, R. (2016). Medicinal Cannabis: *In Vitro* Validation of Vaporizers for the Smoke-free Inhalation of Cannabis. *PLOS ONE* 11 (1), e0147286. doi:10.1371/journal.pone.0147286

Laporte, A. M., Fattaccini, C. M., Lombard, M. C., Chauveau, J., and Hamon, M. (1995). Effects of Dorsal Rhizotomy and Selective Lesion of Serotonergic and Noradrenergic Systems on 5-HT1A, 5-HT1B, and 5-HT3 Receptors in the Rat Spinal Cord. *J. Neural Transm. Gen. Sectgeneral Section* 100 (3), 207–223. doi:10.1007/BF01276459

Lastres-Becker, I., Bizat, N., Boyer, F., Hantraye, P., Fernández-Ruiz, J., and Brouillet, E. (2004). Potential Involvement of Cannabinoid Receptors in 3-nitropropionic Acid Toxicity *In Vivo*. *NeuroReport* 15 (15), 2375–2379. doi:10.1097/00001756-200410250-00015

Lastres-Becker, I., Fezza, F., Cebeira, M., Bisogno, T., Ramos, J. A., Milone, A., et al. (2001). Changes in Endocannabinoid Transmission in the Basal Ganglia in a Rat Model of Huntington's Disease. *Neuroreport* 12 (10), 2125–2129. doi:10.1097/00001756-200107200-00017

Lastres-Becker, I., Hansen, H. H., Berrendero, F., De Miguel, R., Pérez-Rosado, A., Manzanares, J., et al. (2002). Alleviation of Motor Hyperactivity and Neurochemical Deficits by Endocannabinoid Uptake Inhibition in a Rat Model of Huntington's Disease. *Synapse* 44 (1), 23–35. doi:10.1002/syn.10054

Lastres-Becker, I., Molina-Holgado, F., Ramos, J. A., Mechoulam, R., and Fernández-Ruiz, J. (2005). Cannabinoids Provide Neuroprotection against 6-hydroxydopamine Toxicity *In Vivo* and *In Vitro*: Relevance to Parkinson's Disease. *Neurobiol. Dis.* 19 (1), 96–107. doi:10.1016/j.nbd.2004.11.009

Laux, L. C., Bebin, E. M., Checketts, D., Chez, M., Flamini, R., Marsh, E. D., et al. (2019). Long-term Safety and Efficacy of Cannabidiol in Children and Adults with Treatment Resistant Lennox-Gastaut Syndrome or Dravet Syndrome: Expanded Access Program Results. *Epilepsy Res.* 154, 13–20. doi:10.1016/j.eplepsyres.2019.03.015

Ledent, C., Valverde, O., Cossu, G., Petitet, F., Aubert, J. F., Beslot, F., et al. (1999). Unresponsiveness to Cannabinoids and Reduced Addictive Effects of Opiates in CB1 Receptor Knockout Mice. *Science* 283 (5400), 401–404. doi:10.1126/science.283.5400.401

Ledesma, J. C., Manzanedo, C., and Aguilar, M. A. (2021). Cannabidiol Prevents Several of the Behavioral Alterations Related to Cocaine Addiction in Mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 111, 110390. doi:10.1016/j.pnpbp.2021.110390

Lee, J. H., Agacinski, G., Williams, J. H., Wilcock, G. K., Esiri, M. M., Francis, P. T., et al. (2010). Intact Cannabinoid CB1 Receptors in the Alzheimer's Disease Cortex. *Neurochem. Int.* 57 (8), 985–989. doi:10.1016/j.neuint.2010.10.010

Leehey, M. A., Liu, Y., Hart, F., Epstein, C., Cook, M., Sillau, S., et al. (2020). Safety and Tolerability of Cannabidiol in Parkinson Disease: An Open Label, Dose-Escalation Study. *Cannabis Cannabinoid Res.* 5 (4), 326–336. doi:10.1089/can.2019.0068

Lesch, K. P. (2005). Serotonergic Gene Inactivation in Mice: Models for Anxiety and Aggression? *Novartis Found. Symp.* 268, 111–170.

Levin, D. N., Dulberg, Z., Chan, A. W., Hare, G. M., Mazer, C. D., and Hong, A. (2017). A Randomized-Controlled Trial of Nabilone for the Prevention of Acute Postoperative Nausea and Vomiting in Elective Surgery. *Can J Anaesth.* 64, 385–395.

Lichtman, A. H., Lux, E. A., McQuade, R., Rossetti, S., Sanchez, R., Sun, W., et al. (2018). Results of a Double-Blind, Randomized, Placebo-Controlled Study of Nabiximols Oromucosal Spray as an Adjunctive Therapy in Advanced Cancer Patients with Chronic Uncontrolled Pain. *J. Pain Symptom Manage.* 55 (2), 179–e1. e1. doi:10.1016/j.jpainsymman.2017.09.001

Linher-Melville, K., Zhu, Y. F., Sidhu, J., Parzei, N., Shahid, A., Seesankar, G., et al. (2020). Evaluation of the Preclinical Analgesic Efficacy of Naturally Derived, Orally Administered Oil Forms of Δ9-tetrahydrocannabinol (THC), Cannabidiol (CBD), and Their 1:1 Combination. *PLoS One* 15 (6), e0234176. doi:10.1371/journal.pone.0234176

Lintzeris, N., Bhardwaj, A., Mills, L., Dunlop, A., Copeland, J., McGregor, I., et al. (2019). Nabiximols for the Treatment of Cannabis Dependence: A Randomized Clinical Trial. *JAMA Intern Med.* 179, 1242–1253. doi:10.1001/jamainternmed.2019.1993

Little, P. J., Compton, D. R., Johnson, M. R., Melvin, L. S., and Martin, B. R. (1988). Pharmacology and Stereoselectivity of Structurally Novel Cannabinoids in Mice. *J. Pharmacol. Exp. Ther.* 247 (3), 1046–1051.

Liu, J., and Wang, F. (2017). Role of Neuroinflammation in Amyotrophic Lateral Sclerosis: Cellular Mechanisms and Therapeutic Implications. *Front. Immunol.* 8, 1005. doi:10.3389/fimmu.2017.01005

Liu, Q. R., Canseco-Alba, A., Zhang, H. Y., Tagliaferro, P., Chung, M., Dennis, E., et al. (2017). Cannabinoid Type 2 Receptors in Dopamine Neurons Inhibits Psychomotor Behaviors, Alters Anxiety, Depression and Alcohol Preference. *Sci. Rep.* 7 (1), 17410. doi:10.1038/s41598-017-17796-y

Lodzki, M., Godin, B., Rakou, L., Mechoulam, R., Gallily, R., and Touitou, E. (2003). Cannabidiol-transdermal Delivery and Anti-inflammatory Effect in a Murine Model. *J. Control. Release* 93 (3), 377–387. doi:10.1016/j.jconrel.2003.09.001

Long, L. E., Chesworth, R., Huang, X. F., McGregor, I. S., Arnold, J. C., and Karl, T. (2010). A Behavioural Comparison of Acute and Chronic Delta9-tetrahydrocannabinol and Cannabidiol in C57BL/6JArc Mice. *Int. J. Neuropsychopharmacol.* 13 (7), 861–876. doi:10.1017/S1461145709990605

Longinetti, E., and Fang, F. (2019). Epidemiology of Amyotrophic Lateral Sclerosis: An Update of Recent Literature. *Curr. Opin. Neurol.* 32 (5), 771–776. doi:10.1097/WCO.0000000000000730

López-Sendón Moreno, J. L., García Caldentey, J., Trigo Cubillo, P., Ruiz Romero, C., García Ribas, G., Alonso Arias, M. A. A., et al. (2016). A Double-Blind, Randomized, Cross-Over, Placebo-Controlled, Pilot Trial with Sativex in Huntington's Disease. *J. Neurol.* 263 (7), 1390–1400. doi:10.1007/s00415-016-8145-9

Lucas, C. J., Galettis, P., and Schneider, J. (2018). The Pharmacokinetics and the Pharmacodynamics of Cannabinoids. *Br. J. Clin. Pharmacol.* 84 (11), 2477–2482. doi:10.1111/bcp.13710

MacGregor, K. J., Embleton, J. K., Lacy, J. E., Perry, E. A., Solomon, L. J., Seager, H., et al. (1997). Influence of Lipolysis on Drug Absorption from the Gastro-Intestinal Tract. *Adv. Drug Deliv. Rev.* 25 (1), 33–46. doi:10.1016/S0169-409X(96)00489-9

Maggio, N., Shavit Stein, E., and Segal, M. (2018). Cannabidiol Regulates Long Term Potentiation Following Status Epilepticus: Mediation by Calcium Stores and Serotonin. *Front. Mol. Neurosci.* 11, 32. doi:10.3389/fnmol.2018.00032

Mailleux, P., and Vanderhaeghen, J. J. (1992). Distribution of Neuronal Cannabinoid Receptor in the Adult Rat Brain: A Comparative Receptor Binding Radioautography and *In Situ* Hybridization Histochemistry. *Neuroscience* 48 (3), 655–668. doi:10.1016/0306-4522(92)90409-U

Malik, Z., Bayman, L., Valestin, J., Rizvi-Toner, A., Hashmi, S., and Schey, R. (2017). Dronabinol Increases Pain Threshold in Patients with Functional Chest Pain: A Pilot Double-Blind Placebo-Controlled Trial. *Dis. Esophagus* 30 (2), 1–8. doi:10.1111/dote.12455

Mantyh, P. W. (2014). Bone Cancer Pain: From Mechanism to Therapy. *Curr. Opin. Support. Palliat. Care* 8 (2), 83–90. doi:10.1097/SPC.0000000000000048

Manzanares, J., Cabañero, D., Puente, N., García-Gutiérrez, M. S., Grandes, P., and Maldonado, R. (2018). Role of the Endocannabinoid System in Drug Addiction. *Biochem. Pharmacol.* 157, 108–121. doi:10.1016/j.bcp.2018.09.013

Marcinkiewcz, C. A., Mazzone, C. M., D'Agostino, G., Halladay, L. R., Hardaway, J. A., DiBerto, J. F., et al. (2016). Serotonin Engages an Anxiety and Fear-Promoting Circuit in the Extended Amygdala. *Nature* 537 (7618), 97–101. doi:10.1038/nature19318

Maresz, K., Carrier, E. J., Ponomarev, E. D., Hillard, C. J., and Dittel, B. N. (2005). Modulation of the Cannabinoid CB2 Receptor in Microglial Cells in Response to Inflammatory Stimuli. *J. Neurochem.* 95 (2), 437–445. doi:10.1111/j.1471-4159.2005.03380.x

Markos, J. R., Harris, H. M., Gul, W., ElSohly, M. A., and Sufka, K. J. (2018). Effects of Cannabidiol on Morphine Conditioned Place Preference in Mice. *Planta Med.* 84 (4), 221–224. doi:10.1055/s-0043-117838

Markovà, J., Essner, U., Akmaz, B., Marinelli, M., Trompke, C., Lentschat, A., et al. (2019). Sativex® as Add-On Therapy vs. Further Optimized First-Line ANTispastics (SAVANT) in Resistant Multiple Sclerosis Spasticity: a Double-Blind, Placebo-Controlled Randomised Clinical Trial. *Int. J. Neurosci.* 129 (2), 119–128. doi:10.1080/00207454.2018.1481066

Marras, C., Beck, J. C., Bower, J. H., Roberts, E., Ritz, B., Ross, G. W., et al. (2018). Prevalence of Parkinson's Disease across North America. *NPJ Parkinsons Dis.* 4, 21. doi:10.1038/s41531-018-0058-0

Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., et al. (2003). CB1 Cannabinoid Receptors and On-Demand Defense against Excitotoxicity. *Science* 302 (5642), 84–88. doi:10.1126/science.1088208

Martín-Moreno, A. M., Reigada, D., Ramírez, B. G., Mechoulam, R., Innamorato, N., Cuadrado, A., et al. (2011). Cannabidiol and Other Cannabinoids Reduce Microglial Activation *In Vitro* and *In Vivo*: Relevance to Alzheimer's Disease. *Mol. Pharmacol.* 79 (6), 964–973. doi:10.1124/mol.111.071290

Masrori, P., and Van Damme, P. (2020). Amyotrophic Lateral Sclerosis: A Clinical Review. *Eur. J. Neurol.* 27 (10), 1918–1929. doi:10.1111/ene.14393

McCaw, E. A., Hu, H., Gomez, G. T., Hebb, A. L., Kelly, M. E., and Denovan-Wright, E. M. (2004). Structure, Expression and Regulation of the Cannabinoid Receptor Gene (CB1) in Huntington's Disease Transgenic Mice. *Eur. J. Biochem.* 271 (23–24), 4909–4920. doi:10.1111/j.1432-1033.2004.04460.x

McColgan, P., and Tabrizi, S. J. (2018). Huntington's Disease: a Clinical Review. *Eur. J. Neurol.* 25 (1), 24–34. doi:10.1111/ene.13413

McGregor, I. S., Dam, K. D., Mallet, P. E., and Gallate, J. E. (2005). Delta9-THC Reinstates Beer- and Sucrose-Seeking Behaviour in Abstinent Rats: Comparison with Midazolam, Food Deprivation and Predator Odour. *Alcohol Alcohol* 40 (1), 35–45. doi:10.1093/alcalc/agh113

Micale, V., Di Marzo, V., Sulcova, A., Wotjak, C. T., and Drago, F. (2013). Endocannabinoid System and Mood Disorders: Priming a Target for New Therapies. *Pharmacol. Ther.* 138 (1), 18–37. doi:10.1016/j.pharmthera.2012.12.002

Miyazaki, I., Asanuma, M., Murakami, S., Takeshima, M., Torigoe, N., Kitamura, Y., et al. (2013). Targeting 5-HT(1A) Receptors in Astrocytes to Protect Dopaminergic Neurons in Parkinsonian Models. *Neurobiol. Dis.* 59, 244–256. doi:10.1016/j.nbd.2013.08.003

Morgan, C. J., Das, R. K., Joye, A., Curran, H. V., and Kamboj, S. K. (2013). Cannabidiol Reduces Cigarette Consumption in Tobacco Smokers: Preliminary Findings. *Addict. Behav.* 38 (9), 2433–2436. doi:10.1016/j.addbeh.2013.03.011

Morisset, V., and Urban, L. (2001). Cannabinoid-Induced Presynaptic Inhibition of Glutamatergic EPSCs in Substantia Gelatinosa Neurons of the Rat Spinal Cord. *J. Neurophysiol.* 86 (1), 40–48. doi:10.1152/jn.2001.86.1.40

Mulder, J., Zilberter, M., Pasquaré, S. J., Alpár, A., Schulte, G., Ferreira, S. G., et al. (2011). Molecular Reorganization of Endocannabinoid Signalling in Alzheimer's Disease. *Brain* 134 (4), 1041–1060. doi:10.1093/brain/awr046

Müller, C. P., and Homberg, J. R. (2015). The Role of Serotonin in Drug Use and Addiction. *Behav. Brain Res.* 277, 146–192. doi:10.1016/j.bbr.2014.04.007

Müller-Vahl, K. R., Schneider, U., and Emrich, H. M. (1999). Nabilone Increases Choreatic Movements in Huntington's Disease. *Movement Disord. Official J. Movement Disord. Soc.* 14 (6), 1038–1040. doi:10.1002/1531-8257(199911)14:6<1038::aid-mds1024>3.0.co;2-7

Munro, S., Thomas, K. L., and Abu-Shaar, M. (1993). Molecular Characterization of a Peripheral Receptor for Cannabinoids. *Nature* 365 (6441), 61–65. doi:10.1038/365061a0

Murty, R. B., and Murty, S. B. (2012). *An Improved Oral Dosage Form of Tetrahydrocannabinol and a Method of Avoiding And/or Suppressing Hepatic First Pass Metabolism via Targeted Chylomicron/lipoprotein Delivery (World Intellectual Property Organization Patent No. WO2012033478A1)*. Availableat: https://patents.google.com/patent/WO2012033478A1/en.

Muzerelle, A., Scotto-Lomassese, S., Bernard, J. F., Soiza-Reilly, M., and Gaspar, P. (2016). Conditional Anterograde Tracing Reveals Distinct Targeting of Individual Serotonin Cell Groups (B5-B9) to the Forebrain and Brainstem. *Brain Struct. Funct.* 221 (1), 535–561. doi:10.1007/s00429-014-0924-4

Navarrete, F., García-Gutiérrez, M. S., Aracil-Fernández, A., Lanciego, J. L., and Manzanares, J. (2018). Cannabinoid CB1 and CB2 Receptors, and Monoacylglycerol Lipase Gene Expression Alterations in the Basal Ganglia of Patients with Parkinson's Disease. *Neurotherapeutics* 15 (2), 459–469. doi:10.1007/s13311-018-0603-x

Nelson, P. T., Braak, H., and Markesbery, W. R. (2009). Neuropathology and Cognitive Impairment in Alzheimer Disease: A Complex but Coherent Relationship. *J. Neuropathol. Exp. Neurol.* 68 (1), 1–14. doi:10.1097/NEN.0b013e3181919a48

Newton, C. A., Chou, P. J., Perkins, I., and Klein, T. W. (2009). CB(1) and CB(2) Cannabinoid Receptors Mediate Different Aspects of delta-9-tetrahydrocannabinol (THC)-induced T Helper Cell Shift Following Immune Activation by *Legionella pneumophila* Infection. *J. Neuroimmune Pharmacol.* 4 (1), 92–102. doi:10.1007/s11481-008-9126-2

Notcutt, W., Price, M., Miller, R., Newport, S., Phillips, C., Simmons, S., et al. (2004). Initial Experiences with Medicinal Extracts of Cannabis for Chronic

Pain: Results from 34 'N of 1' Studies. *Anaesthesia* 59 (5), 440–452. doi:10.1111/j.1365-2044.2004.03674.x

Novotna, A., Mares, J., Ratcliffe, S., Novakova, I., Vachova, M., Zapletalova, O., et al. and the S. S. S. (2011). A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Enriched-Design Study of Nabiximols* (Sativex(®)), as Add-On Therapy, in Subjects with Refractory Spasticity Caused by Multiple Sclerosis. *Eur. J. Neurol.* 18 (9), 1122–1131. doi:10.1111/j.1468-1331.2010.03328.x

Nurmikko, T. J., Serpell, M. G., Hoggart, B., Toomey, P. J., Morlion, B. J., and Haines, D. (2007). Sativex Successfully Treats Neuropathic Pain Characterised by Allodynia: A Randomised, Double-Blind, Placebo-Controlled Clinical Trial. *Pain* 133 (1–3), 210–220. doi:10.1016/j.pain.2007.08.028

O'Connell, M., Sandgren, M., Frantzen, L., Bower, E., and Erickson, B. (2019). Medical Cannabis: Effects on Opioid and Benzodiazepine Requirements for Pain Control. *Ann. Pharmacother.* 53 (11), 1081–1086. doi:10.1177/1060028019854221

Ögren, S. O., Eriksson, T. M., Elvander-Tottie, E., D'Addario, C., Ekström, J. C., Svenningsson, P., et al. (2008). The Role of 5-HT1A Receptors in Learning and Memory. *Behav. Brain Res.* 195 (1), 54–77. doi:10.1016/j.bbr.2008.02.023

Oh, D. A., Parikh, N., Khurana, V., Cognata Smith, C., and Vetticaden, S. (2017). Effect of Food on the Pharmacokinetics of Dronabinol Oral Solution versus Dronabinol Capsules in Healthy Volunteers. *Clin. Pharmacol.* 9, 9–17. doi:10.2147/CPAA.S119676

Ohlsson, A., Lindgren, J. E., Wahlén, A., Agurell, S., Hollister, L. E., and Gillespie, H. K. (1982). Single Dose Kinetics of Deuterium Labelled delta 1-tetrahydrocannabinol in Heavy and Light Cannabis Users. *Biomed. Mass. Spectrom.* 9 (1), 6–10. doi:10.1002/bms.1200090103

Ohno-Shosaku, T., Maejima, T., and Kano, M. (2001). Endogenous Cannabinoids Mediate Retrograde Signals from Depolarized Postsynaptic Neurons to Presynaptic Terminals. *Neuron* 29 (3), 729–738. doi:10.1016/S0896-6273(01)00247-1

Oleson, E. B., Hamilton, L. R., and Gomez, D. M. (2021). Cannabinoid Modulation of Dopamine Release during Motivation, Periodic Reinforcement, Exploratory Behavior, Habit Formation, and Attention. *Front. Synaptic Neurosci.* 13, 660218. doi:10.3389/fnsyn.2021.660218

Onaivi, E. S., Carpio, O., Ishiguro, H., Schanz, N., Uhl, G. R., and Benno, R. (2008a). Behavioral Effects of Cb2 Cannabinoid Receptor Activation and its Influence on Food and Alcohol Consumption. *Ann. N. Y Acad. Sci.* 1139, 426–433. doi:10.1196/annals.1432.035

Onaivi, E. S., Green, M. R., and Martin, B. R. (1990). Pharmacological Characterization of Cannabinoids in the Elevated Plus Maze. *J. Pharmacol. Exp. Ther.* 253 (3), 1002–1009.

Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Meozzi, P. A., Myers, L., et al. (2008b). Functional Expression of Brain Neuronal CB2 Cannabinoid Receptors Are Involved in the Effects of Drugs of Abuse and in Depression. *Ann. N. Y Acad. Sci.* 1139, 434–449. doi:10.1196/annals.1432.036

Palazuelos, J., Davoust, N., Julien, B., Hatterer, E., Aguado, T., Mechoulam, R., et al. (2008). The CB(2) Cannabinoid Receptor Controls Myeloid Progenitor Trafficking: Involvement in the Pathogenesis of an Animal Model of Multiple Sclerosis. *J. Biol. Chem.* 283 (19), 13320–13329. doi:10.1074/jbc.M707960200

Parikh, N., Kramer, W. G., Khurana, V., Cognata Smith, C., and Vetticaden, S. (2016). Bioavailability Study of Dronabinol Oral Solution versus Dronabinol Capsules in Healthy Volunteers. *Clin. Pharmacol.* 8, 155–162. doi:10.2147/CPAA.S115679

Patel, R. R., Barbosa, C., Brustovetsky, T., Brustovetsky, N., and Cummins, T. R. (2016). Aberrant Epilepsy-Associated Mutant Nav1.6 Sodium Channel Activity Can Be Targeted with Cannabidiol. *Brain* 139 (Pt 8), 2164–2181. doi:10.1093/brain/aww129

Patel, S., Hill, M. N., Cheer, J. F., Wotjak, C. T., and Holmes, A. (2017). The Endocannabinoid System as a Target for Novel Anxiolytic Drugs. *Neurosci. Biobehav Rev.* 76, 56–66. doi:10.1016/j.neubiorev.2016.12.033

Peball, M., Krismer, F., Knaus, H. G., Djamshidian, A., Werkmann, M., Carbone, F., et al. (2020). Non-Motor Symptoms in Parkinson's Disease Are Reduced by Nabilone. *Ann. Neurol.* 88 (4), 712–722. doi:10.1002/ana.25864

Pelloux, Y., Dilleen, R., Economidou, D., Theobald, D., and Everitt, B. J. (2012). Reduced Forebrain Serotonin Transmission Is Causally Involved in the Development of Compulsive Cocaine Seeking in Rats. *Neuropsychopharmacology* 37 (11), 2505–2514. doi:10.1038/npp.2012.111

Penninx, B. W., Pine, D. S., Holmes, E. A., and Reif, A. (2021). Anxiety Disorders. *The Lancet* 397 (10277), 914–927. doi:10.1016/S0140-6736(21)00359-7

Perrin, F. E., Gerber, Y. N., Teigell, M., Lonjon, N., Boniface, G., Bauchet, L., et al. (2011). Anatomical Study of Serotonergic Innervation and 5-HT(1A) Receptor in the Human Spinal Cord. *Cell Death Dis* 2, e218. doi:10.1038/cddis.2011.98

Politis, M., and Niccolini, F. (2015). Serotonin in Parkinson's Disease. *Behav. Brain Res.* 277, 136–145. doi:10.1016/j.bbr.2014.07.037

Portenoy, R. K., Ganae-Motan, E. D., Allende, S., Yanagihara, R., Shaiova, L., Weinstein, S., et al. (2012). Nabiximols for Opioid-Treated Cancer Patients with Poorly-Controlled Chronic Pain: A Randomized, Placebo-Controlled, Graded-Dose Trial. *J. Pain* 13 (5), 438–449. doi:10.1016/j.jpain.2012.01.003

Price, T. J., Helesic, G., Parghi, D., Hargreaves, K. M., and Flores, C. M. (2003). The Neuronal Distribution of Cannabinoid Receptor Type 1 in the Trigeminal Ganglion of the Rat. *Neuroscience* 120 (1), 155–162. doi:10.1016/S0306-4522(03)00333-6

Pryce, G., Ahmed, Z., Hankey, D. J., Jackson, S. J., Croxford, J. L., Pocock, J. M., et al. (2003). Cannabinoids Inhibit Neurodegeneration in Models of Multiple Sclerosis. *Brain* 126 (10), 2191–2202. doi:10.1093/brain/awg224

Racz, I., Nadal, X., Alferink, J., Baños, J. E., Rehnelt, J., Martín, M., et al. (2008). Crucial Role of CB(2) Cannabinoid Receptor in the Regulation of central Immune Responses during Neuropathic Pain. *J. Neurosci.* 28 (46), 12125–12135. doi:10.1523/JNEUROSCI.3400-08.2008

Rahn, E. J., Thakur, G. A., Wood, J. A., Zvonok, A. M., Makriyannis, A., and Hohmann, A. G. (2011). Pharmacological Characterization of AM1710, a Putative Cannabinoid CB2 Agonist from the Cannabilactone Class: Antinociception without central Nervous System Side-Effects. *Pharmacol. Biochem. Behav.* 98 (4), 493–502. doi:10.1016/j.pbb.2011.02.024

Raman, C., McAllister, S. D., Rizvi, G., Patel, S. G., Moore, D. H., and Abood, M. E. (2004). Amyotrophic Lateral Sclerosis: Delayed Disease Progression in Mice by Treatment with a Cannabinoid. *Comp. Study* 5 (1), 33–39. doi:10.1080/14660820310016813

Ramirez, B. G., Blázquez, C., Pulgar, T. G. del., Guzmán, M., and Ceballos, M. L. de. (2005). Prevention of Alzheimer's Disease Pathology by Cannabinoids: Neuroprotection Mediated by Blockade of Microglial Activation. *J. Neurosci.* 25 (8), 1904–1913. doi:10.1523/JNEUROSCI.4540-04.2005

Rao, S. D., and Weiss, J. H. (2004). Excitotoxic and Oxidative Cross-Talk between Motor Neurons and Glia in ALS Pathogenesis. *Trends Neurosci.* 27 (1), 17–23. doi:10.1016/j.tins.2003.11.001

Redmond, W. J., Goffaux, P., Potvin, S., and Marchand, S. (2008). Analgesic and Antihyperalgesic Effects of Nabilone on Experimental Heat Pain. *Curr. Med. Res. Opin.* 24 (4), 1017–1024. doi:10.1185/030079908X280635

Rekand, T. (2014). THC:CBD Spray and MS Spasticity Symptoms: Data from Latest Studies. *Eur. Neurol.* 71 (Suppl. 1), 4–9. doi:10.1159/000357742

Ren, Y., Whittard, J., Higuera-Matas, A., Morris, C. V., and Hurd, Y. L. (2009). Cannabidiol, a Nonpsychotropic Component of Cannabis, Inhibits Cue-Induced Heroin Seeking and Normalizes Discrete Mesolimbic Neuronal Disturbances. *J. Neurosci.* 29 (47), 14764–14769. doi:10.1523/JNEUROSCI.4291-09.2009

Riad, M., Garcia, S., Watkins, K. C., Jodoin, N., Doucet, E., Langlois, X., et al. (2000). Somatodendritic Localization of 5-HT1A and Preterminal Axonal Localization of 5-HT1B Serotonin Receptors in Adult Rat Brain. *J. Comp. Neurol.* 417 (2), 181–194. doi:10.1002/(SICI)1096-9861(20000207)417:2<181::AID-CNE4>3.0.CO;2-A

Riad, M., Mestikawy, S. E., Verge, D., Gozlan, H., and Hamon, M. (1991). Visualization and Quantification of central 5-HT1A Receptors with Specific Antibodies. *Neurochem. Int.* 19 (4), 413–423. doi:10.1016/0197-0186(91)90058-L

Richardson, J. D., Kilo, S., and Hargreaves, K. M. (1998). Cannabinoids Reduce Hyperalgesia and Inflammation via Interaction with Peripheral CB1 Receptors. *Pain* 75 (1), 111–119. doi:10.1016/S0304-3959(97)00213-3

Richfield, E. K., and Herkenham, M. (1994). Selective Vulnerability in Huntington's Disease: Preferential Loss of Cannabinoid Receptors in

Lateral Globus Pallidus. *Ann. Neurol.* 36 (4), 577–584. doi:10.1002/ana.410360406

Rissardo, J. P., and Caprara, A. L. F. (2020). Buspirone-associated Movement Disorder: A Literature Review. *Prague Med. Rep.* 121 (1), 5–24. doi:10.14712/23362936.2020.1

Riva, N., Mora, G., Sorarù, G., Lunetta, C., Ferraro, O. E., Falzone, Y., et al. (2019). Safety and Efficacy of Nabiximols on Spasticity Symptoms in Patients with Motor Neuron Disease (CANALS): A Multicentre, Double-Blind, Randomised, Placebo-Controlled, Phase 2 Trial. *Lancet Neurol.* 18 (2), 155–164. doi:10.1016/S1474-4422(18)30406-X

Robinson, J. D., Cinciripini, P. M., Karam-Hage, M., Aubin, H. J., Dale, L. C., Niaura, R., et al. (2018). Pooled Analysis of Three Randomized, Double-Blind, Placebo Controlled Trials with Rimonabant for Smoking Cessation. *Addict. Biol.* 23 (1), 291–303. doi:10.1111/adb.12508

Rodrı´guez, J. J., Mackie, K., and Pickel, V. M. (2001). Ultrastructural Localization of the CB1 Cannabinoid Receptor in μ-Opioid Receptor Patches of the Rat Caudate Putamen Nucleus. *J. Neurosci.* 21 (3), 823–833. doi:10.1523/JNEUROSCI.21-03-00823.2001

Rog, D. J., Nurmikko, T. J., Friede, T., and Young, C. A. (2005). Randomized, Controlled Trial of Cannabis-Based Medicine in central Pain in Multiple Sclerosis. *Neurology* 65 (6), 812–819. doi:10.1212/01.wnl.0000176753.45410.8b

Rudroff, T. (2020). *Medical Marijuana and its Effects on Motor Function in People with Multiple Sclerosis: An Observational Case-Control Study (Clinical Trial Registration No. NCT02898974)*. Availableat: https://clinicaltrials.gov/ct2/show/NCT02898974.

Russo, E. B. (2018). Cannabis Therapeutics and the Future of Neurology. *Front. Integr. Neurosci.* 12, 51. doi:10.3389/fnint.2018.00051

Russo, E. B., and Marcu, J. (2017). Cannabis Pharmacology: The Usual Suspects and a Few Promising Leads. *Adv. Pharmacol.* 80, 67–134. doi:10.1016/bs.apha.2017.03.004

Ruthirakuhan, M., Herrmann, N., Andreazza, A. C., Verhoeff, N. P. L. G., Gallagher, D., Black, S. E., et al. (2020). Agitation, Oxidative Stress, and Cytokines in Alzheimer Disease: Biomarker Analyses from a Clinical Trial with Nabilone for Agitation. *J. Geriatr. Psychiatry Neurol.* 33 (4), 175–184. doi:10.1177/0891988719874118

Sagredo, O., Ramos, J. A., Decio, A., Mechoulam, R., and Fernández-Ruiz, J. (2007). Cannabidiol Reduced the Striatal Atrophy Caused 3-nitropropionic Acid *In Vivo* by Mechanisms Independent of the Activation of Cannabinoid, Vanilloid TRPV1 and Adenosine A2A Receptors. *Eur. J. Neurosci.* 26 (4), 843–851. doi:10.1111/j.1460-9568.2007.05717.x

Salzman, C. (2001). Treatment of the Agitation of Late-Life Psychosis and Alzheimer's Disease. *Eur. Psychiatry* 16 (S1), 25s–28S. doi:10.1016/S0924-9338(00)00525-3

Sandyk, R. (2006). Serotonergic Mechanisms in Amyotrophic Lateral Sclerosis. *Int. J. Neurosci.* 116 (7), 775–826. doi:10.1080/00207450600754087

Sato, S., Mizukami, K., and Asada, T. (2007). A Preliminary Open-Label Study of 5-HT1A Partial Agonist Tandospirone for Behavioural and Psychological Symptoms Associated with Dementia. *Int. J. Neuropsychopharmacol.* 10 (2), 281–283. doi:10.1017/S1461145706007000

Schatz, A. R., Lee, M., Condie, R. B., Pulaski, J. T., and Kaminski, N. E. (1997). Cannabinoid Receptors CB1 and CB2: A Characterization of Expression and Adenylate Cyclase Modulation within the Immune System. *Toxicol. Appl. Pharmacol.* 142 (2), 278–287. doi:10.1006/taap.1996.8034

Schechter, L. E., Smith, D. L., Rosenzweig-Lipson, S., Sukoff, S. J., Dawson, L. A., Marquis, K., et al. (2005). Lecozotan (SRA-333): A Selective Serotonin 1A Receptor Antagonist that Enhances the Stimulated Release of Glutamate and Acetylcholine in the Hippocampus and Possesses Cognitive-Enhancing Properties. *J. Pharmacol. Exp. Ther.* 314 (3), 1274–1289. doi:10.1124/jpet.105.086363

Scheltens, P., De Strooper, B., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C. E., et al. (2021). Alzheimer's Disease. *The Lancet* 397 (10284), 1577–1590. doi:10.1016/S0140-6736(20)32205-4

Schiavon, A. P., Bonato, J. M., Milani, H., Guimarães, F. S., and Weffort de Oliveira, R. M. (2016). Influence of Single and Repeated Cannabidiol Administration on Emotional Behavior and Markers of Cell Proliferation and Neurogenesis in Non-stressed Mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 64, 27–34. doi:10.1016/j.pnpbp.2015.06.017

Schimrigk, S., Marziniak, M., Neubauer, C., Kugler, E. M., Werner, G., and Abramov-Sommariva, D. (2017). Dronabinol Is a Safe Long-Term Treatment Option for Neuropathic Pain Patients. *Eur. Neurol.* 78 (5–6), 320–329. doi:10.1159/000481089

Schoffelmeer, A. N., Hogenboom, F., Wardeh, G., and De Vries, T. J. (2006). Interactions between CB1 Cannabinoid and Mu Opioid Receptors Mediating Inhibition of Neurotransmitter Release in Rat Nucleus Accumbens Core. *Neuropharmacology* 51 (4), 773–781. doi:10.1016/j.neuropharm.2006.05.019

Shannon, S., Lewis, N., Lee, H., and Hughes, S. (2019). Cannabidiol in Anxiety and Sleep: A Large Case Series. *Perm J.* 23, 18–041. doi:10.7812/TPP/18-041

Silverdale, M. A., McGuire, S., McInnes, A., Crossman, A. R., and Brotchie, J. M. (2001). Striatal Cannabinoid CB1 Receptor mRNA Expression Is Decreased in the Reserpine-Treated Rat Model of Parkinson's Disease. *Exp. Neurol.* 169 (2), 400–406. doi:10.1006/exnr.2001.7649

Skirzewski, M., Hernandez, L., Schechter, L. E., and Rada, P. (2010). Acute Lecozotan Administration Increases Learning and Memory in Rats without Affecting Anxiety or Behavioral Depression. *Pharmacol. Biochem. Behav.* 95 (3), 325–330. doi:10.1016/j.pbb.2010.02.008

Soares, Vde. P., Campos, A. C., Bortoli, V. C., Zangrossi, H., Guimarães, F. S., and Zuardi, A. W. (2010). Intra-dorsal Periaqueductal gray Administration of Cannabidiol Blocks Panic-like Response by Activating 5-HT1A Receptors. *Behav. Brain Res.* 213 (2), 225–229. doi:10.1016/j.bbr.2010.05.004

Solas, M., Francis, P. T., Franco, R., and Ramirez, M. J. (2013). CB2 Receptor and Amyloid Pathology in Frontal Cortex of Alzheimer's Disease Patients. *Neurobiol. Aging* 34 (3), 805–808. doi:10.1016/j.neurobiolaging.2012.06.005

Solinas, M., Panlilio, L. V., and Goldberg, S. R. (2004). Exposure to delta-9-tetrahydrocannabinol (THC) Increases Subsequent Heroin Taking but Not Heroin's Reinforcing Efficacy: a Self-Administration Study in Rats. *Neuropsychopharmacology* 29 (7), 1301–1311. doi:10.1038/sj.npp.1300431

Solís-Guillén, R., Leopoldo, M., Meneses, A., and Centurión, D. (2021). Activation of 5-HT1A and 5-HT7 Receptors Enhanced a Positively Reinforced Long-Term Memory. *Behav. Brain Res.* 397, 112932. doi:10.1016/j.bbr.2020.112932

Solowij, N., Broyd, S. J., van Hell, H. H., and Hazekamp, A. (2014). A Protocol for the Delivery of Cannabidiol (CBD) and Combined CBD and Δ9-tetrahydrocannabinol (THC) by Vaporisation. *BMC Pharmacol. Toxicol.* 15, 58. doi:10.1186/2050-6511-15-58

Stein, D. J., Scott, K. M., de Jonge, P., and Kessler, R. C. (2017). Epidemiology of Anxiety Disorders: From Surveys to Nosology and Back. *Dialogues Clin. Neurosci.* 19 (2), 127–136. doi:10.31887/DCNS.2017.19.2/dstein

Stempel, A. V., Stumpf, A., Zhang, H. Y., Özdoğan, T., Pannasch, U., Theis, A. K., et al. (2016). Cannabinoid Type 2 Receptors Mediate a Cell Type-specific Plasticity in the Hippocampus. *Neuron* 90 (4), 795–809. doi:10.1016/j.neuron.2016.03.034

Sussman, N., and Joffe, R. T. (1998). Antidepressant Augmentation: Conclusions and Recommendations. *J. Clin. Psychiatry* 59 (Suppl. 5), 70–73.

Svendsen, K. B., Jensen, T. S., and Bach, F. W. (2004). Does the Cannabinoid Dronabinol Reduce central Pain in Multiple Sclerosis? Randomised Double Blind Placebo Controlled Crossover Trial. *BMJ* 329 (7460), 253. doi:10.1136/bmj.38149.566979.AE

Szabó, Á., Helyes, Z., Sándor, K., Bite, A., Pintér, E., Németh, J., et al. (2005). Role of Transient Receptor Potential Vanilloid 1 Receptors in Adjuvant-Induced Chronic Arthritis: *In Vivo* Study Using Gene-Deficient Mice. *J. Pharmacol. Exp. Ther.* 314 (1), 111–119. doi:10.1124/jpet.104.082487

Tao, R., and Auerbach, S. B. (1994). Increased Extracellular Serotonin in Rat Brain after Systemic or Intraraphe Administration of Morphine. *J. Neurochem.* 63 (2), 517–524. doi:10.1046/j.1471-4159.1994.63020517.x

Thanos, P. K., Dimitrakakis, E. S., Rice, O., Gifford, A., and Volkow, N. D. (2005). Ethanol Self-Administration and Ethanol Conditioned Place Preference Are Reduced in Mice Lacking Cannabinoid CB1 Receptors. *Behav. Brain Res.* 164 (2), 206–213. doi:10.1016/j.bbr.2005.06.021

Therapeutic Goods Administration (2013). *Australian Public Assessment Report for Nabiximols*, 204.

Thielen, R. J., Bare, D. J., McBride, W. J., Lumeng, L., and Li, T. K. (2002). Ethanol-stimulated Serotonin Release in the Ventral hippocampus: An Absence of Rapid Tolerance for the Alcohol-Preferring P Rat and Insensitivity in the Alcohol-Nonpreferring NP Rat. *Pharmacol. Biochem. Behav.* 71 (1), 111–117. doi:10.1016/S0091-3057(01)00633-5

Thompson, A. J., Baranzini, S. E., Geurts, J., Hemmer, B., and Ciccarelli, O. (2018). Multiple Sclerosis. *Lancet* 391 (10130), 1622–1636. doi:10.1016/S0140-6736(18)30481-1

Tohgi, H., Abe, T., Takahashi, S., Kimura, M., Takahashi, J., and Kikuchi, T. (1992). Concentrations of Serotonin and its Related Substances in the Cerebrospinal Fluid in Patients with Alzheimer Type Dementia. *Neurosci. Lett.* 141 (1), 9–12. doi:10.1016/0304-3940(92)90322-x

Trigo, J. M., Soliman, A., Quilty, L. C., Fischer, B., Rehm, J., Selby, P., et al. (2018). Nabiximols Combined with Motivational Enhancement/Cognitive Behavioral Therapy for the Treatment of Cannabis Dependence: A Pilot Randomized Clinical Trial. *PLoS One*, e0190768

University of California, Davis (2018). *Cannabis for Spasticity in Multiple Sclerosis: A Placebo-Controlled Study (Clinical Trial Registration No. NCT00682929)*. Availableat: https://clinicaltrials.gov/ct2/show/NCT00682929.

Valjent, E., Mitchell, J. M., Besson, M. J., Caboche, J., and Maldonado, R. (2002). Behavioural and Biochemical Evidence for Interactions between Delta 9-tetrahydrocannabinol and Nicotine. *Br. J. Pharmacol.* 135 (2), 564–578. doi:10.1038/sj.bjp.0704479

van Hecke, O., Austin, S. K., Khan, R. A., Smith, B. H., and Torrance, N. (2014). Neuropathic Pain in the General Population: A Systematic Review of Epidemiological Studies. *Pain* 155 (4), 654–662. doi:10.1016/j.pain.2013.11.013

Van Laere, K., Casteels, C., Lunskens, S., Goffin, K., Grachev, I. D., Bormans, G., et al. (2012). Regional Changes in Type 1 Cannabinoid Receptor Availability in Parkinson's Disease *In Vivo*. *Neurobiol. Aging* 33 (3), 620–8620. e8. doi:10.1016/j.neurobiolaging.2011.02.009

Van Sickle, M. D., Duncan, M., Kingsley, P. J., Mouihate, A., Urbani, P., Mackie, K., et al. (2005). Identification and Functional Characterization of Brainstem Cannabinoid CB2 Receptors. *Science* 310 (5746), 329–332. doi:10.1126/science.1115740

van Vliet, S. A., Vanwersch, R. A., Jongsma, M. J., Olivier, B., and Philippens, I. H. (2008). Therapeutic Effects of Delta9-THC and Modafinil in a Marmoset Parkinson Model. *Eur. Neuropsychopharmacol.* 18 (5), 383–389. doi:10.1016/j.euroneuro.2007.11.003

Vela, G., Martín, S., García-Gil, L., Crespo, J. A., Ruiz-Gayo, M., Fernández-Ruiz, J. J., et al. (1998). Maternal Exposure to delta9-tetrahydrocannabinol Facilitates Morphine Self-Administration Behavior and Changes Regional Binding to central Mu Opioid Receptors in Adult Offspring Female Rats. *Brain Res.* 807 (1), 101–109. doi:10.1016/S0006-8993(98)00766-5

Vigil, J. M., Stith, S. S., Adams, I. M., and Reeve, A. P. (2017). Associations between Medical Cannabis and Prescription Opioid Use in Chronic Pain Patients: A Preliminary Cohort Study. *PLOS ONE* 12 (11), e0187795. doi:10.1371/journal.pone.0187795

Viudez-Martínez, A., García-Gutiérrez, M. S., Navarrón, C. M., Morales-Calero, M. I., Navarrete, F., Torres-Suárez, A. I., et al. (2018). Cannabidiol Reduces Ethanol Consumption, Motivation and Relapse in Mice. *Addict. Biol.* 23 (1), 154–164. doi:10.1111/adb.12495

Volkow, N. D., Baler, R. D., Compton, W. M., and Weiss, S. R. (2014). Adverse Health Effects of Marijuana Use. *N. Engl. J. Med.* 370 (23), 2219–2227. doi:10.1056/NEJMra1402309

Volpi-Abadie, J., Kaye, A. M., and Kaye, A. D. (2013). Serotonin Syndrome. *Ochsner J.* 13 (4), 533–540.

Vos, T., Barber, R. M., Bell, B., Bertozzi-Villa, A., Biryukov, S., Bolliger, I., et al. (2015). Global, Regional, and National Incidence, Prevalence, and Years Lived with Disability for 301 Acute and Chronic Diseases and Injuries in 188 Countries, 1990-2013: a Systematic Analysis for the Global Burden of Disease Study 2013. *Lancet* 386 (9995), 743–800. doi:10.1016/S0140-6736(15)60692-4

Wade, D. T., Makela, P., Robson, P., House, H., and Bateman, C. (2004). Do cannabis-based Medicinal Extracts Have General or Specific Effects on Symptoms in Multiple Sclerosis? A Double-Blind, Randomized, Placebo-Controlled Study on 160 Patients. *Mult. Scler.* 10 (4), 434–441. doi:10.1191/1352458504ms1082oa

Wade, D. T., Makela, P. M., House, H., Bateman, C., and Robson, P. (2006). Long-term Use of a Cannabis-Based Medicine in the Treatment of Spasticity and Other Symptoms in Multiple Sclerosis. *Mult. Scler.* 12 (5), 639–645. doi:10.1177/1352458505070618

Wallace, M. J., Blair, R. E., Falenski, K. W., Martin, B. R., and DeLorenzo, R. J. (2003). The Endogenous Cannabinoid System Regulates Seizure Frequency and Duration in a Model of Temporal Lobe Epilepsy. *J. Pharmacol. Exp. Ther.* 307 (1), 129–137. doi:10.1124/jpet.103.051920

Walsh, S., Mnich, K., Mackie, K., Gorman, A. M., Finn, D. P., and Dowd, E. (2010). Loss of Cannabinoid CB1 Receptor Expression in the 6-Hydroxydopamine-Induced Nigrostriatal Terminal Lesion Model of Parkinson's Disease in the Rat. *Brain Res. Bull.* 81 (6), 543–548. doi:10.1016/j.brainresbull.2010.01.009

Walton, C., King, R., Rechtman, L., Kaye, W., Leray, E., Marrie, R. A., et al. (2020). Rising Prevalence of Multiple Sclerosis Worldwide: Insights from the Atlas of MS, Third Edition. *Mult. Scler.* 26 (14), 1816–1821. doi:10.1177/1352458520970841

Wang, H. T., Han, F., and Shi, Y. X. (2009). Activity of the 5-HT1A Receptor Is Involved in the Alteration of Glucocorticoid Receptor in hippocampus and Corticotropin-Releasing Factor in Hypothalamus in SPS Rats. *Int. J. Mol. Med.* 24 (2), 227–231. doi:10.3892/ijmm_00000225

Wang, Q., Liu, Y., and Zhou, J. (2015). Neuroinflammation in Parkinson's Disease and its Potential as Therapeutic Target. *Transl Neurodegener* 4 (1), 19. doi:10.1186/s40035-015-0042-0

Ward, S. J., McAllister, S. D., Kawamura, R., Murase, R., Neelakantan, H., and Walker, E. A. (2014). Cannabidiol Inhibits Paclitaxel-Induced Neuropathic Pain through 5-HT(1A) Receptors without Diminishing Nervous System Function or Chemotherapy Efficacy. *Br. J. Pharmacol.* 171 (3), 636–645. doi:10.1111/bph.12439

Ward, S. J., Ramirez, M. D., Neelakantan, H., and Walker, E. A. (2011). Cannabidiol Prevents the Development of Cold and Mechanical Allodynia in Paclitaxel-Treated Female C57Bl6 Mice. *Anesth. Analg* 113 (4), 947–950. doi:10.1213/ANE.0b013e3182283486

Ware, M. A., Wang, T., Shapiro, S., Collet, J. P., Boulanger, A., Esdaile, J. M., et al. (2015). Cannabis for the Management of Pain: Assessment of Safety Study (COMPASS). *J. Pain* 16 (12), 1233–1242. doi:10.1016/j.jpain.2015.07.014

Ware, M. A., Wang, T., Shapiro, S., Robinson, A., Ducruet, T., Huynh, T., et al. (2010). Smoked Cannabis for Chronic Neuropathic Pain: A Randomized Controlled Trial. *CMAJ* 182 (14), E694–E701. doi:10.1503/cmaj.091414

Weber, M., Goldman, B., and Truniger, S. (2010). Tetrahydrocannabinol (THC) for Cramps in Amyotrophic Lateral Sclerosis: A Randomised, Double-Blind Crossover Trial. *J. Neurol. Neurosurg. Psychiatry* 81 (10), 1135–1140. doi:10.1136/jnnp.2009.200642

Weissman, M. M. (1988). The Epidemiology of Anxiety Disorders: Rates, Risks and Familial Patterns. *J. Psychiatr. Res.* 22 (Suppl. 1), 99–114. doi:10.1016/0022-3956(88)90071-4

Weydt, P., Hong, S., Witting, A., Möller, T., Stella, N., and Kliot, M. (2005). Cannabinol Delays Symptom Onset in SOD1 (G93A) Transgenic Mice without Affecting Survival. *Amyotroph. Lateral Scler. Other Mot. Neuron Disord* 6 (3), 182–184. doi:10.1080/14660820510030149

Wise, R. A., and Robble, M. A. (2020). Dopamine and Addiction. *Annu. Rev. Psychol.* 71, 79–106. doi:10.1146/annurev-psych-010418-103337

Wotherspoon, G., Fox, A., McIntyre, P., Colley, S., Bevan, S., and Winter, J. (2005). Peripheral Nerve Injury Induces Cannabinoid Receptor 2 Protein Expression in Rat Sensory Neurons. *Neuroscience* 135 (1), 235–245. doi:10.1016/j.neuroscience.2005.06.009

Xi, Z. X., Peng, X. Q., Li, X., Song, R., Zhang, H. Y., Liu, Q. R., et al. (2011). Brain Cannabinoid CB_2 Receptors Modulate Cocaine's Actions in Mice. *Nat. Neurosci.* 14 (9), 1160–1166. doi:10.1038/nn.2874

Xu, D. H., Cullen, B. D., Tang, M., and Fang, Y. (2020). The Effectiveness of Topical Cannabidiol Oil in Symptomatic Relief of Peripheral Neuropathy of the Lower Extremities. *Curr. Pharm. Biotechnol.* 21 (5), 390–402. doi:10.2174/1389201020666191202111534

Yagishita, S. (2020). Transient and Sustained Effects of Dopamine and Serotonin Signaling in Motivation-Related Behavior. *Psychiatry Clin. Neurosci.* 74 (2), 91–98. doi:10.1111/pcn.12942

Yiangou, Y., Facer, P., Durrenberger, P., Chessell, I. P., Naylor, A., Bountra, C., et al. (2006). COX-2, CB2 and P2X7-Immunoreactivities Are Increased in Activated Microglial Cells/macrophages of Multiple Sclerosis and Amyotrophic Lateral Sclerosis Spinal Cord. *BMC Neurol.* 6, 12. doi:10.1186/1471-2377-6-12

Yohrling IV, G. J., Jiang, G. C., DeJohn, M. M., Robertson, D. J., Vrana, K. E., and Cha, J. H. (2002). Inhibition of Tryptophan Hydroxylase Activity and Decreased 5-HT1A Receptor Binding in a Mouse Model of Huntington's Disease. *J. Neurochem.* 82 (6), 1416–1423. doi:10.1046/j.1471-4159.2002.01084.x

Yoshimoto, K., McBride, W. J., Lumeng, L., and Li, T. K. (1992). Alcohol Stimulates the Release of Dopamine and Serotonin in the Nucleus Accumbens. *Alcohol* 9 (1), 17–22. doi:10.1016/0741-8329(92)90004-T

Zajicek, J. P., Hobart, J. C., Slade, A., Barnes, D., and Mattison, P. G., and MUSEC Research Group. (2012). Multiple Sclerosis and Extract of Cannabis: Results of the MUSEC Trial. *J. Neurol. Neurosurg. Psychiatry* 83 (11), 1125–1132. doi:10.1136/jnnp-2012-302468

Zarei, S., Carr, K., Reiley, L., Diaz, K., Guerra, O., Altamirano, P. F., et al. (2015). A Comprehensive Review of Amyotrophic Lateral Sclerosis. *Surg. Neurol. Int.* 6, 171. doi:10.4103/2152-7806.169561

Zhang, J., Hoffert, C., Vu, H. K., Groblewski, T., Ahmad, S., and O'Donnell, D. (2003). Induction of CB2 Receptor Expression in the Rat Spinal Cord of Neuropathic but Not Inflammatory Chronic Pain Models. *Eur. J. Neurosci.* 17 (12), 2750–2754. doi:10.1046/j.1460-9568.2003.02704.x

Zhao, P., Ignacio, S., Beattie, E. C., and Abood, M. E. (2008). Altered Presymptomatic AMPA and Cannabinoid Receptor Trafficking in Motor Neurons of ALS Model Mice: Implications for Excitotoxicity. *Eur. J. Neurosci.* 27 (3), 572–579. doi:10.1111/j.1460-9568.2008.06041.x

Zou, Z., Wang, H., d'Oleire Uquillas, F., Wang, X., Ding, J., and Chen, H. (2017). Definition of Substance and Non-substance Addiction. *Adv. Exp. Med. Biol.* 1010, 21–41. doi:10.1007/978-981-10-5562-1_2

Zuardi, A. W., Crippa, J. A., Hallak, J. E., Pinto, J. P., Chagas, M. H., Rodrigues, G. G., et al. (2009). Cannabidiol for the Treatment of Psychosis in Parkinson's Disease. *J. Psychopharmacol.* 23 (8), 979–983. doi:10.1177/0269881108096519

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Pharmacology

REVIEW
published: 25 April 2022
doi: 10.3389/fphar.2022.894960

Cannabis for Medical Use: Versatile Plant Rather Than a Single Drug

*Shiri Procaccia, Gil Moshe Lewitus, Carni Lipson Feder, Anna Shapira, Paula Berman and David Meiri**

The Laboratory of Cancer Biology and Cannabinoid Research, Faculty of Biology, Technion- Israel Institute of Technology, Haifa, Israel

Medical *Cannabis* and its major cannabinoids (–)-*trans*-Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are gaining momentum for various medical purposes as their therapeutic qualities are becoming better established. However, studies regarding their efficacy are oftentimes inconclusive. This is chiefly because *Cannabis* is a versatile plant rather than a single drug and its effects do not depend only on the amount of THC and CBD. Hundreds of *Cannabis* cultivars and hybrids exist worldwide, each with a unique and distinct chemical profile. Most studies focus on THC and CBD, but these are just two of over 140 phytocannabinoids found in the plant in addition to a milieu of terpenoids, flavonoids and other compounds with potential therapeutic activities. Different plants contain a very different array of these metabolites in varying relative ratios, and it is the interplay between these molecules from the plant and the endocannabinoid system in the body that determines the ultimate therapeutic response and associated adverse effects. Here, we discuss how phytocannabinoid profiles differ between plants depending on the chemovar types, review the major factors that affect secondary metabolite accumulation in the plant including the genotype, growth conditions, processing, storage and the delivery route; and highlight how these factors make *Cannabis* treatment highly complex.

Keywords: cannabis, chemovar, phytocannabinoids, terpenoids, secondary metabolites

OPEN ACCESS

Edited by:
Francesca Baratta, University of Turin, Italy

Reviewed by:
Denis J. Dupré, Dalhousie University, Canada
Ethan Budd Russo, CReDO Science, United States

****Correspondence:***
David Meiri
dmeiri@technion.ac.il

Specialty section:
This article was submitted to Experimental Pharmacology and Drug Discovery, a section of the journal Frontiers in Pharmacology

Received: *12 March 2022*
Accepted: *28 March 2022*
Published: *25 April 2022*

Citation:
Procaccia S, Lewitus GM, Lipson Feder C, Shapira A, Berman P and Meiri D (2022) Cannabis for Medical Use: Versatile Plant Rather Than a Single Drug. Front. Pharmacol. 13:894960. doi: 10.3389/fphar.2022.894960

INTRODUCTION

The past 2 decades have seen a major increase in the use of medical *Cannabis* as its therapeutic virtues are becoming better known and accepted (Bridgeman and Abazia, 2017). These therapeutic qualities were attributed to a naturally-occurring unique family of secondary metabolites termed phytocannabinoids. The most abundant and best-known phytocannabinoids are the psychoactive (–)-*trans*-Δ^9-tetrahydrocannabinol (THC), which was first isolated and structurally elucidated by Mechoulam and colleagues in 1964 (Gaoni and Mechoulam, 1964); and cannabidiol (CBD), which was extracted in 1940 (Adams et al., 1940) and its full chemical structure was elucidated in 1963 by the same Mechoulam (Mechoulam and Shvo, 1963). CBD has been gaining interest since the 1980s when CBD oil was found to possess anti-epileptic properties (Consroe et al., 1982), and the CBD molecule was later shown to possess a wide range of therapeutic effects (Mechoulam et al., 2007; Zuardi, 2008). However, THC and CBD are just two of more than 140 distinctive phytocannabinoids that have been identified so far in different *Cannabis* plants (Hanuš et al., 2016; Berman et al., 2018).

The isolation of phytocannabinoids from the *Cannabis* plant has led to the discovery of endogenous cannabinoids (endocannabinoids, eCBs) in vertebrates (Devane et al., 1992). THC was found to bind a specific G-protein-coupled receptor, which was named cannabinoid receptor 1 (CB1) (Matsuda et al., 1990). A second receptor, which was named CB2, was identified by homology

(Munro et al., 1993; Onaivi et al., 2006). Following the discovery of the receptors, their endogenous lipid ligands were identified. The first two and best-studied are N-arachidonoylethanolamine (anandamide) (Devane et al., 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam et al., 1995). These eCBs and their specific receptors, CB1 and CB2, form the classical endocannabinoid system (eCBS) (De Petrocellis and Di Marzo, 2009; Lu and Mackie, 2016), a ubiquitous neuromodulatory signaling system that has widespread functions in the brain and throughout the body. Since its inception, the term eCBS was expanded and now additional cannabinoid receptors, additional eCBs and cannabimimetic lipids as well as the enzymes involved in their synthesis and degradation are recognized as part of the extended eCBS (De Petrocellis et al., 2004; Mackie, 2008). Many of the pharmacological and therapeutic properties of phytocannabinoids rely on their interactions with the eCBS. The numerous and versatile effects of *Cannabis* result from the involvement of the eCBS in multiple processes. It regulates many physiological processes in health and disease (Di Marzo et al., 2004; de Fonseca et al., 2005). It is involved in the maintenance and homeostasis of many vital functions including immune response (Pandey et al., 2009), cardiovascular activity (Pacher and Steffens, 2009; Montecucco and Di Marzo, 2012), memory (Marsicano and Lafenêtre, 2009; Maroso et al., 2016; Lunardi et al., 2020) and pain sensation (Woodhams et al., 2015; Woodhams et al., 2017). This makes *Cannabis* treatment especially valuable since targeting the eCBS and its modulation by phytocannabinoids has been emerging as novel pharmacotherapy, with therapeutic potential suggested in a multitude of diseases affecting humans.

In the last decade, there has been a rapid growth in the discovery and use of pure THC, pure CBD and *Cannabis*-based extracts for various medical purposes. Results regarding the efficacy of *Cannabis*-based extracts are oftentimes inconclusive and sometimes even conflicting. That is because the effects of *Cannabis* extracts do not depend merely on the amount of THC and CBD (Maccarrone, 2020). *Cannabis* is a versatile plant rather than a single drug and importantly, studies involving pure THC or CBD do not reflect the potential benefits of full-spectrum extracts (Maayah et al., 2020b). For example, THC and CBD were both effective in reducing neuropathic pain in various mice and rat models (Comelli et al., 2008; Casey et al., 2017; King et al., 2017; Belardo et al., 2019; Abraham et al., 2020). However, the pain-relieving effects were enhanced by their combination (Casey et al., 2017; King et al., 2017). Moreover, a controlled high-CBD extract with additional secondary metabolites from the plant was more effective than purified CBD or THC at the same dose as in the extract (Comelli et al., 2008). In studies involving patients with multiple sclerosis, full-spectrum extracts demonstrated more beneficial effects for pain relief and reducing inflammation than pure THC and CBD (Maayah et al., 2020a; Maayah et al., 2020b). We have recently shown that both high-THC and high-CBD extracts were effective in reducing chronic pain, however, specific phytocannabinoid compositions were associated with more adverse effects (Aviram et al., 2021a). We also found *Cannabis* extracts effective in reducing migraine frequency, and here again, the presence of a few minor phytocannabinoids in the extracts made some more effective than others regardless of their THC or CBD content (Aviram et al., 2020b).

BIOACTIVE SECONDARY METABOLITES FROM *CANNABIS* AS THERAPEUTIC AGENTS

Phytocannabinoids are conventionally classified into 10 subclasses based on their chemical structure and an 11th miscellaneous types group (**Figure 1**) (Hanuš et al., 2016; Berman et al., 2018). They are lipophilic compounds biosynthesized by the convergence of two main plant pathways: the polyketide and the plastidial non-mevalonate-dependent isoprenoid (MEP) pathways. Phytocannabinoids are made of a resorcinyl core with a carboxyl group (COOH) on the aromatic ring, an alkyl side-chain of varying length that typically contains an odd number of carbon atoms (one to seven carbons), and a terpene moiety (Hanuš et al., 2016; Gülck and Møller, 2020). The most abundant type of phytocannabinoids in *Cannabis* are those with a pentyl side-chain (five carbons), with cannabigerolic acid (CBGA) as the first cannabinoid compound, made by the prenylation of olivetolic acid with the isoprenoid geranyl pyrophosphate (GPP) (Gülck and Møller, 2020). Other phytocannabinoid subclasses, including (−)-*trans*-Δ^9-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA) and cannabichromenic acid (CBCA) are derived from CBGA-type phytocannabinoids via specific enzymatic reactions (Berman et al., 2018). Thus, only these four subclasses are biosynthesized in the plant while the remaining subclasses are the result of different degradation routes and chemical processes such as oxidation, photochemical reaction, double bond isomerization, and others. The well-known neutral phytocannabinoids result from the decarboxylation of the acid compounds, where the carboxyl group is removed and carbon dioxide is released. In the less common cases, instead of olivetolic acid other molecules with different length alkyl side-chain serve as precursors. These undergo the same enzymatic and chemical reactions, resulting in a range of additional phytocannabinoids (Gülck and Møller, 2020) such as the three-carbon cannabigerovarinic acid (CBGVA), (−)-*trans*-Δ^9-tetrahydrocannabivarinic acid (THCVA) and cannabidivarinic acid (CBDVA), or the seven-carbon (-)-trans-Δ^9-tetrahydrocannabiphorol (THCP) and cannabidiphorol (CBDP) (Citti et al., 2019b) and others. Cannabinoid derivatives that were previously detected by MS methods are presented in **Figure 1** (Berman et al., 2018; Citti et al., 2019a; Citti et al., 2019b; Linciano et al., 2020). Though initially considered unique to the *Cannabis* plant, other plant-derived natural products that are able to interact with ECS receptors were later discovered in other types of plants, such as *Radula marginata* and *Piper nigrum* (Gertsch et al., 2010; Russo, 2016).

In addition to phytocannabinoids, the other major active secondary metabolites of *Cannabis* are terpenes and terpenoids (generally termed terpenoids). Terpenes are naturally occurring volatile unsaturated hydrocarbon biomolecules built up by

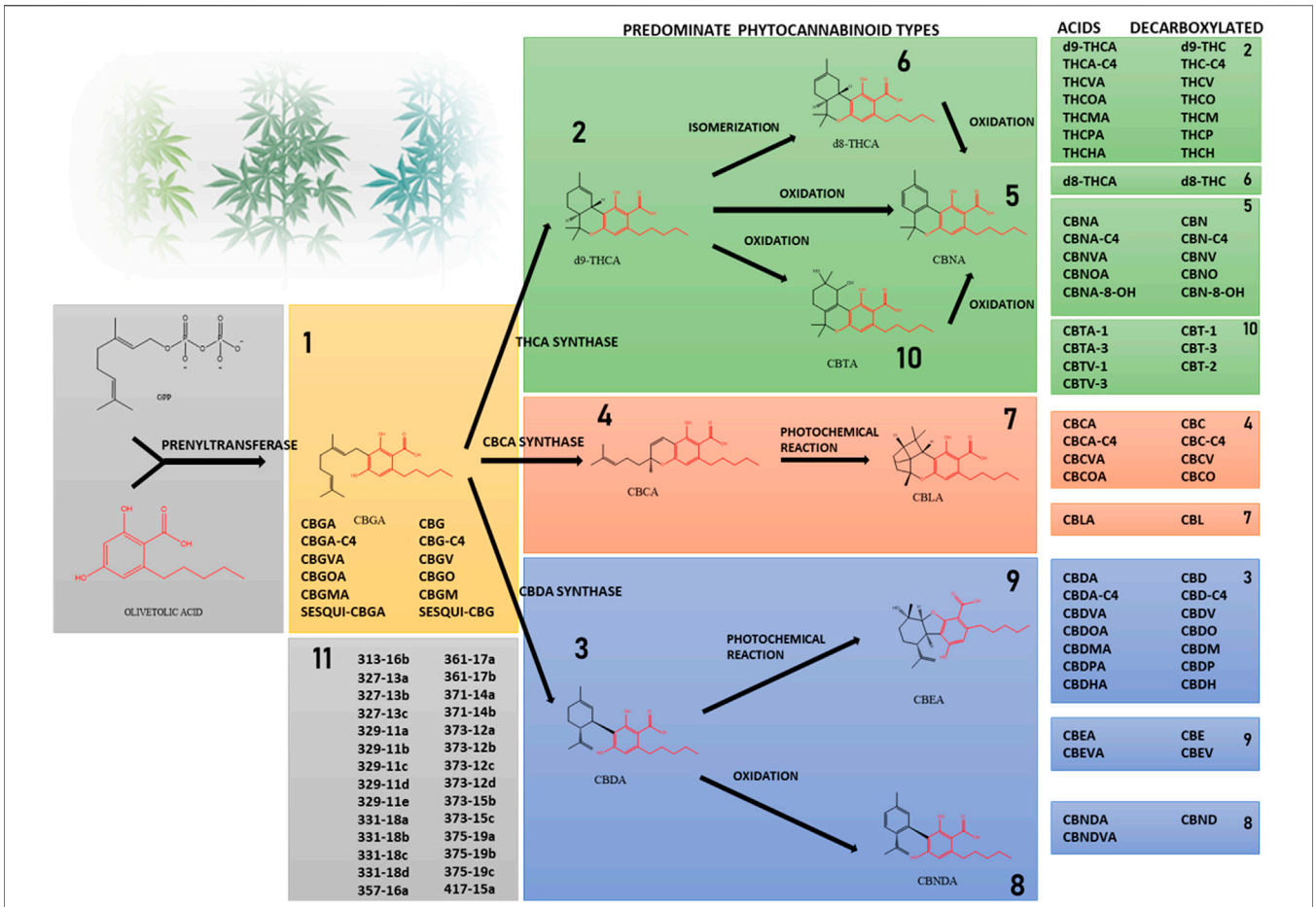


FIGURE 1 | Phytocannabinoids are divided into subclasses according to their structure. Prenylation of olivetolic acid with the isoprenoid geranyl pyrophosphate forms CBGA. Less frequently, instead of olivetolic acid other molecules with different length alkyl side-chain serve as precursors. The acid forms THCA, CBDA and CBCA are synthesized in the *Cannabis* plant from CBGA. The neutral forms and other subclasses of phytocannabinoids are the result of chemical processes such as decarboxylation, isomerization and others. The shared core of olivetolic acid by the different subclasses is depicted in red. Subclass 11 includes phytocannabinoids identified by mass spectrometry in different *Cannabis* chemovars, whose structures have not been elucidated yet.

branched 5-carbon isoprene units, sharing the same isoprenoid precursor as phytocannabinoids. Terpenoids are modified terpenes that contain additional functional groups, usually varying oxygen arrangements or oxidation states. Monoterpenoids are built by two isoprene units (10 carbons) and sesquiterpenoids are built up by three isoprene units (15 carbons) (Shapira et al., 2019). Monoterpenoids and phytocannabinoids share the common biosynthetic precursor GPP and are both biosynthesized in the plastid, while sesquiterpenoids are synthesized in the cytosol from farnesyl pyrophosphate (Booth et al., 2020; Lipson Feder et al., 2021). Terpenoids are responsible for the fragrance and taste of plants as they are characterized by a strong and pleasant aroma (Gershenzon and Dudareva, 2007). Terpenoids are also suggested to have roles in protection from predation and attraction of pollinators. Terpenoids were shown to exert synergistic effects when combined with the phytocannabinoids in *Cannabis* and contribute crucially to its therapeutic effects (Downer, 2020; Ferber et al., 2020; Hanuš and Hod, 2020), and were also suggested to possess therapeutic effects of their own (Russo, 2011). Terpenoids are widely distributed in plants and a few are also present in other species including some animals and microorganisms (Gershenzon and Dudareva, 2007).

Various flavonoids are also found in *Cannabis* and may give the plant some of its exclusive medicinal benefits (Russo et al., 2003). Flavonoids are hydroxylated polyphenolic compounds consisting of two benzene rings linked via a heterocyclic pyran ring (Bautista et al., 2021). Three specific prenylated flavonoids, termed cannflavins A-C, are unique to *Cannabis* and show potent anti-inflammatory capabilities (Calzolari et al., 2017; Erridge et al., 2020). *Cannabis* plants produce additional kinds of secondary metabolites including various alkaloids, stilbenoids and others (Flores-Sanchez and Verpoorte, 2008a), but little is known regarding their biosynthesis and regulation and whether they possess any therapeutic value remains to be elucidated.

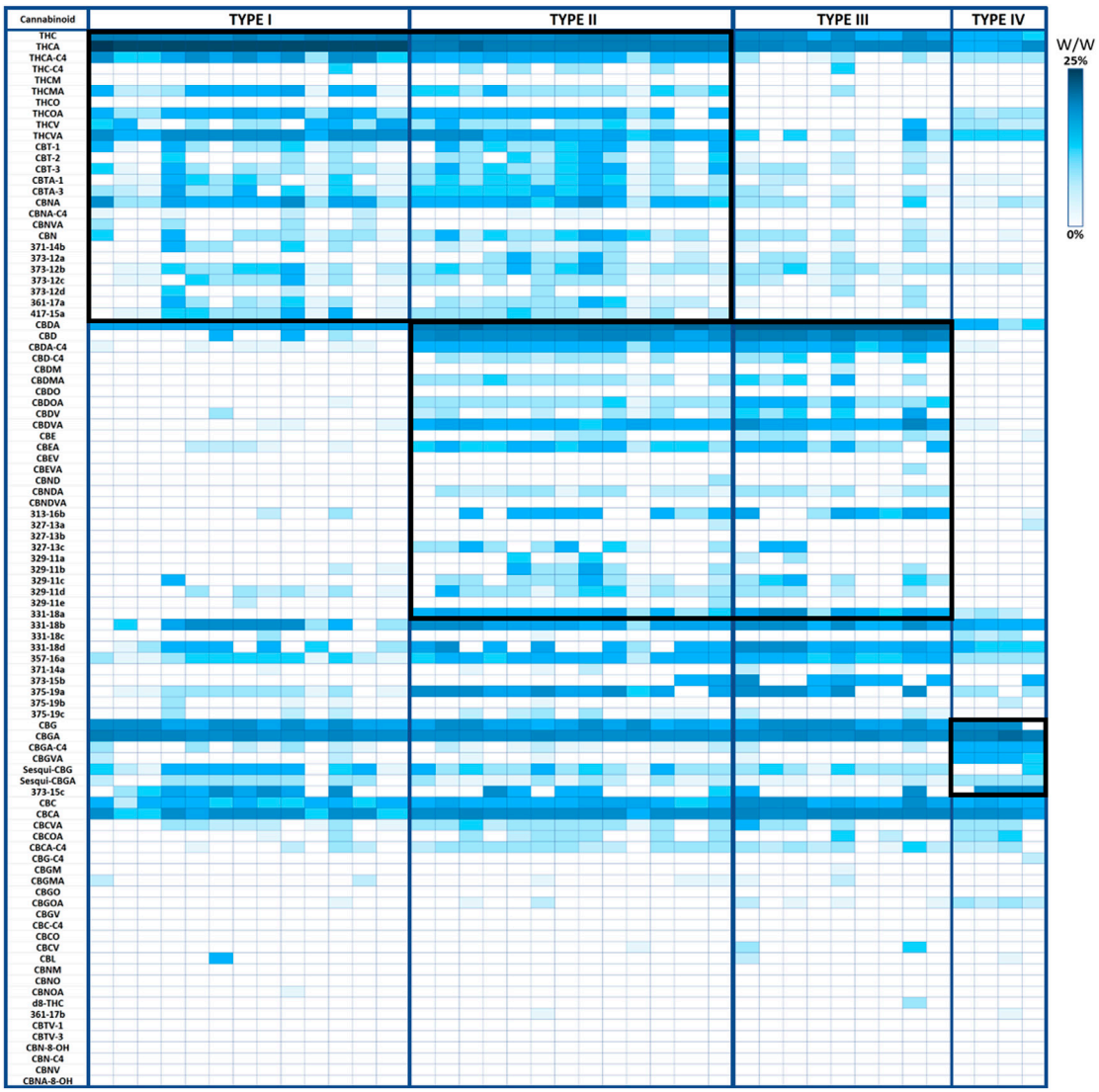


FIGURE 2 | Minor phytocannabinoids are associated with Type I, Type III and Type IV chemovars. Heatmap presenting the concentration of phytocannabinoids (% weight per weight) divided by chemovars. Type I chemovars defined THCA >20% (*n* = 13), Type III chemovars defined CBDA >15% (*n* = 9), Type II defined THCA >4% and CBDA >10% (*n* = 13), type IV defined CBGA >6% (*n* = 4). Groups of unique phytocannabinoids are depicted by a surrounding black square.

NEW ANALYTICAL APPROACHES FOR SECONDARY METABOLITES PROFILING

It is the phytocannabinoids, terpenoids, flavonoids and other constituents in *Cannabis*, as well as their interplay, that determines the medicinal outcomes and adverse effects. As there is wide variability in their contents in different *Cannabis* plants (Delgado-Povedano et al., 2019; Bautista et al., 2021), there is a great need for their accurate chemical analyses that will help better understand the complexity and diversity of *Cannabis* compounds. Identification and quantification of phytocannabinoids and flavonoids can be achieved via gas chromatography (GC), either coupled to a flame ionization detector or a mass-spectrometer (MS). However, there are a few limitations to this method, as some analytes may not be sufficiently separated and decomposition is required for accurate quantification. Therefore, an alternative method using ultra-high-performance liquid chromatography with an ultraviolet detector (UHPLC/UV) and electrospray ionization-liquid chromatography/mass spectrometry (ESI-LC/MS) (Berman et al., 2018) allows for a high-resolution separation of components, without decomposition or derivatization prior to analysis. While UV detection is more appropriate for abundant components having analytical standards (such as THC, CBD and their corresponding acids), the use of mass spectrometry allows comprehensive identification and quantification of additional molecules, both abundant and rare. Additionally, MS and MS/MS analyses enable the identification of unknown molecules and their semi-quantification. Reference MS/MS data for identification of phytocannabinoids is available for labs and experts for putative identification (Berman et al., 2018).

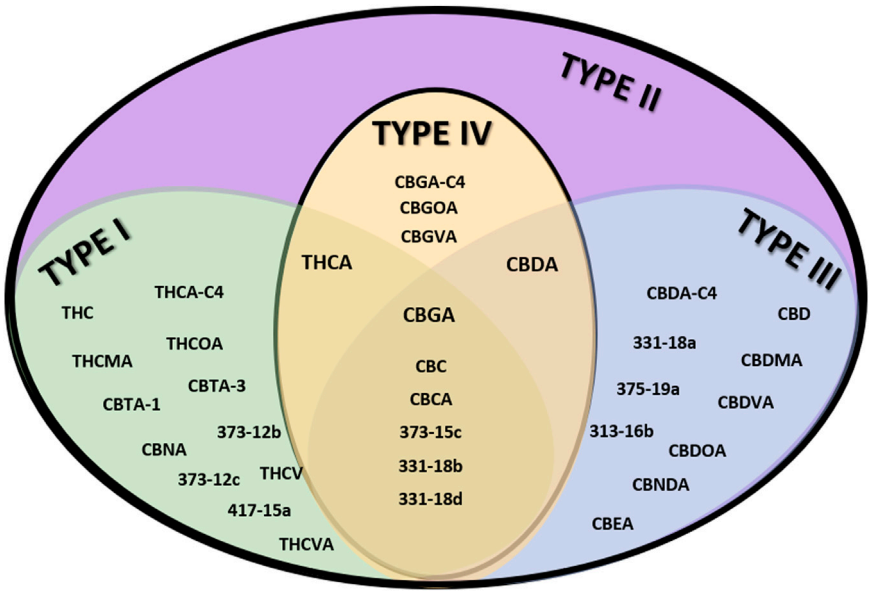


FIGURE 3 | Venn diagram of the distribution of particular phytocannabinoids to specific chemovars. Examples of unique phytocannabinoids per chemovar type are shown in the appropriate subgroup.

Terpenoids can be detected using static headspace gas chromatography-tandem MS (SHS-GC/MS/MS) (Shapira et al., 2019). Similar to phytocannabinoids, terpenoids with no commercially available analytical standards can still be semi-quantified relying on the calibration curves of molecules with standards and relying on both similar MS spectral characteristics and similar retention times (Lipson Feder et al., 2021).

STRAINS, CULTIVARS AND CHEMOVARS

Cannabis is one genus with one species, *sativa* L. (ElSohly and Slade, 2005), which is sometimes divided into subspecies including in addition to *sativa* also *indica* and *ruderalis*. These *Cannabis* subspecies are divided into hundreds of different *Cannabis* cultivars and hybrids. Cultivar stands for cultivated variety, a plant that has been selected for cultivation. A *Cannabis* strain refers to plants reproduced asexually from a cultivar through clonal propagation. *Cannabis* cultivars worldwide vary significantly in their chemical compositions. Therefore, a *Cannabis* chemovar refers to the chemical profile of the plant and is considered a more useful classification in medicine (Hazekamp and Fischedick, 2012). Medical *Cannabis* has been divided into three phenotypic chemovar groups according to its content of THC and CBD: Type I which is THC-predominant, Type II in which the two are balanced and Type III which is CBD-predominant (Hazekamp and Fischedick, 2012).

From the genotypic perspective, *Cannabis* chemovar classification involves two codominant alleles on locus B, allele B_T is specific to THCA and allele B_D is specific to CBDA (De Meijer et al., 2003). Thus, Type I chemovar is B_T/B_T, Type III is B_D/B_D and Type II is B_T/B_D. The nonfunctional allele B_0 does not allow for the conversion of the precursor CBGA into THCA or CBDA, and is sometimes referred to as Type IV chemovar, which is CBGA-predominant. An independent gene at locus C codes for CBCA synthase that produces CBCA from CBGA (Hand et al., 2016). Studies showed that type I chemovar dominates the markets, but often it is not as beneficial as the other chemovars in achieving the desired symptom relief (Lewis et al., 2018; Aviram et al., 2020a; Aviram et al., 2021b). Moreover, the minor phytocannabinoid are not randomly distributed between the different chemovar types. As is shown in the heatmap presented in **Figure 2**, phytocannabinoids from cannabitriol (CBT) and cannabinol (CBN) families are more abundant in Type I chemovars, as they are predominantly the degradation products of THC. They can also be found in type II chemovars, though their concentration would generally be lower due to limitation in the amount of available precursor. Similarly, phytocannabinoids from cannabielsoin (CBE) family are more abundant in Type III chemovars as they are the degradation products of CBD and can also be found in type II chemovars to a lesser extent. Type-IV chemovars contain unique phytocannabinoids from the cannabigerol (CBG) family and high levels of phytocannabinoids from the cannabichromene (CBC) family, as CBCA synthase is intact. These selective distributions among chemovars are the result of metabolic pathways unique to either THC or CBD, which are not found in type IV chemovars. The distribution of particular phytocannabinoids according to chemovar is presented in **Figure 3**. Variations in the minor phytocannabinoid contents of different *Cannabis* extracts lead to varied effects on the eCBS, stressing the importance of their characterization

TABLE 1 | Variability of phytocannabinoids in 320 different cultivars.

Presented as concentration values (%w/w)		Max	Min	Average	Std dev
1. Cannabigerol (CBG) type					
Acids	CBGA	6.182	0.012	0.400	0.464
	CBGA-C4	0.028	0.000	0.001	0.002
	CBGVA	0.024	0.000	0.000	0.002
	CBGOA	0.004	0.000	0.000	0.000
	CBGMA	0.003	0.000	0.000	0.000
	Sesqui-CBGA	0.006	0.000	0.001	0.001
Neutrals	CBG	0.735	0.000	0.084	0.067
	CBG-C4	0.001	0.000	0.000	0.000
	CBGV	0.003	0.000	0.000	0.000
	CBGO	0.000	0.000	0.000	0.000
	CBGM	0.000	0.000	0.000	0.000
	Sesqui-CBG	0.042	0.000	0.008	0.006
2. Δ^9-trans-tetrahydrocannabinol (Δ^9-THC) type					
Acids	THCA	24.325	0.124	10.390	6.425
	THCA-C4	0.192	0.000	0.044	0.036
	THCVA	1.120	0.000	0.124	0.135
	THCOA	0.113	0.000	0.021	0.021
	THCMA	0.062	0.000	0.011	0.011
Neutrals	THC′	7.058	0.000	0.948	1.076
	THC-C4	0.062	0.000	0.003	0.008
	THCV	0.147	0.000	0.007	0.016
	THCO	0.000	0.000	0.000	0.000
	THCM	0.000	0.000	0.000	0.000
3. Cannabidiol (CBD) type					
Acids	CBDA	18.351	0.000	3.085	4.968
	CBDA-C4	0.094	0.000	0.009	0.016
	CBDVA	1.096	0.000	0.041	0.129
	CBDOA	0.053	0.000	0.003	0.007
	CBDMA	0.011	0.000	0.001	0.002
Neutrals	CBD	2.676	0.000	0.166	0.363
	CBD-C4	0.068	0.000	0.001	0.004
	CBDV	0.105	0.000	0.002	0.010
	CBDO	0.003	0.000	0.000	0.000
	CBDM	0.007	0.000	0.000	0.000
4. Cannabichromene (CBC) type					
Acids	CBCA	2.835	0.003	0.251	0.284
	CBCA-C4	0.006	0.000	0.001	0.001
	CBCVA	0.083	0.000	0.003	0.010
	CBCOA	0.018	0.000	0.001	0.002
Neutrals	CBC	0.830	0.000	0.034	0.055
	CBC-C4	0.001	0.000	0.000	0.000
	CBCV	0.014	0.000	0.000	0.001
	CBCO	0.000	0.000	0.000	0.000
5. Cannabinol (CBN) type					
Acids	CBNA	0.499	0.000	0.066	0.081
	CBNA-C4	0.002	0.000	0.000	0.000
	CBNVA	0.006	0.000	0.000	0.001
	CBNOA	0.001	0.000	0.000	0.000
	CBNA-8-OH	0.000	0.000	0.000	0.000
Neutrals	CBN	0.721	0.000	0.017	0.049
	CBN-C4	0.001	0.000	0.000	0.000
	CBNV	0.002	0.000	0.000	0.000
	CBNO	0.000	0.000	0.000	0.000
	CBNM	0.000	0.000	0.000	0.000
	CBN-8-OH	0.001	0.000	0.000	0.000

(Continued on following page)

TABLE 1 | (*Continued*) Variability of phytocannabinoids in 320 different cultivars.

Presented as concentration values (%w/w)		Max	Min	Average	Std dev
6. Δ^8-trans-tetrahydrocannabinol (Δ^8-THC) type					
Neutral	d8-THC	0.137	0.000	0.001	0.012
7. Cannabicyclol (CBL) type					
Neutral	CBL	0.040	0.000	0.000	0.003
8. Cannabinodiol (CBND) type					
Acids	CBNDA	0.014	0.000	0.001	0.002
	CBNDVA	0.000	0.000	0.000	0.000
Neutral	CBND	0.127	0.000	0.002	0.011
9. Cannabielsoin (CBE) type					
Acids	CBEA	0.056	0.000	0.004	0.008
	CBEVA	0.001	0.000	0.000	0.000
Neutrals	CBE	0.007	0.000	0.000	0.001
	CBEV	0.008	0.000	0.000	0.000
10. Cannabitriol (CBT) type					
Acids	CBTA-1	0.203	0.000	0.005	0.013
	CBTA-3	0.084	0.000	0.009	0.012
Neutrals	CBT-1	0.220	0.000	0.013	0.020
	CBTV-1	0.011	0.000	0.000	0.001
	CBT-3	0.172	0.000	0.009	0.015
	CBTV-3	0.010	0.000	0.000	0.001
	CBT-2	0.046	0.000	0.004	0.007

n = 320 *inflorescences from cultivars*; *results are concentration values of phytocannabinoids per plant (%w/w).*

in assessing cannabis effectivity (Berman et al., 2020). The high variability in the concentration of phytocannabinoid from 10 subclasses in their acidic and neutral forms in the inflorescences of 320 different cultivars is presented in **Table 1**.

In addition, the *Cannabis* plant contains an overwhelming milieu of terpenoids, but only a limited number are currently reported and used for metabolic analyses of *Cannabis* chemovars (Shapira et al., 2019). Terpenoids content in different cultivars of *Cannabis* is highly variable, with some terpenoids being more associated with specific cultivars (Hillig, 2004; Casano et al., 2011). Studies that assessed terpenoid metabolism found the monoterpenoids limonene, β-myrcene, terpinolene and α-pinene, and the sesquiterpenoids β-caryophyllene and humulene, were abundant in the majority of *Cannabis* chemovars (Henry et al., 2018; Lewis et al., 2018). Some terpenoids were predominantly found only in Type I chemovars and others only in Type III, suggesting joint metabolic pathways and chemovar-specific aroma and effects (Lewis et al., 2018). **Table 2** summarizes the variability of monoterpenoids and sesquiterpenoids in 79 distinct *Cannabis* inflorescences (out of the 320 described for phytocannabinoids in **Table 1**).

Each *Cannabis* cultivar contains a different profile of more than 500 secondary metabolites (ElSohly and Slade, 2005; Andre et al., 2016; Berman et al., 2018; Piper, 2018). The fact that hundreds of different *Cannabis* cultivars and hybrids exist worldwide, varying significantly in their chemical compositions, makes *Cannabis* treatment highly complex. Moreover, sometimes the outcome of treatment with medical *Cannabis* depends on the way its secondary metabolites act together synergistically, in a mechanism first described by Ben-Shabat and Mechoulam for eCBs (Ben-Shabat et al., 1998) and later postulated by Russo as the 'entourage effect' for phytocannabinoids (Russo, 2011). Thus, phytocannabinoids that are found together in a *Cannabis* chemovar modulate each other's activity and thus the overall effect. The entourage effect postulates that the presence of minor phytocannabinoids, terpenoids and other plant metabolites contributes to the overall response in a way that significantly modulates the effects of the main active components, THC and CBD, and thereby produces more potent or more selective effects. Several studies have shown whole extracts or a combination of THC and CBD, with either each other, minor phytocannabinoids or terpenoids, are more effective than the corresponding major phytocannabinoid in producing the same response (Russo, 2011; Velasco et al., 2016; Blasco-Benito et al., 2018; Baram et al., 2019; Namdar et al., 2019; Ferber et al., 2020). However, other studies did not find evidence that common terpenoids can bind eCBS receptors or modulate the effect of phytocannabinoids on the receptors (Santiago et al., 2019; Finlay et al., 2020; Heblinski et al., 2020). A better understanding of the different components in *Cannabis* and the way they act together is required to fully utilize its therapeutic potential to the fullest.

TABLE 2 | Variability of terpenoids in 79 different cultivars.

Compound	Max (ppm)	Min (ppm)	Average (ppm)	Std dev (ppm)	V (%)
α-Pinene	1903.4	2.3	181.7	357.5	196.8
Camphene	161.6	1.7	18.3	34.0	186.3
Sabinene	3.7	0.0	0.9	1.1	126.3
β-Pinene	1705.3	2.2	132.1	259.9	196.8
β-Myrcene	>2,706	5.1	444.3	706.4	159.0
3δ-Carene	530.3	0.0	10.1	62.7	622.3
α-Phellandrene	701.5	0.0	14.0	80.5	574.7
α-Terpinene	379.0	0.0	14.9	51.1	343.1
Limonene	>2,760	2.7	247.5	577.7	233.5
β-Phellandrene	421.1	0.0	16.9	55.8	330.6
cis-Ocimene	101.6	0.0	4.3	12.9	302.4
Eucalyptol	63.6	0.0	7.3	11.9	162.9
p-Cymene	28.7	0.0	2.2	4.3	192.5
trans-Ocimene	1,648.5	0.0	62.9	237.6	377.8
γ-Terpinene	512.2	1.5	16.4	60.7	369.2
Terpinolene	>2,433	2.4	96.2	394.4	410.1
Linalool	1,204.4	0.0	214.1	249.8	116.7
Fenchone	68.0	0.0	8.9	12.5	139.5
Fenchol	953.7	0.0	118.1	145.4	123.1
$C_{10}H_{18}O$-154 (99/93/79/121)-1*	222.6	0.0	25.8	43.3	168.3
$C_{10}H_{18}O$-154 (99/93/79/121)-2*	29.3	0.0	0.4	3.3	0.0
Menthol	62.1	0.0	5.6	12.9	230.7
Borneol	941.0	0.0	59.8	123.7	206.7
Camphor	20.4	0.0	1.0	2.6	257.5
Terpinen-4-ol	149.3	0.0	17.9	29.3	163.2
α-Terpineol	1,027.8	0.0	98.1	148.3	151.1
Citronellol	129.1	0.0	12.7	25.9	204.3
Nerol	26.2	0.0	2.5	5.4	218.6
Geraniol	93.8	0.0	4.1	14.1	347.1
Bornyl acetate	37.2	0.0	2.8	6.6	236.5
α-Cubebene*	8.2	0.0	2.2	1.9	110.8
Isoledene	7.7	0.0	0.1	0.9	885.1
Cyclosativene	11.6	0.0	0.2	1.3	728.5
Ylangene*	74.5	0.0	5.6	9.8	176.4
α-Copaene*	12.9	0.0	2.2	2.4	118.0
α-Funedrene	1.8	0.0	0.9	0.5	146.0
7-epi-Sesquithujene*	76.3	0.0	13.3	15.4	116.3
$C_{15}H_{24}$-204 (105/(120+119)/161)*	13.4	0.0	2.8	3.2	114.7
Sativene	2.5	0.0	1.0	1.1	106.4
β-Cubebene*	5.2	0.0	0.1	0.6	886.1
Sesquithujene*	116.0	0.0	14.9	17.6	118.4
β-Isocomene*	41.3	0.0	6.7	8.5	126.1
α-Santalene*	71.5	0.0	7.7	11.0	142.8
cis-α-Bergamotene*	23.6	0.0	3.9	4.4	113.2
α-Cedrene	3.9	0.0	1.1	1.1	104.7
trans-α-Bergamotene*	63.6	0.0	7.0	13.8	196.5
β-Caryophyllene	>3,631.5	9.0	670.8	781.0	116.4
Geranyl acetate	1.8	0.0	0.4	0.6	144.7
β-Cedrene	18.5	0.0	1.0	2.9	284.5
α-Guaiene*	567.3	0.0	58.6	107.6	183.7
γ-Elemene*	161.1	0.0	11.9	24.1	202.5
Aromadendrene	8.3	0.0	1.9	2.2	120.3
β-Santalene*	39.2	0.0	3.0	5.7	187.8
Guaia-6,9-diene*	65.4	0.0	7.3	12.5	171.3
trans-β-Farnesene	617.3	3.2	44.5	71.7	161.4
$C_{15}H_{24}$-204 (69/91/105/161)*	25.7	0.0	3.9	6.3	160.6
$C_{15}H_{24}$-204 (91/105/161)*	302.3	0.0	12.3	34.2	279.0
$C_{15}H_{24}$-204 (161/105/133/91)*	44.4	0.0	9.1	11.0	121.5
$C_{15}H_{24}$-204 (105/91/133/161/189)*	44.2	0.0	9.5	11.0	115.9
α-Humulene	2,134.2	12.5	255.8	283.9	111.0
Alloaromadendrene	104.1	0.0	12.0	17.3	143.7
Acoradiene*	10.5	0.0	1.2	2.3	195.4
$C_{15}H_{24}$-204 (105)-1*	35.4	0.0	8.3	10.3	123.6
γ-Curcumene*	432.5	0.0	9.7	48.6	500.5

(Continued on following page)

TABLE 2 | (*Continued*) Variability of terpenoids in 79 different cultivars.

Compound	Max (ppm)	Min (ppm)	Average (ppm)	Std dev (ppm)	V (%)
$C_{15}H_{24}$-204 (189/133)-1*	101.4	0.0	19.9	25.9	130.2
Sesquisabinene*	56.8	0.0	5.0	8.8	173.6
γ-Muurelene*	60.9	0.0	7.6	10.7	140.2
α-Amorphene*	27.0	0.0	6.7	7.5	112.5
Aristolochene*	14.6	0.0	1.8	2.7	152.4
Germacrene D*	28.3	0.0	4.3	7.7	180.4
β-Chamigrene	16.3	0.0	0.5	2.6	488.0
$C_{15}H_{24}$-204 (189/133)-2*	196.3	0.0	44.0	46.9	106.6
$C_{15}H_{24}$-204 (119/93/161)*	28.1	0.0	3.6	5.7	157.4
α-Selinene*	92.0	0.0	16.7	21.1	126.4
Ledene	6.0	0.0	0.1	0.7	688.9
α-Curcumene	69.9	0.0	9.8	17.6	180.2
Valencene	402.8	0.0	26.6	80.5	302.9
β-Selinene*	716.9	0.0	133.0	184.3	138.5
α-Farnesene*	88.7	0.0	6.8	13.4	196.4
β-Bisabolene*	663.2	0.0	42.7	87.0	204.0
δ-Guaiene*	560.0	0.0	47.2	96.5	204.3
$C_{15}H_{24}$-204 (119/161/105/134)*	32.6	0.0	6.2	7.8	125.4
β-Curcumene	27.9	0.0	5.0	6.6	130.0
Dihydroagarofuran*	15.3	0.0	2.2	3.2	145.7
$C_{15}H_{24}$-204 (similar Germarcene B)*	32.7	0.0	8.5	9.5	111.7
Sesquicineole*	135.1	0.0	9.4	16.5	175.2
Eremophilene*	38.6	0.0	9.0	11.5	128.6
β-Sesquiphellandrene*	77.7	0.0	9.3	14.0	149.9
γ-Cadinene*	22.3	0.0	4.5	5.8	128.7
δ-Cadinene*	27.4	0.0	7.0	6.7	95.8
$C_{15}H_{24}$-204 (105)-2*	28.9	0.0	7.3	8.7	118.6
α-Panasinsene*	31.3	0.0	1.3	3.9	288.6
trans-α-Bisabolene*	512.1	0.0	86.2	97.9	113.6
Selina-3,7 (11)-diene*	>1,334.1	0.0	249.3	361.5	145.0
trans-Nerolidol	1,637.2	0.0	102.1	240.3	235.3
Germacrene B*	923.0	0.0	25.7	107.1	417.0
Globulol	31.2	0.0	0.5	3.6	698.6
Guaiol	>2099	0.0	568.1	765.5	134.7
Caryophyllene oxide	>1890	11.2	308.5	488.4	158.3
α-epi-7-epi-5-Eudesmol*	319.1	0.0	30.8	47.5	154.2
$C_{15}H_{26}O$-222 (similar γ-Eudesmol)*	>2099	0.0	541.8	751.1	138.6
Selina-6-en-4-ol*	180.3	0.0	29.8	46.0	154.2
γ-Eudesmol*	>1,588	0.0	296.1	478.4	161.6
Hinesol*	196.1	0.0	33.1	39.2	118.5
$C_{15}H_{26}O$-222 (105/161/59)-1*	496.1	0.0	63.8	108.8	170.5
Agarospirol*	158.1	0.0	14.7	26.2	178.5
$C_{15}H_{26}O$-222 (105/161/59)-2*	812.2	0.0	78.3	135.2	172.5
$C_{15}H_{26}O$-222 (59/81/107/149/161)*	566.6	0.0	66.1	96.2	145.6
α-Eudesmol*	>1,588	0.0	377.3	541.5	143.5
β-Eudesmol	>1,588	0.0	434.2	573.0	132.0
7-epi-α-Eudesmol*	573.7	0.0	61.0	108.5	177.9
Bulnesol*	>2099	0.0	159.9	318.0	198.9
α-Bisabolol	>3,791	0.0	1,515.7	1,592.2	105.0
Total monoterpenoids [ppm]	18,783.3	44.5	1842.0	2,896.2	157.2
Total monoterpenoids [%]	1.88	0.00	0.18	0.29	0.02
Total sesquiterpenoids [ppm]	25,135.2	147.6	6,678.2	5,089.5	76.2
Total sesquiterpenoids [%]	2.51	0.01	0.67	0.51	0.01
Total terpenoids [ppm]	26,501.4	196.1	8,520.2	6,047.6	71.0
Total terpenoids [%]	2.65	0.02	0.85	0.60	0.01

n = *79 inflorescences from cultivars; ppm–parts per million, > values above upper limit of detection, % represents concentration values of terpenoids per plant, * terpenoids that were semi-quantified.*

PRE- AND POST-HARVEST CONDITIONS

The concentrations of the different compounds in the plant depend on many factors. There is a strong genotypic influence on the composition of secondary metabolites in different *Cannabis* chemovars (Aizpurua-Olaizola et al., 2016; Welling et al., 2018; McGarvey et al., 2020). However, a very large variation exists also in the profiles of genetically identical

plants grown under different conditions (De Backer et al., 2009). For example, we previously showed the differences in phytocannabinoids profiles of a high-CBD *Cannabis* chemovar that was used to treat refractory childhood epilepsy in Israel (Berman et al., 2018). While the genetically identical plants from four different greenhouses were planted and harvested in the same way and at the same time, and considered as the same treatment, their CBDA contents were similar but they portrayed substantial differences in many other phytocannabinoids.

In addition to the genetic variety, many environmental factors affect the composition of the secondary metabolites in the *Cannabis* plant (Tang et al., 2016). These include growth conditions such as humidity, light quality and intensity, CO_2 concentration and mineral nutrition (Chandra et al., 2008; Chandra et al., 2017; Bernstein et al., 2019a). The tissue type is also an important factor as within the plant there is a location- and organ-specific distribution of the active secondary metabolites (Happyana et al., 2013; Bernstein et al., 2019a; Bernstein et al., 2019b). Phytocannabinoids are synthesized in glandular trichomes that are located in the highest density on the inflorescences of unfertilized female plants (Lipson Feder et al., 2021), and their accumulation varies in the different aerial parts (flowers, fan leaves, inflorescence leaves, stalk and stem). Accumulation patterns also depend on the age of that part (Flores-Sanchez and Verpoorte, 2008b; Hazekamp and Fischedick, 2012). A study that tested phytocannabinoid and terpenoid content in the plant from the rooting until the end of the flowering stage (Aizpurua-Olaizola et al., 2016) found that the accumulation of some major phytocannabinoids and monoterpenoids requires longer growth time in plants from Type II and Type III chemovars than in Type I. The functional roles of phytocannabinoids and terpenoids *in planta* are still not fully elucidated as well as the biosynthesis pathways involved in their production and the mechanisms of localization and secretion. Cannflavins accumulation also varies depending on the part of the plant, they are found in most parts, including the leaves and inflorescences, but are undetectable in roots and seeds (Flores-sanchez and Verpoorte, 2008b). Interestingly, all three cannflavins A-C were found in greater amounts in genetically identical *Cannabis* plants grown at a higher altitude (Giupponi et al., 2020).

Importantly, the composition and concentration of the different secondary metabolites are also affected by harvest time (Happyana and Kayser, 2016) and change over time postharvest as a result of different degradation routes, depending on the storage conditions and its duration (Trofin et al., 2011; Jin et al., 2019; Zamengo et al., 2019; Milay et al., 2020). The concentrations of terpenoids rapidly decline in storage due to their volatile nature (Milay et al., 2020). For phytocannabinoids, one of the main processes that occur during storage is decarboxylation. Over time due to heat and light, the acidic forms undergo spontaneous decarboxylation, but the extent of which is not uniform. For example, THC is the neutral counterpart of THCA. However, THCA is only partially converted to THC and to varying degrees (Dussy et al., 2005; Jung et al., 2009). THCA has different biological characteristics than THC, it is not psychoactive and has a distinctive pharmacological activity (Moreno-Sanz, 2016). Several studies reported on the therapeutic activities of phytocannabinoids in their acidic form. For example, CBDA was found to be a more potent antiemetic and anticonvulsant agent than CBD *in-vivo* (Bolognini et al., 2013; Anderson et al., 2019), as well as a better inhibitor of breast cancer cell migration *in-vitro* (Takeda et al., 2012). Therefore, the relative ratio between THCA and THC, or between CBDA and CBD, has a therapeutic implication that has yet to be fully elucidated. For phytocannabinoids, the content of CBN is used as a marker for *Cannabis* aging, however, it is not a relevant marker in Type III chemovars (Milay et al., 2020) as it is formed mainly via the oxidation of THC or the decarboxylation of its acidic form cannabinolic acid (CBNA), which in turn rises from the oxidation of THCA.

In a study that tested the optimal postharvest processing, solvents and a range of temperatures, it was concluded that the conditions that best preserved the composition of the secondary metabolites relative to their pre-storage composition were unextracted whole inflorescences at 4°C (Milay et al., 2020). The duration of storage, as well as of drying and curing before storage, varies greatly; as a consequence, a very large variation exists in the phytocannabinoid and terpenoid profiles of *Cannabis* chemovars that are considered the same.

DELIVERY ROUTES

As the active biomolecules in *Cannabis* such as phytocannabinoids are highly lipophilic and therefore present poor oral bioavailability, various administration routes have been investigated for the therapeutic use of *Cannabis*, including the pulmonary, sublingual, oral, dermal and rectal routes (Bruni et al., 2018). Currently, the common administration routes of whole-plant and plant-derived *Cannabis* products are either by inhalation (smoking or vaporization) or ingestion of edibles (Hazekamp et al., 2013; Bridgeman and Abazia, 2017). However, the pharmacokinetics and the effects observed with *Cannabis* administration vary significantly as a function of the delivery route, formulation, and the ratios between the multiple active compounds. For example, the acidic pH of the stomach further reduces bioavailability via the oral route (Grotenhermen, 2003). Moreover, to be used via the oral or sublingual routes, the active secondary metabolites in the plant must be extracted. The extraction method and choice of extracting solvent affect the secondary metabolite profile (Křížek et al., 2018), a phenomenon which was shown for phytocannabinoids (Turner et al., 2017; Namdar et al., 2019), terpenoids (Shapira et al., 2019) and flavonoids (Isidore et al., 2021).

Inhalation provides a rapid and efficient method of drug delivery. Symptom relief is immediate and effective, the dosage can be more controlled than via the alternative routes, and a lower dose can be used to get the desired effect (Foster et al., 2019). However, inhalation has several considerable disadvantages; it leads to high and prompt peak plasma concentration of cannabinoids such as THC and CBD post inhalation (Huestis, 2005), causing a more intense and shorter-lasting effect than other routes, which in turn may be associated with higher toxicity (Dinis-oliveira, 2016). Smoking is associated with health risks and the formation of toxic and carcinogenic substances during combustion (Gates et al., 2014), vaporizers do not heat

Cannabis to the point of combustion (i.e., less than 170–190°C), but still induce heat and expose to a variety of undesirable chemicals (Grotenhermen, 2003; Shiplo et al., 2016). All the bioactive molecules of *Cannabis* are susceptible to degradation processes such as decarboxylation when *Cannabis* is heated above 120°C by smoking or vaping, as well as by cooking (Dussy et al., 2005).

The pharmacokinetics of the current consumption options modulates and limits the therapeutic bioavailability of *Cannabis* metabolites. For example, when THC is ingested rather than inhaled, it is metabolized by the liver before entering the bloodstream and hydroxylated to 11-hydroxy-THC, which is equally potent (Perez-Reyes et al., 1972; Hollister, 1974) or might be even more potent than THC (Christensen et al., 1971; Schwilke et al., 2009), and then further oxidized to the inactive metabolite 11-COOH-THC. This makes the consideration of the *Cannabis* delivery system vital for its effective administration and treatment (Uziel et al., 2020).

New analytical approaches now allow for more accurate profiling of *Cannabis* metabolites both in the plant itself and in the tissues they affect, allowing to better investigate their disposition over time by the body of the organism (Huestis, 2007). Many of the alternative routes to inhalation and digestion are aimed at improving the bioavailability via avoiding degradation with first-pass metabolism by the liver. Other delivery routes that have yet to be explored are intravenous, intramuscular and intranasal. Emulsions via nanotechnology advances are also aimed at improving the bioavailability of the active molecules in *Cannabis* (Holgado et al., 2017; Adusumilli et al., 2021).

DISCUSSION AND FUTURE PERSPECTIVES

The use of medical *Cannabis* is ever increasing in the treatment of numerous conditions as it has been proven to be both effective and safe, but the *Cannabis* plant contains more than 500 different components, each with potential therapeutic qualities. The components of *Cannabis* act together, hitting several targets at once and mutually enhancing each other's activity so that the overall outcome is greater than that of their additive effect. The concentrations and combinations of the various secondary metabolites, including the way they complement each other, determine both the final medicinal response and adverse effects.

Cannabis can treat a multitude of very different conditions as it exerts its effects via the ECS, which is involved in many physiological processes. *Cannabis* treatment can be personalized to both the condition and the person to improve treatment outcomes while also reducing the drug load and minimizing the adverse effects. Most patients do not receive *Cannabis*-based medication but rather whole plants or extracts that contain many active bio-compounds in different proportions. Each has a different profile of components, undergoing different drug interactions. It is still unknown which molecules in the whole extract are responsible for its overall effect and via which ECS receptors, effectors and metabolic pathways. Further research is needed to find which whole extracts or specific molecules are best suited to treat a given condition.

Physicians and patients require more information to guide them in choosing the most appropriate cultivar or molecules, in the correct dose and via the optimal delivery route. The number of studies that tested different cannabinoids or tried to recognize the specific bioactive molecules from whole extracts is low and should be addressed to fulfill the full potential of *Cannabis* and improve human health.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

We thank Almog Uziel for comments on the manuscript.

REFERENCES

Abraham, A. D., Leung, E. J. Y., Wong, B. A., Rivera, Z. M. G., Kruse, L. C., Clark, J. J., et al. (2020). Orally Consumed Cannabinoids Provide Long-Lasting Relief of Allodynia in a Mouse Model of Chronic Neuropathic Pain. *Neuropsychopharmacology* 45, 1105–1114. doi:10.1038/s41386-019-0585-3

Adams, R., Hunt, M., and Clark, J. H. (1940). Structure of Cannabidiol, a Product Isolated from the Marihuana Extract of Minnesota Wild Hemp. I. *J. Am. Chem. Soc.* 62, 196–200. doi:10.1021/ja01858a058

Adusumilli, N. C., Hazuka, E. L., and Friedman, A. J. (2021). Nanotechnology to Deliver Cannabinoids in Dermatology. *Precis. Nanomedicine* 4, 787–794. doi:10.33218/001c.24597

Aizpurua-Olaizola, O., Soydaner, U., Öztürk, E., Schibano, D., Simsir, Y., Navarro, P., et al. (2016). Evolution of the Cannabinoid and Terpene Content during the Growth of Cannabis Sativa Plants from Different Chemotypes. *J. Nat. Prod.* 79, 324–331. doi:10.1021/acs.jnatprod.5b00949

Anderson, L. L., Low, I. K., Banister, S. D., McGregor, I. S., and Arnold, J. C. (2019). Pharmacokinetics of Phytocannabinoid Acids and Anticonvulsant Effect of Cannabidiolic Acid in a Mouse Model of Dravet Syndrome. *J. Nat. Prod.* 82, 3047–3055. doi:10.1021/acs.jnatprod.9b00600

Andre, C. M., Hausman, J. F., and Guerriero, G. (2016). Cannabis Sativa: The Plant of the Thousand and One Molecules. *Front. Plant Sci.* 7, 19. doi:10.3389/fpls.2016.00019

Aviram, J., Lewitus, G. M., Vysotski, Y., Uribayev, A., Procaccia, S., Cohen, I., et al. (2020a). Short-term Medical Cannabis Treatment Regimens Produced Beneficial Effects Among Palliative Cancer Patients. *Pharmaceuticals (Basel)* 13, 1–16. doi:10.3390/ph13120435

Aviram, J., Vysotski, Y., Berman, P., Lewitus, G. M., Eisenberg, E., and Meiri, D. (2020b). Migraine Frequency Decrease Following Prolonged Medical Cannabis Treatment: A Cross-Sectional Study. *Brain Sci.* 10, 360. doi:10.3390/brainsci10060360

Aviram, J., Lewitus, G. M., Pud, D., Procaccia, S., Berman, P., Yellin, B., et al. (2021a). Specific Phytocannabinoid Compositions Are Associated with Analgesic Response and Adverse Effects in Chronic Pain Patients Treated with Medical Cannabis. *Pharmacol. Res.* 169, 105651. doi:10.1016/j.phrs.2021.105651

Aviram, J., Pud, D., Gershoni, T., Schiff-Keren, B., Ogintz, M., Vulfsons, S., et al. (2021b). Medical Cannabis Treatment for Chronic Pain: Outcomes and Prediction of Response. *Eur. J. Pain* 25, 359–374. doi:10.1002/ejp.1675

Baram, L., Peled, E., Berman, P., Yellin, B., Besser, E., Benami, M., et al. (2019). The Heterogeneity and Complexity of *Cannabis* Extracts as Antitumor Agents. *Oncotarget* 10, 4091–4106. doi:10.18632/oncotarget.26983

Bautista, J. L., Yu, S., and Tian, L. (2021). Flavonoids in Cannabis Sativa: Biosynthesis, Bioactivities, and Biotechnology. *ACS Omega* 6, 5119–5123. doi:10.1021/acsomega.1c00318

Belardo, C., Iannotta, M., Boccella, S., Rubino, R. C., Ricciardi, F., Infantino, R., et al. (2019). Oral Cannabidiol Prevents Allodynia and Neurological Dysfunctions in a Mouse Model of Mild Traumatic Brain Injury. *Front. Pharmacol.* 10, 352–411. doi:10.3389/fphar.2019.00352

Ben-Shabat, S., Fride, E., Sheskin, T., Tamiri, T., Rhee, M. H., Vogel, Z., et al. (1998). An Entourage Effect: Inactive Endogenous Fatty Acid Glycerol Esters Enhance 2-Arachidonoyl-Glycerol Cannabinoid Activity. *Eur. J. Pharmacol.* 353, 23–31. doi:10.1016/S0014-2999(98)00392-6

Berman, P., Futoran, K., Lewitus, G. M., Mukha, D., Benami, M., Shlomi, T., et al. (2018). A New ESI-LC/MS Approach for Comprehensive Metabolic Profiling of Phytocannabinoids in Cannabis. *Sci. Rep.* 8, 14280–14315. doi:10.1038/s41598-018-32651-4

Berman, P., Sulimani, L., Gelfand, A., Amsalem, K., Lewitus, G. M., and Meiri, D. (2020). Cannabinoidomics - An Analytical Approach to Understand the Effect of Medical Cannabis Treatment on the Endocannabinoid Metabolome. *Talanta* 219, 121336. doi:10.1016/j.talanta.2020.121336

Bernstein, N., Gorelick, J., and Koch, S. (2019a). Interplay between Chemistry and Morphology in Medical Cannabis (Cannabis Sativa L.). *Ind. Crops Prod.* 129, 185–194. doi:10.1016/j.indcrop.2018.11.039

Bernstein, N., Gorelick, J., Zerahia, R., and Koch, S. (2019b). Impact of N, P, K, and Humic Acid Supplementation on the Chemical Profile of Medical Cannabis (Cannabis Sativa L). *Front. Plant Sci.* 10, 736. doi:10.3389/fpls.2019.00736

Blasco-Benito, S., Seijo-Vila, M., Caro-Villalobos, M., Tundidor, I., Andradas, C., García-Taboada, E., et al. (2018). Appraising the "entourage Effect": Antitumor Action of a Pure Cannabinoid versus a Botanical Drug Preparation in Preclinical Models of Breast Cancer. *Biochem. Pharmacol.* 157, 285–293. doi:10.1016/j.bcp.2018.06.025

Bolognini, D., Rock, E. M., Cluny, N. L., Cascio, M. G., Limebeer, C. L., Duncan, M., et al. (2013). Cannabidiolic Acid Prevents Vomiting in Suncus Murinus and Nausea-Induced Behaviour in Rats by Enhancing 5-HT1A Receptor Activation. *Br. J. Pharmacol.* 168, 1456–1470. doi:10.1111/bph.12043

Booth, J. K., Yuen, M. M. S., Jancsik, S., Madilao, L. L., Page, J. E., and Bohlmann, J. (2020). Terpene Synthases and Terpene Variation in Cannabis Sativa. *Plant Physiol.* 184, 130–147. doi:10.1104/PP.20.00593

Bridgeman, M. B., and Abazia, D. T. (2017). Medicinal Cannabis: History, Pharmacology, and Implications for the Acute Care Setting. *P T* 42, 180–188.

Bruni, N., Della Pepa, C., Oliaro-Bosso, S., Pessione, E., Gastaldi, D., and Dosio, F. (2018). Cannabinoid Delivery Systems for Pain and Inflammation Treatment. *Molecules* 23, 2478. doi:10.3390/molecules23102478

Calzolari, D., Magagnini, G., Lucini, L., Grassi, G., Appendino, G. B., and Amaducci, S. (2017). High Added-Value Compounds from Cannabis Threshing Residues. *Ind. Crops Prod.* 108, 558–563. doi:10.1016/j.indcrop.2017.06.063

Casano, S., Grassi, G., Martini, V., and Michelozzi, M. (2011). Variations in Terpene Profiles of Different Strains of Cannabis Sativa L. *Acta Hortic.* 925, 115–121. doi:10.17660/actahortic.2011.925.15

Casey, S. L., Atwal, N., and Vaughan, C. W. (2017). Cannabis Constituent Synergy in a Mouse Neuropathic Pain Model. *Pain* 158, 2452–2460. doi:10.1097/j.pain.0000000000001051

Chandra, S., Lata, H., Khan, I. A., and Elsohly, M. A. (2008). Photosynthetic Response of Cannabis Sativa L. To Variations in Photosynthetic Photon Flux Densities, Temperature and CO2 Conditions. *Physiol. Mol. Biol. Plants* 14, 299–306. doi:10.1007/s12298-008-0027-x

Chandra, S., Lata, H., Khan, I. A., and ElSohly, M. A. (2017). "Cannabis Sativa L.: Botany and Horticulture," in *Cannabis Sativa L.-Botany and Biotechnology* (Cham: Springer), 79–100. doi:10.1007/978-3-319-54564-6_3

Christensen, H. D., Freudenthal, R. I., Gidley, J. T., Rosenfeld, R., Boegli, G., Testino, L., et al. (1971). Activity of delta8- and delta9-tetrahydrocannabinol and Related Compounds in the Mouse. *Science* 172, 165–167. doi:10.1126/science.172.3979.165

Citti, C., Linciano, P., Panseri, S., Vezzalini, F., Forni, F., Vandelli, M. A., et al. (2019a). Cannabinoid Profiling of Hemp Seed Oil by Liquid Chromatography Coupled to High-Resolution Mass Spectrometry. *Front. Plant Sci.* 10, 120–217. doi:10.3389/fpls.2019.00120

Citti, C., Linciano, P., Russo, F., Luongo, L., Iannotta, M., Maione, S., et al. (2019b). A Novel Phytocannabinoid Isolated from Cannabis Sativa L. With an *In Vivo* Cannabimimetic Activity Higher Than Δ9-tetrahydrocannabinol: Δ9-Tetrahydrocannabiphorol. *Sci. Rep.* 9, 20335–20413. doi:10.1038/s41598-019-56785-1

Comelli, F., Giagnoni, G., Bettoni, I., Colleoni, M., and Costa, B. (2008). Antihyperalgesic Effect of a Cannabis Sativa Extract in a Rat Model of Neuropathic Pain: Mechanisms Involved. *Phytother Res.* 22, 1017–1024. doi:10.1002/ptr.2401

Consroe, P., Benedito, M. A., Leite, J. R., Carlini, E. A., and Mechoulam, R. (1982). Effects of Cannabidiol on Behavioral Seizures Caused by Convulsant Drugs or Current in Mice. *Eur. J. Pharmacol.* 83, 293–298. doi:10.1016/0014-2999(82)90264-3

De Backer, B., Debrus, B., Lebrun, P., Theunis, L., Dubois, N., Decock, L., et al. (2009). Innovative Development and Validation of an HPLC/DAD Method for the Qualitative and Quantitative Determination of Major Cannabinoids in Cannabis Plant Material. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 877, 4115–4124. doi:10.1016/j.jchromb.2009.11.004

de Fonseca, F. R., del Arco, I., Bermudez-Silva, F. J., Bilbao, A., Cippitelli, A., and Navarro, M. (2005). The Endocannabinoid System: Physiology and Pharmacology. *Alcohol Alcohol* 40, 2–14. doi:10.1093/alcalc/agh110

De Meijer, E. P., Bagatta, M., Carboni, A., Crucitti, P., Moliterni, V. M., Ranalli, P., et al. (2003). The Inheritance of Chemical Phenotype in Cannabis Sativa L. *Genetics* 163, 335–346. doi:10.1093/genetics/163.1.335

De Petrocellis, L., and Di Marzo, V. (2009). An Introduction to the Endocannabinoid System: from the Early to the Latest Concepts. *Best Pract. Res. Clin. Endocrinol. Metab.* 23, 1–15. doi:10.1016/j.beem.2008.10.013

De Petrocellis, L., Cascio, M. G., and Di Marzo, V. (2004). The Endocannabinoid System: A General View and Latest Additions. *Br. J. Pharmacol.* 141, 765–774. doi:10.1038/sj.bjp.0705666

Delgado-Povedano, M. M., Sánchez-Carnerero Callado, C., Priego-Capote, F., and Ferreiro-Vera, C. (2019). Untargeted Characterization of Extracts from Cannabis Sativa L. Cultivars by Gas and Liquid Chromatography Coupled to Mass Spectrometry in High Resolution Mode. *Talanta* 208, 120384. doi:10.1016/j.talanta.2019.120384

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., et al. (1992). Isolation and Structure of a Brain Constituent that Binds to the Cannabinoid Receptor. *Science* 258, 1946–1949. doi:10.1126/science.1470919

Di Marzo, V., Bifulco, M., and De Petrocellis, L. (2004). The Endocannabinoid System and its Therapeutic Exploitation. *Nat. Rev. Drug Discov.* 3, 771–784. doi:10.1038/nrd1495

Dinis-oliveira, R. J. (2016). Metabolomics of Δ9-tetrahydrocannabinol: Implications in Toxicity. *Drug Metab. Rev.* 48, 80–87. doi:10.3109/03602532.2015.1137307

Downer, E. J. (2020). Anti-inflammatory Potential of Terpenes Present in Cannabis Sativa L. *ACS Chem. Neurosci.* 11, 659–662. doi:10.1021/acschemneuro.0c00075

Dussy, F. E., Hamberg, C., Luginbühl, M., Schwerzmann, T., and Briellmann, T. A. (2005). Isolation of Delta9-THCA-A from Hemp and Analytical Aspects Concerning the Determination of Delta9-THC in Cannabis Products. *Forensic Sci. Int.* 149, 3–10. doi:10.1016/j.forsciint.2004.05.015

ElSohly, M. A., and Slade, D. (2005). Chemical Constituents of Marijuana: the Complex Mixture of Natural Cannabinoids. *Life Sci.* 78, 539–548. doi:10.1016/j.lfs.2005.09.011

Erridge, S., Mangal, N., Salazar, O., Pacchetti, B., and Sodergren, M. H. (2020). Cannflavins - from Plant to Patient: A Scoping Review. *Fitoterapia* 146, 104712. doi:10.1016/j.fitote.2020.104712

Ferber, S. G., Namdar, D., Hen-Shoval, D., Eger, G., Koltai, H., Shoval, G., et al. (2020). The "Entourage Effect": Terpenes Coupled with Cannabinoids for the Treatment of Mood Disorders and Anxiety Disorders. *Curr. Neuropharmacol.* 18, 87–96. doi:10.2174/1570159X17666190903103923

Finlay, D. B., Sircombe, K. J., Nimick, M., Jones, C., and Glass, M. (2020). Terpenoids from Cannabis Do Not Mediate an Entourage Effect by Acting at Cannabinoid Receptors. *Front. Pharmacol.* 11, 359. doi:10.3389/fphar.2020.00359

Flores-Sanchez, I. J., and Verpoorte, R. (2008a). PKS Activities and Biosynthesis of Cannabinoids and Flavonoids in Cannabis Sativa L. Plants. *Plant Cell Physiol.* 49, 1767–1782. doi:10.1093/pcp/pcn150

Flores-Sanchez, I. J., and Verpoorte, R. (2008b). Secondary Metabolism in Cannabis. *Phytochem. Rev.* 7, 615–639. doi:10.1007/s11101-008-9094-4

Foster, B. C., Abramovici, H., and Harris, C. S. (2019). Cannabis and Cannabinoids: Kinetics and Interactions. *Am. J. Med.* 132, 1266–1270. doi:10.1016/j.amjmed.2019.05.017

Gaoni, Y., and Mechoulam, R. (1964). Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. *J. Am. Chem. Soc.* 86, 1646–1647. doi:10.1021/ja01062a046

Gates, P., Jaffe, A., and Copeland, J. (2014). Cannabis Smoking and Respiratory Health: Consideration of the Literature. *Respirology* 19, 655–662. doi:10.1111/resp.12298

Gershenzon, J., and Dudareva, N. (2007). The Function of Terpene Natural Products in the Natural World. *Nat. Chem. Biol.* 3, 408–414. doi:10.1038/nchembio.2007.5

Gertsch, J., Pertwee, R. G., and Di Marzo, V. (2010). Phytocannabinoids beyond the Cannabis Plant - Do They Exist? *Br. J. Pharmacol.* 160, 523–529. doi:10.1111/j.1476-5381.2010.00745.x

Giupponi, L., Leoni, V., Pavlovic, R., and Giorgi, A. (2020). Influence of Altitude on Phytochemical Composition of Hemp Inflorescence: A Metabolomic Approach. *Molecules* 25, 1381. doi:10.3390/molecules25061381

Grotenhermen, F. (2003). Pharmacokinetics and Pharmacodynamics of Cannabinoids. *Clin. Pharmacokinet.* 42, 327–360. doi:10.2165/00003088-200342040-00003

Gülck, T., and Møller, B. L. (2020). Phytocannabinoids: Origins and Biosynthesis. *Trends Plant Sci.* 25, 985–1004. doi:10.1016/j.tplants.2020.05.005

Hand, A., Blake, A., Kerrigan, P., and Samuel, P. (2016). History of Medical Cannabis. *J. Pain Manag.* 9, 387–394.

Hanuš, L. O., and Hod, Y. (2020). Terpenes/Terpenoids in Cannabis: Are They Important? *Med. Cannabis Cannabinoids* 3, 25–60. doi:10.1159/000509733

Hanuš, L. O., Meyer, S. M., Muñoz, E., Taglialatela-Scafati, O., and Appendino, G. (2016). Phytocannabinoids: A Unified Critical Inventory. *Nat. Prod. Rep.* 33, 1357–1392. doi:10.1039/c6np00074f

Happyana, N., and Kayser, O. (2016). Monitoring Metabolite Profiles of Cannabis Sativa L. Trichomes during Flowering Period Using 1H NMR-Based Metabolomics and Real-Time PCR. *Planta Med.* 82, 1217–1223. doi:10.1055/s-0042-108058

Happyana, N., Agnolet, S., Muntendam, R., Van Dam, A., Schneider, B., and Kayser, O. (2013). Analysis of Cannabinoids in Laser-Microdissected Trichomes of Medicinal Cannabis Sativa Using LCMS and Cryogenic NMR. *Phytochemistry* 87, 51–59. doi:10.1016/j.phytochem.2012.11.001

Hazekamp, A., and Fischedick, J. T. (2012). Cannabis - from Cultivar to Chemovar. *Drug Test. Anal.* 4, 660–667. doi:10.1002/dta.407

Hazekamp, A., Ware, M. A., Muller-Vahl, K. R., Abrams, D., and Grotenhermen, F. (2013). The Medicinal Use of Cannabis and Cannabinoids-Aan International Cross-Sectional Survey on Administration Forms. *J. Psychoactive Drugs* 45, 199–210. doi:10.1080/02791072.2013.805976

Heblinski, M., Santiago, M., Fletcher, C., Stuart, J., Connor, M., Mcgregor, I. S., et al. (2020). Terpenoids Commonly Found in Cannabis Sativa Do Not Modulate the Actions of Phytocannabinoids or Endocannabinoids on TRPA1 and TRPV1 Channels. *Cannabis Cannabinoid Res.* 5, 305–317. doi:10.1089/can.2019.0099

Henry, P., Hilyard, A., Johnson, S., and Orser, C. (2018). Predicting Chemovar Cluster and Variety Verification in Vegetative Cannabis Accessions Using Targeted Single Nucleotide Polymorphisms. *PeerJ Prepr* 6, e27442v1. doi:10.7287/peerj.preprints.27442v1

Hillig, K. W. (2004). A Chemotaxonomic Analysis of Terpenoid Variation in Cannabis. *Biochem. Syst. Ecol.* 32, 875–891. doi:10.1016/j.bse.2004.04.004

Holgado, M. A., Martín-Banderas, L., Álvarez-Fuentes, J., and Fernández-Arévalo, M. (2017). Neuroprotective Effect of Cannabinoids Nanoplatforms in Neurodegenerative Diseases. *J. Drug Deliv. Sci. Technol.* 42, 84–93. doi:10.1016/j.jddst.2017.04.023

Hollister, L. E. (1974). Structure-activity Relationships in Man of Cannabis Constituents, and Homologs and Metabolites of delta9-tetrahydrocannabinol. *Pharmacology* 11, 3–11. doi:10.1159/000136462

Huestis, M. A. (2005). "Pharmacokinetics and Metabolism of the Plant Cannabinoids, Δ 9-tetrahydrocannibinol, Cannabidiol and Cannabinol," in *Cannabinoids*. Editor R. G. Pertwee (Berlin, Heidelberg: Springer), 657–690.

Huestis, M. A. (2007). Human Cannabinoid Pharmacokinetics. *Chem. Biodivers.* 4, 1770–1804. doi:10.1002/cbdv.200790152

Isidore, E., Karim, H., and Ioannou, I. (2021). Extraction of Phenolic Compounds and Terpenes from Cannabis Sativa L. By-Products: From Conventional to Intensified Processes. *Antioxidants (Basel)* 10, 942. doi:10.3390/antiox10060942

Jin, D., Jin, S., and Chen, J. (2019). Cannabis Indoor Growing Conditions, Management Practices, and Post-Harvest Treatment: A Review. *Am. J. Plant Sci.* 10, 925–946. doi:10.4236/ajps.2019.106067

Jung, J., Meyer, M. R., Maurer, H. H., Neusüß, C., Weinmann, W., and Auwärter, V. (2009). Studies on the Metabolism of the Δ9-tetrahydrocannabinol Precursor Δ9-tetrahydrocannabinolic Acid A (Δ9-THCA-A) in Rat Using LC-MS/MS, LC-QTOF MS and GC-MS Techniques. *J. Mass. Spectrom.* 44, 1423–1433. doi:10.1002/jms.1624

King, K. M., Myers, A. M., Soroka-Monzo, A. J., Tuma, R. F., Tallarida, R. J., Walker, E. A., et al. (2017). Single and Combined Effects of Δ9 -tetrahydrocannabinol and Cannabidiol in a Mouse Model of Chemotherapy-Induced Neuropathic Pain. *Br. J. Pharmacol.* 174, 2832–2841. doi:10.1111/bph.13887

Křížek, T., Bursová, M., Horsley, R., Kuchař, M., Tůma, P., Čabala, R., et al. (2018). Menthol-based Hydrophobic Deep Eutectic Solvents: Towards Greener and Efficient Extraction of Phytocannabinoids. *J. Clean. Prod.* 193, 391–396. doi:10.1016/j.jclepro.2018.05.080

Lewis, M. A., Russo, E. B., and Smith, K. M. (2018). Pharmacological Foundations of Cannabis Chemovars. *Planta Med.* 84, 225–233. doi:10.1055/s-0043-122240

Linciano, P., Citti, C., Russo, F., Tolomeo, F., Laganà, A., Capriotti, A. L., et al. (2020). Identification of a New Cannabidiol N-Hexyl Homolog in a Medicinal Cannabis Variety with an Antinociceptive Activity in Mice: Cannabidihexol. *Sci. Rep.* 10, 22019–22111. doi:10.1038/s41598-020-79042-2

Lipson Feder, C., Cohen, O., Shapira, A., Katzir, I., Peer, R., Guberman, O., et al. (2021). Fertilization Following Pollination Predominantly Decreases Phytocannabinoids Accumulation and Alters the Accumulation of Terpenoids in Cannabis Inflorescences. *Front. Plant Sci.* 2426, 753847. doi:10.3389/fpls.2021.753847

Lu, H. C., and Mackie, K. (2016). An Introduction to the Endogenous Cannabinoid System. *Biol. Psychiatry* 79, 516–525. doi:10.1016/j.biopsych.2015.07.028

Lunardi, P., de Souza, L. W., dos Santos, B., Popik, B., and de Oliveira Alvares, L. (2020). Effect of the Endocannabinoid System in Memory Updating and Forgetting. *Neuroscience* 444, 33–42. doi:10.1016/j.neuroscience.2020.07.045

Maayah, Z. H., Takahara, S., Ferdaoussi, M., and Dyck, J. R. B. (2020a). The Anti-inflammatory and Analgesic Effects of Formulated Full-Spectrum Cannabis Extract in the Treatment of Neuropathic Pain Associated with Multiple Sclerosis. *Inflamm. Res.* 69, 549–558. doi:10.1007/s00011-020-01341-1

Maayah, Z. H., Takahara, S., Ferdaoussi, M., and Dyck, J. R. B. (2020b). The Molecular Mechanisms that Underpin the Biological Benefits of Full-Spectrum Cannabis Extract in the Treatment of Neuropathic Pain and Inflammation. *Biochim. Biophys. Acta Mol. Basis Dis.* 1866, 165771. doi:10.1016/j.bbadis.2020.165771

Maccarrone, M. (2020). Phytocannabinoids and Endocannabinoids: Different in Nature. *Rend. Fis. Acc. Lincei* 31, 931–938. doi:10.1007/s12210-020-00957-z

Mackie, K. (2008). Cannabinoid Receptors: Where They Are and what They Do. *J. Neuroendocrinol.* 20, 10–14. doi:10.1111/j.1365-2826.2008.01671.x

Maroso, M., Szabo, G. G., Kim, H. K., Alexander, A., Bui, A. D., Lee, S. H., et al. (2016). Cannabinoid Control of Learning and Memory through HCN Channels. *Neuron* 89, 1059–1073. doi:10.1016/j.neuron.2016.01.023

Marsicano, G., and Lafenêtre, P. (2009). "Roles of the Endocannabinoid System in Learning and Memory," in *Behavioral Neurobiology of the Endocannabinoid System* (Berlin, Heidelberg: Springer), Vol. 1, 201–230. doi:10.1007/978-3-540-88955-7_8

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., and Bonner, T. I. (1990). Structure of a Cannabinoid Receptor and Functional Expression of the Cloned cDNA. *Nature* 346, 561–564. doi:10.1038/346561a0

McGarvey, P., Huang, J., McCoy, M., Orvis, J., Katsir, Y., Lotringer, N., et al. (2020). De Novo assembly and Annotation of Transcriptomes from Two Cultivars of Cannabis Sativa with Different Cannabinoid Profiles. *Gene* 762, 145026. doi:10.1016/j.gene.2020.145026

Mechoulam, R., and Shvo, Y. (1963). Hashish. I. The Structure of Cannabidiol. *Tetrahedron* 19, 2073–2078. doi:10.1016/0040-4020(63)85022-x

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., et al. (1995). Identification of an Endogenous 2-monoglyceride, Present in Canine Gut, that Binds to Cannabinoid Receptors. *Biochem. Pharmacol.* 50, 83–90. doi:10.1016/0006-2952(95)00109-d

Mechoulam, R., Peters, M., Murillo-Rodriguez, E., and Hanus, L. O. (2007). Cannabidiol--recent Advances. *Chem. Biodivers.* 4, 1678–1692. doi:10.1002/cbdv.200790147

Milay, L., Berman, P., Shapira, A., Guberman, O., and Meiri, D. (2020). Metabolic Profiling of Cannabis Secondary Metabolites for Evaluation of Optimal Postharvest Storage Conditions. *Front. Plant Sci.* 11, 583605–583615. doi:10.3389/fpls.2020.583605

Montecucco, F., and Di Marzo, V. (2012). At the Heart of the Matter: The Endocannabinoid System in Cardiovascular Function and Dysfunction. *Trends Pharmacol. Sci.* 33, 331–340. doi:10.1016/j.tips.2012.03.002

Moreno-Sanz, G. (2016). Can You Pass the Acid Test? Critical Review and Novel Therapeutic Perspectives of Δ9-Tetrahydrocannabinolic Acid A. *Cannabis Cannabinoid Res.* 1, 124–130. doi:10.1089/can.2016.0008

Munro, S., Thomas, K. L., and Abu-Shaar, M. (1993). Molecular Characterization of a Peripheral Receptor for Cannabinoids. *Nature* 365, 61–65. doi:10.1038/365061a0

Namdar, D., Voet, H., Ajjampura, V., Nadarajan, S., Mayzlish-Gati, E., Mazuz, M., et al. (2019). Terpenoids and Phytocannabinoids Co-produced in Cannabis Sativa Strains Show Specific Interaction for Cell Cytotoxic Activity. *Molecules* 24, 3031. doi:10.3390/molecules24173031

Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Perchuk, A., Meozzi, P. A., et al. (2006). Discovery of the Presence and Functional Expression of Cannabinoid CB2 Receptors in Brain. *Ann. N. Y. Acad. Sci.* 1074, 514–536. doi:10.1196/annals.1369.052

Pacher, P., and Steffens, S. (2009). The Emerging Role of the Endocannabinoid System in Cardiovascular Disease. *Semin. Immunopathol.* 31, 63–77. doi:10.1007/s00281-009-0145-8

Pandey, R., Mousawy, K., Nagarkatti, M., and Nagarkatti, P. (2009). Endocannabinoids and Immune Regulation. *Pharmacol. Res.* 60, 85–92. doi:10.1016/j.phrs.2009.03.019

Perez-Reyes, M., Timmons, M. C., Lipton, M. A., Davis, K. H., and Wall, M. E. (1972). Intravenous Injection in Man of 9 -tetrahydrocannabinol and 11-OH- 9 -tetrahydrocannabinol. *Science* 177, 633–635. doi:10.1126/science.177.4049.633

Piper, B. J. (2018). Mother of Berries, ACDC, or Chocolope: Examination of the Strains Used by Medical Cannabis Patients in New England. *J. Psychoactive Drugs* 50, 95–104. doi:10.1080/02791072.2017.1390179

Russo, E. B., McPartland, J. M., ElSohly, M. A., Wachtel, S. R., and Wit, H. (2003). Cannabis Is More Than Simply delta(9)-tetrahydrocannabinol. *Psychopharmacology (Berl)* 165, 431–434. doi:10.1007/s00213-002-1348-z

Russo, E. B. (2011). Taming THC: Potential Cannabis Synergy and Phytocannabinoid-Terpenoid Entourage Effects. *Br. J. Pharmacol.* 163, 1344–1364. doi:10.1111/j.1476-5381.2011.01238.x

Russo, E. B. (2016). Beyond Cannabis: Plants and the Endocannabinoid System. *Trends Pharmacol. Sci.* 37, 594–605. doi:10.1016/j.tips.2016.04.005

Santiago, M., Sachdev, S., Arnold, J. C., Mcgregor, I. S., and Connor, M. (2019). Absence of Entourage: Terpenoids Commonly Found in Cannabis Sativa Do Not Modulate the Functional Activity of Δ9-THC at Human CB1 and CB2 Receptors. *Cannabis Cannabinoid Res.* 4, 165–176. doi:10.1089/can.2019.0016

Schwilke, E. W., Schwope, D. M., Karschner, E. L., Lowe, R. H., Darwin, W. D., Kelly, D. L., et al. (2009). Delta9-tetrahydrocannabinol (THC), 11-Hydroxy-THC, and 11-Nor-9-Carboxy-THC Plasma Pharmacokinetics during and after Continuous High-Dose Oral THC. *Clin. Chem.* 55, 2180–2189. doi:10.1373/clinchem.2008.122119

Shapira, A., Berman, P., Futoran, K., Guberman, O., and Meiri, D. (2019). Tandem Mass Spectrometric Quantification of 93 Terpenoids in Cannabis Using Static Headspace Injections. *Anal. Chem.* 91, 11425–11432. doi:10.1021/acs.analchem.9b02844

Shiplo, S., Asbridge, M., Leatherdale, S. T., and Hammond, D. (2016). Medical Cannabis Use in Canada: Vapourization and Modes of Delivery. *Harm Reduct. J.* 13, 30–10. doi:10.1186/s12954-016-0119-9

Takeda, S., Okajima, S., Miyoshi, H., Yoshida, K., Okamoto, Y., Okada, T., et al. (2012). Cannabidiolic Acid, a Major Cannabinoid in Fiber-type Cannabis, Is an Inhibitor of MDA-MB-231 Breast Cancer Cell Migration. *Toxicol. Lett.* 214, 314–319. doi:10.1016/j.toxlet.2012.08.029

Tang, K., Struik, P. C., Yin, X., Thouminot, C., Bjelková, M., Stramkale, V., et al. (2016). Comparing Hemp (Cannabis Sativa L.) Cultivars for Dual-Purpose Production under Contrasting Environments. *Ind. Crops Prod.* 87, 33–44. doi:10.1016/j.indcrop.2016.04.026

Trofin, I. G., Vlad, C. C., Dabija, G., and Filipescu, L. (2011). Influence of Storage Conditions on the Chemical Potency of Herbal Cannabis. *Rev. Chim.* 62, 639–645.

Turner, S. E., Williams, C. M., Iversen, L., and Whalley, B. J. (2017). "Molecular Pharmacology of Phytocannabinoids," in *Phytocannabinoids* (Cham: Springer), 61–101. doi:10.1007/978-3-319-45541-9_3

Uziel, A., Gelfand, A., Amsalem, K., Berman, P., Lewitus, G. M., Meiri, D., et al. (2020). Full-Spectrum Cannabis Extract Microdepots Support Controlled Release of Multiple Phytocannabinoids for Extended Therapeutic Effect. *ACS Appl. Mater. Inter.* 12, 23707–23716. doi:10.1021/acsami.0c04435

Velasco, G., Sánchez, C., and Guzmán, M. (2016). Anticancer Mechanisms of Cannabinoids. *Curr. Oncol.* 23, S23–S32. doi:10.3747/co.23.3080

Welling, M. T., Liu, L., Raymond, C. A., Ansari, O., and King, G. J. (2018). Developmental Plasticity of the Major Alkyl Cannabinoid Chemotypes in a Diverse Cannabis Genetic Resource Collection. *Front. Plant Sci.* 9, 1510. doi:10.3389/fpls.2018.01510

Woodhams, S. G., Sagar, D. R., Burston, J. J., and Chapman, V. (2015). The Role of the Endocannabinoid System in Pain. *Handb Exp. Pharmacol.* 227, 119–143. doi:10.1007/978-3-662-46450-2_7

Woodhams, S. G., Chapman, V., Finn, D. P., Hohmann, A. G., and Neugebauer, V. (2017). The Cannabinoid System and Pain. *Neuropharmacology* 124, 105–120. doi:10.1016/j.neuropharm.2017.06.015

Zamengo, L., Bettin, C., Badocco, D., Di Marco, V., Miolo, G., and Frison, G. (2019). The Role of Time and Storage Conditions on the Composition of Hashish and Marijuana Samples: a Four-Year Study. *Forensic Sci. Int.* 298, 131–137. doi:10.1016/j.forsciint.2019.02.058

Zuardi, A. W. (2008). Cannabidiol: from an Inactive Cannabinoid to a Drug with Wide Spectrum of Action. *Braz. J. Psychiatry* 30, 271–280. doi:10.1590/s1516-44462008000300015

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Pharmacology

REVIEW
published: 27 April 2022
doi: 10.3389/fphar.2022.883987

What Do We Know About Medical Cannabis in Neurological Disorders and What Are the Next Steps?

Clémence Lacroix, Isabelle Alleman-Brimault, Arnaud Zalta, Frank Rouby, Catherine Cassé-Perrot, Elisabeth Jouve, Laurence Attolini, Romain Guilhaumou, Joëlle Micallef and Olivier Blin*

APHM, INSERM, Inst Neurosci Syst, UMR 1106, Aix Marseille Univ, University Hospital Federation DHUNE, Service de Pharmacologie Clinique et Pharmacovigilance, Marseille, France

Medical use of cannabis has been receiving growing attention over the last few decades in modern medicine. As we know that the endocannabinoid system is largely involved in neurological disorders, we focused on the scientific rationale of medical cannabis in three neurological disorders: amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease through pharmacological plausibility, clinical studies, and patients' view. Clinical studies (randomized controlled trials, open-label studies, cohorts, and case reports) exploring medical cannabis in these disorders show different results depending on the methods and outcomes. Some show benefits on motor symptoms and others on non-motor symptoms and quality of life. Concerning patients' view, several web surveys were collected, highlighting the real use of cannabis to relieve symptoms of neurological disorders, mostly outside a medical pathway. This anarchic use keeps questioning particularly in terms of risks: consumption of street cannabis, drug–drug interactions with usual medical treatment, consideration of medical history, and adverse reactions (psychiatric, respiratory, cardiovascular disorders, etc.), underlining the importance of a medical supervision. To date, most scientific data support the therapeutic potential of cannabis in neurological disorders. As far as patients and patients' associations are calling for it, there is an urgent need to manage clinical studies to provide stronger evidence and secure medical cannabis use.

Keywords: medical cannabis, neurological disorders, amyotrophic lateral sclerosis, Parkinson, Alzheimer, pharmacology, scientific research

OPEN ACCESS

Edited by:
Francesca Baratta, University of Turin, Italy

Reviewed by:
Alexandre Quintas, Egas Moniz Interdisciplinary Research Center, Portugal
Renata Solimini, National Institute of Health (ISS), Italy

****Correspondence:***
Clémence Lacroix
clemence.lacroix@ap-hm.fr

Specialty section:
This article was submitted to Experimental Pharmacology and Drug Discovery, a section of the journal Frontiers in Pharmacology

Received: *25 February 2022*
Accepted: *28 March 2022*
Published: *27 April 2022*

Citation:
Lacroix C, Alleman-Brimault I, Zalta A, Rouby F, Cassé-Perrot C, Jouve E, Attolini L, Guilhaumou R, Micallef J and Blin O (2022) What Do We Know About Medical Cannabis in Neurological Disorders and What Are the Next Steps? Front. Pharmacol. 13:883987. doi: 10.3389/fphar.2022.883987

INTRODUCTION

Medical use of cannabis has been receiving growing attention over the last few decades in modern medicine. As cannabis is a complex plant containing hundreds of cannabinoids, we keep questioning about its therapeutic benefits, justified by its pleiotropic pharmacological activity. As a result, it has been reported that changes in endocannabinoid levels may be related to neurological diseases such as Parkinson's disease, Huntington's disease, Alzheimer's disease, and multiple sclerosis (Fraguas-Sánchez and Torres-Suárez, 2018). As we know that the endocannabinoid system (ECS) is largely involved in neurological disorders, we here chose to focus on the scientific rationale of medical cannabis through a narrative review in three neurological disorders: amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Alzheimer's disease (AD) through pharmacological

plausibility, clinical studies, and patients' view. Developing medical cannabis could be an important issue to better control neurodegeneration.

PHARMACOLOGICAL PLAUSIBILITY

ECS is largely expressed in the cerebellum, basal ganglia, and hippocampus and is thus an area of choice for molecular targets. Characterization of the ECS and detection of widespread cannabinoid receptors in the brain and peripheral tissues have opened the door to a vast field of research. The ECS is formed by cannabinoid receptors 1 and 2 (CB1 and CB2), the two endocannabinoids anandamide and 2-arachidonoylglycerol, and endocannabinoid anabolic and catabolic enzymes. Manipulation of the ECS may have beneficial disease-modifying potential in neurological disorders. Exogenous cannabinoids play a pleiotropic activity mostly through two cannabinoid receptors: CB1 is predominantly expressed in the brain, and CB2 is primarily found in the cells of the immune system (Lucas et al., 2018). Since the pathophysiology of motor neuron degeneration in ALS may involve mitochondrial dysfunction, excessive glutamate activity, oxidative stress, neuroinflammation, and growth factor deficiency, cannabis could be effective in modulating these processes (Bilsland and Greensmith, 2008; Carter et al., 2010; Papadimitriou et al., 2010; Appel et al., 2011). To support these hypotheses, a recent meta-analysis of preclinical studies in murine ALS models conducted by Urbi and colleagues suggests that cannabinoid receptor agonists may improve survival time (Urbi et al., 2019b).

PD mostly involves dopaminergic and cholinergic systems. The interactions between cannabinoids and dopamine in the basal ganglia may involve both the modulation of other neurotransmitters (GABA, glutamate) and the activation of CB1 and CB2 (Stampanoni Bassi et al., 2017; Patricio et al., 2020). Preclinical studies in the animal model of PD have shown various influences of cannabis on motor and non-motor behaviors: reducing motor fluctuations and levodopa-induced dyskinesias (Segovia et al., 2003; Morgese et al., 2007; Song et al., 2014). Activation of CB2 has shown a reduction in dopamine depletion in PD rats (García-Arencibia et al., 2007). In a preclinical study investigating the role of a CB2 receptor agonist on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity in a mouse model of PD managed in 2017, the use of a CB2 agonist reversed the depletion of CB2 and thus increased the levels of dopamine and improved the behavior of PD mice (Shi et al., 2017). Cannabinoids seem to be protective by binding to the CB1 receptor, inhibiting the dopamine beta hydroxylase activity and decreasing glutamate levels or by binding to CB2, reducing neuroinflammation (Ferreira et al., 2020). All these considerations suggest therapeutic benefits of cannabis in PD.

AD is characterized by extracellular deposits of β-amyloid plaques and neurofibrillary tangles of tau protein (Selkoe, 2011). Cannabis promotes neuroprotection through different signal pathways mediated indirectly by CB receptors by reducing the β-amyloid peptide action and tau phosphorylation, as well as modulating oxidative stress and inflammation (Esposito et al., 2006; Aso and Ferrer, 2014). CB1 and CB2 agonists ameliorated memory and cognitive impairment in mice that have received intracerebral injection of β-amyloid peptide (Ramirez, 2005). CB2 activation also reduced levels of neurotoxic factors and pro-inflammatory mediators produced by reactive astrocytes and microglial cells, stimulated microglial proliferation and migration, and decreased β-amyloid peptide levels (Cristino et al., 2020). To resume, cannabis improved immune function, amyloidogenesis, and reduced behavioral symptoms and pain but also stimulated appetite and inhibited acetylcholinesterase in animal models of AD (Cooray et al., 2020; Li et al., 2020).

These pharmacological considerations concerning cannabis in neurological disorders suggest mechanism-based therapeutic targets for future clinical studies.

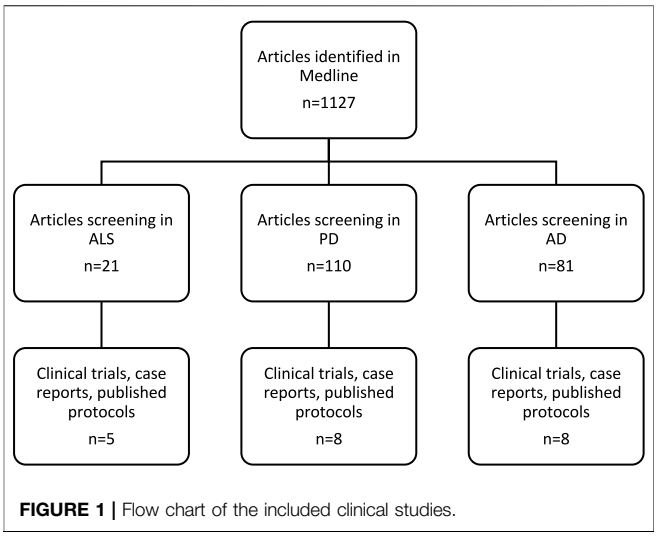


FIGURE 1 | Flow chart of the included clinical studies.

CLINICAL STUDIES

We managed a literature search on Medline using the keywords "medical cannabis" and "neurological disorders," "medical cannabis" and "amyotrophic lateral sclerosis," "medical cannabis" and "Parkinson," and "medical cannabis" and "Alzheimer". The articles were thoroughly screened by reviewing each article with titles, abstracts, and content of the full articles. We only included the studies published between 1986 and 2021 and human studies (clinical trials, case reports, and published protocols) in the English language, including adults of 18 years of age and older, and we excluded review articles and position studies (**Figure 1**). An additional search on clinicaltrials.gov was also performed using "ALS" and "cannabis," "Parkinson" and "cannabis," and "Alzheimer" and "cannabis".

Only sparse data on the benefits of medical cannabis in neurological disorders are available from clinical studies. As we know that cannabis is a complex plant with hundreds of phytocannabinoids, several components are studied in the following clinical studies. Tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most studied in therapeutic use as

TABLE 1 | Summary of clinical studies exploring medical cannabis in ALS.

Reference	Study design	Number and the type of patients	Molecule explored and the route of administration	Dose/frequency duration	Outcomes and efficacy	Safety
Weber et al. (2010)	Randomized, double-blind, placebo-controlled crossover study	*n* = 22 ALS patients suffering from daily cramps (completed)	Dronabinol, Marinol®; per os	5 mg of dronabinol twice daily during 2 weeks Wash-out: 2 weeks	-No significant effect on cramp intensity (primary outcome) -No significant effect on number of cramps per day, number of cramps during daytime and bedtime, fasciculations, quality of life, quality of sleep, appetite, and depression (secondary outcomes)	Two AEs non–study-related: one pneumonia and one deep venous thrombosis
Joerger et al. (2012)	Randomized, double-blind, placebo-controlled study	*n* = 9 ALS patients suffering from cramps	Dronabinol; per os	1) 5 mg (single dose) 2) wash-out: 2 weeks 3) 10 mg (single dose)	-PK linear with a doubling of the AUC -High inter-individual PK variability -Heart rate peaked approximately together with the plasma concentrations of THC-OH	-Drowsiness, euphoria, orthostasis, sleepiness, vertigo, and weakness: significantly more frequent in patients receiving 10 mg THC as compared to 5 mg THC per day -No association between drug exposure and the occurrence of AE
Meyer et al. (2019)	Observational, retrospective, monocentric, cross-sectional cohort study	*n* = 32 ALS patients suffering from spasticity	Mix of THC:CBD (2.7 mg:2.5 mg), Sativex®; oromucosal spray	three groups: >7 sprays (*n* = 11) <7 sprays (*n* = 16) Infrequent use (*n* = 5)	-Severe spasticity related to high doses of Sativex® -High treatment satisfaction -*n* = 16 discontinued the treatment during observation period	No AE reported
Riva et al. (2019)	Randomized, double-blind, placebo-controlled study	*n* = 59 ALS patients Sativex®, *n* = 29 Placebo, *n* = 30	Mix of THC:CBD (2.7 mg:2.5 mg), Sativex®; oromucosal spray	14 days titration, duration 6 weeks	-Significant reduction of ALS-related spasticity -Significant effect of the patient's impression of change -No significant reduction of the global impression of change (caregivers and physicians), pain, spasm frequency, sleep, timed 10-m walk, scores on the amyotrophic lateral sclerosis functional rating scale—revised, forced vital capacity, scores on the barthel activities of daily living index, and body mass index	-three temporarily discontinuations of AEs one nausea and anxiety event, one influenza and accidental fall, and one disease progression event -No SAEs

AE, adverse event; ALS, amyotrophic lateral sclerosis; CBD, cannabidiol; SAE, severe adverse event; THC, tetrahydrocannabinol.

they are the two major compounds of the *Cannabis sativa* L. plant. THC could be synthetized from CBD acid extracted from the plant as dronabinol. Nabilone is completely synthetized and is an analog of THC whereas Sativex®, which is a commercialized medication, contains a mix of THC and CBD directly extracted from the plant of cannabis (nabiximol).

TABLE 2 | Summary of clinical studies exploring medical cannabis in PD.

Reference	Study design	Number and the type of patients	Molecule explored and the route of administration	Dose/frequency duration	Outcomes and efficacy	Safety
Consroe et al. (1986)	Open-label study	*n* = 5 PD patients with dystonia	CBD; per os	From 100 mg/day to 600 mg/day increased 100 mg/week, duration 6 weeks	Improvements in dystonia, dose-related in a 20–50% range	-four hypotension events -three exacerbations of hypokinesia and/or tremor with a higher dose of CBD -two dry mouth -two sedation events -two lightheadedness
Frankel et al. (1990)	Case report	*n* = 5 PD patients resistant to common therapies	Cannabis; smoking	1 g; 2,9% THC, duration not reported	No effects in reducing the tremor	Drowsiness and mild euphoria
Sieradzan et al. (2001)	Randomized, double-blind, placebo-controlled crossover study	*n* = 9 PD patients with L-Dopa–induced dyskinesia	Nabilone; per os	0,03 mg/kg, duration not reported	Significant improvement in L-Dopa dyskinesia	-Two AEs with withdrawn: one vertigo and one hypotension -Other AEs (*n* = 5 patients): mild sedation, "floating sensation", dizziness, hyperacusis, partial disorientation, and visual hallucinations
Carroll et al. (2004)	Randomized, double-blind, placebo-controlled crossover study	*n* = 19 PD patients with L-Dopa–induced dyskinesia	Mix of THC:CBD (2.5 mg:1.25 mg); per os	Max 0.25 mg/kg/day THC, administration twice daily, during 4 weeks Wash-out: 2 weeks	-No significant effects reported on UPDRS, Rush, Bail, PDQ-39 scales -No significant effects in improving the quality of life	No SAE reported
Zuardi et al. (2009)	Open-label study	*n* = 6 PD patients with at least 3-month-old psychosis	CBD; per os	150 mg/day to 400 mg/day, duration 4 weeks	-Significant improvements in BPRS and psychotic symptoms, in sleep quality, less hallucinations, and disorientations (PPQ) -Significant improvement in UPDRS and CGI-I	No AE reported
Chagas et al. (2014a)	Randomized, double-blind, placebo-controlled study	*n* = 21 PD patients without dementia or psychiatric symptoms and no occasional cannabis consumers	CBD; per os	75 mg/day or 300 mg/day, duration 6 weeks	Significant difference in PDQ39 between placebo and CBD 300 mg/day groups	No AE reported
Chagas et al. (2014b)	Case reports	*n* = 4 PD patients with sleep behavioral problems	CBD; per os	75 mg/day (*n* = 3); 300 mg/day (*n* = 1), duration 6 weeks	Four patients described an improvement in sleep behavioral disorders	No AE reported
Lotan et al. (2014)	Open-label study	*n* = 22 PD patients	Cannabis; smoking	0, 5 g, duration not reported	-Significant improvements in UPRDS, in tremor, in rigidity, and bradykinesia -Significant improvement in sleep and pain scores	-Two AEs: one hypoglycaemia and one dizziness

BPRS, Brief Psychiatric Rating Scale; CBD, cannabidiol; CGI-I, clinical global impression–improvement; PD, Parkinson's disease; PDQ-39, Parkinson's disease questionnaire; THC, tetrahydrocannabinol; UPDRS, Unified Parkinson's Disease Rating Scale.

To date, we have only found four clinical studies exploring the use of medical cannabis in ALS (Weber et al., 2010; Joerger et al., 2012; Meyer et al., 2019; Riva et al., 2019). Data from these studies are summarized in **Table 1**. The use of dronabinol alone did not demonstrate improvement in cramp intensity, cramp frequency, and fasciculation intensity neither on quality of life, sleep, appetite, and depression in a randomized controlled trial (RCT) of 2010 (Weber et al., 2010). The lack of treatment effect could be due to the short duration treatment (2 weeks). In parallel, an equilibrated mix of tetrahydrocannabinol (THC)

TABLE 3 | Summary of clinical studies exploring medical cannabis in AD.

Reference	Study design	Number and the type of patients	Molecule explored and the route of administration	Dose/frequency duration	Outcomes and efficacy	Safety
Volicer et al. (1997)	Randomized, double-blind, placebo-controlled crossover study	*n* = 15 AD patients with behavioral disorders and anorexia	Dronabinol; per os	2.5 mg twice daily, duration 6 weeks	-Significant improvement in body weight -Significant improvement in the severity of behavioral disorders -Significant improvement in the negative affect score	-Nine tiredness and eight somnolence events Seven euphoria events -No SAE reported
Walther et al. (2006)	Open-label study	*n* = 6 patients in the late stages of dementia and suffering from circadian and behavioral disorders	Dronabinol; per os	2.5 mg/day, duration 2 weeks	-Significant reduction in the nocturnal motor activity -Significant improvement of the NPI score (agitation, aberrant motor, and night-time behaviors) -Significant reduction in appetite disturbances and irritability	No AE reported
Mahlberg and Walther (2007)	Randomized, placebo-controlled study	*n* = 24 AD patients suffering from agitated behavior	Dronabinol; per os	2.5 mg/day, duration 2 weeks	-Significant reduction in nocturnal motor activity -Significant improvement of the NPI score	No AE reported
Walther et al. (2011)	Randomized, double-blind, placebo-controlled crossover study	*n* = 2 AD patients with night time agitation	Dronabinol, Marinol®; per os	2.5 mg/day, duration 2 weeks	-Reduction in nocturnal motor activity	No AE reported
Ahmed et al. (2015)	Randomized, double-blind, placebo-controlled crossover study	*n* = 10 patients suffering from dementia	THC, Namisol®; per os	-Weeks 1–6: 0.75 mg twice daily -Weeks 7–12: 1.5 mg twice daily Wash-out period: 4 days Duration: 12 weeks	—	-Two AEs at 0.75 mg: one dizziness and one fatigue -Four AEs at 1.5 mg: three agitation and one fatigue -No SAEs
Shelef et al. (2016)	Open-label study	*n* = 11 patients with dementia and NPS	THC; per os	-2.5 mg twice daily -5 mg twice daily if 2.5 mg ineffective -7.5 mg twice daily if 5 mg ineffective Duration: 4 weeks	-Significant improvement in CGI and NPI scores (delusions, agitation/aggression, irritability, apathy, sleep, and caregiver distress)	Three AEs: one fall, one confusion, and one dysphagia
Herrmann et al. (2019)	Randomized, double-blind, placebo-controlled crossover study	*n* = 39 AD patients suffering from agitation	Nabilone; per os	1 mg/day; 1,5 mg/day; 2 mg/day according to tolerance, duration 14 weeks Wash-out: 1 week	-Significant improvement in agitation (CMAI) -Significant improvement in NPI (caregiver distress, behavior) -Significant improvement in the sMMSE score -Significant improvement in the nutritional status without weight gain	-36 AEs: 22 sedation, eight falls, one bradycardia, one myoclonic jerk, one elevated urea level, one rash, one NPS increase, and one dizziness -Five SAEs: two lethargy, one death, one high INR, and one myocardial infarction

AD, Alzheimer's disease; AE, adverse event; CGI, clinical global impression of change; CMAI, Cohen-Mansfield agitation inventory; sMMSE, standardized mini-mental status examination; NPI, neuropsychiatric inventory; NPS, neuropsychiatric symptoms; SAE, severe adverse event, THC, tetrahydrocannabinol.

and cannabidiol (CBD) in the oromucosal spray Sativex® seems to be effective on ALS-related spasticity and on the patients' global impression of change in a 6-week RCT (Riva et al., 2019) and also in a cohort study (Meyer et al., 2019). Noticeably, Sativex® (an equilibrated mix of THC and CBD) is already commercialized and indicated for symptom improvement in adult patients with resistant spasticity due to multiple sclerosis. All these studies reported good tolerability of medical cannabis. Therefore, these modest but encouraging results suggest the need for further studies enrolling a higher number of patients.

Concerning PD patients, eight clinical studies were published (Consroe et al., 1986; Frankel et al., 1990; Sieradzan et al., 2001; Carroll et al., 2004; Zuardi et al., 2009; Chagas et al., 2014a, 2014b; Lotan et al., 2014), as shown in **Table 2**. Medical cannabis could be effective both on motor symptoms (dystonia, dyskinesia, and fluctuations), and non-motor symptoms (anxiety, sleep quality, hallucinations, and disorientation) (Consroe et al., 1986; Frankel et al., 1990; Sieradzan et al., 2001; Zuardi et al., 2009; Chagas et al., 2014a; Lotan et al., 2014). Two studies (one RCT and one case report of five PD patients) show that there is not any reduction of motor and non-motor symptoms. One open-label study shows that there is an improvement of motor and non-motor symptoms only at the highest dose of CBD (400 mg/day for 4 weeks). Case reports of four PD patients show that there is an improvement of the quality of sleep without nightmares and reduction of agitation. Five studies show improvement of motor and non-motor symptoms and quality of life (three open-label studies and two RCTs). Anyway, all studies demonstrate that there are no serious adverse effects. The main limitations to these findings are short study duration and small sample sizes. Another limitation may be due to the low bioavailability of THC and CBD in oral preparations. This means that there is an obvious need for larger well-conducted studies.

To our knowledge, five RCTs and two open-label studies were published in AD regarding medical cannabis effectiveness and safety (Volicer et al., 1997; Walther et al., 2006; Mahlberg and Walther, 2007; Walther et al., 2011; Ahmed et al., 2015; Shelef et al., 2016; Herrmann et al., 2019). Results of these studies are available in **Table 3**. Only dronabinol and nabilone were experimented in AD patients. The benefits published in these studies were improving in agitation, nocturnal motor activity, disturbed behavior, anorexia, and the patient's global impression of change (Volicer et al., 1997; Walther et al., 2006; Walther et al., 2011; Shelef et al., 2016; Herrmann et al., 2019).

To resume, among these 19 clinical studies, nine were randomized double-blind placebo-controlled designed. Open-label design has inherent limitations of a placebo effect and rater bias. Moreover, as the experimental products and the routes of administration used were different (synthetic or natural and mix of cannabinoids or only one cannabinoid; per os or smoked), it adds an additional difficulty to compare results. According to the experimental product, it could also be difficult to perform a placebo-controlled design because of the conspicuous and characteristic smell of a cannabis cigarette, for example. It still underlines that more well-conducted studies would be necessary to further strengthen evidence of effectiveness. Nevertheless, these results are hopeful for patients suffering from these neurological disorders. Moreover, adverse effects reported with the use of medical cannabis do not seem to be limiting for its clinical use. Reported adverse effects were expected ones compared to the knowledge of cannabis use in general population (drowsiness, euphoria, sleepiness, weakness, dizziness, hypotension, and dry mouth). Due to pharmacokinetics variability of medical cannabis (and its numerous metabolites), future studies should apply parallel group study design rather than crossover design.

To date, 13 studies are registered in clinicaltrials.gov; two protocols are already published in Medline (Urbi et al., 2019a; Timler et al., 2020). Urbi et al. published a protocol of a randomized double-blind placebo-controlled study in ALS patients to evaluate the efficacy of a mix oil of CBD:THC (25 mg CBD: <2 mg THC) in slowing the disease progression. Secondary objectives are safety and tolerability. Timler et al. carried a randomized double-blind crossover study experimenting a mix oil of THC:CBD (3:2) in patients with dementia, on behavior symptoms, quality of life, and discomfort by pain.

Overall, clinical studies exploring medical cannabis in neurological disorders show different results depending on the methods and outcomes. Some show benefits on motor symptoms of neurological diseases, some on non-motor symptoms, and others no benefit at all. Therefore, it is becoming essential to conduct more and larger clinical studies in order to scientifically enlighten clinicians and first and foremost patients.

WHAT ABOUT PATIENTS' VIEW?

As cannabis has been presented as a treatment for many medical conditions for few years, patients experiment this plant in many ways to manage their neurological disorders. Nevertheless, very few surveys have been conducted to describe 1) the consumers (medical condition and demographics); 2) the consumption (the cannabinoid type, form, route of administration, frequency, duration, and way of acquisition); 3) the relief symptoms (duration and level of the relief); 4) the adverse effects (type, duration, and frequency). It is unavoidable to understand the motivations and experiences of cannabis use among people living with neurological disorders to better orient clinical trials.

In 2004, Amtmann and colleagues published a worldwide anonymous web survey analyzing the answers of 131 ALS patients (Amtmann et al., 2004). The mean age was 54 years, and patients were mostly male (75%). Respondents reported a stable family life and a high education level for the majority. The median time since ALS diagnosis was 3 years, and the mean duration was 4 years. About 10% of the respondents (n = 13) reported the use of cannabis to relieve symptoms of ALS in the last 12 months. They mostly consume smoking cannabis. Only three of them reported using medical cannabis (dronabinol). Concerning relieve symptoms, patients reported cannabis as moderately effective in symptoms of appetite loss, depression, pain, spasticity, and drooling and ineffective in reducing difficulties with speech and swallowing and sexual dysfunction. The longest relief was reported for depression. In 2004,

Venderova and colleagues also sent an anonymous questionnaire to all patients attending the Prague Movement Disorder Centre (Venderová et al., 2004). In total, 339 questionnaires were returned, and 25% of the respondents declared having taken cannabis. The mean age of cannabis users was 63.9 years, and the mean duration of PD was 8.3 years. They mostly reported an oral consumption once a day. Interestingly, none of them reported their doctor. PD patients described alleviations, especially in motor symptoms: 44.7% in bradykinesia, 37.7% in muscle rigidity, 30% in tremor, and 14.1% in L-dopa-induced dyskinesias. Another anonymous web survey managed on PD patients in the United States in 2020 analyzed the answers of 1,064 patients (Feeney et al., 2021). The mean age of the respondents was 71.2 years, male accounted for 52.5%, and the mean PD duration was 7.4 years. They were mostly highly educated with 78% of retired, evolving in a stable family life. About 25% of them reported the use of cannabis in the previous 6 months, and 35.6% of them considered themselves as regular users. They most frequently reported spraying or drooping, smoking, and eating as their primary method of cannabis use. The ways of acquisition were medical dispensary (38.7%) and family/friend gift (24.5%). When known, patients reported products with a high THC dosage in 21.2%, to get a better efficacy for both motor and non-motor symptoms. The reported relief symptoms were non-motor symptoms insufficiently controlled by classic medications: anxiety (45.5%), pain (44%), sleep disorders (44%), and specific motor symptoms such as stiffness (43%) or tremor (42%). Only 12.6% of PD cannabis users reported adverse effects (anxiety, impaired coordination, and dizziness). Interestingly, cannabis non-users (75.5%) reported two major reasons for not using cannabis: the lack of evidence (59.9%) and the fear of cannabis adverse effects (34.9%). In 2021, a German nationwide questionnaire survey described the used of medical cannabis in PD patients (Yenilmez et al., 2021). A total of 1,348 questionnaires were analyzed. The mean age of the patients was 71.6 years, and the mean PD duration was 11.6 years. Cannabis use was reported in 8.4% of the questionnaires, with a reduction of pain and muscle cramps in more than 40% of users (respectively, 43.9 and 41.4%). Moreover, more than 20% of them described an improvement in depression (28.1%), stiffness/akinesia (27.3%), sleep disorders (27.1%), freezing (25.0%), tremor (24.3%), anxiety (24.0%), and restless legs syndrome (21.4%). The improvements were related to 54.1% of oral CBD use and to 68.2% inhaling THC-containing cannabis. In the majority of patients (85%), cannabis was well-tolerated. Adverse effects reported were mainly fatigue, dizziness, and ravenous appetite. Another recent survey showed that 95% of movement disorders specialist neurologists reported to be asked to prescribe medical cannabis to their patients (Bega et al., 2017).

Concerning AD patients, a recent Polish anonymous web survey addressed to caregivers identified the attitudes and beliefs of caregivers of individuals with AD toward CBD oil in Poland (Leszko and Meenrajan, 2021). A total of 73 caregivers answered the questionnaire. They reported an effective use of CBD oil in behavioral symptoms of AD, to slow memory loss, agitation, anxiety, and insomnia. Most of the caregivers (84%) answered that CBD oil improved their care recipient's quality of life. None of them reported adverse effects with the use of CBD oil. It is also interesting to note that only 63% of them informed their physician about this habit. In this survey, people also reported lack of information about the legacy, the medical use of cannabis as far as a lack of scientific data.

Despite being great sources of information, these surveys present several limitations. First, the results are based on small sample sizes compared to the affected population; respondents may not have been representative of the entire ALS, PD, and AD population. Second, internet users' population constitutes a selection bias because all patients with neurological disorders could not use the internet, and internet users tend to be highly educated. Third, cannabis users may have been more inclined to answer the surveys and inflated the number of users and benefits, leading to a possible answer bias. Another limitation is the country of survey and/or residence because cannabis could be legal or not.

WHAT ARE THE MAIN RISKS OF CANNABIS USE IN NEUROLOGICAL DISORDERS?

The main risk is that most of the therapeutic uses of cannabis are performed outside of a medical pathway exposing patients to uncontrolled drugs (street cannabis) and unexpected drug–drug interactions. Published case reports show CBD interactions with antiepileptic drugs (Anderson et al., 2019; Gilmartin et al., 2021), warfarin (Grayson et al., 2018), immunosuppressants such as tacrolimus (Leino et al., 2019), and methadone (Madden et al., 2020). In all these case reports, the consequence of the drug–drug interaction was an increase in plasma concentrations of co-administered medications and potential associated complications. Cannabis could also cause well-known psychiatric adverse effects (psychosis, paranoia, anxiety, disorientation, etc.). Therefore, it is important to supervise and regulate the consumption of medical cannabis. In the majority of clinical studies exploring medical cannabis, exclusion criteria included history of psychiatric disorders (Collin et al., 2007; Wallace et al., 2015; van de Donk et al., 2019). In addition to the respiratory adverse effects, cardiovascular complications are poorly known but also reported with the use of cannabis. Jouanjus et al. conducted an observational retrospective study in 2011 in patients admitted to a French hospital with a relation of cannabis use. In total, 200 patients were included, and 619 adverse effects were reported with 9.5% of cardiovascular ones (Jouanjus et al., 2011). Serious cardiovascular complications described with the use of cannabis are arrythmia including ventricular tachycardia, acute coronary syndromes, peripheral complications (arteriopathies), and cerebral complications (acute cerebral angiopathy, transient cortical blindness, and spasm of the cerebral artery) (Jouanjus et al., 2014; Goyal et al., 2017). There is a lack of consensus that likely reflects a general knowledge gap and paucity of data to guide clinical practice. Nevertheless, it is essential to supervise cannabis consumption and consider the medical history and the

concomitant medication use in these patients to avoid serious complications.

DISCUSSION/CONCLUSION

In recent decades, the endocannabinoid system has attracted considerable interest as a potential therapeutic target in numerous pathological conditions. Medical cannabis has clearly demonstrated several benefits on neurological disorders, owing to its pleiotropic pharmacological activity. Preclinical, clinical, and real-life experiences described in ALS, PD, and AD are even more important cues to develop research on medical cannabis. Acceptable safety and tolerability profiles are also strong arguments to be considered in the development of medical cannabis. It is now essential to answer several questions to broadly develop medical cannabis in neurological disorders: 1) which cannabinoids (THC, CBD, mix of THC and CBD, and others)? 2) What dosage? 3) What frequency? 4) Which route of administration? 5) In which symptoms? Answers to these questions will be helpful for patients and clinicians to manage care pathways with medical cannabis treatment.

As all pharmacological substance and a fortiori with its pleiotropic activity, clinicians should be cautious with the use of medical cannabis because of drug–drug interactions and with the medical history of their patients. Only few patients inform their clinicians of cannabis experience, and very few data are available on these interactions.

Nevertheless, there is a call for more clinical studies to secure cannabis consumption in a medical enrollment. As far as patients and patients' associations are calling for it, there is an urgent need to manage clinical studies to provide stronger evidence and secure medical cannabis use. Therefore, more controlled clinical studies with larger neuropsychiatric populations should be prioritized to bring important cues in the near future and support the translation of research findings to clinical settings.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

REFERENCES

Ahmed, A. I., van den Elsen, G. A., Colbers, A., Kramers, C., Burger, D. M., van der Marck, M. A., et al. (2015). Safety, Pharmacodynamics, and Pharmacokinetics of Multiple Oral Doses of delta-9-tetrahydrocannabinol in Older Persons with Dementia. *Psychopharmacology (Berl)* 232, 2587–2595. doi:10.1007/s00213-015-3889-y

Amtmann, D., Weydt, P., Johnson, K. L., Jensen, M. P., and Carter, G. T. (2004). Survey of Cannabis Use in Patients with Amyotrophic Lateral Sclerosis. *Am. J. Hosp. Palliat. Care* 21, 95–104. doi:10.1177/104990910402100206

Anderson, L. L., Absalom, N. L., Abelev, S. V., Low, I. K., Doohan, P. T., Martin, L. J., et al. (2019). Coadministered Cannabidiol and Clobazam: Preclinical Evidence for Both Pharmacodynamic and Pharmacokinetic Interactions. *Epilepsia* 60, 2224–2234. doi:10.1111/epi.16355

Appel, S. H., Zhao, W., Beers, D. R., and Henkel, J. S. (2011). The Microglial-Motoneuron Dialogue in ALS. *Acta Myol* 30, 4–8.

Aso, E., and Ferrer, I. (2014). Cannabinoids for Treatment of Alzheimer's Disease: Moving toward the Clinic. *Front. Pharmacol.* 5, 37. doi:10.3389/fphar.2014.00037

Bega, D., Simuni, T., Okun, M. S., Chen, X., and Schmidt, P. (2017). Medicinal Cannabis for Parkinson's Disease: Practices, Beliefs, and Attitudes Among Providers at National Parkinson Foundation Centers of Excellence. *Mov Disord. Clin. Pract.* 4, 90–95. doi:10.1002/mdc3.12359

Bilsland, L. G., and Greensmith, L. (2008). The Endocannabinoid System in Amyotrophic Lateral Sclerosis. *Curr. Pharm. Des.* 14, 2306–2316. doi:10.2174/138161208785740081

Carroll, C. B., Bain, P. G., Teare, L., Liu, X., Joint, C., Wroath, C., et al. (2004). Cannabis for Dyskinesia in Parkinson Disease: A Randomized Double-Blind Crossover Study. *Neurology* 63, 1245–1250. doi:10.1212/01.WNL.0000140288.48796.8E

Carter, G. T., Abood, M. E., Aggarwal, S. K., and Weiss, M. D. (2010). Cannabis and Amyotrophic Lateral Sclerosis: Hypothetical and Practical Applications, and a Call for Clinical Trials. *Am. J. Hosp. Palliat. Care* 27, 347–356. doi:10.1177/1049909110369531

Chagas, M. H., Eckeli, A. L., Zuardi, A. W., Pena-Pereira, M. A., Sobreira-Neto, M. A., Sobreira, E. T., et al. (2014a). Cannabidiol Can Improve Complex Sleep-Related Behaviours Associated with Rapid Eye Movement Sleep Behaviour Disorder in Parkinson's Disease Patients: a Case Series. *J. Clin. Pharm. Ther.* 39, 564–566. doi:10.1111/jcpt.12179

Chagas, M. H., Zuardi, A. W., Tumas, V., Pena-Pereira, M. A., Sobreira, E. T., Bergamaschi, M. M., et al. (2014b). Effects of Cannabidiol in the Treatment of Patients with Parkinson's Disease: an Exploratory Double-Blind Trial. *J. Psychopharmacol.* 28, 1088–1098. doi:10.1177/0269881114550355

Collin, C., Davies, P., Mutiboko, I. K., and Ratcliffe, S.,and for the Sativex Spasticity in MS Study Group (2007). Randomized Controlled Trial of Cannabis-Based Medicine in Spasticity Caused by Multiple Sclerosis. *Eur. J. Neurol.* 14, 290–296. doi:10.1111/j.1468-1331.2006.01639.x

Consroe, P., Sandyk, R., and Snider, S. R. (1986). Open Label Evaluation of Cannabidiol in Dystonic Movement Disorders. *Int. J. Neurosci.* 30, 277–282. doi:10.3109/00207458608985678

Cooray, R., Gupta, V., and Suphioglu, C. (2020). Current Aspects of the Endocannabinoid System and Targeted THC and CBD Phytocannabinoids as Potential Therapeutics for Parkinson's and Alzheimer's Diseases: a Review. *Mol. Neurobiol.* 57, 4878–4890. doi:10.1007/s12035-020-02054-6

Cristino, L., Bisogno, T., and Di Marzo, V. (2020). Cannabinoids and the Expanded Endocannabinoid System in Neurological Disorders. *Nat. Rev. Neurol.* 16, 9–29. doi:10.1038/s41582-019-0284-z

Esposito, G., De Filippis, D., Carnuccio, R., Izzo, A. A., and Iuvone, T. (2006). The Marijuana Component Cannabidiol Inhibits Beta-Amyloid-Induced Tau Protein Hyperphosphorylation through Wnt/beta-Catenin Pathway rescue in PC12 Cells. *J. Mol. Med. (Berl)* 84, 253–258. doi:10.1007/s00109-005-0025-1

Feeney, M. P., Bega, D., Kluger, B. M., Stoessl, A. J., Evers, C. M., De Leon, R., et al. (2021). Weeding through the Haze: a Survey on Cannabis Use Among People Living with Parkinson's Disease in the US. *Npj Parkinsons Dis.* 7, 21. doi:10.1038/s41531-021-00165-y

Ferreira, C., Almeida, C., Tenreiro, S., and Quintas, A. (2020). Neuroprotection or Neurotoxicity of Illicit Drugs on Parkinson's Disease. *Life (Basel)* 10, E86. doi:10.3390/life10060086

Fraguas-Sánchez, A. I., and Torres-Suárez, A. I. (2018). Medical Use of Cannabinoids. *Drugs* 78, 1665–1703. doi:10.1007/s40265-018-0996-1

Frankel, J. P., Hughes, A., Lees, A. J., and Stern, G. M. (1990). Marijuana for Parkinsonian Tremor. *J. Neurol. Neurosurg. Psychiatry* 53, 436. doi:10.1136/jnnp.53.5.436

García-Arencibia, M., González, S., de Lago, E., Ramos, J. A., Mechoulam, R., and Fernández-Ruiz, J. (2007). Evaluation of the Neuroprotective Effect of Cannabinoids in a Rat Model of Parkinson's Disease: Importance of

Antioxidant and Cannabinoid Receptor-independent Properties. *Brain Res.* 1134, 162–170. doi:10.1016/j.brainres.2006.11.063

Gilmartin, C. G. S., Dowd, Z., Parker, A. P. J., and Harijan, P. (2021). Interaction of Cannabidiol with Other Antiseizure Medications: A Narrative Review. *Seizure* 86, 189–196. doi:10.1016/j.seizure.2020.09.010

Goyal, H., Awad, H. H., and Ghali, J. K. (2017). Role of Cannabis in Cardiovascular Disorders. *J. Thorac. Dis.* 9, 2079–2092. doi:10.21037/jtd.2017.06.104

Grayson, L., Vines, B., Nichol, K., and Szaflarski, J. P.UAB CBD Program (2018). An Interaction between Warfarin and Cannabidiol, a Case Report. *Epilepsy Behav. Case Rep.* 9, 10–11. doi:10.1016/j.ebcr.2017.10.001

Herrmann, N., Ruthirakuhan, M., Gallagher, D., Verhoeff, N. P. L. G., Kiss, A., Black, S. E., et al. (2019). Randomized Placebo-Controlled Trial of Nabilone for Agitation in Alzheimer's Disease. *Am. J. Geriatr. Psychiatry* 27, 1161–1173. doi:10.1016/j.jagp.2019.05.002

Joerger, M., Wilkins, J., Fagagnini, S., Baldinger, R., Brenneisen, R., Schneider, U., et al. (2012). Single-dose Pharmacokinetics and Tolerability of Oral delta-9- Tetrahydrocannabinol in Patients with Amyotrophic Lateral Sclerosis. *Drug Metab. Lett.* 6, 102–108. doi:10.2174/187231281120602010 2

Jouanjus, E., Leymarie, F., Tubery, M., and Lapeyre-Mestre, M. (2011). Cannabis-related Hospitalizations: Unexpected Serious Events Identified through Hospital Databases. *Br. J. Clin. Pharmacol.* 71, 758–765. doi:10.1111/j.1365-2125.2010.03897.x

Jouanjus, E., Lapeyre-Mestre, M., and Micallef, J.The French Association of the Regional Abuse and Dependence Monitoring Centres (CEIP-A) Working Group on Cannabis Complications (2014). Cannabis Use: Signal of Increasing Risk of Serious Cardiovascular Disorders. *J. Am. Heart Assoc.* 3, 638. doi:10.1161/JAHA.113.000638

Leino, A. D., Emoto, C., Fukuda, T., Privitera, M., Vinks, A. A., and Alloway, R. R. (2019). Evidence of a Clinically Significant Drug-Drug Interaction between Cannabidiol and Tacrolimus. *Am. J. Transpl.* 19, 2944–2948. doi:10.1111/ajt.15398

Leszko, M., and Meenrajan, S. (2021). Attitudes, Beliefs, and Changing Trends of Cannabidiol (CBD) Oil Use Among Caregivers of Individuals with Alzheimer's Disease. *Complement. Ther. Med.* 57, 102660. doi:10.1016/j.ctim.2021.102660

Li, H., Liu, Y., Tian, D., Tian, L., Ju, X., Qi, L., et al. (2020). Overview of Cannabidiol (CBD) and its Analogues: Structures, Biological Activities, and Neuroprotective Mechanisms in Epilepsy and Alzheimer's Disease. *Eur. J. Med. Chem.* 192, 112163. doi:10.1016/j.ejmech.2020.112163

Lotan, I., Treves, T. A., Roditi, Y., and Djaldetti, R. (2014). Cannabis (Medical Marijuana) Treatment for Motor and Non-motor Symptoms of Parkinson Disease: an Open-Label Observational Study. *Clin. Neuropharmacol* 37, 41–44. doi:10.1097/WNF.0000000000000016

Lucas, C. J., Galettis, P., and Schneider, J. (2018). The Pharmacokinetics and the Pharmacodynamics of Cannabinoids. *Br. J. Clin. Pharmacol.* 84, 2477–2482. doi:10.1111/bcp.13710

Madden, K., Tanco, K., and Bruera, E. (2020). Clinically Significant Drug-Drug Interaction between Methadone and Cannabidiol. *Pediatrics* 145, e20193256. doi:10.1542/peds.2019-3256

Mahlberg, R., and Walther, S. (2007). Actigraphy in Agitated Patients with Dementia. Monitoring Treatment Outcomes. *Z. Gerontol. Geriatr.* 40, 178–184. doi:10.1007/s00391-007-0420-z

Meyer, T., Funke, A., Münch, C., Kettemann, D., Maier, A., Walter, B., et al. (2019). Real World Experience of Patients with Amyotrophic Lateral Sclerosis (ALS) in the Treatment of Spasticity Using Tetrahydrocannabinol: cannabidiol (THC:CBD). *BMC Neurol.* 19, 222. doi:10.1186/s12883-019-1443-y

Morgese, M. G., Cassano, T., Cuomo, V., and Giuffrida, A. (2007). Anti-dyskinetic Effects of Cannabinoids in a Rat Model of Parkinson's Disease: Role of CB(1) and TRPV1 Receptors. *Exp. Neurol.* 208, 110–119. doi:10.1016/j.expneurol.2007.07.021

Papadimitriou, D., Le Verche, V., Jacquier, A., Ikiz, B., Przedborski, S., and Re, D. B. (2010). Inflammation in ALS and SMA: Sorting Out the Good from the Evil. *Neurobiol. Dis.* 37, 493–502. doi:10.1016/j.nbd.2009.10.005

Patricio, F., Morales-Andrade, A. A., Patricio-Martínez, A., and Limón, I. D. (2020). Cannabidiol as a Therapeutic Target: Evidence of its Neuroprotective and Neuromodulatory Function in Parkinson's Disease. *Front. Pharmacol.* 11, 595635. doi:10.3389/fphar.2020.595635

Ramírez, B. G., Blázquez, C., Gómez del Pulgar, T., Guzmán, M., and de Ceballos, M. L. (2005). Prevention of Alzheimer's Disease Pathology by Cannabinoids: Neuroprotection Mediated by Blockade of Microglial Activation. *J. Neurosci.* 25, 1904–1913. doi:10.1523/JNEUROSCI.4540-04.2005

Riva, N., Mora, G., Sorarù, G., Lunetta, C., Ferraro, O. E., Falzone, Y., et al. (2019). Safety and Efficacy of Nabiximols on Spasticity Symptoms in Patients with Motor Neuron Disease (CANALS): a Multicentre, Double-Blind, Randomised, Placebo-Controlled, Phase 2 Trial. *Lancet Neurol.* 18, 155–164. doi:10.1016/S1474-4422(18)30406-X

Segovia, G., Mora, F., Crossman, A. R., and Brotchie, J. M. (2003). Effects of CB1 Cannabinoid Receptor Modulating Compounds on the Hyperkinesia Induced by High-Dose Levodopa in the Reserpine-Treated Rat Model of Parkinson's Disease. *Mov Disord.* 18, 138–149. doi:10.1002/mds.10312

Selkoe, D. J. (2011). Alzheimer's Disease. *Cold Spring Harb Perspect. Biol.* 3, a004457. doi:10.1101/cshperspect.a004457

Shelef, A., Barak, Y., Berger, U., Paleacu, D., Tadger, S., Plopsky, I., et al. (2016). Safety and Efficacy of Medical Cannabis Oil for Behavioral and Psychological Symptoms of Dementia: An-Open Label, Add-On, Pilot Study. *J. Alzheimers Dis.* 51, 15–19. doi:10.3233/JAD-150915

Shi, J., Cai, Q., Zhang, J., He, X., Liu, Y., Zhu, R., et al. (2017). AM1241 Alleviates MPTP-Induced Parkinson's Disease and Promotes the Regeneration of DA Neurons in PD Mice. *Oncotarget* 8, 67837–67850. doi:10.18632/oncotarget.18871

Sieradzan, K. A., Fox, S. H., Hill, M., Dick, J. P., Crossman, A. R., and Brotchie, J. M. (2001). Cannabinoids Reduce Levodopa-Induced Dyskinesia in Parkinson's Disease: a Pilot Study. *Neurology* 57, 2108–2111. doi:10.1212/WNL.57.11.2108

Song, L., Yang, X., Ma, Y., Wu, N., and Liu, Z. (2014). The CB1 Cannabinoid Receptor Agonist Reduces L-DOPA-Induced Motor Fluctuation and ERK1/2 Phosphorylation in 6-OHDA-Lesioned Rats. *Drug Des. Devel Ther.* 8, 2173–2179. doi:10.2147/DDDT.S60944

Stampanoni Bassi, M., Sancesario, A., Morace, R., Centonze, D., and Iezzi, E. (2017). Cannabinoids in Parkinson's Disease. *Cannabis Cannabinoid Res.* 2, 21–29. doi:10.1089/can.2017.0002

Timler, A., Bulsara, C., Bulsara, M., Vickery, A., Smith, J., and Codde, J. (2020). Use of Cannabinoid-Based Medicine Among Older Residential Care Recipients Diagnosed with Dementia: Study Protocol for a Double-Blind Randomised Crossover Trial. *Trials* 21, 188. doi:10.1186/s13063-020-4085-x

Urbi, B., Broadley, S., Bedlack, R., Russo, E., and Sabet, A. (2019a). Study Protocol for a Randomised, Double-Blind, Placebo-Controlled Study Evaluating the Efficacy of Cannabis-Based Medicine Extract in Slowing the Disease pRogression of Amyotrophic Lateral Sclerosis or Motor Neurone Disease: the EMERALD Trial. *BMJ Open* 9, e029449. doi:10.1136/bmjopen-2019-029449

Urbi, B., Owusu, M. A., Hughes, I., Katz, M., Broadley, S., and Sabet, A. (2019b). Effects of Cannabinoids in Amyotrophic Lateral Sclerosis (ALS) Murine Models: a Systematic Review and Meta-Analysis. *J. Neurochem.* 149, 284–297. doi:10.1111/jnc.14639

van de Donk, T., Niesters, M., Kowal, M. A., Olofsen, E., Dahan, A., and van Velzen, M. (2019). An Experimental Randomized Study on the Analgesic Effects of Pharmaceutical-Grade Cannabis in Chronic Pain Patients with Fibromyalgia. *Pain* 160, 860–869. doi:10.1097/j.pain.0000000000001464

Venderová, K., Růžička, E., Vorříšek, V., and Višňovský, P. (2004). Survey on Cannabis Use in Parkinson's Disease: Subjective Improvement of Motor Symptoms. *Mov. Disord.* 19, 1102–1106. doi:10.1002/mds.20111

Volicer, L., Stelly, M., Morris, J., McLaughlin, J., and Volicer, B. J. (1997). Effects of Dronabinol on Anorexia and Disturbed Behavior in Patients with Alzheimer's Disease. *Int. J. Geriatr. Psychiatry* 12, 913–919. doi:10.1002/(sici)1099-1166(199709)12:9<913::aid-gps663>3.0.co;2-d

Wallace, M. S., Marcotte, T. D., Umlauf, A., Gouaux, B., and Atkinson, J. H. (2015). Efficacy of Inhaled Cannabis on Painful Diabetic Neuropathy. *J. Pain* 16, 616–627. doi:10.1016/j.jpain.2015.03.008

Walther, S., Mahlberg, R., Eichmann, U., and Kunz, D. (2006). Delta-9-tetrahydrocannabinol for Nighttime Agitation in Severe Dementia. *Psychopharmacology (Berl)* 185, 524–528. doi:10.1007/s00213-006-0343-1

Walther, S., Schüpbach, B., Seifritz, E., Homan, P., and Strik, W. (2011). Randomized, Controlled Crossover Trial of Dronabinol, 2.5 Mg, for Agitation in 2 Patients with Dementia. *J. Clin. Psychopharmacol.* 31, 256–258. doi:10.1097/JCP.0b013e31820e861c

Weber, M., Goldman, B., and Truniger, S. (2010). Tetrahydrocannabinol (THC) for Cramps in Amyotrophic Lateral Sclerosis: a Randomised, Double-Blind Crossover Trial. *J. Neurol. Neurosurg. Psychiatry* 81, 1135–1140. doi:10.1136/jnnp.2009.200642

Yenilmez, F., Fründt, O., Hidding, U., and Buhmann, C. (2021). Cannabis in Parkinson's Disease: The Patients' View. *J. Parkinsons Dis.* 11, 309–321. doi:10.3233/JPD-202260

Zuardi, A. W., Crippa, J. A., Hallak, J. E., Pinto, J. P., Chagas, M. H., Rodrigues, G. G., et al. (2009). Cannabidiol for the Treatment of Psychosis in Parkinson's Disease. *J. Psychopharmacol.* 23, 979–983. doi:10.1177/0269881108096519

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Pharmacology

MINI REVIEW
published: 09 May 2022
doi: 10.3389/fphar.2022.908198

Medical Cannabis Activity Against Inflammation: Active Compounds and Modes of Action

Seegehalli M. Anil†, *Hadar Peeri*† *and Hinanit Koltai* *

Institute of Plant Science, Agriculture Research Organization, Volcani Center, Rishon LeZion, Israel

Inflammation often develops from acute, chronic, or auto-inflammatory disorders that can lead to compromised organ function. Cannabis (*Cannabis sativa*) has been used to treat inflammation for millennia, but its use in modern medicine is hampered by a lack of scientific knowledge. Previous studies report that cannabis extracts and inflorescence inhibit inflammatory responses *in vitro* and in pre-clinical and clinical trials. The endocannabinoid system (ECS) is a modulator of immune system activity, and dysregulation of this system is involved in various chronic inflammations. This system includes cannabinoid receptor types 1 and 2 (CB1 and CB2), arachidonic acid-derived endocannabinoids, and enzymes involved in endocannabinoid metabolism. Cannabis produces a large number of phytocannabinoids and numerous other biomolecules such as terpenes and flavonoids. In multiple experimental models, both *in vitro* and *in vivo*, several phytocannabinoids, including Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabigerol (CBG), exhibit activity against inflammation. These phytocannabinoids may bind to ECS and/or other receptors and ameliorate various inflammatory-related diseases by activating several signaling pathways. Synergy between phytocannabinoids, as well as between phytocannabinoids and terpenes, has been demonstrated. Cannabis activity can be improved by selecting the most active plant ingredients (API) while eliminating parts of the whole extract. Moreover, in the future cannabis components might be combined with pharmaceutical drugs to reduce inflammation.

Keywords: inflammation, medicinal cannabis, phytocannabinoids, Δ 9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG)

OPEN ACCESS

Edited by:
Francesca Baratta, University of Turin, Italy

Reviewed by:
Ethan Budd Russo, CReDO Sciecne, United States

***Correspondence:**
Hinanit Koltai
hkoltai@agri.gov.il

†*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Experimental Pharmacology and Drug Discovery, a section of the journal Frontiers in Pharmacology

Received: *30 March 2022*
Accepted: *08 April 2022*
Published: *09 May 2022*

Citation:
Anil SM, Peeri H and Koltai H (2022) Medical Cannabis Activity Against Inflammation: Active Compounds and Modes of Action. Front. Pharmacol. 13:908198. doi: 10.3389/fphar.2022.908198

INTRODUCTION

The immune system consists of both adaptive and innate immunity. Innate immunity is the rapid and non-specific response to pathogens mediated by myeloid cells and natural killer (NK) cells. On the other hand, adaptive immunity is a slower but specific response that generates immunological memory, involving the activation of B and T lymphocytes (Netea et al., 2019). During normal inflammation, innate immunity is activated within minutes to hours as a first line of defense against pathogen infection, followed by the elimination of the threats carried out by both the innate and the adaptive immune responses (Netea et al., 2019). Ending inflammation and returning to homeostasis is a process known as resolution. However, failure to remove the inciting stimulus efficiently can lead

Abbreviations: CBD, cannabidiol; CBG, cannabigerol; Δ^9-THC, Δ^9-tetrahydrocannabinol.

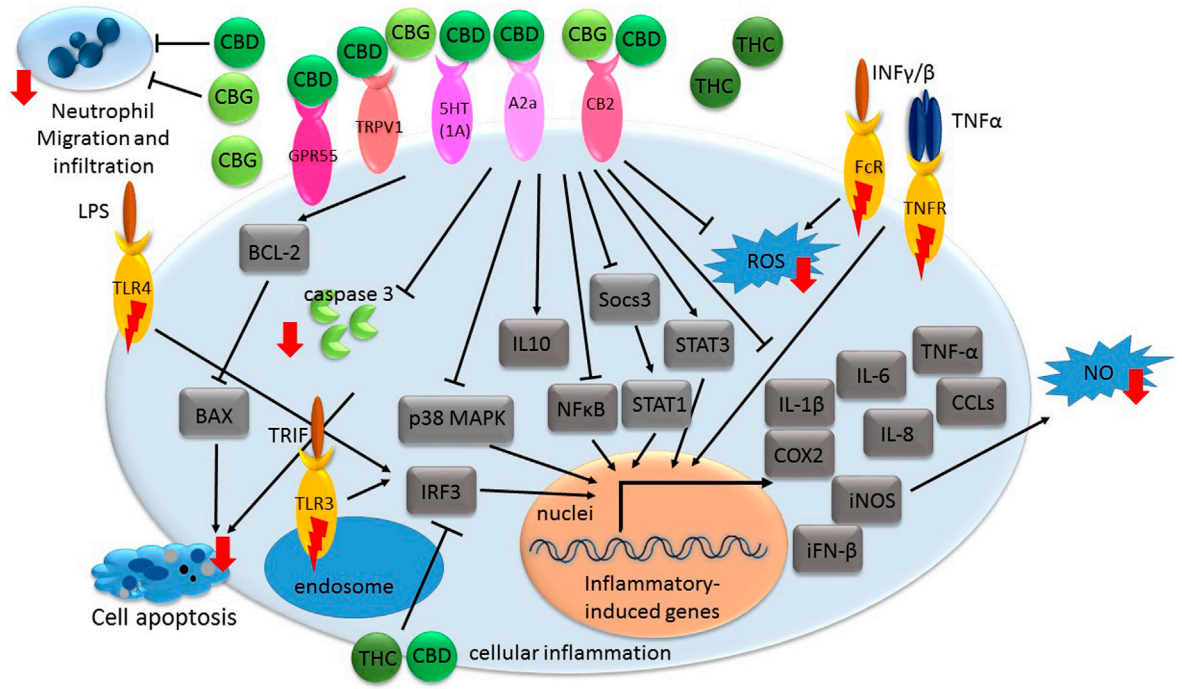


FIGURE 1 | A general illustration of some of the signaling pathways suggested being associated with phytocannabinoid-mediated inflammation suppression. Receptors with inflammatory-inducing activity are marked with a red lightning bolt. Other receptors interact with phytocannabinoids to convey anti-inflammatory responses. Genes or proteins are designated in rectangular boxes. Red arrows denote reduction in biological processes or components following cannabinoid treatments. CBD-cannabidiol; CBG-cannabigerol; THC- Δ^9-tetrahydrocannabinol; CB- cannabinoid receptor; GPR- G protein-coupled receptor; TRPV- transient receptor potential vanilloid; A2a-adenosine receptor; iFN- interferon; TNF- tumor necrosis factor; CCL- C-C motif chemokine; IL-interleukin; COX-cyclooxygenase; iNOS- nitric oxide synthase; ROS- reactive oxygen species; NO- nitric oxide; MAPK- mitogen-activated protein kinase; LPS- bacterial lipopolysaccharide; NFκB- nuclear factor kappa B; IRF3- regulatory factor 3; FcR- Fc receptor; TNFR- TNF receptor; INF- interferon; 5HT(1A)- serotonin receptor; TLR-toll-like receptor; TRIF- Toll-Interleukin-1 Receptor (TIR)-domain-containing adaptor-inducing interferon-β.

to the development of chronic inflammation and progression of tissue damage (Feehan and Gilroy, 2019). This kind of chronic, unresolved inflammation contributes significantly to various pathogeneses, including that of asthma (Pothen et al., 2015), COVID-19 (Effenberger et al., 2021), atherosclerosis (Galkina and Ley, 2009), chronic obstructive pulmonary disease (Sevenoaks and Stockley, 2006), inflammatory bowel disease (Actis et al., 2019), neurodegenerative disease (Perry, 2004), multiple sclerosis (Sá, 2012) and rheumatoid arthritis (Masoumi et al., 2021).

Cannabis (*Cannabis sativa*) has been used as medicine for the treatment of inflammation for millennia, but its use in modern medicine has been hampered by a lack of scientific knowledge (Ryz et al., 2017). Previous studies reported that cannabis extracts and inflorescence inhibited inflammatory responses *in vitro* and in pre-clinical and clinical studies. For example, a high-CBD cannabis ethanolic extract reduced the release of skin inflammation mediators in keratinocytes (Sangiovanni et al., 2019). Similarly, a study on a mouse model of colitis showed that oral or intraperitoneally treatment with high-CBD cannabis extract led to a reduction in intestinal inflammation and hypermotility, in contrast to pure CBD treatment at matched doses (Pagano et al., 2016). Moreover, two clinical trials on patients with Crohn's disease reported that daily treatment with THC-rich cannabis inflorescence had beneficial effects against the disease symptoms with no significant side effects and reduced the need for other medications (Naftali et al., 2011; Naftali et al., 2013). In another clinical trial, daily cannabis treatment was associated with lower levels of pro-inflammatory biomarkers in cerebral fluid (CSF) of HIV patients (Watson et al., 2021).

Great efforts have been made to suppress chronic inflammation. Cannabis and its compounds were shown to have anti-inflammatory activity (see **Appendix A** for methodology), but to exploit the full potential of cannabis it is important to define the active molecules and understand the cellular and molecular mechanisms that underlie its anti-inflammatory activity.

A BRIEF DESCRIPTION OF THE CORNERSTONES OF INFLAMMATION

Monocytes are the major starting entities of inflammation. Once released from bone marrow, monocytes migrate through the blood into various tissues and undergo the tissue-specific maturation required to become inflammatory macrophages that respond to infection, injury, or damage. The various sub-populations of activated macrophages may differ in morphology, release of inflammatory mediators and functional properties, but in inflammation they have three major functions: phagocytosis,

antigen presentation and immunomodulation (Fujiwara and Kobayashi, 2005). The process of inflammation is orchestrated via inflammatory mediators. Pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-1β are released from activated macrophages in response to infection (**Figure 1**; Abdulkhaleq et al., 2018). TNF-α and IL-1β act through specific cell membrane-bound receptors and participate in the recruitment of polymorphonuclear neutrophils (PMNs) into the site of infection and their activation (Hackel et al., 2021).

TNF-α facilitates the release of other pro-inflammatory cytokines from immune effector cells, including interferon alpha (IFN-α), interferon gamma (IFN-γ), IL-1β, IL-6, IL-8, Transforming growth factor beta (TGF-β) and chemokines (Silva et al., 2019). Further, in cases of enhanced inflammation, when the cell is stimulated, typically by bacterial lipopolysaccharide (LPS) or pro-inflammatory cytokines, there is induction of inducible nitric oxide synthase (iNOS). An increase in iNOS levels generates significant amounts of nitric oxide (NO) radicals or cyclooxygenase 2 (COX2); COX2 catalyzes the conversion of arachidonic acid to prostaglandins (PGs), prostacyclin and thromboxane A_2 (Salvemini et al., 2013; Cinelli et al., 2020).

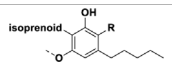
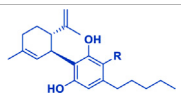
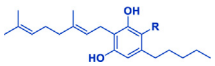
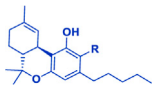
The signal transduction of inflammatory responses involves several signaling pathways including mitogen-activated protein kinase (MAPK), toll-like receptor (TLR), Janus kinase/signal transducers and activators of transcription (JAK-STAT), and nuclear factor kappa B (NFκB) pathways (**Figure 1**; Zhao et al., 2021). The activation of these pathways involves a series of phosphorylation events leading to the induction of various anti-apoptotic target genes and the expression of cytokines, chemokines, and adhesion molecules (Taniguchi and Karin, 2018; Fitzgerald and Kagan, 2020). Moreover, during inflammatory processes, reactive oxygen species (ROS) are commonly multiplied and can contribute to host cell and organ damage. Further, intracellular redox changes induced by ROS augment NF-κB activation through the phosphorylation and degradation of IκB by increasing IkB kinase β (IKK) or Akt kinase activity (Haddad, 2002).

Resolution of inflammation may involve increased production of IL-10, among others. IL-10 is an anti-inflammatory cytokine, which inhibits the release of lipid mediators and pro-inflammatory cytokines (e.g., IL-1β, IL-6, and TNF-α; **Figure 1**; Panigrahy et al., 2021).

THE ENDOCANNABINOID SYSTEM AND INFLAMMATION

The endocannabinoid system (ECS) is a modulator of multiple physiological activities, including in the nervous, endocrine, immune, blood circulation, gastrointestinal tract and reproductive systems (Di Marzo et al., 1998). Accordingly, dysregulation of the ECS is involved with various pathological conditions, including inflammation among others (Di Marzo and Piscitelli, 2015; Hillard, 2018), whereas therapeutic modulation of ECS activity has beneficial effects on various medical conditions,

TABLE 1 | Representative structures of three major phytocannabinoids.

Phytocannabinoid	
	
CBD	
CBG	
Δ^9-THC	

Abbreviations: CBD, cannabidiol; CBG, cannabigerol; Δ^9-THC, Δ^9-tetrahydrocannabinol.

including those associated with inflammation (Ambrose and Simmons, 2019; Giacobbe et al., 2021). ECS is involved in both innate and adaptive immunity and in several chronic inflammatory diseases (Chiurchiù et al., 2015). ECS includes cannabinoid receptors types 1 and 2 (CB1 and CB2, respectively) and multiple other receptors such as the peroxisome proliferator-activated receptors (PPARs) and ion channels (e.g., the transient receptor potential ankyrin [TRPA] family and the transient receptor potential vanilloid [TRPV] family) (Biringer, 2021). Also included in the ECS are the receptors' ligand, arachidonic acid derived endocannabinoids, and enzymes for endocannabinoid metabolism (Di Marzo et al., 1998).

Most immune cells express endocannabinoids, the enzymes regulating their biosynthesis and degradation, and endocannabinoid receptors (Chiurchiù et al., 2015). Both CB1 and CB2 are expressed in immune cells, with CB2 being expressed 10–100 times higher than CB1 in these cells (Jean-Gilles et al., 2010; Rahaman and Ganguly, 2021). Moreover, CB receptor activation regulates anti-inflammatory responses. For example, activation of CB2 receptors by its agonist inhibited the release of the pro-inflammatory cytokine IL-12 and IL-23 and enhanced the release of the anti-inflammatory cytokine IL-10 from cultured activated macrophages. This study suggested that the inhibitory effect of CB2 on IL-12 production was mediated by ERK1/2-MAPK (Correa et al., 2009).

In another example, a CB2 receptor agonist reduced in human peripheral blood mononuclear cells LPS-induced ERK1/2 and NF-kB-p65 phosphorylation and release of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and IL-8 (Capozzi et al., 2021). A selective/inverse agonist of CB2 induced the differentiation of Th0 cells into regulatory T cells (Treg) cell phenotypes in a naïve $CD4^+$ T lymphocyte population isolated from a mouse spleen. The Treg phenotype is important for suppressing immune response by inhibiting T cell proliferation and cytokine production. The Treg phenotype was induced via P38 phosphorylation and STAT5A activation and was characterized by the expression of FoxP3, TGF-β and IL-10. Accordingly, treatment with this CB2 selective/inverse agonist reduced colitis severity *in vivo* (Gentili et al., 2019).

CANNABIS BIOMOLECULES

Cannabis produces a large number of phytocannabinoids (Hanuš et al., 2016). Phytocannabinoids are aromatic oxygenated hydrocarbons, derived from meroterpenoids with a resorcinyl core structure with isoprenyl, alkyl or aralkyl substitutions. The characteristic alkyl side chain typically contains an odd number of carbon atoms (Hanuš et al., 2016; Gülck and Møller, 2020). They are produced in the plant in their acid form and are decarboxylated to the active form (Gülck and Møller, 2020). Among the phytocannabinoids, Δ^9-trans-tetrahydrocannabinols (Δ^9-THCs) and cannabidiols (CBDs) are the most abundant (**Table 1**). Cannabigerol (CBG) in its acid form (CBGA) serves as a core intermediate that diverges to provide the phytocannabinolic acids (**Table 1**; Hanuš et al., 2016; Tahir et al., 2021).

In addition to phytocannabinoids, cannabis produces a plethora of non-cannabinoid constituents including a vast array of terpenes as the second-largest class of cannabis constituents (El Sohly et al., 2017). Cannabis biosynthesizes flavonoids as well, among them cannflavins, which are prenylated (C5) and geranylated (C10) flavones (Bautista et al., 2021).

KNOWN PHYTOCANNABINOID ACTIVITY AGAINST INFLAMMATION

Cannabidiol

CBD was demonstrated in multiple experimental models, *in vitro* and *in vivo*, to exert anti-inflammatory activities and ameliorate various inflammatory-related degenerative diseases. The mechanism of this anti-inflammatory activity is, however, not completely understood. CBD treatment of hypoxic-ischemic (HI) immature brains of newborn mice was shown to significantly reduce IL-6, TNF-α, COX-2 and iNOS expression in brain slices. This activity was suggested to be mediated via CB2 and adenosine A_{2A} receptors (**Figure 1**; Castillo et al., 2010). Likewise, treatments of lipopolysaccharide-treated mice with a low dose of CBD decreased TNF-α production; this effect was abolished in A_{2A} receptor knockout mice and reversed with an A_{2A} adenosine receptor antagonist, supporting the notion that CBD may enhance adenosine signaling (Carrier et al., 2006). Further, in a murine model of acute lung injury, CBD, via the A_{2A} adenosine receptor, significantly reduced leukocyte migration into the lungs and reduced the levels of albumin, TNF-α, IL-6 and other chemokines in bronchoalveolar lavage fluid (**Figure 1**; Ribeiro et al., 2012). CBD also reduced the activity of myeloperoxidase (MOP, an index of neutrophil infiltration) in lung tissue (Ribeiro et al., 2012).

In newborn pigs with HI brain injury, CBD administration reduced inflammation and prevented the increase in brain IL-1 levels. It also prevented the decrease in the number of viable neurons and the increase of excitotoxicity and oxidative stress. This activity was suggested to be mediated via CB2 and 5HT1A receptors (**Figure 1**; Pazos et al., 2013). In liver filtrate from mice with acute hepatitis, CBD was shown to trigger Myeloid-derived suppressor cells (MDSCs); these cells are regulators of the immune system that suppress T cell functions. MDSCs induction by CBD was mediated through activation of TRPV1 (**Figure 1**). CBD also significantly reduced blood levels of IL-2, TNF-α, IFN-γ, IL-6, IL-12, IL-17, MCP-1 and C-C motif chemokine (CCL)-11 in this model (Hegde et al., 2011).

CBD is also a selective antagonist of GPR55, another G protein-coupled receptor present in human macrophages (**Figure 1**). Pharmacological activation of GPR55 by its selective agonist O-1602 enhanced pro-inflammatory responses in macrophages-derived foam cells associated with a reduction in IL-10 levels and induction in TNF-α levels (Lanuti et al., 2015).

CBD treatment completely inhibited TNF-α production via p38 MAPK pathway (**Figure 1**) in microglial cells isolated from the retinas of newborn rats treated with endotoxin or LPS for acute ocular inflammation. In addition, LPS-treated rat retinas accumulated macrophages and activated microglia, increased levels of ROS and nitrotyrosine, and activated p38 MAPK and neuronal apoptosis. Treatment with CBD blocked all these effects (El-Remessy et al., 2008).

CBD decreases the production and release of IL-1β, IL-6 and IFN-β from LPS-activated microglial cells of BV-2 mice. CBD reduced the activity of the NF-κB pathway and the levels of IL-1β and IL-6. CBD also decreased *Socs3* gene expression; Socs3 is a main negative regulator of STATs. In accordance, CBD treatment up-regulated the STAT3 transcription factor phosphorylation, needed for its activation (**Figure 1**; Kozela et al., 2010). However, NF-κB and STAT3 are likely to play important and in some cases, overlapping roles in pro-inflammatory and cancer processes (He & Karin, 2011). In contrast, CBD decreased the phosphorylation of the LPS-induced STAT1 transcription factor, a key player in pro-inflammatory processes that are IFN-β-dependent (Kozela et al., 2010).

Cannabigerol

The anti-inflammatory activity of CBG is less studied than that of CBD. Yet, several studies demonstrated significant anti-inflammatory activity of CBG. For example, CBG treatment was shown to reduce nitric oxide production in macrophages via the CB2 receptor and reduce ROS formation in intestinal epithelial cells and iNOS expression (**Figure 1**) in the inflamed colons. Treatment with CBG also reduced oedema in colon submucosa. This treatment also reduced the colon weight/length ratio; this ratio is a reliable marker of intestinal inflammation in a murine model of colitis glands (Borrelli et al., 2013). In addition, CBG decreased dinitrobenzene sulfonic acid (DNBS)-induced neutrophil infiltration, as evaluated by MOP activity (Borrelli et al., 2013).

In a study that characterized the anti-inflammatory properties of CBG on human skin cells *in vitro*, it was demonstrated that CBG treatment reduced ROS levels in human dermal fibroblasts, better than vitamin C. CBG also protected human epidermal keratinocytes by inhibiting pro-inflammatory cytokines that were released following induction using UVA, UVB or *Cutibacterium acnes* exposure, including TNF-α, IL-1β, IL-6 and IL-8 (**Figure 1**; Perez et al., 2022). Furthermore, the researchers performed a single-blind clinical study on 20 healthy volunteers with sodium lauryl sulfate (SLS)-induced contact dermatitis and found that topical application of 0.1% CBG serum showed significantly lower trans-epidermal

water loss (TEWL) values compared to placebo and untreated sites. Moreover, the CBG serum reduced redness and inflammation following 48 h treatment, and after 2 weeks of application, the skin condition almost returned to baseline levels of visual grade (Perez et al., 2022).

Several studies have described the neuroprotective properties of CBG against inflammation. It was demonstrated that CBG pre-treatment of cultured motor neurons not only reduced the levels of pro-inflammatory cytokines, including IL-1β, TNF-α and IFN-γ (**Figure 1**), but also inhibited apoptosis in LPS-stimulated macrophages, via suppression of caspase-3 and Bax expression and induction of Bcl-2 levels (Gugliandolo et al., 2018). In addition, in a study that examined the effects of CBG on Huntington's disease pathology in 3-nitropropionate model *in vivo*, it was found that treatment with the phytocannabinoid reduced neuronal death by half and significantly attenuated the upregulation of expression of COX-2, iNOS and pro-inflammatory cytokines such as TNF-α and IL-6 (**Figure 1**; Valdeolivas et al., 2015).

Δ^9-Tetrahydrocannabinol

Several experiments suggest that THC has anti-inflammatory effects. For example, topical treatment of THC on DNFB-mediated allergic contact dermatitis in mice revealed that THC effectively decreased myeloid immune cell infiltration and contact allergic ear swelling (Gaffal et al., 2013). These anti-inflammatory effects were evident in both wild-type and CB1/2 receptor-deficient mice suggesting that these activities of THC were not mediated via CB1 or CB2 receptors. In addition, THC reduced the production by epidermal keratinocytes of CCL8 and CCL2 induced by IFNγ and the production of IFNγ by T cells (**Figure 1**). As a result, in a CB1/2 receptor-independent way, THC limited the recruitment of myeloid immune cells *in vitro* (Gaffal et al., 2013).

Interestingly, in LPS-induced macrophages, THC (and CBD) attenuated TLR3/4 signaling in a MyD88-independent manner (Fitzpatrick et al., 2020). TLR3 signaling is mediated via a toll-interleukin-1 receptor (TIR)-domain-containing adaptor-inducing interferon-β (TRIF). TLR4-induced expression of regulatory factor 3 (IRF3) activation, and CXCL10 and IFN-β were repressed by the THC and/or CBD (alone or in combination) treatments. However, these phytocannabinoid treatments did not impact TNF-α/CXCL8 expression and TLR4-induced IκB-α degradation. These activities of THC and CBD were independent of the cannabinoid receptors or PPARγ (**Figure 1**; Fitzpatrick et al., 2020). Finally, THC, dose-dependently, protected against diclofenac-induced gastric inflammation, hemorrhagic streaks and gastric ulcers in male mice, and protected against tissue damage at doses insufficient to cause common cannabinoid side effects (Kinsey and Cole, 2013).

SYNERGY BETWEEN CANNABIS MOLECULES AND FORMULATIONS OF ACTIVE PLANT INGREDIENTS

The synergy between phytocannabinoids (Mazuz et al., 2020; Anis et al., 2021) as well as between phytocannabinoids and terpenes (Namdar et al., 2019) has been demonstrated. Pre-clinical evidence suggests an 'entourage effect' might be inferred from the superior medical activities of full-spectrum cannabis extracts versus single molecules (Koltai et al., 2019). Furthermore, in some cases a "parasitage effect" might be detected, as there might also be negative molecular interactions *in vitro* (Namdar et al., 2020).

Indeed, as detailed above, phytocannabinoids are potent anti-inflammatory and immunomodulatory agents and in some cases they act via different signaling pathways. For example, although both THC and CBD decreased inflammation in LPS-activated microglial cells of a BV-2 mouse, they acted through different, although partially overlapping, mechanisms. CBD but not THC inhibited the NF-κB-dependent pathway, yet both CBD and THC regulated the IFNβ pathway activity (Kozela et al., 2010).

In order to take advantage of the synergy and to diminish negative interactions between phytomolecules, activity might be improved by selecting the most active phytomolecules while eliminating parts of the whole extract. This approach was demonstrated in the reduction of inflammation in colon cells and tissues. A THCA-rich fraction from the cannabis strain was shown to have superior activity against inflammation over the crude extract (Nallathambi et al., 2017) suggesting that the selection of active compounds may reduce the presence of inactive compounds or even those that have pro-inflammatory effects.

Moreover, in some cases, the activity of a combination of phytomolecules was found to be superior over that of a single molecule. This was demonstrated in an *in vivo* study on inflammation, where treatment with CBD combined with cannabis extract overcame the bell-shaped dose-response of purified CBD, suggesting that components found in the extract synergize with CBD to achieve the desired anti-inflammatory action (Gallily et al., 2015). In addition, a phytocannabinoid formulation showed superior activity reducing lung inflammation over the cannabis-derived fraction *in vitro*. Moreover, this particular phytocannabinoid and CBD formulation had superior activity over CBD alone (Anil et al., 2021).

DISCUSSION

Cannabis compounds, in some cases via the endocannabinoids system, were shown to affect some of the cornerstones of chronic inflammation. However, in light of the large number of active molecules produced by cannabis and their sometimes-synergistic interactions, there is a need to better specify cannabis-based treatments and the active compounds, while utilizing the synergy identified between cannabis phytomolecules. Thus, even if CBD or THC are considered potentially leading molecules, additional cannabis-derived compounds may be selected for improved activity.

Future approaches for improved usage of cannabis demand the development, transformation and formulation of full-spectrum cannabis extracts into active plant ingredients (APIs) to achieve higher effectivity. This might be done via careful

selection of phytomolecules composition (Koltai et al., 2019; Koltai and Namdar, 2020). Notably, selecting only a few compounds for drug formulation may be compatible with modern medicine due to the potential for standardization, and careful dosing of API-based products. Importantly, once the mode of action of phytocannabinoids and that of their combination is known, APIs might be targeted towards specific mechanisms involved with inflammation.

Moreover, it might be that cannabis components can be combined with other pharmaceutical drugs to reduce inflammation. On the one hand, complementary effects might be identified due to different and perhaps complementary modes of action of cannabis compounds and pharmaceutical drugs. For example, THC was shown to reduce gastric inflammation caused by diclofenac, which may facilitate diclofenac's effective usage against inflammation (Kinsey and Cole, 2013). On the other, CBD and THC were shown to have metabolism-dependent inhibition for Cytochrome P450 (CYP) enzymes. CYPs are responsible for drug metabolism, including detoxication and metabolic activation of xenobiotics (Yamaori et al., 2011). Hence, combined treatment with cannabis and anti-inflammatory drugs should be carefully considered.

AUTHOR CONTRIBUTIONS

SA composed the first draft of the manuscript, HP improved the draft and HK composed the final draft of the manuscript.

FUNDING

SA and HP are research scholars of ARO and HK is an employee of ARO. No external funding supported this manuscript.

ACKNOWLEDGMENTS

We thank Zach Dunseth for English editing.

REFERENCES

Abdulkhaleq, L. A., Assi, M. A., Abdullah, R., Zamri-Saad, M., Taufiq-Yap, Y. H., and Hezmee, M. N. M. (2018). The Crucial Roles of Inflammatory Mediators in Inflammation: A Review. *Vet. World* 11 (5), 627–635. doi:10.14202/vetworld.2018.627-635

Actis, G. C., Pellicano, R., Fagoonee, S., and Ribaldone, D. G. (2019). History of Inflammatory Bowel Diseases. *J. Clin. Med.* 8 (11), 1970. doi:10.3390/jcm8111970

Ambrose, T., and Simmons, A. (2019). Cannabis, Cannabinoids, and the Endocannabinoid System-is There Therapeutic Potential for Inflammatory Bowel Disease? *J. Crohns Colitis* 13 (4), 525–535. doi:10.1093/ecco-jcc/jjy185

Anil, S. M., Shalev, N., Vinayaka, A. C., Nadarajan, S., Namdar, D., Belausov, E., et al. (2021). Cannabis Compounds Exhibit Anti-inflammatory Activity *In Vitro* in COVID-19-Related Inflammation in Lung Epithelial Cells and Pro-inflammatory Activity in Macrophages. *Sci. Rep.* 11 (1), 1462–1514. doi:10.1038/s41598-021-81049-2

Anis, O., Vinayaka, A. C., Shalev, N., Namdar, D., Nadarajan, S., Anil, S. M., et al. (2021). Cannabis-Derived Compounds Cannabichromene and Δ9-Tetrahydrocannabinol Interact and Exhibit Cytotoxic Activity against Urothelial Cell Carcinoma Correlated with Inhibition of Cell Migration and Cytoskeleton Organization. *Molecules* 26 (2), 465. doi:10.3390/molecules26020465

Bautista, J. L., Yu, S., and Tian, L. (2021). Flavonoids in *Cannabis Sativa*: Biosynthesis, Bioactivities, and Biotechnology. *ACS omega* 6 (8), 5119–5123. doi:10.1021/acsomega.1c00318

Biringer, R. G. (2021). Endocannabinoid Signaling Pathways: beyond CB1R and CB2R. *J. Cel Commun. Signal.* 15 (3), 335–360. doi:10.1007/s12079-021-00622-6

Borrelli, F., Fasolino, I., Romano, B., Capasso, R., Maiello, F., Coppola, D., et al. (2013). Beneficial Effect of the Non-psychotropic Plant Cannabinoid Cannabigerol on Experimental Inflammatory Bowel Disease. *Biochem. Pharmacol.* 85 (9), 1306–1316. doi:10.1016/j.bcp.2013.01.017

Capozzi, A., Caissutti, D., Mattei, V., Gado, F., Martellucci, S., Longo, A., et al. (2021). Anti-inflammatory Activity of a CB2 Selective Cannabinoid Receptor Agonist: Signaling and Cytokines Release in Blood Mononuclear Cells. *Molecules* 27 (1), 64. doi:10.3390/molecules27010064

Carrier, E. J., Auchampach, J. A., and Hillard, C. J. (2006). Inhibition of an Equilibrative Nucleoside Transporter by Cannabidiol: a Mechanism of Cannabinoid Immunosuppression. *Proc. Natl. Acad. Sci. U S A.* 103 (20), 7895–7900. doi:10.1073/pnas.0511232103

Castillo, A., Tolón, M. R., Fernández-Ruiz, J., Romero, J., and Martinez-Orgado, J. (2010). The Neuroprotective Effect of Cannabidiol in an *In Vitro* Model of Newborn Hypoxic-Ischemic Brain Damage in Mice Is Mediated by CB(2) and Adenosine Receptors. *Neurobiol. Dis.* 37 (2), 434–440. doi:10.1016/j.nbd.2009.10.023

Chiurchiù, V., Lanuti, M., De Bardi, M., Battistini, L., and Maccarrone, M. (2015). The Differential Characterization of GPR55 Receptor in Human Peripheral Blood Reveals a Distinctive Expression in Monocytes and NK Cells and a Proinflammatory Role in These Innate Cells. *Int. Immunol.* 27 (3), 153–160. doi:10.1093/intimm/dxu097

Cinelli, M. A., Do, H. T., Miley, G. P., and Silverman, R. B. (2020). Inducible Nitric Oxide Synthase: Regulation, Structure, and Inhibition. *Med. Res. Rev.* 40 (1), 158–189. doi:10.1002/med.21599

Correa, F., Docagne, F., Mestre, L., Clemente, D., Hernangómez, M., Loría, F., et al. (2009). A Role for CB2 Receptors in Anandamide Signalling Pathways Involved in the Regulation of IL-12 and IL-23 in Microglial Cells. *Biochem. Pharmacol.* 77 (1), 86–100. doi:10.1016/j.bcp.2008.09.014

Di Marzo, V., and Piscitelli, F. (2015). The Endocannabinoid System and its Modulation by Phytocannabinoids. *Neurotherapeutics* 12 (4), 692–698. doi:10.1007/s13311-015-0374-6

Di Marzo, V., Melck, D., Bisogno, T., and De Petrocellis, L. (1998). Endocannabinoids: Endogenous Cannabinoid Receptor Ligands with Neuromodulatory Action. *Trends Neurosci.* 21 (12), 521–528. doi:10.1016/s0166-2236(98)01283-1

Effenberger, M., Grander, C., Grabherr, F., Griesmacher, A., Ploner, T., Hartig, F., et al. (2021). Systemic Inflammation as Fuel for Acute Liver Injury in COVID-19. *Dig. Liver Dis.* 53 (2), 158–165. doi:10.1016/j.dld.2020.08.004

El Sohly, M. A., Radwan, M. M., Gul, W., Chandra, S., and Galal, A. (2017). "Phytochemistry of *Cannabis Sativa* L," in *Phytocannabinoids: Unraveling the Complex Chemistry and Pharmacology of Cannabis Sativa*. Editors A. D. Kinghorn, H. Falk, S. Gibbons, and J. I. Kobayashi (Cham: Springer International Publishing), 1–36. doi:10.1007/978-3-319-45541-9_1

El-Remessy, A. B., Tang, Y., Zhu, G., Matragoon, S., Khalifa, Y., Liu, E. K., et al. (2008). Neuroprotective Effects of Cannabidiol in Endotoxin-Induced Uveitis: Critical Role of P38 MAPK Activation. *Mol. Vis.* 14, 2190–2203.

Feehan, K. T., and Gilroy, D. W. (2019). Is Resolution the End of Inflammation? *Trends Mol. Med.* 25 (3), 198–214. doi:10.1016/j.molmed.2019.01.006

Fitzgerald, K. A., and Kagan, J. C. (2020). Toll-like Receptors and the Control of Immunity. *Cell* 180 (6), 1044–1066. doi:10.1016/j.cell.2020.02.041

Fitzpatrick, J. M., Minogue, E., Curham, L., Tyrrell, H., Gavigan, P., Hind, W., et al. (2020). MyD88-dependent and -independent Signalling via TLR3 and TLR4 Are Differentially Modulated by Δ9-tetrahydrocannabinol and Cannabidiol in

Human Macrophages. *J. Neuroimmunol.* 343, 577217. doi:10.1016/j.jneuroim.2020.577217

Fujiwara, N., and Kobayashi, K. (2005). Macrophages in Inflammation. *Curr. Drug Targets Inflamm. Allergy* 4 (3), 281–286. doi:10.2174/1568010054022024

Gaffal, E., Cron, M., Glodde, N., and Tüting, T. (2013). Anti-inflammatory Activity of Topical THC in DNFB-Mediated Mouse Allergic Contact Dermatitis Independent of CB1 and CB2 Receptors. *Allergy* 68 (8), 994–1000. doi:10.1111/all.12183

Galkina, E., and Ley, K. (2009). Immune and Inflammatory Mechanisms of Atherosclerosis. *Annu. Rev. Immunol.* 27, 165–197. doi:10.1146/annurev.immunol.021908.132620

Gallily, R., Yekhtin, Z., and Hanuš, L. O. (2015). Overcoming the Bell-Shaped Dose-Response of Cannabidiol by Using Cannabis Extract Enriched in Cannabidiol. *Pharmacol. Pharm.* 06 (02), 75–85. doi:10.4236/pp.2015.62010

Gentili, M., Ronchetti, S., Ricci, E., Di Paola, R., Gugliandolo, E., Cuzzocrea, S., et al. (2019). Selective CB2 Inverse Agonist JTE907 Drives T Cell Differentiation towards a Treg Cell Phenotype and Ameliorates Inflammation in a Mouse Model of Inflammatory Bowel Disease. *Pharmacol. Res.* 141, 21–31. doi:10.1016/j.phrs.2018.12.005

Giacobbe, J., Marrocu, A., Di Benedetto, M. G., Pariante, C. M., and Borsini, A. (2021). A Systematic, Integrative Review of the Effects of the Endocannabinoid System on Inflammation and Neurogenesis in Animal Models of Affective Disorders. *Brain Behav. Immun.* 93, 353–367. doi:10.1016/j.bbi.2020.12.024

Gülck, T., and Møller, B. L. (2020). Phytocannabinoids: Origins and Biosynthesis. *Trends Plant Sci.* 25 (10), 985–1004. doi:10.1016/j.tplants.2020.05.005

Gugliandolo, A., Pollastro, F., Grassi, G., Bramanti, P., and Mazzon, E. (2018). *In Vitro* model of Neuroinflammation: Efficacy of Cannabigerol, a Non-psychoactive Cannabinoid. *Int. J. Mol. Sci.* 19 (7), 1992. doi:10.3390/ijms19071992

Hackel, A., Aksamit, A., Bruderek, K., Lang, S., and Brandau, S. (2021). TNF-α and IL-1β Sensitize Human MSC for IFN-γ Signaling and Enhance Neutrophil Recruitment. *Eur. J. Immunol.* 51 (2), 319–330. doi:10.1002/eji.201948336

Haddad, J. J. (2002). Redox Regulation of Pro-inflammatory Cytokines and IκB-α/NF-κB Nuclear Translocation and Activation. *Biochem. Biophys. Res. Commun.* 296 (4), 847–856. doi:10.1016/s0006-291x(02)00947-6

Hanuš, L. O., Meyer, S. M., Munoz, E., Taglialatela-Scafiti, O., and Appendino, G. (2016). Phytocannabinoids: a Unified Critical Inventory. *Nat. Prod. Rep.* 33, 1357–1392. doi:10.1039/C6NP00074F

He, G., and Karin, M. (2011). NF-κB and STAT3 - Key Players in Liver Inflammation and Cancer. *Cell Res.* 21 (1), 159–168. doi:10.1038/cr.2010.183

Hegde, V. L., Nagarkatti, P. S., and Nagarkatti, M. (2011). Role of Myeloid-Derived Suppressor Cells in Amelioration of Experimental Autoimmune Hepatitis Following Activation of TRPV1 Receptors by Cannabidiol. *PloS one* 6 (4), e18281. doi:10.1371/journal.pone.0018281

Hillard, C. J. (2018). Circulating Endocannabinoids: from Whence Do They Come and where Are They Going? *Neuropsychopharmacology* 43 (1), 155–172. doi:10.1038/npp.2017.130

Jean-Gilles, L., Gran, B., and Constantinescu, C. S. (2010). Interaction between Cytokines, Cannabinoids and the Nervous System. *Immunobiology* 215, 606–610. doi:10.1016/j.imbio.2009.12.006

Kinsey, S. G., and Cole, E. C. (2013). Acute Δ(9)-tetrahydrocannabinol Blocks Gastric Hemorrhages Induced by the Nonsteroidal Anti-inflammatory Drug Diclofenac Sodium in Mice. *Eur. J. Pharmacol.* 715 (1-3), 111–116. doi:10.1016/j.ejphar.2013.06.001

Koltai, H., and Namdar, D. (2020). Cannabis Phytomolecule 'Entourage': From Domestication to Medical Use. *Trends Plant Sci.* 25 (10), 976–984. doi:10.1016/j.tplants.2020.04.007

Koltai, H., Poulin, P., and Namdar, D. (2019). Promoting Cannabis Products to Pharmaceutical Drugs. *Eur. J. Pharm. Sci.* 132, 118–120. doi:10.1016/j.ejps.2019.02.027

Kozela, E., Pietr, M., Juknat, A., Rimmerman, N., Levy, R., and Vogel, Z. (2010). Cannabinoids Δ9-Tetrahydrocannabinol and Cannabidiol Differentially Inhibit the Lipopolysaccharide-Activated NF-κB and Interferon-β/STAT Proinflammatory Pathways in BV-2 Microglial Cells. *J. Biol. Chem.* 285 (3), 1616–1626. doi:10.1074/jbc.m109.069294

Lanuti, M., Talamonti, E., Maccarrone, M., and Chiurchiù, V. (2015). Activation of GPR55 Receptors Exacerbates oxLDL-Induced Lipid Accumulation and Inflammatory Responses, while Reducing Cholesterol Efflux from Human Macrophages. *PLoS One* 10 (5), e0126839. doi:10.1371/journal.pone.0126839

Masoumi, M., Bashiri, H., Khorramdelazad, H., Barzaman, K., Hashemi, N., Sereshki, H. A., et al. (2021). Destructive Roles of Fibroblast-like Synoviocytes in Chronic Inflammation and Joint Damage in Rheumatoid Arthritis. *Inflammation* 44 (2), 466–479. doi:10.1007/s10753-020-01371-1

Mazuz, M., Tiroler, A., Moyal, L., Hodak, E., Nadarajan, S., Vinayaka, A. C., et al. (2020). Synergistic Cytotoxic Activity of Cannabinoids from Cannabis Sativa against Cutaneous T-Cell Lymphoma (CTCL) *In-Vitro* and *Ex-Vivo*. *Oncotarget* 11, 1141–1156. þ. doi:10.18632/oncotarget.27528

Naftali, T., Lev, L. B., Yablecovitch, D., Yablekovitz, D., Half, E., and Konikoff, F. M. (2011). Treatment of Crohn's Disease with Cannabis: an Observational Study. *Isr. Med. Assoc. J.* 13, 455–458.

Naftali, T., Bar-Lev Schleider, L., Dotan, I., Lansky, E. P., Sklerovsky Benjaminov, F., and Konikoff, F. M. (2013). Cannabis Induces a Clinical Response in Patients with Crohn's Disease: a Prospective Placebo-Controlled Study. *Clin. Gastroenterol. Hepatol.* 11 (10), 1276. doi:10.1016/j.cgh.2013.04.034

Nallathambi, R., Mazuz, M., Ion, A., Selvaraj, G., Weininger, S., Fridlender, M., et al. (2017). Anti-Inflammatory Activity in Colon Models Is Derived from Δ9-Tetrahydrocannabinolic Acid that Interacts with Additional Compounds in Cannabis Extracts. *Cannabis Cannabinoid Res.* 2 (1), 167–182. doi:10.1089/can.2017.0027

Namdar, D., Voet, H., Ajjampura, V., Nadarajan, S., Mayzlish-Gati, E., Mazuz, M., et al. (2019). Terpenoids and Phytocannabinoids Co-produced in *Cannabis Sativa* Strains Show Specific Interaction for Cell Cytotoxic Activity. *Molecules* 24, 3031. doi:10.3390/molecules24173031

Namdar, D., Anis, O., Poulin, P., and Koltai, H. (2020). Chronological Review and Rational and Future Prospects of Cannabis-Based Drug Development. *Molecules* 25 (20), 4821. doi:10.3390/molecules25204821

Netea, M. G., Schlitzer, A., Placek, K., Joosten, L. A. B., and Schultze, J. L. (2019). Innate and Adaptive Immune Memory: an Evolutionary Continuum in the Host's Response to Pathogens. *Cell Host Microbe* 25 (1), 13–26. doi:10.1016/j.chom.2018.12.006

Pagano, E., Capasso, R., Piscitelli, F., Romano, B., Parisi, O. A., Finizio, S., et al. (2016). An Orally Active Cannabis Extract with High Content in Cannabidiol Attenuates Chemically-Induced Intestinal Inflammation and Hypermotility in the Mouse. *Front. Pharmacol.* 7, 341. doi:10.3389/fphar.2016.00341

Panigrahy, D., Gilligan, M. M., Serhan, C. N., and Kashfi, K. (2021). Resolution of Inflammation: An Organizing Principle in Biology and Medicine. *Pharmacol. Ther.* 227, 107879. doi:10.1016/j.pharmthera.2021.107879

Pazos, M. R., Mohammed, N., Lafuente, H., Santos, M., Martínez-Pinilla, E., Moreno, E., et al. (2013). Mechanisms of Cannabidiol Neuroprotection in Hypoxic-Ischemic Newborn Pigs: Role of 5HT(1A) and CB2 Receptors. *Neuropharmacology* 71, 282–291. doi:10.1016/j.neuropharm.2013.03.027

Perez, E., Fernandez, J. R., Fitzgerald, C., Rouzard, K., Tamura, M., and Savile, C. (2022). *In Vitro* and Clinical Evaluation of Cannabigerol (CBG) Produced via Yeast Biosynthesis: a Cannabinoid with a Broad Range of Anti-inflammatory and Skin Health-Boosting Properties. *Molecules* 27 (2), 491. doi:10.3390/molecules27020491

Perry, V. H. (2004). The Influence of Systemic Inflammation on Inflammation in the Brain: Implications for Chronic Neurodegenerative Disease. *Brain Behav. Immun.* 18 (5), 407–413. doi:10.1016/j.bbi.2004.01.004

Pothen, J. J., Poynter, M. E., and Bates, J. H. (2015). A Computational Model of Unresolved Allergic Inflammation in Chronic Asthma. *Am. J. Physiol. Lung Cel Mol. Physiol.* 308 (4), L384–L390. doi:10.1152/ajplung.00268.2014

Rahaman, O., and Ganguly, D. (2021). Endocannabinoids in Immune Regulation and Immunopathologies. *Immunology* 164 (2), 242–252. doi:10.1111/imm.13378

Ribeiro, A., Ferraz-de-Paula, V., Pinheiro, M. L., Vitoretti, L. B., Mariano-Souza, D. P., Quinteiro-Filho, W. M., et al. (2012). Cannabidiol, a Non-psychotropic Plant-Derived Cannabinoid, Decreases Inflammation in a Murine Model of Acute Lung Injury: Role for the Adenosine A(2A) Receptor. *Eur. J. Pharmacol.* 678 (1-3), 78–85. doi:10.1016/j.ejphar.2011.12.043

Ryz, N. R., Remillard, D. J., and Russo, E. B. (2017). Cannabis Roots: A Traditional Therapy with Future Potential for Treating Inflammation and Pain. *Cannabis Cannabinoid Res.* 2 (1), 210–216. doi:10.1089/can.2017.0028

Sá, M. J. (2012). Physiopathology of Symptoms and Signs in Multiple Sclerosis. *Arq. Neuropsiquiatr.* 70, 733–740. doi:10.1590/s0004-282x2012000900016

Salvemini, D., Kim, S. F., and Mollace, V. (2013). Reciprocal Regulation of the Nitric Oxide and Cyclooxygenase Pathway in Pathophysiology: Relevance and Clinical Implications. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 304 (7), R473–R487. doi:10.1152/ajpregu.00355.2012

Sangiovanni, E., Fumagalli, M., Pacchetti, B., Piazza, S., Magnavacca, A., Khalilpour, S., et al. (2019). *Cannabis Sativa* L. Extract and Cannabidiol Inhibit *In Vitro* Mediators of Skin Inflammation and Wound Injury. *Phytother. Res.* 33 (8), 2083–2093. doi:10.1002/ptr.6400

Sevenoaks, M. J., and Stockley, R. A. (2006). Chronic Obstructive Pulmonary Disease, Inflammation and Co-morbidity--a Common Inflammatory Phenotype? *Respir. Res.* 7 (1), 70–79. doi:10.1186/1465-9921-7-70

Silva, L. B., dos Santos Neto, A. P., Maia, S. M. A. S., dos Santos Guimarães, C., Quidute, I. L., Carvalho, A. d. A. T., et al. (2019). The Role of TNF-α as a Proinflammatory Cytokine in Pathological Processes. *Todent J* 13 (1), 332–338. doi:10.2174/1874210601913010332

Tahir, M. N., Shahbazi, F., Rondeau-Gagné, S., and Trant, J. F. (2021). The Biosynthesis of the Cannabinoids. *J. Cannabis Res.* 3 (1), 7–12. doi:10.1186/s42238-021-00062-4

Taniguchi, K., and Karin, M. (2018). NF-κB, Inflammation, Immunity and Cancer: Coming of Age. *Nat. Rev. Immunol.* 18 (5), 309–324. doi:10.1038/nri.2017.142

Valdeolivas, S., Navarrete, C., Cantarero, I., Bellido, M. L., Muñoz, E., and Sagredo, O. (2015). Neuroprotective Properties of Cannabigerol in Huntington's Disease: Studies in R6/2 Mice and 3-Nitropropionate-Lesioned Mice. *Neurotherapeutics* 12 (1), 185–199. doi:10.1007/s13311-014-0304-z

Watson, C. W., Campbell, L. M., Sun-Suslow, N., Hong, S., Umlauf, A., Ellis, R. J., et al. (2021). Daily Cannabis Use Is Associated with Lower CNS Inflammation in People with HIV. *J. Int. Neuropsychol. Soc.* 27 (6), 661–672. doi:10.1017/S1355617720001447

Yamaori, S., Maeda, C., Yamamoto, I., and Watanabe, K. (2011). Differential Inhibition of Human Cytochrome P450 2A6 and 2B6 by Major Phytocannabinoids. *Forensic Toxicol.* 29 (2), 117–124. doi:10.1007/s11419-011-0112-7

Zhao, H., Wu, L., Yan, G., Chen, Y., Zhou, M., Wu, Y., et al. (2021). Inflammation and Tumor Progression: Signaling Pathways and Targeted Intervention. *Signal. Transduct. Target. Ther.* 6 (1), 1–46. doi:10.1038/s41392-021-00658-5

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

APPENDIX A

Methodology: To reflect on the effect of cannabis and its derived compounds on acute or chronic inflammation and the accumulating knowledge regarding cannabis active compounds and their mode of action, we have conducted a literature review using the following terms: "inflammation ", "acute inflammation", "chronic inflammation", "medical use of cannabis", "therapy" "*Cannabis sativa*", "*C. sativa*", "cannabis", "cannabinoids", "terpenes", "cannabis oil", "adverse effects", "endocannabinoid", "phytocannabinoid" and "entourage effect". The search was conducted on general and multidisciplinary research databases for peer-reviewed scientific manuscripts, including PubMed, Google Scholar, Scopus, and Web of Science.

frontiers | Frontiers in Pharmacology

ORIGINAL RESEARCH
published: 10 May 2022
doi: 10.3389/fphar.2022.885655

Medicinal Cannabis Prescribing in Australia: An Analysis of Trends Over the First Five Years

Sara L. MacPhail[1,2], *Miguel A. Bedoya-Pérez*[1,2], *Rhys Cohen*[3,4], *Vicki Kotsirilos*[5], *Iain S. McGregor*[1,2] *and Elizabeth A. Cairns*[1,2]*

[1]*The Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, Sydney, NSW, Australia,* [2]*School of Psychology, The University of Sydney, Sydney, NSW, Australia,* [3]*School of Social Sciences, Department of Sociology, Macquarie University, Sydney, NSW, Australia,* [4]*Cannabis Consulting Australia, Sydney, NSW, Australia,* [5]*NICM Health Research Institute, Western Sydney University, Sydney, NSW, Australia*

A regulatory framework allowing legal access to medicinal cannabis (MC) products has operated in Australia since November 2016. MC prescribing by healthcare practitioners (HCPs) is primarily conducted through the Special Access Scheme - Category B (SAS-B) pathway, through which prescribers apply to the Therapeutic Goods Administration (TGA–the federal regulator) for approval to prescribe a category of product to an individual patient suffering from a specific indication. The dataset collected by the TGA provides a unique opportunity to examine MC prescribing trends over time in the Australian population. Here we analysed this TGA SAS-B dataset since inception with respect to age, gender, product type (e.g., oil, flower, etc.), CBD content, indication treated, and prescriber location. Results are presented descriptively as well as being analysed using non-linear regression models. Relationship between variables were explored via correspondence analyses. Indications were classified with reference to the International Statistical Classification of Diseases and Related Health Problems (10th Revision). As of 31 August 2021, a total of 159,665 SAS-B approvals had been issued for MC products, 82.4% of were since January 2020. Leading indications for approvals were for pain, anxiety, and sleep disorders. Oil products were the most popular product type, while CBD-dominant products (≥98% CBD) accounted for 25.1% of total approvals. Approvals for flower products increased markedly during 2020–2021, as did approvals involving younger age groups (18–31 years old), male patients, and non-CBD dominant products. A disproportionate number of SAS-B MC applications (around 50%) came from HCPs in the state of Queensland. Associations between patient gender and age and/or indication with product type were found. For example, approvals for oil products were commonly associated with approvals for pain. While, overall prescribing increased dramatically over the last 2 years of analysis, stabilization of approval numbers is evident for some indications, such as pain. Current prescribing practices do not always reflect provided TGA guidance documents for MC prescribing. While acknowledging some limitations around the SAS-B dataset, it provides a unique and valuable resource with which to better understand current prescribing practices and utilisation of MC products within Australia.

Keywords: medicinal cannabis, prescribing trends, Australia, authorised prescriber scheme, special access scheme, cannabinoid, regulation, therapeutic goods administration (TGA)

OPEN ACCESS

Edited by:
Francesca Baratta, University of Turin, Italy

Reviewed by:
E. Alfonso Romero-Sandoval, Wake Forest School of Medicine, United States
Mary Kathryn Dahlgren, McLean Hospital, United States

***Correspondence:**
Elizabeth A. Cairns
elizabeth.cairns@sydney.edu.au

Specialty section:
This article was submitted to Drugs Outcomes Research and Policies, a section of the journal Frontiers in Pharmacology

Received: *01 March 2022*
Accepted: *31 March 2022*
Published: *10 May 2022*

Citation:
MacPhail SL, Bedoya-Pérez MA, Cohen R, Kotsirilos V, McGregor IS and Cairns EA (2022) Medicinal Cannabis Prescribing in Australia: An Analysis of Trends Over the First Five Years. Front. Pharmacol. 13:885655. doi: 10.3389/fphar.2022.885655

1 INTRODUCTION

The use of cannabis as a medicine can be traced as far back as 2000 BCE in Central Asia, where it has documented use in treating a significant array of health problems (Crocq, 2020). The recent worldwide renaissance in the use of cannabis for medical purposes is supported by evidence of efficacy, albeit somewhat variable, across a range of conditions such as chronic pain (Stella et al., 2021), muscle spasticity in multiple sclerosis (Fragoso et al., 2020), chemotherapy-induced nausea and vomiting (Grimison et al., 2020), palliative care (Herbert and Hardy, 2021), and severe forms of childhood epilepsy (Nabbout and Thiele, 2020).

The cannabis plant contains hundreds of bioactive molecules, of which two plant-derived cannabinoids (phytocannabinoids) are the best studied: Δ^9-tetrahydrocannabinol (THC), the main intoxicating constituent; and cannabidiol (CBD), which is non-intoxicating. THC and CBD have distinct pharmacological actions and different, but partly overlapping, therapeutic applications. THC influences pain, spasticity, sedation, appetite, and mood in animal and human studies, primarily through agonist action on cannabinoid receptors 1 (CB1) and 2 (CB2) (Banister et al., 2019). CBD is more "promiscuous", having activity at a large number of targets, and with anxiolytic, anti-convulsant and anti-inflammatory effects reported, in at least preclinical models (Nelson et al., 2020).

Evidence around efficacy of cannabis in certain health conditions continues to evolve, with rapidly increasing global numbers of randomized controlled trials (RCTs) and preclinical research (Schlag et al., 2021). However, there remain many conditions for which clinical evidence is minimal or ambiguous, with systematic reviews often highlighting a paucity of high quality randomized RCTs to support current prescribing (Alexander, 2020).

Legal availability of medicinal cannabis (MC) varies by location, and even within the same country there can be differential regulation at state and federal levels (reviewed in Gleeson, 2020). Recent reviews of MC programs can be found elsewhere (Decorte et al., 2020; Corva and Meisel, 2021). Prescribing MC is a relatively new phenomenon in Australia, with the government legalizing a framework for MC access in November 2016 (Gleeson, 2020). Patient access pathways exist under the regulatory power of the *Therapeutic Goods Administration (TGA),* within the federal Department of Health. Almost all currently available MC products are classified as "unregistered medicines" as they have not undergone the rigorous assessment of safety, quality, and efficacy that would allow entry into the *Australian Registry of Therapeutic Goods (ARTG).* The only two current exceptions are *Sativex* (nabiximols, an oromucosal spray containing equivalent amounts of THC and CBD) and *Epidyolex* (also known as *Epidiolex* in other jurisdictions, a 100 mg/ml CBD solution; Therapeutic Goods Administration, 2020a), although other products, such as *Marinol* (THC capsules) and *Cesamet* (a THC analogue called nabilone) are also approved in other countries.

There are three routes through which a healthcare practitioner[1] (HCP) can request permission from the TGA to prescribe an unregistered MC product to a patient. The *Special Access Scheme Category A* (*SAS-A*) allows practitioners to prescribe MC products to a patient that is seriously ill or likely to die, with only post-hoc notification of prescribing to the TGA being required. Most patient access, however, is through the *Special Access Scheme Category B* (*SAS-B*) which allows practitioners to make an application to the TGA to allow an individual patient to be prescribed a certain type of MC product to treat a specific condition (Therapeutic Goods Administration, 2020b). Prior to November 2021, SAS-B applications were required to nominate a specific product to be prescribed, and so if a patient required more than one product, then multiple applications were needed (Gleeson, 2020). A refinement to the scheme in November 2021 allowed prescribers to request a general class of product, based on cannabinoid content and product format, rather than an individual product. The third and final route is *The Authorised Prescriber* (*AP*) scheme, which allows HCPs an authority to prescribe a specific MC product to multiple patients in their care if the patients all suffer from the same condition.

Although not registered medicines, the TGA do regulate supply and access to MC products to ensure appropriate practices in manufacturing. The one exception to this is compounded medicines, which have been largely exempt from therapeutic goods regulations (Falconer and Steadman, 2017) and currently do not require prescribers to seek TGA approval or post-hoc notification (Therapeutic Goods Administration, 2020c), as described above. However, the government has announced reforms to the regulation of compounded products that are to commence in April 2022 (Therapeutic Goods Administration, 2022).

Medical conditions allowed for MC prescribing are not specified by the TGA although a series of guidance documents were published in 2017 by the TGA, outlining the evidence-base for use of MC products in multiple sclerosis, palliative care, epilepsy (pediatric and young adult patients), nausea and vomiting, and chronic non-cancer pain (Therapeutic Goods Administration, 2017). In general, however, any practitioner can apply to the TGA to prescribe a MC product to a patient for any condition, provided they can justify prescribing based on the available evidence (Benson and Cohen, 2019).

Since the inception of MC access in Australia in November 2016, the TGA has collected detailed information on approvals issued under the SAS-A, SAS-B, and AP schemes. These datasets are accessible and provide a unique repository of detailed patient-level information around MC approvals that covers almost the entire population of Australian MC patients. It is the largest known dataset of its kind with few other countries/regions systematically capturing approvals in this way (see Banerjee et al., 2022).

To date, little systematic analysis of these TGA datasets have been undertaken (although see Benson and Cohen, 2019; Arnold

[1]In this study the term "healthcare practitioner" (HCP) refers to the Medical Practitioners and Nurse Practitioners who are authorized to gain access to MC products for these patients. Criteria determining eligibility to prescribe varies across states and territories (RACGP, 2019).

et al., 2020; Henderson et al., 2021). The purpose of the current study was to provide more detailed analysis of these datasets to allow insights into current trends in MC prescribing in Australia, including indications, patient demographics, and product categories. Our analysis of trends stretches back to when a framework for MC access became legally available in Australia (November 2016). Elucidating trends over time may yield novel and valuable insights into current clinical practice and contribute to a more comprehensive understanding of patterns of prescribing.

2 MATERIALS AND METHODS

2.1 Data Acquisition

An email request was made to the TGA under *The Freedom of Information Act (1982) (FOI)* for the release of documents pertaining to applications through the SAS-B and AP schemes since inception (2016; **Supplementary Figure S1**). The scope of this request was informed by previously released FOI datasets (FOI 2013, 2250, 2275, 2370, 2419). The data received via FOI request were supplemented (where specified) with data from the new TGA Medicinal Cannabis Access Data Dashboard, which contains publicly available SAS-A and SAS-B data summaries (Therapeutic Goods Administration, 2021a). No alternative source of AP data is currently publicly available. Human ethics approval was not required for this study as it involved data already collected.

2.2 Data Preparation

FOI data were systematically ordered on Microsoft Excel (version 16.54). Three applications were excluded from temporal analysis due to application dates being listed as prior to 2016. Age groups were determined by calculating septiles based on frequencies of approvals for ages 18 and older. MC products were grouped into 9 formats (capsules, crystal, flower, lozenge, oil, spray, tablet, topical, and wafer) based on those reported by Freshleaf Analytics (2021). Indications were classified with reference to the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10; WHO, version 2019—English) to improve consistency and validity of indication analysis. These classifications were then verified for appropriateness by a Medical Practitioner with significant clinical experience and expertise in this area. Where provided indications were ambiguous, or indications were multifactorial, the nearest possible indication classification was chosen.

Applications and patient numbers reported per consulting location were also normalized according to the Australian Bureau of Statistics population statistics in March 2021 (Australian Bureau of Statistics, 2021), reported here as approvals per 100,000 people.

2.3 Statistical Analysis

Data were considered at a general descriptive level (e.g., overall numbers of approvals over time by patients, products, indications), as well as being analyzed using best fit non-linear regression models. Statistical analyses were done in R version 4.1.1 (R Core Team, 2021). Data was processed for each analyses using the packages "tidyverse" (Wickham et al., 2019), "padr" (Thoen, 2020) and "dplyr" (Wickham et al., 2021). Examinations of changes over time were performed by non-linear regression fitting using the *glm* and *glm.nb* functions from the package "MASS" (Venables and Ripley, 2002) and graphs were constructed using the packages "ggplot" (Wickham, 2016), "cowplot" (Wilke, 2020) and "ggpubr" (Kassambara, 2020). Residuals plots and Pearson's dispersion test were used to choose the appropriate error distribution for each regression fit—i.e., Poisson or Negative binomial. A stepwise comparison of non-linear polynomial regression of the 1st, 2nd, 3rd, and 4th degree was carried out comparing the Corrected Akaike Information Criterion (AICc) for each regression using the package "MuMIn" (Bartoń, 2020) to determine the curve with comparatively best fit, without overfitting the data. Raising the functions to a higher degree allowed the model to fit more turning points or fluctuations in the data, indicating increasing complexity in the pattern of change. Here, Δm was calculated between models and excluded models with $\Delta m > 2$ as having substantially less support (Burnham and Anderson, 2002). To estimate goodness of fit, R^2 was calculated for each of the best fitted regressions by the equation: 1—deviance/residual deviance, and using the classification established by Moore and Kirkland (2013). Averages are listed as means ± standard error unless otherwise specified.

Correspondence analyses were performed using packages "Factoshiny" and "FactoMineR" (Le et al., 2008; Vaissie et al., 2021). These display associations between two categorical variables, taking into account weighting according to frequency. A graphical representation of the relationship between categorical variables is constructed by plotting the two most explanatory dimensions based on residuals. These dimensions explain the proportion of variance that is displayed along a horizontal or vertical axis, the sum of which explains the total variance represented in the graph. The point at which the two-axis intercept (origin) represents the point of least differentiation, and those categories close to this point can be considered to deviate the least from expected proportions.

Three separate insights into the properties of the two categorical variables can be interpreted from this analysis. First, the categories further away from the origin are considered more differentiated. Second, the proximity of two categories from the same variable, for example two different product formats, indicates that they are probably similar. Finally, a greater association is demonstrated by a smaller angle between the vectors connecting two categorical variables to the origin (e.g., between an indication and product).

Graphpad Prism (version 9.2.0) was also used to present the remaining distributions of data across categorical variables.

3 RESULTS

On 20 October 2021 the TGA granted the FOI request (FOI 2989), releasing a number of Excel documents by email. The SAS-B dataset contained a chronological record of 159,665 approved applications that were submitted by HCPs between 10 February 2016 and 31 August 2021, and approved between 26 February 2016 and 8 September 2021. Data included indication treated, product format sought, duration of supply, demographic

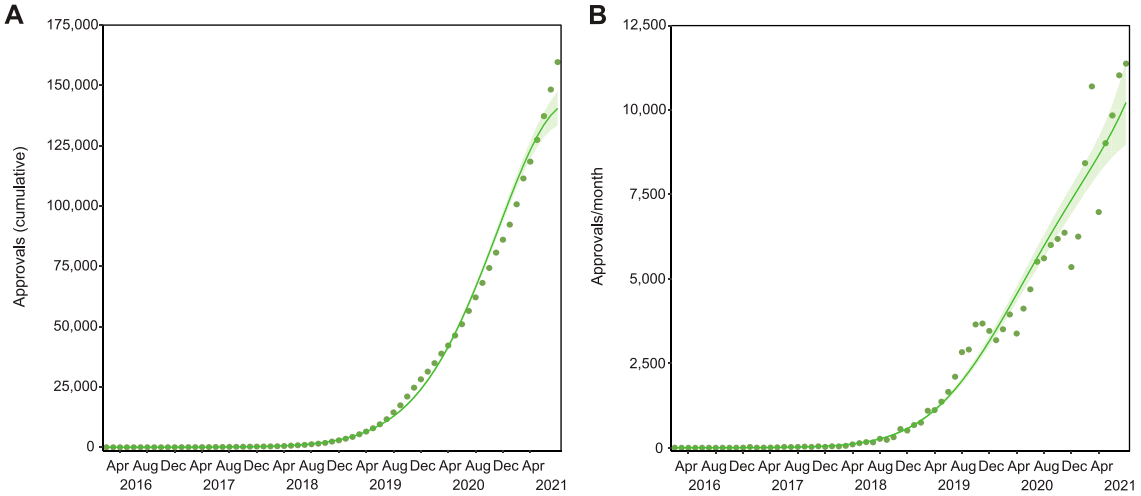


FIGURE 1 | SAS-B approvals over time. There have been 159,665 cumulative SAS-B approvals since 2016, with growth that followed a Negative binomial, 3rd degree polynomial curve (R^2 = 0.998, Δm = 44.398; **(A)**. Applications per month increase by around 12,000 a month, following a Negative binomial, 4th degree polynomial curve (R^2 = 0.984, Δm = 22.590; **(B)**. Solid lines represent the best fit with shaded standard error of the mean (SEM).

information and whether the patient had received a prior SAS approval for MC. Two applications had been rejected by the TGA and were not included with the dataset (TGA, *personal communication*). Some data points in this set were missing: schedule, 18 applications (0.01%); patient gender, 641 applications (0.40%); state, 52 applications (0.03%); indication, 2 applications (0.00%); product, 18 applications (0.01%); age, 44 applications (0.02%).

In addition to the SAS-B data, AP data were released by the TGA in two additional documents. One document contained the consulting location (state or territory) of Authorised Prescribers and the number of patients either commencing or continuing treatment across five 6-month time periods from June 2016 to December 2020 (N = 25,933). The other document listed the indications for which Authorised Prescribers had been approved from May 2018 until September 2021. It included the approval and expiration dates of the authorizations, the number of new patients commenced treatment (N = 7,137), and the total number of patients treated over that time (N = 10,323).

SAS-A information was not requested via the FOI but is available on the Medicinal Cannabis Access Data Dashboard. As of 23 March 2022, 1,398 SAS-A notifications have been logged by the TGA (Therapeutic Goods Administration, 2021a).

3.1 Approved SAS-B Applications Over Time

The number of SAS-B approvals has been increasing in a non-linear pattern over time (3rd degree polynomial, R^2 = 0.998, Δm = 44.398) with a dramatic increase in the last 2 years of analysis. Indeed, only 1.80% of total cumulative approvals were granted in the first 3 years of the legal access (2016–2018; **Figure 1A**). The rate of growth throughout the entire period (2016–2021) was non-linear (**Figure 1B**; 4th degree polynomial, R^2 = 0.984, Δm = 22.590), and does not yet appear to be slowing. For example, applications submitted between January and August 2021 were 2.2 times greater compared to the equivalent period in 2020.

3.2 Indications

There were 202 unique entries for indications specified by practitioners in their SAS-B applications. Reclassifying these according to the ICD-10 found 149 distinct indications, covering 121 different categories within 17 diagnostic groups (**Supplementary Table S1**). For example, indications listed as "Autism" were reclassified as "childhood autism (F84.0)" as indication, "pervasive developmental disorders (F84)" as category, which falls under the "mental and behavioral disorders (V)" diagnostic group. An additional 5 indications were insufficiently described to be coded (see "inadequate information", **Supplementary Table S1**).

Almost all indications fell into 6 diagnostic groups: "symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified" (symptoms and signs; N = 100,744); "mental and behavioral disorders" (N = 31,315); "diseases of the nervous system" (N = 18,463); "neoplasms" (N = 6,984); "diseases of the musculoskeletal system and connective tissue" (musculoskeletal; N = 1,616); and "factors influencing health status and contact with health services" (health services; N = 476; **Figure 2**). While the number of monthly applications for indications falling in these first three groups continues to rise (symptoms and signs, 4th degree polynomial, R^2 = 0.989, Δm = 7.055; mental and behavioral disorders, 4th degree polynomial, R^2 = 0.993, Δm = 18.903; diseases of the nervous system, 4th degree polynomial, R^2 = 0.976, Δm = 6.037), the change in the number of monthly applications for the next three groups appears to be more stochastic (neoplasms, 4th degree polynomial, R^2 = 0.976, Δm = 20.462; musculoskeletal, 3rd degree polynomial, R^2 = 0.894, Δm = 83.058; health services, 3rd degree polynomial, R^2 = 0.831, Δm = 49.676).

Nine ICD-10 indication categories had over 1,000 cumulative approvals in the SAS-B dataset (**Figure 3**), representing 94.1% of total approvals. These conditions were "pain, not elsewhere classified" (61.0% of total approvals); "other anxiety disorders"

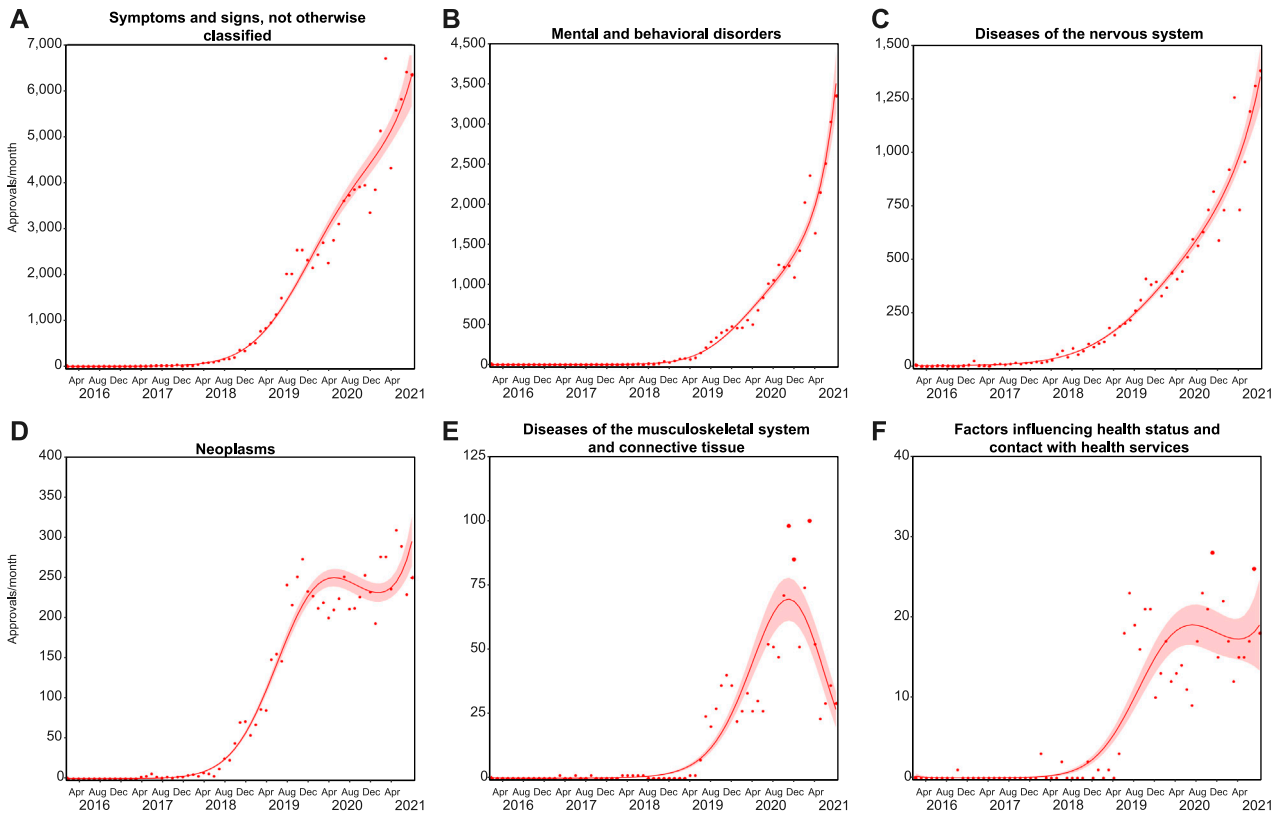


FIGURE 2 | Growth across indication groups differs. Trends in monthly approvals over time were analyzed in six ICD-10 groups representing almost all cumulative approvals (**Supplementary Table S1**). Approvals for symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified **(A)**; mental and behavioral disorders **(B)**; neoplasms **(C)**; and diseases of the nervous system **(D)** followed Negative binomial, 4th degree polynomial curves (R^2 = 0.989, 0.993, 0.976, and 0.976, and Δm = 7.055, 18.903, 20.462, and 6.037, respectively). Approvals for diseases of the musculoskeletal system and connective tissue **(E)**; and factors influencing health status and contact with health services **(F)** followed Negative binomial 3rd degree polynomial curves (R^2 = 0.894 and 0.831, and Δm = 83.058 and 49.676, respectively). Solid lines represent the best fit with shaded standard error of the mean.

(16.0%); "sleep disorders" (5.7%); "neoplasm of uncertain or unknown behavior of other and unspecified sites" (4.4%); "other polyneuropathies" (3.0%); "reaction to severe stress, and adjustment disorders" (1.6%); "epilepsy" (0.8%); "pervasive developmental disorders" (0.8%); and "convulsions, not elsewhere classified" (0.8%). These are referred to henceforth as pain; anxiety; sleep disorders; cancer and related symptoms; neuropathy; PTSD; epilepsy; ASD; and convulsions, respectively, for the remainder of the text.

The majority of these indication categories show continued growth in SAS-B approvals over time, with the exception of an apparent slowing of approvals for pain (**Figure 3A**; 2nd degree polynomial; R^2 = 0.988, Δm = 13,202.020); a trend towards decreasing monthly approvals in epilepsy (**Figure 3G**; 4th degree polynomial, R^2 = 0.587, Δm = 7.922); and an increase following a prior decrease in the number of monthly approvals for convulsions (**Figure 3I**; 3rd degree polynomial, R^2 = 0.918, Δm = 31.812).

3.3 Products

There are currently at least 375 different unregistered MC products in Australia that can be supplied via the SAS-B and AP schemes (Therapeutic Goods Administration, 2021b; Freshleaf Analytics, 2021). These include different formulations and composition (e.g., capsules, sprays, oils, flower). Different routes of administration may be optimal for different conditions (Bruni et al., 2018). For example, inhaled routes of administration have rapid absorption, and onset of action within seconds to a few minutes which may be useful for breakthrough pain (Malcolm, 2018). On the other hand, oral products such as oils and capsules have much slower onset to action, and more persistent effects (Vandrey et al., 2017).

In Australian regulation, the THC and CBD content of MC products determines the "Schedule" they fall under in the "Poisons Standards", based on the potential risks and harms associated with their use (Therapeutic Goods Administration, 2020b). Products comprising ≥98% CBD in total cannabinoid content are in Schedule 4 (*Prescription Only Medicine*) reflecting an acceptable safety profile (Iffland and Grotenhermen, 2017; World Health Organization, 2018). By contrast, THC has intoxicating properties and has abuse potential (Banister et al., 2019). During the project period, MC products that contain <98% CBD, and therefore likely higher THC content, were classified as

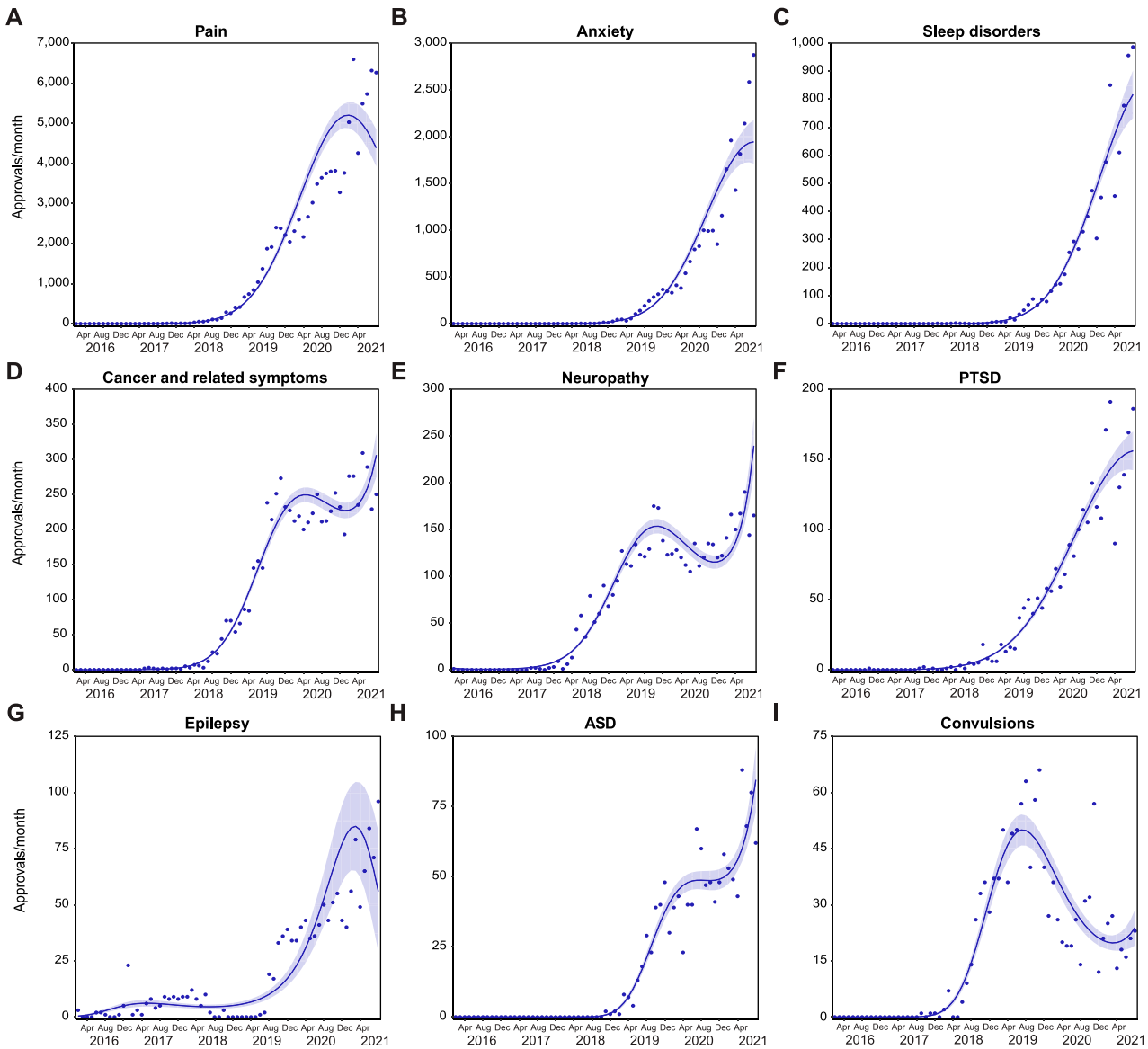


FIGURE 3 | Growth is varied across indication categories. Trends in monthly approvals over time were analyzed in ICD-10 indication categories with >1,000 cumulative approvals (**Supplementary Table S1**). Approvals for pain **(A)**; anxiety **(B)**; sleep disorders **(C)**; and PTSD **(F)** followed Negative binomial, 2nd degree polynomial curves (R^2 = 0.988, 0.989, 0.987, and 0.973, and Δm = 13,202.020, 1,413.481, 51.913, and 54.544, respectively). Approvals for cancer and related symptoms **(D)**; and neuropathy **(E)** followed Negative binomial 4th degree polynomial curve (R^2 = 0.981 and 0.962, and Δm = 17.271 and 21.698, respectively). Approvals for epilepsy **(G)** moderately followed a Negative binomial 4th degree polynomial curve (R2 = 0.587, Δm = 7.922). Approvals for ASD **(H)** and convulsions **(I)** followed Negative binomial 3rd degree polynomial curves (R^2 = 0.977 and 0.918, and Δm = 27.715 and 31.812, respectively). Solid lines represent the best fit with shaded standard error of the mean.

Schedule 8 (*Controlled Drug*) in most states in Australia. In addition to federal approval by the TGA, prescribers may also need approval from their state or territory health department, although the conditions under which state/territory approval is required varies considerably between jurisdictions.

The FOI data received did not include information on individual products, only the Schedule (i.e., Schedule 4 [S4] or 8 [S8]) and product format (e.g., oil, flower, capsule). There were 45 variations of product format that were specified in approved applications to the TGA, with oil and flower products representing >90.0% of total cumulative approvals (**Supplementary Table S2**; **Figure 4**). On average, 79.8 ± 2.1% of applications each month were for oil products, while 9.1 ± 1.5% were for flower (**Figure 4B**). The number of applications for oil increased over time (**Figure 4A**; 4th degree polynomial, R^2 = 0.980, Δm = 18.292), while applications for flower products showed a more rapid increase from the end of 2019 (3rd order polynomial, R^2 = 0.991, Δm = 8.267).

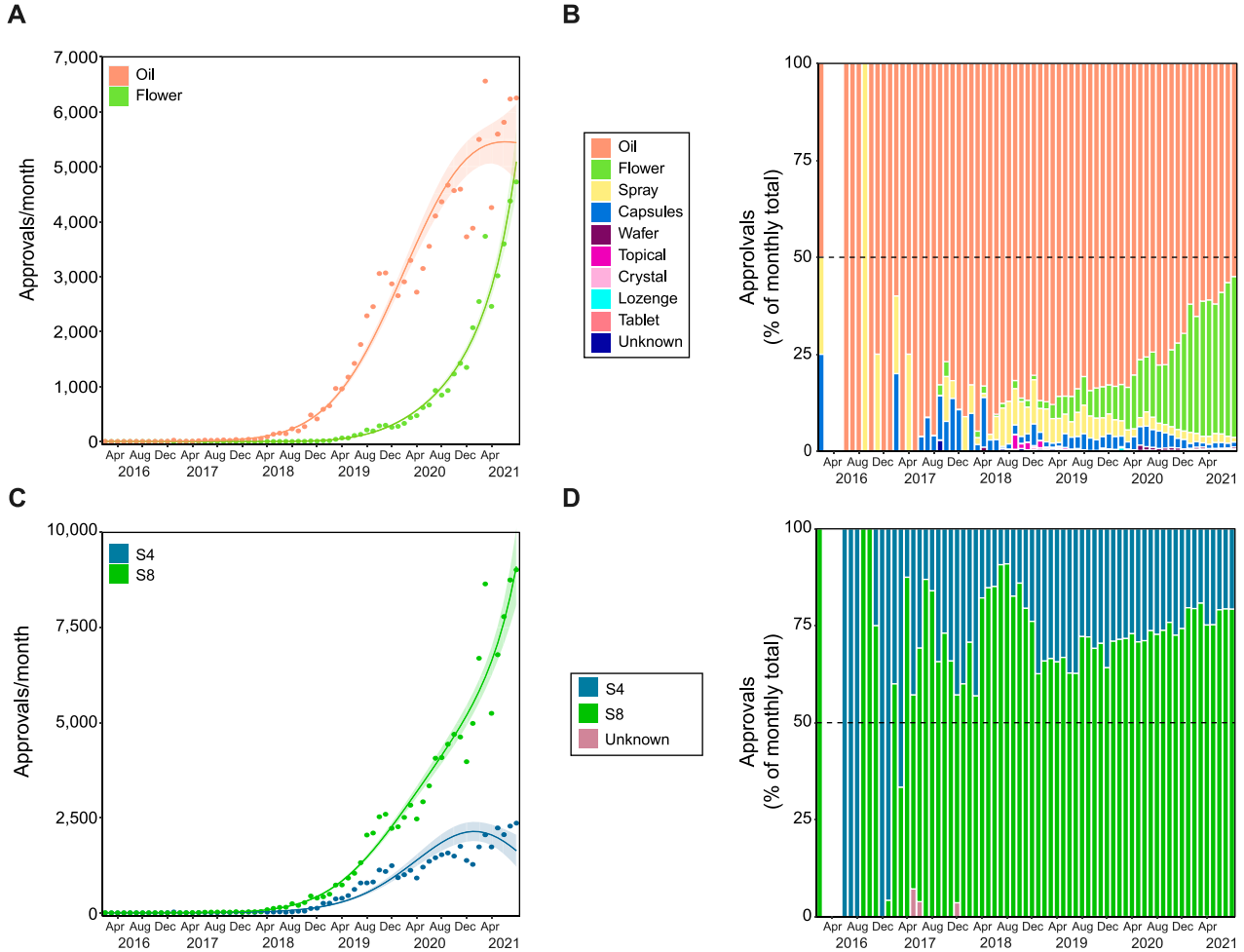


FIGURE 4 | Flower and Schedule 8 products have disproportionate growth. Growth trends in product format **(A,B)** and product schedule **(C,D)**. Approvals for oil products followed a Negative binomial 4th degree polynomial curve (R^2 = 0.980, Δm = 18.292), while approvals for flower followed a Negative binomial 3rd degree polynomial curve (R^2 = 0.991, Δm = 8.267). The proportion (%) of approvals per product category (see legend) are shown in panel **(B)**. Approvals for S4 and S8 products followed Negative binomial 4th degree polynomial curves (C; R^2 = 0.934 and 0.987, and Δm = 11.691 and 15.707, respectively). The proportion (%) of approvals per product schedule (see legend) are presented in panel **(D)**. The gap (between February 2016 and June 2016 in panels **(B)** and **(D)**, indicates no applications submitted in this period. In panels **(A)** and **(C)** Solid lines represent best fit with shaded standard error of the mean.

Overall, the majority of approvals were for S8 products (74.8%), with a pronounced non-linear growth (4th degree polynomial, R^2 = 0.987, Δm = 15.707; **Figures 4C,D**), while growth in S4 approvals appears to be slowing (4th degree polynomial, R^2 = 0.934, Δm = 11.691). This is consistent with trends seen with the top nine indications (**Supplementary Figure S2**). Approvals for S8 products gradually increased over time for anxiety conditions (1st degree polynomial, R^2 = 0.742; **Supplementary Figure S2B**). Until early 2019, approvals for anxiety conditions were primarily for S4 products, but by August 2021, 78.2% were for S8 products. Additionally, while S4 approvals were more common overall in epilepsy (**Supplementary Figure S2G**), ASD (**Supplementary Figure S2H**) and convulsions (**Supplementary Figure S2I**), current prescribing patterns appear to be trending toward majority S8 approvals in epilepsy (3rd degree polynomial, R^2 = 0.656, Δm = 4.482) and convulsions (3rd degree polynomial, R^2 = 0.375, Δm = 7.864, weak fit). The remaining indications were either relatively stable or stochastic.

3.4 Patient Demographics

Patient ages ranged from 0–103, which were grouped into the following categories: <18 (*N* = 2,978); 18–30 (*N* = 23,635); 31–37 (*N* = 21,968); 38–44 (*N* = 22,667); 45–52 (*N* = 23,390); 53–60 (*N* = 20,848); 61–71 (*N* = 22,599); >71 (*N* = 21,537), and unknown (*N* = 43). Prior to 2019, the majority of approvals were granted for patients >45 years old (**Figures 5A,B**). However, since 2019, the proportion of approvals for younger patients is increasing, with the exception of patients <18 (3rd degree polynomial, R^2 = 0.888, Δm = 6.750). Approvals for patients aged 18–30 have been steadily increasing (2nd degree polynomial, R^2 = 0.986, Δm = 58.345), and comprised the

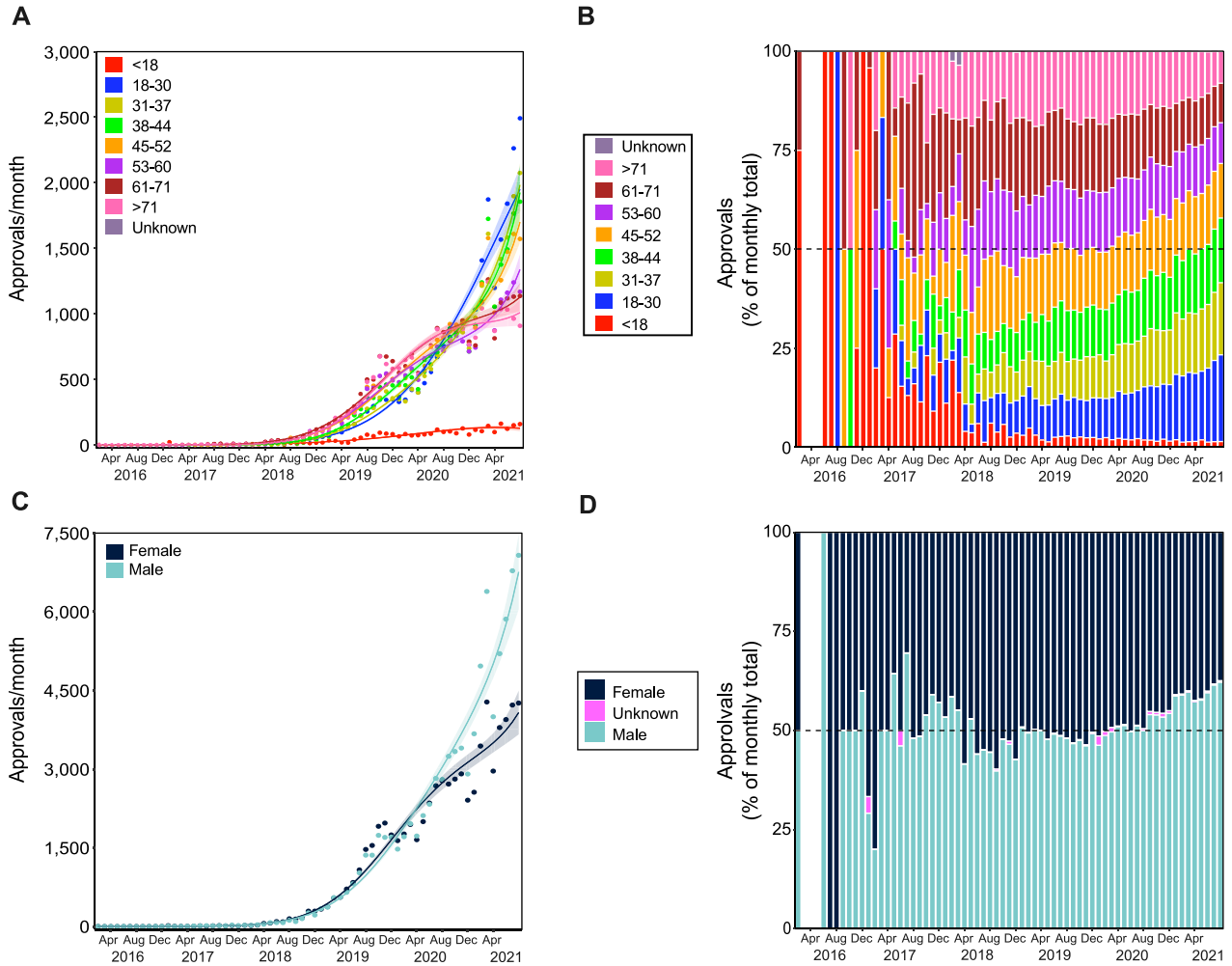


FIGURE 5 | Patient demographics for SAS-B approvals. Growth trends in age groups **(A,B)** and in patient genders **(C,D)**. Approvals for patients younger than 18 years and between 31 and 37 years of age followed a Negative binomial 3rd degree polynomial curve (R^2 = 0.888 and 0.993, and Δm = 6.750 and 22.056, respectively); approvals for patients between 18 and 30 years of age followed a Negative binomial 2nd degree polynomial curve (R^2 = 0.986, and Δm = 58.345); approvals for patients in age groups 38 to 44, 45 to 52, 53 to 60, 61 to 71, and over 71 years of age followed a Negative binomial 4th degree polynomial curve (R^2 = 0.990, 0.990, 0.991, 0.985, and 0.989, and Δm = 10.929, 16.765, 10.162, 27.937, and 8.374, respectively). Panel **(B)** shows the proportion of approvals for each age category (see legend) per month. Approvals for females and males followed Negative binomial 4th degree polynomial curves (**C**; R^2 = 0.986 and 0.989, and Δm = 32.522 and 16.797, respectively). The proportion (%) of approvals by patient gender per month are shown in panel **(D)**. Solid lines in panels **(A)** and **(C)** represent best fit with shaded standard error of the mean. The gap (between February 2016 and June 2016 in panels **(B)** and **(D)**, indicates no applications submitted in this period.

largest number of approvals for 2021 (18.6%), particularly in August 2021, where they represented 21.9% of all approvals. Approvals for patients between 31 and 37 are increasing rapidly (3rd degree polynomial, R^2 = 0.993, Δm = 22.056), making up 16.1% of all approvals in 2021. These proportional gains are likely achieved from the relative lack of growth in the number of applications for patients aged 61–71 (4th degree polynomial, R^2 = 0.985, Δm = 27.937), and >71 (4th degree polynomial, R^2 = 0.989, Δm = 8.374).

Since early 2020, the rate of increase in approvals has also been greater in males compared with females (**Figure 5C**; 4th degree polynomial, R^2 = 0.989, Δm = 16.797, and 4th degree polynomial, R^2 = 0.986, Δm = 32.522, respectively), which is reflected in the observed proportional gains. In January 2020, 46.3% of approvals were for males, but in August 2021 was 62.2% (**Figure 5D**).

3.5 Prescriber Consulting Location

The SAS-B prescribing trends also varied by prescriber consulting location. Australia has 6 states and 2 territories, which, as described above, can sometimes differ in how scheduled drugs are regulated. Prior to 2019, the rate of prescribing normalized to population (per 100,000) was relatively comparable between states. However, the normalized rate of growth of approvals from the state of Queensland far outnumbers all other states and territories (**Figure 6**; 4th degree polynomial, R^2 = 0.986, Δm = 73.955). A trend toward continued growth was observed in the remaining

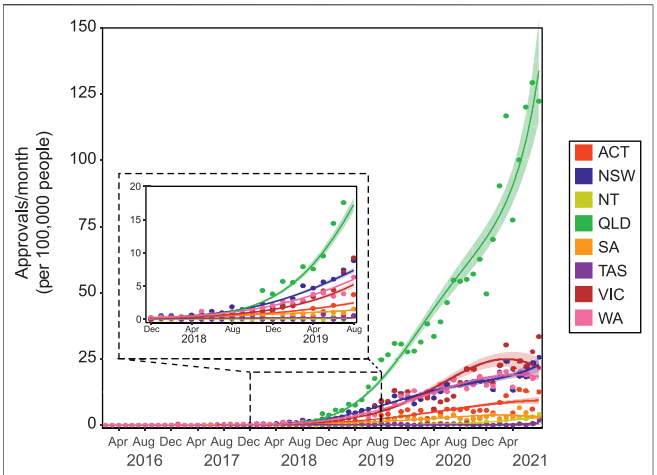


FIGURE 6 | SAS-B approvals across states and territories. The number of SAS-B approvals per month across states and territories represented as per 100,000 persons. Approvals from the Northern Territory (NT) followed a Poisson 1st degree polynomial curve (R^2 = 0.772). Approvals from Australian Capital Territory (ACT) and Western Australia (WA) followed Negative binomial, 2nd degree polynomial curves (R^2 = 0.926 and 0.986, and Δm = 24.998, and 1,525.398, respectively). Approvals Victoria (VIC) followed a Negative binomial, 3rd degree polynomial curves (R^2 = 0.934, Δm = 11.759). Approvals New South Wales (NSW), Queensland (QLD) and South Australia (SA) followed Negative binomial, 4th degree polynomial curves (R^2 = 0.982, 0.986, and 0.926, and Δm = 6.783, 73.955, and 4.143, respectively). Approvals from Tasmania (TAS) moderately followed a Negative binomial, 3rd degree polynomial curve (R^2 = 0.402, Δm = 2.611). Solid lines represent best fit with shaded standard error of the mean.

states and territories, with the exception of Victoria (3rd degree polynomial, R^2 = 0.934, Δm = 11.759).

3.6 Authorised Prescribers

Authorised Prescribers are required to report to the TGA twice a year on the number of patients they have treated in the prior 6 months. AP data were provided by the TGA in two documents that were grouped differently, either by indication, or by state. They also contained overlapping, but non-matching time frames. Further, when selecting for approximately the same time frame (2018–2020), these documents contained different reporting numbers. When sorting by indication, there were 6,748 and 9,687 reports for new and continuing patients, respectively. When sorting by location, there were 15,333 new patient reports, and 10,210 continuing. When asked to clarify the discrepancies between these two datasets, a TGA spokesperson commented that "this is incomplete data and it is the best we could provide with what information is available to us. They are not linked" (TGA, *personal communication*). In general, pain was the most common indication category, and most approvals originated from prescribers in Queensland. Given the unreliability and low quality of these data, and the lack of patient demographics and product information, a more meaningful analysis of this dataset could not be completed. However, monitoring of information released by the TGA over time indicates that the number of registered APs has been increasing significantly in the last year, with 194 active APs in January 2021, rising to 715 in January 2022 (data not shown).

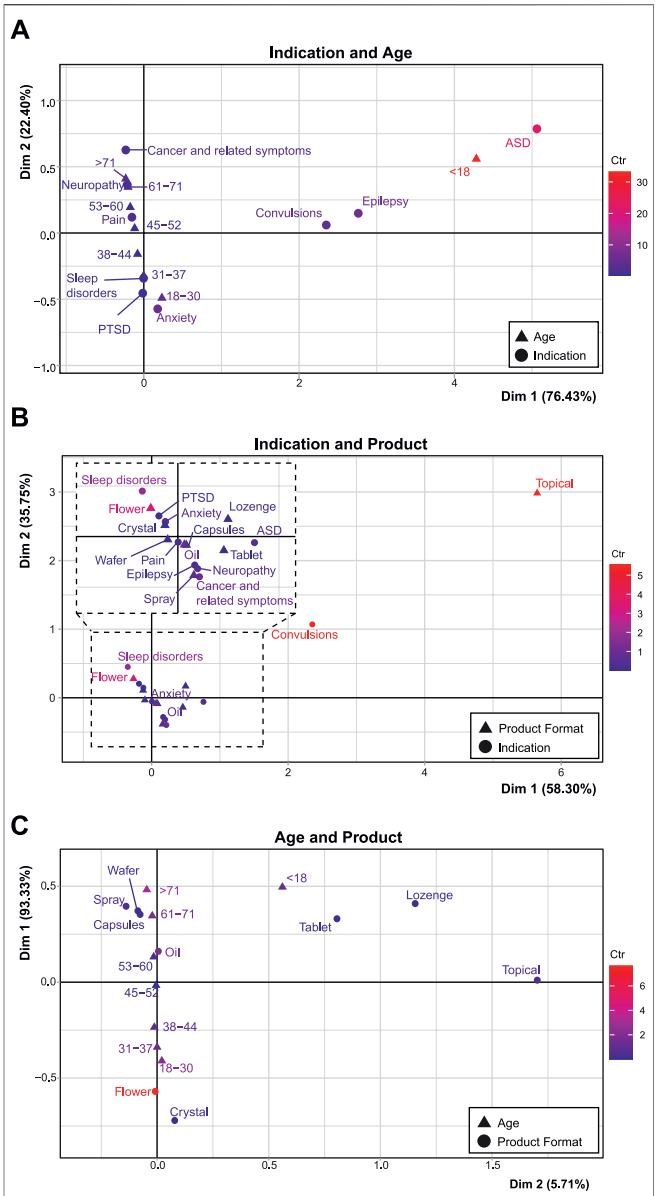


FIGURE 7 | Associations between age, product format, and indication. Correspondence analyses with groups of age compared with indication **(A)**, indication compared with product preference (with an inset representing an expanded view of the dashed area; **(B)**, and age compared with product preference **(C)**. Description of deviation from independence is labelled with the according axes, while the red to blue color gradient indicates the scaled contribution of these factors to the overall variance (the inertia*100; "Ctr") for each graph. The maximum inertia*100 are 33.07, 8.47, and 5.56, respectively. See **Supplementary Tables S3–S5** for the contribution of variance related to these graphs.

3.7 Associations

To probe the relationship between key variables, a two-dimensional correspondence analysis was used. This analysis captured distinct patient subgroups with attributes of indication category, product

category, and age group. Associations were found between indication category and patient age group (χ^2_{56} = 69,422.45, $p < 0.001$; **Figure 7A**), indication and product format (χ^2_{64} = 16,143.57, $p < 0.001$; **Figure 7B**), and product format and age group (χ^2_{56} = 69,490.8, $p < 0.001$; **Figure 7C**). In each instance, variables with a larger contribution than the expected average were considered distinct subgroups. Cut off for age was 12.5%, and for indication and product type was 11.1%. A summary of the contribution to variance in each of these studies is included in **Supplementary Tables S3–S5**.

In comparing indication and age, patients aged <18 deviated from the average with a large contribution to overall variance (Dim 1: 92.01%, inertia*100 = 33.12) and were largely associated with ASD (Dim 1: 60.91%, inertia*100: 22.06), as well as epilepsy (Dim 1: 19.38%, inertia*100 = 6.87), and convulsions (Dim 1: 12.77%, inertia*100 = 4.52). There were also associations along the Dim 2 axis, between patients aged 18–30 (Dim 2: 34.88%, inertia*100 = 4.53) and approvals for anxiety (Dim 2: 54.0%, inertia*100 = 6.22). Another subgroup were patients aged >71, who were more associated with approvals for neuropathy and cancer and related symptoms (Dim 2: 21.11%, inertia*100 = 2.98), as well as patients aged 61–71 (Dim 2: 16.04%, inertia*100 = 2.37). Each age category was associated with a particular indication with no average profile observed.

Approvals for topical products contributed greatly to the Dim 1 axis construction (68.53%, inertia*100 = 5.56) when investigating product vs. indication. This group was associated with approvals for convulsions (Dim 1: 70.93%, inertia*100 = 5.45). Approvals for flower products also represented a distinct patient subgroup (Dim 2: 47.15% inertia*100 = 3.44), that was significantly related to sleep disorders (Dim 2: 32.11%, inertia*100 = 2.03). In this analysis, pain represented the average profile of application across all products (inertia*100 = 0.2, coordinate for Dim 1: 0.00 and Dim 2: 0.04).

Approvals for flower products were the most distinct patient subgroup in relation to age group, as indicated by the distance from the origin and the highest relative contribution to inertia (contribution to Dim 1: 73.80%, inertia*100 = 7.66). This was associated with patients aged 18–30 (contribution to variance Dim 1: 23.70%, inertia*100 = 2.48) and to a lesser extent 31–37 (contribution to variance Dim 1: 15.01%, inertia*100 = 1.56). Approvals for oil products also contributed to variance (contribution to variance Dim 1: 17.58%, inertia*100 = 1.84), and was associated with patients aged 53–60. Sprays, wafers, and capsules were associated with applications for patients aged 61–71 (contribution to variance Dim 1: 16.58%, inertia*100 = 1.74), and >71 who were a distinct patient subgroup (contribution to variance Dim 1: 30.57%, inertia*100 = 3.24). Patients aged 45–52 represented the average profile, having little contribution to the overall variance and being in close proximity to the origin (inertia*100 = 0.01, coordinate for Dim 1: 0.02 and Dim 2: 0.05), with no association to a specific product choice.

4 DISCUSSION

The current report characterizes the prescribing of MC products under the SAS-B scheme in Australia since the inception of a legal MC framework in November 2016. The availability of a unique large dataset with detailed patient-level data provides an unparalleled opportunity to examine MC prescribing trends in Australia. This analysis represents the first step in what could possibly be a series of future analyses, including expanded analysis within particular subsets of data, incorporating additional information as it becomes available, as described below.

4.1 Trends Over Time

The SAS-B dataset shows dynamic and evolving prescribing trends, not necessarily foreseeable at the inception of the scheme in 2016. The dramatic escalation of prescribing over time is unlikely to reflect greater population morbidity (with notable exceptions, *vide infra*), but is more likely to reflect improved patient access pathways and greater familiarity and acceptance of MC prescribing amongst HCPs. Surveys of Australian health professionals report a shifting attitude towards acceptance of MC as a treatment option, as more educational material and evidence becomes available, and prescribers become more confident in their MC prescribing practices (Karanges et al., 2018; Lewis and Flood, 2021). Also salient was the launch of a streamlined online "portal" system for SAS-B applications in 2018, with the intention of improving the speed and simplicity with which clinicians could apply for SAS-B MC approvals (Therapeutic Goods Administration, 2020b).

Policy changes since 2016 are also relevant, as some state and territory-level eligibility and approval requirements have been simplified or removed (Royal Australian College of General Practitioners, 2019). For example, in 2017, Queensland restricted general practitioners from prescribing without the endorsement of a condition-specialist physician, and required state Health Department approval (in addition to TGA approval) for all MC prescriptions (Public Health (Medicinal Cannabis) Act 2016 (Queensland)). Reforms made in July 2019 allowed all HCPs to prescribe, and the requirement for state approval for MC prescribing was reduced to S8 products and only when prescribing to drug-dependent patients (Health Legislation Amendment Regulation (No.2) 2019 (Queensland)). Similar reforms have been made in other jurisdictions, notably in New South Wales and Victoria (Royal Australian College of General Practitioners, 2019; Community Affairs References Committee, 2020).

Some recent increases in prescribing may be reflective of an overall increase in mental health-related morbidity. An overall increase in mental health-related government-subsidised and co-payment prescriptions has been noted in 2020–2021, likely related to the mental health burden of COVID-19 restrictions and lockdowns in Australia (Australian Institute of Health and Welfare, 2021). MC approvals for mental and behavioural disorders also significantly increased during this period.

In contrast, SAS-B applications for epilepsy showed downward trends. It is possible that this trend may be influenced by the CBD-containing medicine *Epidyolex* becoming a registered medicine in Australia (September 2020), obviating the need for access to CBD under the SAS-B schemes (Therapeutic Goods Administration, 2020b). Interestingly, the proportion of approvals for S8 products for epilepsy has increased

since mid-2019, perhaps reflecting inadequate treatment responses in patients with S4 products (Anderson et al., 2020). Future work should compare the changepoints in this (and other) approval data to time-relevant contextual changes (e.g., legislative changes).

Another notable trend is for younger males and females gaining approvals for flower products for indications within the mental and behavioural disorders group, as also noted by Lane and Cohen (2021). This was also evident in correspondence analyses that highlighted this distinct patient subgroup, and a shift toward S8 products in the treatment of anxiety. In some patient scenarios (e.g., breakthrough pain (Bhaskar et al., 2021) and panic attacks (Stith et al., 2020)) vaporized flower is preferred due to the rapid onset of action and shorter duration of action compared to oral products (Huestis, 2007). Future analyses may wish to explore this specific association further.

4.2 Disparities Between Prescribing and Provided Therapeutic Goods Administration Guidance

The SAS-B application process requires HCPs to provide a clinical justification for prescription of MC products, drawing on available evidence. The TGA provides *Clinical Guidance* documents to help support this process, but these are limited to indications deemed to have the highest quality evidence (chronic non-cancer pain; epilepsy; palliative care; chemotherapy-induced nausea and vomiting; and spasticity in multiple sclerosis) (Therapeutic Goods Administration, 2017). Notably, there are no guidance documents for leading conditions in the current dataset such as anxiety (Black et al., 2019), sleep disorders (Suraev et al., 2020), ASD (Fletcher et al., 2022), and PTSD (Hindocha et al., 2020). These conditions, all which have >1,000 cumulative approvals, are characterized by ongoing uncertainty around MC efficacy and poor quality of available evidence. Regardless, HCP justification provided in the SAS-B applications was evidentially sufficient to warrant approval by the TGA.

Practitioners may see MC as a viable treatment option, even with limited or ambiguous clinical evidence of efficacy or as last resort treatment when all other conventional treatments have failed (Hallinan et al., 2021). This may be the case with pain and mental and behavioral disorders in particular, where there has been ever-expanding clinical need, and a high side-effect burden with conventional prescribing options (Deloitte Access Economics, 2019; Braund et al., 2021; Painaustralia, 2021).

4.3 Identifying Gaps in Clinical Evidence

The SAS-B dataset may be particularly useful in identifying indications where MC treatment effects might not be captured in existing RCT data, either negatively or positively. Association analyses might also assist in identifying subset populations, particularly where approvals are not abundant. For example, we were surprised to find an association between topical products and approvals for convulsions; however, on further examination, we found at least one topical CBD product that is being investigated for use for the treatment of seizures, with this route of administration intended to reduce possible side effects seen with oral administration (O'Brien, 2019; Sebree et al., 2016). Future studies may also assess the utility of a multiple correspondence analysis to allow insights into multiple associations within the SAS-B dataset, rather than the restricted two-dimensional correspondence analysis used in this study.

It is important to note that the SAS-B dataset is essentially descriptive and provides no information around efficacy, or the lack thereof, across indications. It might be presumed that the presence of tens of thousands of prescriptions for an individual condition (e.g., pain) must indicate efficacy, but this is not assured. Repeat applications for the same patient for the same condition might also be considered a proxy for perceived efficacy. However, some conditions may resolve and no longer require treatment, meaning that discontinuation outcomes are inherently ambiguous. Prescribing could also be influenced by the now-available public data on the TGA dashboard (Therapeutic Goods Administration, 2021a), which could create a "feed-forward" cycle in prescribing, even in the absence of perceived patient benefit. Furthermore, while the TGA sets quality standards to ensure consistent cannabinoid content in products used in the SAS and AP schemes (Therapeutic Goods Administration, 2020b), limited information on these products is supplied by product companies to the TGA (Therapeutic Goods Administration, 2021b; Office of Drug Control, 2021). The TGA also does not qualify or verify this information, so the database is likely incomplete and inaccurate. Information on cannabinoid content, dose, and how different conditions have been dosed over time, would also be valuable, especially if coupled to readouts of perceived efficacy.

4.4 Caveats and Limitations

Several limitations are important to consider when interpreting this dataset and analysis. While the SAS-B dataset covers the large majority of MC prescribing in Australia, some patient cohorts are not represented, namely, patients receiving products through SAS-A and AP schemes, those receiving compounded products, and those enrolled in clinical trials. To the best of our knowledge, data on patients receiving compounded products or participating in active clinical trials are not collected by the TGA. This changed from March 2022 where the TGA requires prescribers to seek approval via the SAS-B or AP pathways prior to prescribing a compounded MC product. Additionally, while the TGA collects data on the AP scheme, the datasets supplied were incomplete and ambiguous, and so the exact number of patients could not be estimated.

Another significant limitation is the lack of a definitive link between a SAS-B application being approved, a prescription being written, and a product actually being dispensed to a patient (Prof. Nick Lintzeris, *personal communication*). A prescriber may submit multiple SAS-B applications for a single patient but only write a prescription for one product. Alternatively, or in addition, a prescription may be received by a patient but not filled. Until November 2021, the TGA specified that SAS-B and AP applications be for a single named product (Skerritt, 2017; Therapeutic Goods Administration, 2021c), meaning that if a specified product were unavailable from a manufacturer, an

additional application needed to be submitted. To overcome this, multiple simultaneous applications for different products were often submitted for the same patient (Prof. Nick Lintzeris, *personal communication*). This has the capacity to distort the SAS-B dataset to over-estimate number of prescriptions. New TGA regulations as of November 2021 allow prescribers to seek an approval to prescribe any products of the same format (e.g., oral oils) in the same product "category" based on cannabinoid content (Therapeutic Goods Administration, 2021c). These changes were implemented to reduce the administrative burden on prescribers who were seeking multiple approvals for the same patient as a hedge against product unavailability. The data collected and used in this paper pre-dates these changes, but future analysis should consider the impact of these changes on MC approvals. SAS-B approvals and AP registration status also have expiration dates, after which they become invalid and must be renewed/replaced. Variations in these timeframes may also impact observed trends.

The data around indication in the current analysis must also be treated with some caution. The SAS-B application process does not require prescribers to specify strict diagnostic criteria, resulting in potentially ambiguous or erroneous classifications of patients. Some applications note patient symptoms rather than diagnosis, for example, some applications noted "epilepsy" (diseases of the nervous system) while others noted "convulsions" (symptoms and signs). It is likely that these approvals involved the same patient population, which, had they been combined, might have produced different trends. Additionally, many conditions are multi-factorial involving multiple medical classifications; for example, MC for "cancer" is ambiguous as to whether the treatment is intended to be targeted at the tumour itself or the *symptoms* associated with cancer and cancer therapy such as nausea, insomnia, or pain. Implementation of a more rigorous diagnostic and data capture process under the current regulatory framework would be a major advantage to future research.

The context in which these data were collected should also be considered, as the regulatory framework and the study of MC continues to evolve. Further analysis of change points in prescribing trends across a broad range of indications within the context of a timeline of published evidence and policy changes would be informative, but is outside the scope of this current work.

Finally, a polynomial regression line of best fit, which assumes that there is infinite potential growth and restricted from negative intercept (i.e., Poisson and Negative binomial error distribution), imposes certain limitations. Using generated trend lines to predict future usage or to model usage in other jurisdictions may be possible, but should be interpreted with caution. A Bass model which incorporates eventual saturation of the available population, would be ideal for appropriately assessing the capacity for growth in the future (Burnham and Anderson, 2010). However, the point of saturation is difficult to estimate given there is no current model at which to estimate this point. Thus, for the purposes of this current analysis, the polynomial model is appropriate. Approvals in some indications, such as pain, seem to be nearing saturation. By continuing to monitor trends with the data over time, and capturing this saturating data point, the fit of the Bass model to this data could be very informative for other jurisdictions wishing to predict outcomes from their own MC programs.

5 CONCLUSION

Data captured by the TGA in the first 5 years following implementation of a MC prescribing regulatory framework in Australia displays rapidly escalating numbers of approvals, particularly since January 2020, and other highly dynamic trends. These data and associated analyses, provide a unique resource that can be drawn upon by researchers, practitioners, and regulators to better understand current clinical practice around MC in Australia, and to identify where research gaps exist relative to prescribing. The analysis presented here shows the utility of such accessible records as the regulatory framework for MC continues to evolve. Other jurisdictions which have initiated, or are looking to initiate, MC schemes might usefully consider the Australian model.

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 review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SM collected the data. SM, MB-P and EC performed the data analysis, with input from RC, VK, and IM. All authors contributed to the writing and editing of the manuscript.

FUNDING

Supported by the Lambert Initiative for Cannabinoid Therapeutics, a philanthropically funded center for medicinal cannabis research at the University of Sydney.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.885655/full#supplementary-material

REFERENCES

Alexander, S. P. (2020). Barriers to the Wider Adoption of Medicinal Cannabis. *Br. J. Pain* 14, 122–132. doi:10.1177/2049463720922884

Anderson, L. L., Low, I. K., McGregor, I. S., and Arnold, J. C. (2020). Interactions between Cannabidiol and Δ9-tetrahydrocannabinol in Modulating Seizure Susceptibility and Survival in a Mouse Model of Dravet Syndrome. *Br. J. Pharmacol.* 177, 4261–4274. doi:10.1111/bph.15181

Arnold, J. C., Nation, T., and McGregor, I. S. (2020). Prescribing Medicinal Cannabis. *Aust. Prescr* 43, 152–159. doi:10.18773/austprescr.2020.052

Australian Bureau of Statistics (2021). National, State and Territory Population. [Online]. Available at: https://www.abs.gov.au/statistics/people/population/national-state-and-territory-population/mar-2021 (Accessed October, 2021).

Australian Institute of Health and Welfare (2021). *COVID-19 Impact on Mental Health [Online]*. Canberra, ACT: Australian Government. Available at: https://www.aihw.gov.au/reports/mental-health-services/mental-health-services-in-australia/report-contents/mental-health-impact-of-covid-19.

Banerjee, R., Erridge, S., Salazar, O., Mangal, N., Couch, D., Pacchetti, B., et al. (2022). Real World Evidence in Medical Cannabis Research. *Ther. Innov. Regul. Sci.* 56 (1), 8–14. doi:10.1007/s43441-021-00346-0

Banister, S. D., Arnold, J. C., Connor, M., Glass, M., and McGregor, I. S. (2019). Dark Classics in Chemical Neuroscience: Δ9-Tetrahydrocannabinol. *ACS Chem. Neurosci.* 10, 2160–2175. doi:10.1021/acschemneuro.8b00651

Bartoń, K. (2020). *MuMIn: Multi-Model Inference*.

Benson, M., and Cohen, R. (2019). "Does Medicinal Cannabis Actually Help Australian Patients?," in *Med. Cannabis Netw. 1*. Congleton, United Kingdom: Pan European Networks Ltd. Available at: https://www.healtheuropa.eu/does-medicinal-cannabis-actually-help-australian-patients/92921/.

Bhaskar, A., Bell, A., Boivin, M., Briques, W., Brown, M., Clarke, H., et al. (2021). Consensus Recommendations on Dosing and Administration of Medical Cannabis to Treat Chronic Pain: Results of a Modified Delphi Process. *J. Cannabis Res.* 3, 22. doi:10.1186/s42238-021-00073-1

Black, N., Stockings, E., Campbell, G., Tran, L. T., Zagic, D., Hall, W. D., et al. (2019). Cannabinoids for the Treatment of Mental Disorders and Symptoms of Mental Disorders: A Systematic Review and Meta-Analysis. *Lancet Psychiatry* 6, 995–1010. doi:10.1016/S2215-0366(19)30401-8

Braund, T. A., Tillman, G., Palmer, D. M., Gordon, E., Rush, A. J., and Harris, A. W. F. (2021). Antidepressant Side Effects and Their Impact on Treatment Outcome in People with Major Depressive Disorder: An iSPOT-D Report. *Transl. Psychiatry* 11, 417. doi:10.1038/s41398-021-01533-1

Bruni, N., Della Pepa, C., Oliaro-Bosso, S., Pessione, E., Gastaldi, D., and Dosio, F. (2018). Cannabinoid Delivery Systems for Pain and Inflammation Treatment. *Molecules* 23, 2478. doi:10.3390/molecules23102478

Burnham, K. P., and Anderson, D. R. (2002). *A Practical Information-Theoretic Approach*. New York, NY: Springer.

Burnham, K. P., and Anderson, D. R. (2010). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. New York: Springer.

Community Affairs References Committee (2020). *Current Barriers to Patient Access to Medicinal Cannabis in Australia*. Australia: Commonwealth of Australia.

Corva, D., and Meisel, J. S. (2021). *The Routledge Handbook of Post-Prohibition Cannabis Research*. Abingdon, Oxen; New York, NY: Routledge.

Crocq, M. A. (2020). History of Cannabis and the Endocannabinoid System. *Dialogues Clin. Neurosci.* 22, 223–228. doi:10.31887/DCNS.2020.22.3/mcrocq

Decorte, T., Lenton, S., and Wilkins, C. (2020). *Legalizing Cannabis: Experiences, Lessons and Scenarios*. Abingdon, Oxen; New York, NY: Routledge.

Deloitte Access Economics (2019). *The Cost of Pain in Australia: Report for Painaustralia*. Canberra, ACT.

Falconer, J. R., and Steadman, K. J. (2017). Extemporaneously Compounded Medicines. *Aust. Prescr.* 40, 5–8. doi:10.18773/austprescr.2017.001

Fletcher, S., Pawliuk, C., Ip, A., Huh, L., Rassekh, S. R., Oberlander, T. F., et al. (2022). Medicinal Cannabis in Children and Adolescents with Autism Spectrum Disorder: A Scoping Review. *Child* 48, 33–44. doi:10.1111/cch.12909

Fragoso, Y. D., Carra, A., and Macias, M. A. (2020). Cannabis and Multiple Sclerosis. *Expert Rev. Neurother.* 20, 849–854. doi:10.1080/14737175.2020.1776610

Freshleaf Analytics (2021). *Australian Medicinal Cannabis Market Patient, Product and Pricing Analysis*. Sydney NSW.

Gleeson, P. (2020). *The Challenge of Medicinal Cannabis to the Political Legitimacy of Therapeutic Goods Regulation in Australia*, 43. Melbourne: Melbourne University Law Review, 558–604.

Grimison, P., Mersiades, A., Kirby, A., Lintzeris, N., Morton, R., Haber, P., et al. (2020). Oral THC:CBD Cannabis Extract for Refractory Chemotherapy-Induced Nausea and Vomiting: A Randomised, Placebo-Controlled, Phase II Crossover Trial. *Ann. Oncol.* 31, 1553–1560. doi:10.1016/j.annonc.2020.07.020

Hallinan, C. M., Gunn, J. M., and Bonomo, Y. A. (2021). Implementation of Medicinal Cannabis in Australia: Innovation or Upheaval? Perspectives from Physicians as Key Informants, a Qualitative Analysis. *BMJ Open* 11, e054044. doi:10.1136/bmjopen-2021-054044

Henderson, L. A., Kotsirilos, V., Cairns, E. A., Ramachandran, A., Peck, C. C., and McGregor, I. S. (2021). Medicinal Cannabis in the Treatment of Chronic Pain. *Aust. J. Gen. Pract.* 50, 724–732. doi:10.31128/ajgp-04-21-5939

Herbert, A., and Hardy, J. (2021). Medicinal Cannabis Use in Palliative Care. *Aust. J. Gen. Pract.* 50, 363–368. doi:10.31128/ajgp-02-21-5831

Hindocha, C., Cousijn, J., Rall, M., and Bloomfield, M. A. P. (2020). The Effectiveness of Cannabinoids in the Treatment of Posttraumatic Stress Disorder (PTSD): A Systematic Review. *J. Dual Diagn.* 16, 120–139. doi:10.1080/15504263.2019.1652380

Huestis, M. A. (2007). Human Cannabinoid Pharmacokinetics. *Chem. Biodivers.* 4, 1770–1804. doi:10.1002/cbdv.200790152

Iffland, K., and Grotenhermen, F. (2017). An Update on Safety and Side Effects of Cannabidiol: A Review of Clinical Data and Relevant Animal Studies. *Cannabis Cannabinoid Res.* 2, 139–154. doi:10.1089/can.2016.0034

Karanges, E. A., Suraev, A., Elias, N., Manocha, R., and McGregor, I. S. (2018). Knowledge and Attitudes of Australian General Practitioners Towards Medicinal Cannabis: A Cross-Sectional Survey. *BMJ Open* 8, e022101. doi:10.1136/bmjopen-2018-022101

Kassambara, A. (2020). *Ggpubr: 'ggplot2' Based Publication Ready Plots. R Package*. version 0.4. 0

Lane, M., and Cohen, R. (2021). Younger Men Driving Demand for Flower in Game-Changing Industry Trend. Available at: https://www.cannabiz.com.au/monthly-sas-b-approvals-hit-13666-with-flower-taking-40-market-share/ (Accessed August, 2021).

Le, S., Josse, J., and Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *J. Stat. Softw.* 25, 1–18. doi:10.18637/jss.v025.i01

Lewis, M., and Flood, J. (2021). The Transition of Cannabis into the Mainstream of Australian Healthcare: Framings in Professional Medical Publications. *J. Cannabis Res.* 3, 48. doi:10.1186/s42238-021-00105-w

Malcolm, J., B. (2018). Should Medical Cannabis Administered by Inhalation Be Allowed for Hospitalized Patients? *Can. J. Hosp. Pharm.* 71, 211–214. doi:10.4212/cjhp.v71i3.2588

Moore, D. S., and Kirkland, S. (2013). *The Basic Practice of Statistics*. New York: W. H. Freeman.

Nabbout, R., and Thiele, E. A. (2020). The Role of Cannabinoids in Epilepsy Treatment: A Critical Review of Efficacy Results from Clinical Trials. *Epileptic Disord.* 22, 23–28. doi:10.1684/epd.2019.1124

Nelson, K. M., Bisson, J., Singh, G., Graham, J. G., Chen, S. N., Friesen, J. B., et al. (2020). The Essential Medicinal Chemistry of Cannabidiol (CBD). *J. Med. Chem.* 63, 12137–12155. doi:10.1021/acs.jmedchem.0c00724

O'Brien, T. (2019). Open-Label Extension Study of ZYN002 (Transdermal Gel) in Patients with Partial Onset Seizures. Identifier ACTRN12616001455459. Available at https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=371636 (Accessed March 22, 2022).

Office of Drug Control (2021). "Manufacturers and Suppliers of Medicinal Cannabis Products," in *Australian Government Department of Health: Office of Drug Control*. Canberra, ACT. [Online]. Available at: www.odc.gov.au/manufacturers-and-suppliers-medicinal-cannabis-products (Accessed September, 2021).

Painaustralia (2021). *The National Strategic Action Plan for Pain Management*, 1. Canberra, ACT: The Department of Health.

Queensland Government (2019). *Health Legislation Amendment Regulation*. Brisbane: QLD. (No. 2) 2019.

Queensland Government (2016). *Public Health (Medicinal Cannabis) Act 2016*.

R Core Team (2021). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. [Online]. Available at: https://www.R-project.org/.

Royal Australian College of General Practitioners (2019). The Regulatory Framework for Medicinal Use of Cannabis Products. [Online]. Available at: https://www.racgp.org.au/advocacy/position-statements/view-all-position-statements/clinical-and-practice-management/medical-cannabis.

Schlag, A. K., O'sullivan, S. E., Zafar, R. R., and Nutt, D. J. (2021). Current Controversies in Medical Cannabis: Recent Developments in Human Clinical Applications and Potential Therapeutics. *Neuropharmacology* 191, 108586. doi:10.1016/j.neuropharm.2021.108586

Sebree, T., O'Neill, C., Messenheimer, J., and Dutterman, D. (2016). *Safety and Tolerability of ZYN002 (Synthetic Cannabidiol) Transdermal Permeation-Enhanced Gel in Healthy Subjects and Epilepsy Patients: Three Phase 1, Randomized, Double-Blind, Placebo-Controlled Studies*. Available at: http://www.zynerba.com/wp-content/uploads/2016/12/Zynerba_ZYN002_AES-2016_Safety_Poster.pdf (Accessed March 22, 2022).

Skerritt, J. (2017). "Medicinal Cannabis: An Update from the Regulators," in *Australian Pharmacist*. Canberra, ACT. [Online]. Available at: https://www.australianpharmacist.com.au/medicinal-cannabis-an-update-from-the-regulators/ (Accessed February, 2022).

Stella, B., Baratta, F., Della Pepa, C., Arpicco, S., Gastaldi, D., and Dosio, F. (2021). Cannabinoid Formulations and Delivery Systems: Current and Future Options to Treat Pain. *Drugs* 81, 1513–1557. doi:10.1007/s40265-021-01579-x

Stith, S. S., Li, X., Diviant, J. P., Brockelman, F. C., Keeling, K. S., Hall, B., et al. (2020). The Effectiveness of Inhaled Cannabis Flower for the Treatment of Agitation/Irritability, Anxiety, and Common Stress. *J. Cannabis Res.* 2, 47. doi:10.1186/s42238-020-00051-z

Suraev, A. S., Marshall, N. S., Vandrey, R., Mccartney, D., Benson, M. J., McGregor, I. S., et al. (2020). Cannabinoid Therapies in the Management of Sleep Disorders: A Systematic Review of Preclinical and Clinical Studies. *Sleep Med. Rev.* 53, 101339. doi:10.1016/j.smrv.2020.101339

Therapeutic Goods Administration (2022). *Consultation: Potential Reforms to Medicinal Cannabis Manufacturing, Labelling and Packaging Requirement [Online]*. Woden, ACT: Department of Health. Available at: https://www.tga.gov.au/consultation/consultation-potential-reforms-medicinal-cannabis-manufacturing-labelling-and-packaging-requirement (Accessed March, 2022).

Therapeutic Goods Administration (2021a). *Medicinal Cannabis Access Data Dashboard [Online]*. Woden, ACT: The Department of Health. Available at: https://www.tga.gov.au/medicinal-cannabis-access-data-dashboard (Accessed February, 2022).

Therapeutic Goods Administration (2020a). *Epidyolex [Online]*. Woden, ACT: The Department of Health. (Accessed, 2022).

Therapeutic Goods Administration (2020b). *Medicinal Cannabis [Online]*. Woden, ACT: The Department of Health. Available at: https://www.tga.gov.au/node/769199 (Accessed September, 2021).

Therapeutic Goods Administration (2021b). *Medicinal Cannabis Products by Active Ingredients [Online]*. Available at: https://www.tga.gov.au/medicinal-cannabis-products-active-ingredients (Accessed February 21, 2022).

Therapeutic Goods Administration (2017). *Medicinal Cannabis – Guidance Documents [Online]*. Woden, ACT: The Department of Health. Available at: www.tga.gov.au/medicinal-cannabis-guidance-documents (Accessed September, 2021).

Therapeutic Goods Administration (2021c). *New Streamlined Process for Medicinal Cannabis Applications [Online]*. Woden, ACT: The Department of Health. Available at: https://www.tga.gov.au/media-release/new-streamlined-process-medicinal-cannabis-applications (Accessed February, 2022).

Therapeutic Goods Administration (2020b). *Potential Reforms to Medicinal Cannabis Manufacturing, Labelling and Packaging Requirements [Online]*. Woden, ACT: Department of Health. Available at: https://consultations.tga.gov.au/tga/medicinal-cannabis-reforms-2020/ (Accessed March, 2022).

Thoen, E. (2020). *Padr: Quickly Get Datetime Data Ready for Analysis*. R package. version 0.5.

Vaissie, P., Monge, A., and Husson, F. (2021). *Factoshiny: Perform Factorial Analysis from 'FactoMineR' with a Shiny Application*. R package version 2.4.

Vandrey, R., Herrmann, E. S., Mitchell, J. M., Bigelow, G. E., Flegel, R., Lodico, C., et al. (2017). Pharmacokinetic Profile of Oral Cannabis in Humans: Blood and Oral Fluid Disposition and Relation to Pharmacodynamic Outcomes. *J. Anal. Toxicol.* 41, 83–99. doi:10.1093/jat/bkx012

Venables, W. N., and Ripley, B. D. (2002). *Modern Applied Statistics with S*. New York: Springer.

Wickham, H., Averick, M., Bryan, J., Chang, W., Mcgowan, L., François, R., et al. (2019). Welcome to the Tidyverse. *Joss* 4, 1686. doi:10.21105/joss.01686

Wickham, H., François, R., Henry, L., and Müller, K. (2021). *Dplyr: A Grammar of Data Manipulation*. 2020. R package. version 0.8 4.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York.

Wilke, C. (2020). *Cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'*. New York, NY: Springer-Verlag. R Package. 2017. Version 0.9. 2. Available at: https://CRAN.R-project.org/package=cowplot.

World Health Organization (2018). "CANNABIDIOL (CBD) Critical Review Report," in *Expert Committee on Drug Dependence Fortieth Meeting* (Geneva).

Conflict of Interest: RC is an owner of Cannabis Consulting Australia; however, receives no financial or commercial benefit from the results of 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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Pharmacology

ORIGINAL RESEARCH
published: 25 May 2022
doi: 10.3389/fphar.2022.888903

Cannabis for Medical Use: Analysis of Recent Clinical Trials in View of Current Legislation

F. Baratta, I. Pignata, L. Ravetto Enri and P. Brusa*

Department of Drug Science and Technology, University of Turin, Turin, Italy

Cannabis has long been regarded as a recreational substance in the Western world. The recent marketing authorization of some medicinal products of industrial origin and the introduction onto the market of inflorescences for medical use mean that medical doctors can now prescribe *Cannabis*-based medicines in those countries which allow it. Nevertheless, there is still considerable controversy on this topic in the scientific community. In particular, this controversy concerns: the plant species to be used; the pathologies that can be treated and consequently the efficacy and safety of use; the routes of administration; the methods of preparation; the type and dosage of cannabinoids to be used; and, the active molecules of interest. As such, although medical *Cannabis* has been historically used, the results of currently completed and internationally published studies are inconclusive and often discordant. In light of these considerations, the aim of this work is to analyse the current legislation in countries that allow the use of medical *Cannabis*, in relation to the impact that this legislation has had on clinical trials. First of all, a literature search has been performed (PubMed and SciFinder) on clinical trials which involved the administration of *Cannabis* for medical use over the last 3 years. Of the numerous studies extrapolated from the literature, only about 43 reported data on clinical trials on medical *Cannabis*, with these mainly being performed in Australia, Brazil, Canada, Denmark, Germany, Israel, Netherlands, Switzerland, the United Kingdom and the United States of America. Once the reference countries were identified, an evaluation of the legislation in relation to *Cannabis* for medical use in each was carried out via the consultation of the pertinent scientific literature, but also of official government documentation and that of local regulatory authorities. This analysis provided us with an overview of the different legislation in these countries and, consequently, allowed us to analyse, with greater awareness, the results of the clinical trials published in the last 3 years in order to obtain general interest indications in the prosecution of scientific research in this area.

Keywords: medical *Cannabis*, clinical trials, study protocols, legislation, law

OPEN ACCESS

Edited by:
Claudio Ferrante,
University of Studies G. d'Annunzio Chieti and Pescara, Italy

Reviewed by:
Aviv M. Weinstein,
Ariel University, Israel
Franjo Grotenhermen,
Nova Institut GmbH, Germany

****Correspondence:***
F. Baratta
francesca.baratta@unito.it

Specialty section:
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

Received: *03 March 2022*
Accepted: *09 May 2022*
Published: *25 May 2022*

Citation:
Baratta F, Pignata I, Ravetto Enri L and Brusa P (2022) Cannabis for Medical Use: Analysis of Recent Clinical Trials in View of Current Legislation. Front. Pharmacol. 13:888903. doi: 10.3389/fphar.2022.888903

Abbreviations: ADHD, Attention-Deficit/Hyperactivity Disorder; AIDS, Acquired ImmunoDeficiency Syndrome; CBD, CannaBiDiol; COVID-19, COronaVIrus Disease 2019; FDA, Food and Drug Administration; HIV, Human Immunodeficiency Virus; NICE, National Institute for health and Care Excellence; OCD, Obsessive-Compulsive Disorder; PTSD, Post-Traumatic Stress Disorder; THC, delta-9-TetraHydroCannabinol; United States, United States of America.

1 INTRODUCTION

Cannabis was widely used in the past for its curative properties. The earliest records of its medicinal use date back to China where *Cannabis* has been cultivated for millennia for use as a fiber, food, and medicine. Over time, it spread to the whole of Asia, the Middle East, and Africa. In the West, the plant started to attract scientific interest only in the 20th century. However, in the last century, the cultivation, sale, and use of *Cannabis* was made illegal in the majority of countries (Lafaye, et al., 2017; Pisanti and Bifulco, 2019; Romano and Hazekamp, 2019; Arias, et al., 2021).

In the last few decades, there has been revived support for its decriminalisation, and legalisation for medical uses thanks to new and scientifically founded indications of its potential therapeutic value. This is partly due to the support gained in the media, and to the high expectations for its efficacy, even though these hopes, for many diseases, are not sufficiently supported by scientific research (Hill, 2015; Whiting, et al., 2015).

The phytocomplex of *Cannabis* plants is made up of more than 500 molecules, of which about a hundred belong to the Cannabinoid chemical class. Among these molecules, even small variations in molecular structure can produce significantly different effects. The molecules of greatest interest to pharmacologists are the decarboxylated forms of 9-tetrahydracannabinol (THC) and cannabidiol since these are easily absorbed in the intestine (Grotenhermen, 2003; Gould, 2015; Baratta, et al., 2019; Baratta, et al., 2021).

Recently, *Cannabis* based industrial medicines have been approved for sale, and medical use inflorescences have been made available. This has given medical doctors, in those countries which allow it, the option to prescribe *Cannabis*-based products. At present, the most widely available products are: Marinol® (AbbVie Inc) and Syndros® (Benuvia Therapeutics) which contain dronabinol, an isomer of delta-9-tetrahydrocannabinol; Cesamet® based on nabilone (Meda Pharmaceuticals Inc.), another synthetic cannabinoid; Sativex® (GW Pharma Ltd.), based on an ethanol extraction of *Cannabis sativa*; and Epidiolex®[1] (Greenwich Biosciences), which contains CBD (Casiraghi, et al., 2018).

A variety of pharmaceutical-grade inflorescence products are also available on the market. Usually, the label only indicates the concentrations of THC and CBD. This is a critical point as the phytocomplex of medical *Cannabis* contains many active molecules which contribute to the "Entourage effect," a hypothesis postulating a positive synergic action between cannabinoids and terpenes (Stella, et al., 2021; Baratta, et al., 2022).

Given the increasing availability of the above products, many countries have introduced specific legislation, regulations, and guidelines regarding the use of medical use *Cannabis* in the treatment of various pathologies. Nevertheless, debate continues around this subject within the scientific community. The main points of contention are the correct plant varieties to be used, the pathologies to be treated, and, consequently, the efficacy and safety of their use. There are no universally shared indications on the optimum administration route, the preparation methodology, the definitive types of cannabinoids and dosages to recommend, or even the identity of the active molecule of interest. This controversy stems in large part from the findings of the clinical trials conducted till now. Although the number of studies and publications is growing rapidly, for many diseases the results are often contradictory or inconclusive. All too often, these trials were performed on a non-homogeneous population, and utilising diverse plant material, extraction methods, dosages, pharmaceutical forms, and administration routes. Moreover, the trials were often conducted without a control group (Stella, et al., 2021).

In light of all these considerations, the objective of this work is to analyse the current legislation and regulations in a number of countries where medical use *Cannabis* is permitted in order to evaluate any relationship of these on the design of clinical trials carried out there.

2 MATERIALS AND METHODS

We carried out a literature search (PubMed and SciFinder) for clinical trials with medical *Cannabis* published in the last 3 years (2019/01/01–2021/12/15). We excluded literature reviews, non-clinical trials, and articles about non-medical use *Cannabis*. We also considered published articles about clinical trial protocols to be carried out. The key search terms used were clinical trials, medical *Cannabis*, and medical use.

After the publications had been selected, the countries of origin were identified in order to perform an evaluation of the current regulations in each regarding medical *Cannabis*. The scientific literature, and relevant official publications from government and local authorities were consulted for this analysis.

Finally, the characteristics and the results of the clinical studies were analysed to evaluate any possible link to the state legislation where the studies had been carried out.

3 RESULTS

Of the 400 matches from the literature search, only 10% (43) of the publications reported data from trials or clinical protocols regarding medical *Cannabis*. The relevant trials were carried out in: Australia, Brazil, Canada, Denmark, Germany, Israel, Netherlands, Switzerland, the United Kingdom, and the United States of America. Given their geographical distribution, these countries can be considered of interest despite the small number of studies available.

For each of the countries in question, the current legislation on medical *Cannabis* was analysed, and some specific features are reported such as: prescription procedure, indicated pathologies for medical *Cannabis*, products available for sale, dispensation forms, authorisation to grow *Cannabis* for medical use, and reimbursement procedure.

[1]Epidiolex® has received approval in the European Union under the tradename Epidyolex®.

3.1 Current Legislation

3.1.1 Australia

Although there are some regulatory differences among the federal states regarding the importation of products, and the qualification required to write a prescription, medical *Cannabis* may be prescribed after receiving authorisation from the Therapeutic Goods Administration, through the Special Access Scheme for an individual patient, or through the Authorized Prescriber Scheme for a group of patients with the same condition. Products of industrial origin are exempt from these schemes as approval for sale has already been granted (Sativex® and Epidiolex®).

As well as Sativex® and Epidiolex®, indicated for the treatment of spasticity in multiple sclerosis and paediatric epilepsy, herbal-*Cannabis* based products may also be prescribed. The most common conditions are spasticity in multiple sclerosis, nausea or vomiting caused by anti-tumoral chemotherapy, pain or anxiety in patients with terminal diseases, and refractory child epilepsy. The physician may in any case write a prescription for pathologies other than those indicated.

Pharmacies are authorised to dispense medical *Cannabis*-based products.

The cost of the therapy is not subsidised by the government.

Alcohol and Drug Foundation, 2021; Australian Capital Territory Government, 2021; Australian Government, 2017a; Australian Government, 2017b; Australian Government, 2018; Australian Government, 2020; Australian Government, 2021; Australian Institute of Health and Welfare, 2019; Castle, et al., 2019; Centre for Medicinal Cannabis Research and Innovation, 2021; Health Direct, 2019; Mersiades, et al., 2019; The Health Products Regulatory Authority, 2017; The Office of Drug Control, 2021)

3.1.2 Brazil

Various products of industrial origin are available such as Epidiolex® and Sativex®, and the importation of *Cannabis*-derived products is generally authorised. However, the importation of the raw plant or parts of the plant is not permitted. Products with a concentration of THC greater than 0.2% may only be prescribed when no alternative therapy is available, and the patient has reached the irreversible or terminal stage of their disease. Prescription is under the responsibility of the prescribing medical doctor. The medication may be taken either orally or by inhalation.

The cost of the treatment is generally high and is completely at the patient's expense.

The dispensation may take place in a pharmacy, where *Cannabis* may not be processed, however.

(Crippa, et al., 2018; Marketrealist, 2019; Ministério da Saúde, 2019; Reuters, 2019; Brazilian Government, 2021)

3.1.3 Canada

The situation in Canada is quite different, medical *Cannabis* (with the exception of approved industrial products) is not considered as a medicine; hence, it is not dispensed in pharmacies. Medical doctors or nurses may prescribe it for individual patients. The patient can then acquire it from a licensed vendor; grow a quantity sufficient for personal use in residence after registering with the Ministry for Health; nominate a grower in their place (a grower can only cultivate for two people); or acquire it from a provincial or area level licensed retailer. The patient is allowed to prepare *Cannabis*-based products, but the use of organic solvents such as butane, benzene, methyl-chloride, or chlorinated hydrocarbons is forbidden.

Regarding industrial products, Sativex® is available for sale; it is indicated for the treatment of spasticity in multiple sclerosis. Other recommended uses include additional pain relief for neuropathic pain in adult patients with multiple sclerosis, and additional pain relief for patients with late-stage cancer who experience moderate to serious pain when already undergoing palliative care with the highest tolerable dosages of opioids. Nabilone is approved for treatment of serious nausea and vomiting associated with chemotherapy, while dronabinol is approved for the treatment of AIDS-related anorexia, and for serious nausea and vomiting associated with chemotherapy. Dronabinol was withdrawn for the Canadian market by the producer in February 2012, but not for health risks.

Generally, *Cannabis* may be used for any symptom without demonstrating the inefficacy of the previous therapies.

The approved industrial products may be reimbursed by health insurance companies, while all the others are non-reimbursable.

(Fischer, et al., 2015; Ablin, et al., 2016; Health Canada, 2016; The Health Products Regulatory Authority, 2017; Abuhasira, et al., 2018; Conseil fédéral, 2018; Government of Canada, 2019; Health Canada, 2022)

3.1.4 Denmark

All medical doctors are authorised to prescribe *Cannabis*-based products as part of a 4 years pilot project launched in January 2018. As part of this project, a medical doctor may prescribe medicines that are not approved for distribution or sale in Denmark. However, the medical doctor must take full responsibility for the products they prescribe and must determine the proper dosage for each patient. Medical doctors may refer to the guidelines laid out by the Danish Medicines Agency. The imported plant products available for prescription may vary in content, but they must comply with strict standards and regulations governing the cultivation of the plant species, and the production and standardisation of the *Cannabis*-based product.

Herbal *Cannabis* is available by prescription only in pharmacies, which may also prepare magistral preparations.

Regarding industrial products, neurologists may prescribe Sativex® to treat spasticity from multiple sclerosis. In general, medical doctors may prescribe imported *Cannabis*-derived medicines that have not been approved for sale in Denmark, such as Marinol® and Cesamet® on compassionate grounds, but only if the request is approved by the Danish Medicines Agency.

In general, the Danish Medicines Agency indicates that medical *Cannabis* be considered as a therapy only for the following conditions: painful spasticity in multiple sclerosis, painful spasticity caused by spinal cord damage, chemotherapy-induced nausea, and neuropathic pain. As part

of the pilot project, *Cannabis* may, however, be prescribed to any patient even outside of the guidelines. The use of *Cannabis* is not recommended for patients under 18 years of age.

The prices of the prescribed products within the pilot project are set freely by the manufacturers. It is possible to obtain a reimbursement as of 01/01/2019 (retroactive for 2018). Patients in the terminal stages of a disease are fully reimbursed, while patients with other illnesses receive a 50% reimbursement, up to annual maximum of 10,000 Danish Krone. The reimbursement is automatically deducted at the time of the purchase in a pharmacy.

For prescriptions that are not part of the pilot project, the medical doctor may request a reimbursement for an individual patient from the Danish Medicines Agency. It will consider the request for those patients with pathologies where *Cannabis*-based treatment appears to be effective, and for those whom all other treatments with approved medicines have been used without effect.

(The Health Products Regulatory Authority, 2017; Abuhasira, et al., 2018; Krcevski-Skvarc, et al., 2018; Danish Medicines Agency, 2020; Gustavsen, et al., 2021)

3.1.5 Germany

Medical doctors may prescribe medical *Cannabis* using a specific "narcotics" prescription form. The prescription may be for any condition that has no standard treatment, or the standard treatment cannot be used owing to reactions, or based on the patient's specific condition. Among the industrial products available is Sativex®, which is indicated for spasticity in refractory multiple sclerosis. In addition, it is possible to prescribe dronabinol without particular restrictions regarding its indicated use. Nabilone is approved for nausea and vomiting associated with chemotherapy and unresponsive to conventional therapies. Finally, Epidiolex® and many types of *Cannabis* inflorescences may also be prescribed. Magisterial preparations may be prescribed, and pharmacies may dispense extracts of *Cannabis* and inflorescences.

In the past, *Cannabis* could also be theoretically grown in residence by private individuals if conventional therapies had been inefficacious, no other alternative treatments were available, and/or to reduce the cost of therapy. Actually, this possibility has never been really applied. Since 2019, however, a system of checks on the production and supply of *Cannabis* has been introduced by the government.

The patients may request a reimbursement from health insurance companies. For this purpose the prescribing medical doctor has the task of certifying the seriousness of the disease, that the standard therapies have been ineffective, or cannot be used due to the patient's specific condition, or that there is a reasonable likelihood that medical *Cannabis* will be effective for that subject.

(Grotenhermen and Müller-Vahl, 2012; Ablin, et al., 2016; The Health Products Regulatory Authority, 2017; Abuhasira, et al., 2018; Conseil fédéral, 2018; Federal Institute for Drugs and Medical Devices, 2018; Krcevski-Skvarc, et al., 2018; Rasche, et al., 2019; Federal Institute for Drugs and Medical Devices, 2022a; Federal Institute for Drugs and Medical Devices, 2022b; Federal Institute for Drugs and Medical Devices, 2022c; Federal Institute for Drugs and Medical Devices, 2022d; German Institute for Medical *Cannabis*, 2022)

3.1.6 Israel

In Israel, patients with a prescription may use a licensed pharmacy to obtain medical *Cannabis*. There is a list of conditions for which *Cannabis* may be used, but the medical doctor may also prescribe it for other pathologies: in any case, it may only be used when other therapies have proved ineffective. The list includes neuropathic pain, serious cachexia in AIDS patients, spasticity from multiple sclerosis, pain associated with Parkinson's disease, Tourette's syndrome, treatment of metastatic cancer or chemotherapy-induced symptoms, inflammatory intestinal diseases and post-traumatic stress disorders.

In general, the products available are *Cannabis* inflorescences, Sativex® and Epidiolex®. The number of medical *Cannabis* patients among the Israeli population is one of the highest in the world (on February 2022 about 100,000 Israelis -about 1% of the population-were allowed to consume medical *Cannabis*).

Sativex® is recommended for spasticity from multiple sclerosis unresponsive to other treatments, or as an additional analgesic therapy in adult patients with advanced stage cancer with moderate to severe pain despite being administered the highest tolerable dosage of opioids; Epidiolex® is used to treat convulsions in Dravet syndrome, and Lennox-Gastaut syndrome.

As for herbal *Cannabis*, a government-run programme produces and distributes this product. Medical *Cannabis* is supplied in two forms: as an oil extract for oral administration or sub-lingual deposition, and as the inflorescence which may be smoked or inhaled with vaporisers. The cost of the therapy is reimbursed in part by some private and state health insurance schemes.

(abcNEWS, 2022; Ablin, et al., 2016; Abuhasira, et al., 2018; Krcevski-Skvarc, et al., 2018; State of Israel - Minister of Health, 2017; State of Israel - Minister of Health, 2022; The Health Products Regulatory Authority, 2017)

3.1.7 Netherlands

In Netherlands, all medical doctors may prescribe medical *Cannabis*. The pharmacies may also produce extracts using the plant material produced by the Office of Medical *Cannabis*. These are usually oil extracts to be taken orally or deposited under the tongue. Some types of inflorescences are available for this purpose: the concentration of the active molecules and granulation properties may vary. The inflorescences may also be taken in the decoction form or inhaled through vaporisers.

Sativex® is approved for the treatment of spasticity from multiple sclerosis refractory to conventional therapies.

Cannabis is indicated for the treatment of pain (multiple sclerosis, or spinal cord injuries), chronic pain, nausea and vomiting (in chemotherapy or radiotherapy, HIV therapies, adverse reactions to hepatitis C medication), palliative care for cancer or AIDS (to increase appetite and alleviate pain, nausea and weight loss), Tourette's syndrome, and refractory glaucoma, epilepsy and epileptic syndromes (even in children). In addition, its use is indicated in the reduction in symptomology of the following pathologies: Crohn's disease, ulcerative colitis, itching, migraine, rheumatic conditions, ADHD, post-traumatic stress disorders, agitation in Alzheimer's disease and cerebral trauma. Medical doctors are in any case authorised to prescribe these

therapies for other conditions if they consider it fit. *Cannabis*-based products must, however, be considered only in cases where authorised medicines have inefficacious or provoked unacceptable adverse reactions.

As concerns the available herbal *Cannabis* species, Bediol® (THC 6.3%; CBD 8%) is usually recommended as the first-choice therapy to alleviate pain or as an anti-inflammatory therapy. Bedrocan® (THC 22%; CBD <1.0%), Bedica® (THC 14%; CBD <1.0%) and Bedrobinol® (THC 13.5%; CBD <1.0%) are considered more effective for the treatment of symptoms such as appetite loss, weight loss, nausea, vomiting, anorexia, cachexia, emesis, Tourette's syndrome, and glaucoma. Bedrolite® (THC <1.0%; CBD 7.5%) is employed for certain forms of epilepsy.

The healthcare system does not reimburse the cost of *Cannabis*-based medicines. In some cases, the patient may be able to claim from private insurance schemes.

(The Health Products Regulatory Authority, 2017; Abuhasira, et al., 2018; Conseil fédéral, 2018; Krcevski-Skvarc, et al., 2018; Bedrocan, 2021; Office of Medicinal Cannabis, 2022)

3.1.8 Switzerland

The prescription and use of *Cannabis*-based magistral preparations is authorised for spasticity (multiple sclerosis), chronic pain, appetite loss in AIDS, and nausea, pain, and appetite loss from cancer.

The magistral preparations are prepared in a pharmacy.

Medical doctors may prescribe *Cannabis*-based medicines only after receiving authorisation from the Federal office of the Public Health System.

The cost of the therapy is not reimbursed systematically, but on a case-by-case basis.

As well as the inflorescence, it is possible to use dronabinol and Epidiolex®. Sativex® is also authorised for use and available for treatment of spasticity from multiple sclerosis.

(Abuhasira, et al., 2018; Krcevski-Skvarc, et al., 2018; Swiss Confederation, Federal Office of Public Health, 2020; Swiss Confederation, Federal Office of Public Health, 2021a; Swiss Confederation, Federal Office of Public Health, 2021b; Swiss Confederation, Federal Office of Public Health, 2021c)

3.1.9 United Kingdom

In the United Kingdom, medical *Cannabis* is generally prescribed to adults and children with rare and serious forms of epilepsy, adults suffering from nausea or vomiting from chemotherapy, and adults with muscular stiffness or spasms from multiple sclerosis. This therapy is considered only in cases in which no alternative treatment is available, or other treatments have been inefficacious. The available products are Epidiolex®, prescribed to patients with Lennox-Gastaut syndrome or Dravet syndrome; nabilone, which is authorised for nausea and vomiting associated with chemotherapy; dronabinol is also available, but it has no marketing authorization; and Sativex®, which is prescribed for muscular spasms in multiple sclerosis unresponsive to other treatments (even though it is discouraged by NICE in that it is not cost-effective).

The medical *Cannabis* therapy cannot be obtained from a general practitioner but must be prescribed by a hospital specialist registered with the General Medical Council. The medical doctor may collect data on adverse reactions, which can also be signalled directly by the patient through a yellow card system.

(Department of Health and Social Care, 2018; Medicines and healthcare products Regulatory Agency, 2020; MS Society, 2021; National Health Service, 2021; General Medical Council, 2022; National Health Service, 2022; UK Government, 2022)

3.1.10 United States of America

There are significant legislative differences among the states concerning *Cannabis* in the United States. In some states the legislation in force is extremely limiting, in others significantly less restrictive. Therefore, the state laws may not be completely harmonised with federal laws.

Regarding industrial products, the FDA has approved the prescription of dronabinol and nabilone for the treatment of chemotherapy-induced nausea and vomiting. Dronabinol may also be used for the treatment of appetite and weight loss in HIV patients. Epidiolex® may be prescribed for the treatment of epileptic disorders, Lennox-Gastaut syndrome and Dravet's syndrome.

Concerning herbal *Cannabis*, only 36 states have legalised or decriminalised its use. In general, in those states which have authorised the use of medical use *Cannabis*, there are restrictions on its prescription. Depending to the local laws, therefore, *Cannabis* may be prescribed for pain, anxiety, epilepsy, glaucoma, appetite and weight loss associated with AIDS, inflammatory intestinal disturbances irritable intestine syndrome, motor disturbances due to Tourette's syndrome or multiple sclerosis, nausea and vomiting caused by chemotherapy, sleep disorders, posttraumatic stress disorders. Some states allow the addition, at the prescribing medical doctor's discretion, of pathologies other than those expressly stated.

Generally, medical doctors do not need specific training to prescribe *Cannabis*, but in many states, it is necessary to register before doing so. In other states, medical doctors must attend a short training course to be able to register. In some states, it is enough that the medical doctor gives advice verbally to take medical *Cannabis*, or its use may be recommended by a health care professional who is not a medical doctor. On the other hand, in some states, it is necessary that two medical doctors confirm the need for a *Cannabis*-based treatment for a patient. Depending on the state, *Cannabis* may be supplied to the patient by licensed dispensaries, or it may be grown at home by the patient or by a caregiver.

Smoking medical *Cannabis* is prohibited in some states. Similarly even the edible forms are prohibited in some states. Generally, the administration is performed orally or by vaporiser.

Patients are generally registered so that the possession and use of medical *Cannabis* is not prosecuted.

Abuhasira, et al., 2018; Alharbi, 2020; Carliner, et al., 2017; Choo and Emery, 2017; Corroon and Kight, 2018; Johnson, et al., 2021; Mead, 2017; National Conferences of State Legislatures, 2022; ProCon, 2022; Ryan, et al., 2021; The Health Products Regulatory Authority, 2017)

TABLE 1 | Characteristics of the selected clinical trials.

Clinical trials with a POSITIVE outcome

	Country	Study TITLE	Study type	Administration	Product	Condition	Number of patients
1	AUSTRALIA	A pilot randomised placebo-controlled trial of cannabidiol to reduce severe behavioural problems in children and adolescents with intellectual disability Efron et al. (2021)	Double-blinded Placebo-controlled Randomized	Oral (solution)	CBD	Severe behavioural problems (in children and adolescents with intellectual disability)	8
2	AUSTRALIA	Oral THC:CBD cannabis extract for refractory chemotherapy-induced nausea and vomiting: a randomised, placebo-controlled, phase II crossover trial Grimison et al. (2020)	Double-blinded Multicentre Placebo-controlled Randomized	Oral (capsules)	Herbal *Cannabis*: THC:CBD (1:1 ratio) *Cannabis sativa* L. extract	Refractory chemotherapy-induced nausea and vomiting	80 enrolled 72 completed the study
3	AUSTRALIA	Cannabis use in patients 3 months after ceasing nabiximols for the treatment of cannabis dependence: Results from a placebo-controlled randomised trial Lintzeris et al. (2020)	Double-blinded Multicentre Placebo-controlled Randomized	Oro-mucosal spray	Sativex® (obtained from *Cannabis sativa* L.)	*Cannabis* dependence	128
4	AUSTRALIA	A Phase 1, Randomised, Placebo-Controlled, Dose Escalation Study to Investigate the Safety, Tolerability and Pharmacokinetics of Cannabidiol in Fed Healthy Volunteers Perkins et al. (2020)	Double-blinded Placebo-controlled Randomized	Oral (solution)	CBD	Safety, tolerability and pharmacokinetics evaluations on healthy volunteers	24
5	ISRAEL	The pharmacokinetics, efficacy, and safety of a novel selective dose cannabis inhaler in patients with chronic pain: A randomized, double-blinded, placebo-controlled trial Almog et al. (2020)	Double-blinded Placebo-controlled Randomized	Inhalation	Herbal *Cannabis*: Bedrocan® (22% THC, <1% CBD)	Chronic pain	27 enrolled 25 completed the study
6	ISRAEL	Pharmacokinetic investigation of synthetic cannabidiol oral formulations in healthy volunteers Izgelov et al. (2020)	Blinded	Oral (powder, oil or self-nano-emulsifying drug delivery system)	CBD	Oral absorption processes of synthetic CBD when given in different oral formulations in healthy volunteers	12
7	ISRAEL	The safety, tolerability, and effectiveness of PTL-101, an oral cannabidiol formulation, in pediatric intractable epilepsy: A phase II, open-label, single-center study Mitelpunkt et al. (2019)	Open-label	Oral (capsules)	CBD	Treatment-resistant epilepsy (in paediatric patients)	16 enrolled 11 completed
8	ISRAEL	Effect of adding medical cannabis to analgesic treatment in patients with low back pain related to fibromyalgia: an observational cross-over single centre study Yassin et al. (2019)	Observational	Inhalation	THC:CBD (1:4 ratio) herbal *Cannabis*	Low back pain related to fibromyalgia	31

(Continued on following page)

TABLE 1 | (*Continued*) Characteristics of the selected clinical trials.

Clinical trials with a POSITIVE outcome

	Country	Study TITLE	Study type	Administration	Product	Condition	Number of patients
9	SWITZERLAND	Cannabidiol enhances verbal episodic memory in healthy young participants: A randomized clinical trial Hotz et al. (2021)	Double-blinded Placebo-controlled Randomized	Inhalation	CBD	Verbal episodic memory in healthy young subjects	39
10	UNITED KINGDOM	Cannabidiol for the treatment of cannabis use disorder: a phase 2a, double-blind, placebo-controlled, randomised, adaptive Bayesian trial Freeman et al. (2020)	Double-blinded Placebo-controlled Randomized	Oral (capsules)	CBD	Desire to stop using *Cannabis*	82
11	UNITED KINGDOM	Normalization of mediotemporal and prefrontal activity, and mediotemporal-striatal connectivity, may underlie antipsychotic effects of cannabidiol in psychosis O'Neill et al. (2021)	Double-blinded Placebo-controlled Randomized	Oral (capsules)	CBD	Psychosis	34 enrolled 32 completed
12	UNITED KINGDOM	Effects of cannabidiol on brain excitation and inhibition systems; a randomised placebo-controlled single dose trial during magnetic resonance spectroscopy in adults with and without autism spectrum disorder Pretzsch et al. (2019a)	Double-blinded Placebo-controlled Randomized	Oral (solution)	CBD	Brain excitation and inhibition systems	34
13	UNITED KINGDOM	The effect of cannabidiol (CBD) on low-frequency activity and functional connectivity in the brain of adults with and without autism spectrum disorder (ASD) Pretzsch et al. (2019c)	Double-blinded Placebo-controlled Randomized	Oral (solution)	CBD	Low-frequency activity and functional connectivity in the brain	34
14	UNITED KINGDOM	Dissociable effects of cannabis with and without cannabidiol on the human brain's resting-state functional connectivity Wall et al. (2019)	Double-blinded Placebo-controlled Randomized	Inhalation	Herbal *Cannabis*: *Cannabis* containing both THC and CBD; *Cannabis* containing THC	Human brain's resting-state networks	17
15	United States	Food effect on pharmacokinetics of cannabidiol oral capsules in adult patients with refractory epilepsy Bimbaum et al. (2019)	Open-label	Oral (capsules)	CBD	Refractory epilepsy	11 enrolled 8 completed the study
16	United States	Cannabidiol for the Reduction of Cue-Induced Craving and Anxiety in Drug-Abstinent Individuals With Heroin Use Disorder: A Double-Blind Randomized Placebo-Controlled Trial Hurd et al. (2019)	Double-blinded Placebo-controlled Randomized	Oral (solution)	CBD	Drug cue–induced craving and anxiety in drug-abstinent individuals with heroin use disorder	50 enrolled 42 completed
17	United States	The Effectiveness of Topical Cannabidiol Oil in Symptomatic Relief of Peripheral Neuropathy of the Lower extremities Xu et al. (2020)	Double-blinded Placebo-controlled Randomized	Topical (cream)	CBD	Peripheral neuropathy	29

(Continued on following page)

TABLE 1 | (*Continued*) Characteristics of the selected clinical trials.

Clinical trials with a POSITIVE outcome

Country		Study TITLE	Study type	Administration	Product	Condition	Number of patients
18	United States	A randomized trial of medical cannabis in patients with stage IV cancers to assess feasibility, dose requirements, impact on pain and opioid use, safety, and overall patient satisfaction Zylla et al. (2021)	Placebo-controlled Randomized	Inhalation, oral, topical	A variety of formulations (also based on herbal *Cannabis*) with differing ratios of THC/CBD were provided depending on individual patient symptoms	Pain, opioid use, safety, and satisfaction in cancer patient	30

Clinical Trials With A NEGATIVE Outcome

Country		Title	Study Type	Administration	Administerd Product	Condition	Number Of Patients
1	AUSTRALIA	The CANBACK trial: a randomised, controlled clinical trial of oral cannabidiol for people presenting to the emergency department with acute low back pain Bebee et al. (2021)	Double-blinded Placebo-controlled Randomized	Oral (solution)	CBD	Low back pain	100
2	BRAZIL	Cannabidiol for COVID-19 Patients with Mild to Moderate Symptoms (CANDIDATE Study): A Randomized, Double-Blind, Placebo-Controlled Clinical Trial Crippa et al. (2021)	Double-blinded Placebo-controlled Randomized	Oral (solution)	CBD	COVID-19	105
3	UNITED KINGDOM	The acute effects of cannabidiol on the neural correlates of reward anticipation and feedback in healthy volunteers Lawn et al. (2020)	Double-blinded Placebo-controlled Randomized	Oral (capsules)	CBD	Neural correlates of reward anticipation and feedback	28 enrolled 24 completed the study
4	UNITED STATES	The short-term impact of 3 smoked cannabis preparations versus placebo on PTSD symptoms: A randomized cross-over clinical trial Bonn-Miller et al. (2021)	Double-blinded Placebo-controlled Randomized	Inhalation	Herbal *Cannabis*: *Cannabis* containing 12% THC and <0.05% CBD; *Cannabis* containing 11% CBD and 0.50% THC; *Cannabis* containing 7.9% THC and 8.1% CBD	Posttraumatic Stress Disorder	80 enroled 76 completed stage I 74 completed stage II
5	UNITED STATES	Acute effects of cannabinoids on symptoms of obsessive-compulsive disorder: A human laboratory study Kayser et al. (2020)	Double-blinded Placebo-controlled Randomized	Inhalation	Herbal *Cannabis*: *Cannabis* containing THC 7% and CBD 0.18%; *Cannabis* containing 0.4% THC and 10.4% CBD	Obsessive-compulsive disorder	14 enrolled 12 completed the study

Clinical Trials With an INCONCLUSIVE Outcome

Country		Title	Study Type	Administration	Administerd Product	Condition	Number of patients
1	AUSTRALIA	Citalopram and Cannabidiol: *In Vitro* and *In Vivo* Evidence of Pharmacokinetic Interactions Relevant to the Treatment of Anxiety Disorders in Young People Anderson et al. (2021)	Controlled Randomized	Oral (capsules)	CBD	Anxiety disorders	6

(Continued on following page)

TABLE 1 | (*Continued*) Characteristics of the selected clinical trials.

Clinical Trials With an INCONCLUSIVE Outcome

	Country	Title	Study Type	Administration	Administerd Product	Condition	Number of patients
2	AUSTRALIA	Model-based analysis on systemic availability of co-administered cannabinoids after controlled vaporised administration Liu et al. (2020)	Double-blinded Placebo-controlled Randomized	Inhalation	THC; CBD; THC + low-dose CBD; THC + high-dose CBD	Evaluate the active dosage	36
3	AUSTRALIA	A randomised controlled trial of vaporised Δ9-tetrahydrocannabinol and cannabidiol alone and in combination in frequent and infrequent cannabis users: acute intoxication effects Solowij et al. (2019)	Double-blinded Placebo-controlled Randomized	Inhalation	THC; CBD; THC + low-dose CBD; THC + high-dose CBD	Evaluate the acute intoxication effects	36
4	ISRAEL	Oral CBD-rich Cannabis Induces Clinical but Not Endoscopic Response in Patients with Crohn's Disease, a Randomised Controlled Trial Naftali et al. (2021a)	Double-blinded Placebo-controlled Randomized	Oral (solution)	THC:CBD (1:4 ratio) herbal *Cannabis*	Crohn's Disease	56
5	ISRAEL	Cannabis is associated with clinical but not endoscopic remission in ulcerative colitis: A randomized controlled trial Naftali et al. (2021b)	Double-blinded Placebo-controlled Randomized	Inhalation	Herbal *Cannabis*: *Cannabis* containing 16% THC and 0.1% CBD	Ulcerative colitis	32
6	ISRAEL	Cannabinoid treatment for autism: a proof-of-concept randomized trial Aran et al. (2021)	Double-blinded Placebo-controlled Randomized	Oral (solution)	THC:CBD (1:20 ratio) herbal *Cannabis;* purified CBD: THC (1:20 ratio)	Autism spectrum disorder in children and adolescents	150
7	NETHERLANDS	An experimental randomized study on the analgesic effects of pharmaceutical-grade cannabis in chronic pain patients with fibromyalgia Van de Donk et al. (2019)	Double-blinded Placebo-controlled Randomized	Inhalation	Herbal *Cannabis*: Bedrocan® (22% THC, < 1% CBD); Bediol® (6.3% THC, 8% CBD); Bedrolite® (9% CBD, <1% THC)	Pain in patients with fibromyalgia	20
8	UNITED KINGDOM	Effects of cannabidivarin (CBDV) on brain excitation and inhibition systems in adults with and without Autism Spectrum Disorder (ASD): a single dose trial during magnetic resonance spectroscopy Pretzsch et al. (2019b)	Double-blinded Placebo-controlled Randomized	Oral (solution)	Cannabidivarin	Autism Spectrum Disorder	34
9	UNITED STATES	Effect of Inhaled Cannabis for Pain in Adults With Sickle Cell Disease. A Randomized Clinical Trial Abrams et al. (2020)	Double-blinded Placebo-controlled Randomized	Inhalation	Herbal *Cannabis*: *Cannabis* containing 4.4% THC and 4.9% CBD	Pain in patients with sickle cell disease	23

(Continued on following page)

TABLE 1 | (*Continued*) Characteristics of the selected clinical trials.

Clinical Trials With an INCONCLUSIVE Outcome							
	Country	Title	Study Type	Administration	Administerd Product	Condition	Number of patients
10	UNITED STATES	Effects of oral, smoked, and vaporized cannabis on endocrine pathways related to appetite and metabolism: a randomized, double-blind, placebo-controlled, human laboratory study Farokhnia et al. (2020)	Double-blinded Placebo-controlled Randomized	Inhalation and oral (food)	THC	Appetite and metabolism in healthy valuntaires	20
11	UNITED STATES	Effects of Hemp Extract on Markers of Wellness, Stress Resilience, Recovery and Clinical Biomarkers of Safety in Overweight, But Otherwise Healthy Subjects Lopez et al. (2020)	Double-blinded Placebo-controlled Randomized	Oral (solution)	CBD (from Herbal *Cannabis* oil extract)	Markers of wellness, stress resilience, recovery and clinical biomarkers of safety in overweight, but otherwise healthy subjects	65
12	UNITED STATES	Cognitive function and adaptive skills after a 1-year trial of cannabidiol (CBD) in a pediatric sample with treatment-resistant epilepsy Thompson et al. (2020)	Open-label	Oral (solution)	CBD	Cognitive function and adaptive skills in a paediatric patients with treatment-resistant epilepsy	38

3.2 Study Protocols and Clinical Trials

There are 43 publications of proposed, or executed, clinical trial protocols in those countries whose legislation has been analysed; eight of these regarded proposed clinical trial protocols.

Hence, 35 publications regarded actual clinical trial data. These were sub-divided into three groups: the first, "positive outcome," included those studies which demonstrated the efficacy of the preparation administered, or that the actual results were in line with those expected (18). The second group, "negative outcome," included those studies where the authors reported that the administered product was no more efficacious than the placebo (5). Finally, the third group, "inconclusive outcome," comprised those studies where the results were not conclusive (12).

The characteristics of the taken into account clinical studies are summarized in **Table 1**.

3.2.1 Clinical Trials With a Positive Outcome

Of the 18 studies in this category, 4 were conducted in Australia, 4 in Israel, 1 in Switzerland, 5 in the United Kingdom, and 4 in the United States.

Regarding the study design, 2 were multi-centred, 13 used the double-blind method, 14 had a randomised control design, and 14 used a placebo control group.

The sample size varied greatly, from a minimum of 8 to a maximum of 128 enrolled subjects.

As for the products used in the trials, 12 studies administer CBD, 6 studied herbal *Cannabis* derivatives.

CBD was administered orally in 10 cases, topically and by inhalation in only one study. The herbal *Cannabis* derivatives were administered by inhalation in 3 cases, and by the oral route in 2 cases. One study considered products to be administered orally, by inhalation or topically.

In 9 studies, the *Cannabis* derivatives were administered in addition to a standard therapy.

The most commonly studied conditions were behaviour, cerebral activity, and memory (6), pain (4), addiction or abstinence to drugs (3), epilepsy (2), pharmacokinetic studies, safety, and tolerability (2), and nausea and vomiting (1). Two studies were carried out on a paediatric population.

In general, the studies involving the administration of CBD regarded epilepsy, addiction or abstinence to drugs, behaviour, cerebral activity and memory, peripheral neuropathy, pharmacokinetic studies, and safety and tolerability.

Instead, studies administering herbal *Cannabis* derivatives focused mainly about pain and then about nausea and vomiting, cerebral activity and *Cannabis* dependence. In most cases both THC and CBD were administered in different ratios. In some cases, a herbal *Cannabis* strain was used with a high concentration of THC.

(Almog, et al., 2020; Birnbaum, et al., 2019; Efron, et al., 2021; Freeman, et al., 2020; Grimison, et al., 2020; Hotz, et al., 2021; Hurd, et al., 2019; Izgelov, et al., 2020; Lintzeris, et al., 2020; Mitelpunkt, et al., 2019; O'Neill, et al., 2021; Perkins, et al., 2020; Pretzsch, et al., 2019a; Pretzsch, et al., 2019c; Wall, et al., 2019; Xu, et al., 2020; Yassin, et al., 2019; Zylla, et al., 2021)

3.2.2 Clinical Trials With a Negative Outcome

Five trials had a negative outcome. Two of these were conducted in the United States, 1 in Australia, 1 in Brazil and 1 in the United Kingdom.

All of the trials had a randomised control, used a placebo control group, and a double-blind control. The sample size ranged from 14 to 105 enrolled subjects.

As for the products used, 3 studies administered oral preparations containing CBD. 2 studies were based on the administration of inflorescences by inhalation. 4 studies out of 5 administered the product in addition to a standard therapy.

The conditions studied in these trials with CBD were pain, COVID-19 infection, and the effects on neural correlates of reward anticipation and feedback. Herbal *Cannabis*, in three different forms and different ratios of THC/CBD), was administered to evaluate its efficacy in the treatment of Obsessive-Compulsive Disorder (OCD) and Post-Traumatic Stress Disorder (PTSD).

None of these studies demonstrated that the administered product was more efficacious than the placebo control.

(Kayser, et al., 2020; Lawn, et al., 2020; Bebee, et al., 2021; Bonn-Miller, et al., 2021; Crippa, et al., 2021)

3.2.3 Clinical Trials With an Inconclusive Outcome

12 studies had an inconclusive outcome: 3 were conducted in Australia, 3 in Israel, 1 in the Netherlands, 1 in the United Kingdom and 4 in the United States.

Regarding study design, 10 included a double-blind system, 11 had a randomised control, and 10 utilised a placebo control group. The sample size ranged from a minimum of 6 subjects to a maximum of 150 individuals. Two of the studies were conducted on paediatric subjects.

Concerning the products used, 2 studies administered CBD alone, one study used THC alone, 1 study administered cannabidivarin, 2 studies administered THC and CBD, both alone and in a mixture, 5 studies administered herbal *Cannabis* derivatives, and 1 study administered both THC and CBD as well as a herbal *Cannabis* extract.

CBD and cannabidivarin were administered orally; THC, and the mixtures of THC and CBD were administered by inhalation. THC was also administered orally. The herbal *Cannabis* derivatives were administered by inhalation in 3 studies, while they were for oral use in 2 studies. 1 study used oral administration of a herbal *Cannabis* extract or an equivalent mixture of THC and CBD.

Six trials predicted that the administration was additional to standard therapy.

The conditions to be studied for the efficacy of CBD were anxiety and cognitive function in patients suffering from epilepsy. THC and/or CBD were administered to evaluate the active dosage or to study its effects on problems linked to appetite and metabolism, herbal *Cannabis* derivatives were studied to evaluate their activity in Crohn's disease, ulcerative colitis, pain, haemolytic anaemia, markers of wellness and clinical biomarkers in obese patients. Trials related to autism were conducted with, as well as cannabidivarin, the administration of a herbal *Cannabis* extract or an equivalent mixture of THC and CBD.

When herbal *Cannabis* derivatives were administered, the concentration of THC and CBD, and the ratio of the two varied greatly among the trials. Some used products with a high concentration of THC, while others used products with a high concentration of CBD. In 1 trial, different types of inflorescences were administered to evaluate the most efficacious ratio of THC to CBD concentrations against pain.

(Pretzsch, et al., 2019b; Solowij, et al., 2019; Van de Donk, et al., 2019; Abrams, et al., 2020; Farokhnia, et al., 2020; Liu, et al., 2020; Lopez, et al., 2020; Thompson, et al., 2020; Naftali, et al., 2021a; Anderson, et al., 2021; Aran, et al., 2021; Naftali, et al., 2021b)

3.2.4 Study Protocols

There are 8 examples of published protocols that have not yet initiated the clinical trial phase. 4 are in Australia, and 1 each in Denmark, Canada, Germany, and Netherlands. The number of enrolled subjects is between 10 and 180 in total. One study will be carried out among the paediatric population.

Concerning the study design, 3 will be multi-centre studies, 7 use a double-blind system, 8 are randomised, and 7 use a placebo control group.

Regarding the products to be used, 4 protocols will use the oral administration of THC and CBD. The ratio between the components in question varies from study to study. In 2 protocols, the administration of CBD is also foreseen. One protocol foresees the administration of both CBD and a preparation containing a high concentration of THC.

For those studies using THC and CBD mixtures, the pathologies to be studied are, pain, dementia, spasms, and the activation of the immune system in HIV patients. Instead, the CBD alone preparations will be administered for behavioural problems and phobias. The herbal-*Cannabis* derived product will be administered for chronic tic disorder. The protocol that foresees the administration of both CBD and a preparation with a high concentration of THC will focus on the alleviation of pain.

(Costiniuk, et al., 2019; Hendricks, et al., 2019; Urbi, et al., 2019; Van der Flier, et al., 2019; Efron, et al., 2020; Hardy, et al., 2020; Jakubovski, et al., 2020; Timler, et al., 2020)

4 DISCUSSION

From the analysis of the current legislation in states where clinical trials and proposed protocols on medical *Cannabis* and derived products have been published in the last 3 years, many significant differences have been found regarding the products available, the indicated pathologies for which it may be prescribed, the production of the raw plant material, as well as its reimbursement and prescription. It was evaluated to consider the studies published in the last 3 years supposing that the researchers have benefited from the latest knowledge on

medical *Cannabis* and to make an overview of the pathologies currently under study.

In particular, regarding industrial products, practically every country, with the exception of the United States, has approved the use of Sativex®. However, Epidiolex®, dronabinol.Netherlands, and nabilone are also quite common.

In all the countries, the use of herbal *Cannabis* is also authorised. The only exception is Brazil, which is certainly the country with the most restrictive legislation. Netherlands is the only country to provide directions for use, which are not binding, but quite strict, regarding the plant strain to be used for a determined pathology based on the concentration of active molecules (THC and CBD). Instead, for the other countries, it must be pointed out that the current legislation provides for the use of inflorescences or herbal *Cannabis* extracts without providing specific directions concerning the recommended concentration of active molecules to treat a determined condition.

Regarding the pathologies or symptoms associated with the more or less well-defined conditions, the most common are pain, nausea, vomiting, spasticity, and epilepsy followed by spasms, and weight and appetite loss. The less frequently indicated conditions in this case include Tourette's syndrome, PTSD, and glaucoma. In many countries, additional conditions are considered in more or less detail.

In this regard, it is interesting to note that the country with the greatest number of specifically recommended pathologies not indicated in other countries is the Netherlands: perhaps based on the longstanding use of *Cannabis* both for medical use and recreational purposes. Although the legislation regarding medical *Cannabis* is quite comprehensive in all the countries considered, some of them, namely Australia, Canada, Denmark, Germany, Israel, Netherlands, and the United States, also permit the prescription of *Cannabis* for any therapeutic application at the discretion of the medical doctor. However, in Germany, Netherlands and Israel, this is limited to cases in which other therapies have proved ineffective, excessive adverse reactions to standard treatments have occurred, or valid alternative treatments are not available. Instead, in Australia, Canada, Denmark, and the United States, therapeutic strategies different from those specified are authorised regardless of any prior treatment. The prescription of medical *Cannabis* for any condition certainly does not conform to the procedures generally in force for other medicinal products, and especially products with a psychoactive effect such as those prepared containing THC.

It is interesting to note that in Canada, and in some states in the United States, the medical inflorescences may be grown directly by the patient, and the treatment may be recommended by a health worker, and not only a medical doctor; in the event that the plant species is not home-grown, it is distributed through a licensed dispensary. In Germany, Israel and Netherlands, herbal *Cannabis* is grown locally under the supervision of a government agency. This is significant if one considers that, in these three countries, the prescription process is highly deregulated regarding the recommended pathologies to be treated with *Cannabis*, but the same does not apply to its cultivation.

The normal administration routes are oral or by inhalation. Some countries, such as Israel, authorise smoking *Cannabis* inflorescences as a route of administration, something that is categorically banned in some states of the United States.

In addition, regarding prescription, it is noteworthy that the United Kingdom is the only country where this must be obtained from a hospital specialist. In some states in the United States, on the other hand, the prescribing medical doctor must be registered to prescribe this therapy and have attended a specific training course. In Australia and Switzerland, medical doctors may write the prescription only after receiving authorisation from a specific agency. Therefore, there is a different focus on the prescription process and hence inhomogeneity in this aspect too. The treatment costs are generally borne by the patient, and no reimbursement is foreseen, unless it is from a private health insurance scheme. This certainly restricts access to this kind of therapy to the more privileged members of society.

Concerning the results of the clinical trials, some interesting observations may be made. In the first place, a greater number of studies have been published in certain countries. These countries are the United States (11) and Australia (9), followed by Israel (7) and the United Kingdom (7). In general, the majority of the studies featured randomisation, the use of a double-blind method, and a placebo control group: these are factors which guarantee the quality of the data gathered. On the other hand, the majority of the studies took place with a small sample size. Moreover, the studies made use of a heterogeneous population: healthy and ill volunteers, adults and children, acute and chronically ill patients, and subjects who had previously used or had never used *Cannabis* prior to the study. Factors that, being so numerous, make it particularly challenging to draw any conclusive evaluations of the results of these trials, and more in general, the real efficacy of medical *Cannabis*.

Considering only the studies with a positive outcome, it should be noted that the studied pathologies are coherent with those provided for in current legislation i.e., pain, epilepsy, nausea, and vomiting; on the contrary, psychosis, behavioural problems, memory and cerebral activity represent a novelty. Furthermore, there is a net distinction between the products used based on the different conditions to be treated: the trials on pain, nausea and vomiting with positive outcomes administered herbal *Cannabis* derivatives in which, in 3 cases out of 4, both THC and CBD are present; the other studies with a positive outcome administered CBD alone. In those trials with a negative outcome, CBD was administered for pain, while herbal *Cannabis* derivatives were used for conditions such as OCD or PTSD. This consideration supports the use of herbal *Cannabis* in which both THC and CBD are present for pain, even though it should be stressed that the studies with a positive outcome for this pathology had a maximum of 30 enrolled subjects.

The studies with an inconclusive outcome regarded a variegated list of conditions including anxiety, Crohn's syndrome, ulcerative colitis, pain, and appetite loss. Many of these are already included in some national regulations although the efficacy of *Cannabis* in these cases according to the currently available data is not satisfactorily demonstrated.

It is evident that the only pathology present in all three study categories is pain, for which 4 studies had a positive outcome, 1 had a negative outcome, and 1 had an inconclusive outcome.

Among the study protocols to be trialled, pain and spasticity appear again, approved by legislation in most countries and the object of numerous studies, as well as a number of less-investigated conditions such as dementia, phobias, tic disorders and the activation of the immune system in HIV patients.

Based on the research conducted, it is, therefore, possible to stress that in spite of the growing number of recent studies on medical *Cannabis*, many of which have had a positive outcome while many others have had an inconclusive or negative outcome. The presumed broad spectrum action of *Cannabis* has led to the initiation of many trials and the preparation of many study protocols for a wide range of pathologies with the enrolment of subjects with diverse characteristics from study to study. This means that there is very little data for each pathology or symptomology.

Another important factor is that the products used are very diverse from each other; consequently, a comparison is extremely difficult to make, especially for the herbal products. All of the trials indicate the precise dosages used in terms of active molecules, but when it comes to inflorescences, or extracts derived from them, the concentration is provided only for the THC and CBD content and not for the other active molecules. Furthermore, the diverse administration routes make a comparison based on pharmacokinetics difficult for the molecules of interest.

Therefore, it is difficult to compare the studies and draw conclusions concerning the efficacy of the protocol for the single pathologies. However, for some, substantial evidence is emerging regarding their efficacy and the suitable products to ensure that. From the analysed data, it is clear that the best pain treatment is herbal *Cannabis* derivatives containing both THC and CBD, just as the best way to treat epilepsy is to administer CBD.

One interesting point is that for some of the pathologies approved for treatment with medical *Cannabis* under the current legislation, the data do not paint a definitive picture. This is true for conditions such as anxiety, ulcerative colitis, Crohn's syndrome, and appetite enhancement.

On the other hand, the current legislation often authorises inflorescences or extracts without indicating the exact concentration of the active molecules. In parallel, many studies use different plant strains or study a small number of subjects, making it difficult to compare and consequently interpret the results. Moreover, in many studies, the *Cannabis*-based medicines were administered in addition to other treatments making any evaluation of their efficacy it even more complex.

5 CONCLUSION

Medical *Cannabis* is often considered as if it were a single active component, but, in fact, there are countless possible variations. Hence, it will be some time before the current list of pathologies that each product may be used for can be updated based on definitive clinical data on the efficacy of the various components. Certainly, the development of standardised industrial products will facilitate the execution of more meaningful trials compared to those that involve the administration of inflorescences or derived extracts prepared using a variety of methods and, thus, highly variable in terms of concentration of the active molecules.

The authors want moreover to put in evidence that, despite legislation authorising the use of medical *Cannabis* and instituting the national production centre for inflorescences more than 5 years ago, Italy is still among the states where clinical trials have not been conducted. This gap is due to legal restrictions on the approval and conduction of clinical trials in this field, and the difficulty in sourcing the raw plant material, of which there is always a shortage. The result of this is therapies using inflorescences and extracts which have never undergone specific clinical trialling.

In the end, the influence of the media, economic interests, and the demands of associations representing patients affected by these diseases and conditions, for whom *Cannabis* is a panacea, means that in many countries it is currently possible to use medical *Cannabis* even though the scientific data do not entirely support the signs of efficacy: certainly this is a special case where the consolidated procedures for the administration of any product in the medical field have been either overlooked or ignored. It is time that the regulatory agencies considered whether this is actually safeguarding the health of patients.

LIMITS

The analysis of the current legislation may not be exhaustive in that it refers only to public texts available online.

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.

AUTHOR CONTRIBUTIONS

FB and PB performed the conceptualization of the work. FB, IP and LE performed the investigation and took care of the data. FB wrote the manuscript. PB coordinated the project. All authors approved the final version of the study.

ACKNOWLEDGMENTS

The authors would like to thank Dr Tom O Byrne for the linguistic revision of the text.

REFERENCES

abc NEWS (2022). Israeli Bigwigs Eye Profits from Cannabis Legalization. Available at: https://abcnews.go.com/Health/wireStory/israeli-bigwigs-eye-profits-cannabis-legalization-82617899 (Accessed April 26th, 2022).

Ablin, J., Ste-Marie, P. A., Schäfer, M., Häuser, W., and Fitzcharles, M. A. (2016). Medical Use of Cannabis Products: Lessons to Be Learned from Israel and Canada. *Schmerz* 30 (1), 3–13. doi:10.1007/s00482-015-0083-4

Abrams, D. I., Couey, P., Dixit, N., Sagi, V., Hagar, W., Vichinsky, E., et al. (2020). Effect of Inhaled Cannabis for Pain in Adults with Sickle Cell Disease: A Randomized Clinical Trial. *JAMA Netw. Open* 3 (7), e2010874. doi:10.1001/jamanetworkopen.2020.10874

Abuhasira, R., Shbiro, L., and Landschaft, Y. (2018). Medical Use of Cannabis and Cannabinoids Containing Products - Regulations in Europe and North America. *Eur. J. Intern Med.* 49, 2–6. doi:10.1016/j.ejim.2018.01.001

Alcohol and Drug Foundation (2021). Medicinal Cannabis Products FAQs. Available at: https://adf.org.au/talking-about-drugs/medicinal-cannabis-products/medicinal-cannabis-products-faqs/ (Accessed February 15th, 2022).

Alharbi, Y. N. (2020). Current Legal Status of Medical Marijuana and Cannabidiol in the United States. *Epilepsy Behav.* 112, 107452. doi:10.1016/j.yebeh.2020.107452

Almog, S., Aharon-Peretz, J., Vulfsons, S., Ogintz, M., Abalia, H., Lupo, T., et al. (2020). The Pharmacokinetics, Efficacy, and Safety of a Novel Selective-Dose Cannabis Inhaler in Patients with Chronic Pain: A Randomized, Double-Blinded, Placebo-Controlled Trial. *Eur. J. Pain* 24 (8), 1505–1516. doi:10.1002/ejp.1605

Anderson, L. L., Doohan, P. T., Oldfield, L., Kevin, R. C., Arnold, J. C., Berger, M., et al. (2021). Citalopram and Cannabidiol: *In Vitro* and *In Vivo* Evidence of Pharmacokinetic Interactions Relevant to the Treatment of Anxiety Disorders in Young People. *J. Clin. Psychopharmacol.* 41 (5), 525–533. doi:10.1097/JCP.0000000000001427

Aran, A., Harel, M., Cassuto, H., Polyansky, L., Schnapp, A., Wattad, N., et al. (2021). Cannabinoid Treatment for Autism: a Proof-Of-Concept Randomized Trial. *Mol. Autism* 12 (1), 6. doi:10.1186/s13229-021-00420-2

Arias, S., Leon, M., Jaimes, D., and Bustos, R.-H. (2021). Clinical Evidence of Magistral Preparations Based on Medicinal Cannabis. *Pharmaceuticals* 14 (2), 78. doi:10.3390/ph14020078

Australian Capital Territory Government (2021). Patient Information for Medicinal Cannabis. Available at: https://www.health.act.gov.au/sites/default/files/2018-09/Medicinal%20Cannabis%20-%20Patient%20Information_0.pdf (Accessed February 15th, 2022).

Australian Government, Department of Health (2018). Access to Medicinal Cannabis Products: Frequently Asked Questions (FAQs). Available at: https://www.tga.gov.au/access-medicinal-cannabis-products-frequently-asked-questions-faqs (Accessed February 15th, 2022).

Australian Government, Department of Health (2017a). Guidance for the Use of Medicinal Cannabis in Australia: Overview. Available at: https://www.tga.gov.au/publication/guidance-use-medicinal-cannabis-australia-overview (Accessed February 15th, 2022).

Australian Government, Department of Health (2017b). Guidance for the Use of Medicinal Cannabis in Australia: Patient Information. Available at: https://www.tga.gov.au/publication/guidance-use-medicinal-cannabis-australia-patient-information (Accessed February 15th, 2022).

Australian Government, Department of Health (2020). Medicinal Cannabis: Information for Consumers. Available at: https://www.tga.gov.au/medicinal-cannabis-information-consumers (Accessed February 15th, 2022).

Australian Government, Department of Health (2021). Medicinal Cannabis: Information for Health Professionals. Available at: https://www.tga.gov.au/medicinal-cannabis-information-health-professionals (Accessed February 15th, 2022).

Australian Institute of Health and Welfare (2019). National Drug Strategy Household Survey. Available at: https://www.aihw.gov.au/getmedia/108d1761-b523-492b-81cc-a09db6740e85/aihw-phe-270-Chapter6-Medicinal-cannabis.pdf.aspx#:~:text=Household%20Survey%202019-,Emerging%20topic%3A%20Medicinal%20cannabis,and%20leaves%20of%20the%20plant (Accessed February 15th, 2022).

Baratta, F., Peira, E., Maza, C., Gallarate, M., and Brusa, P. (2022). *Cannabis*-Based Oral Emulsion for Medical Purposes to Meet the Needs of Patients: Formulation, Quality and Stability. *Pharmaceutics* 14, 513. doi:10.3390/pharmaceutics14030513

Baratta, F., Simiele, M., Pignata, I., Ravetto Enri, L., Torta, R., De Luca, A., et al. (2019). Development of Standard Operating Protocols for the Optimization of *Cannabis*-Based Formulations for Medical Purposes. *Front. Pharmacol.* 10, 701. doi:10.3389/fphar.2019.00701

Baratta, F., Simiele, M., Pignata, I., Ravetto Enri, L., D'Avolio, A., Torta, R., et al. (2021). *Cannabis*-Based Oral Formulations for Medical Purposes: Preparation, Quality and Stability. *Pharmaceuticals* 14 (2), 171. doi:10.3390/ph14020171

Bebee, B., Taylor, D. M., Bourke, E., Pollack, K., Foster, L., Ching, M., et al. (2021). The CANBACK Trial: a Randomised, Controlled Clinical Trial of Oral Cannabidiol for People Presenting to the Emergency Department with Acute Low Back Pain. *Med. J. Aust.* 214 (8), 370–375. doi:10.5694/mja2.51014

Bedrocan (2021). Products. Available at: https://bedrocan.com/it/prodotti/ (Accessed February 15th, 2022).

Birnbaum, A. K., Karanam, A., Marino, S. E., Barkley, C. M., Remmel, R. P., Roslawski, M., et al. (2019). Food Effect on Pharmacokinetics of Cannabidiol Oral Capsules in Adult Patients with Refractory Epilepsy. *Epilepsia* 60 (8), 1586–1592. doi:10.1111/epi.16093

Bonn-Miller, M. O., Sisley, S., Riggs, P., Yazar-Klosinski, B., Wang, J. B., Loflin, M. J. E., et al. (2021). The Short-Term Impact of 3 Smoked Cannabis Preparations versus Placebo on PTSD Symptoms: A Randomized Cross-Over Clinical Trial. *PloS one* 16 (3), e0246990. doi:10.1371/journal.pone.0246990

Brazilian Government (2021). Autorização Sanitária de Produtos de Cannabis. Available at: https://www.gov.br/anvisa/pt-br/assuntos/educacaoepesquisa/webinar/medicamentos/arquivos/perguntas-e-respostas-autorizacao-sanitaria-de-produtos-de-cannabis.pdf (Accessed February 15th, 2022).

Carliner, H., Brown, Q. L., Sarvet, A. L., and Hasin, D. S. (2017). Cannabis Use, Attitudes, and Legal Status in the U.S.: A Review. *Prev. Med.* 104, 13–23. doi:10.1016/j.ypmed.2017.07.008

Casiraghi, A., Roda, G., Casagni, E., Cristina, C., Musazzi, U. M., Franzè, S., et al. (2018). Extraction Method and Analysis of Cannabinoids in Cannabis Olive Oil Preparations. *Planta Med.* 84 (4), 242–249. doi:10.1055/s-0043-123074

Castle, D. J., Strauss, N., Norman, A., and Bonomo, Y. (2019). Medical Marijuana: The Australian Experience. *Mo Med.* 116 (4), 270–273.

Centre for Medicinal Cannabis Research and Innovation (2021). Legal Access to Cannabis Based Medicines. Available at: https://www.medicinalcannabis.nsw.gov.au/__data/assets/pdf_file/0020/2864/Legal-Access-To-Cannabis-Based-Medicines-Fact-Sheet-Medical-Practitioners-FINAL.pdf (Accessed February 15th, 2022).

Choo, E. K., and Emery, S. L. (2017). Clearing the Haze: the Complexities and Challenges of Research on State Marijuana Laws. *Ann. N. Y. Acad. Sci.* 1394 (1), 55–73. doi:10.1111/nyas.13093

Conseil fédéral (2018). Traiter les personnes gravement malades avec du cannabis. Rapport du Conseil fédéral en réponse à la motion Kessler (14.4164). Available at: https://www.aramis.admin.ch/Default?DocumentID=49994&Load=true (Accessed February 15th, 2022).

Corroon, J., and Kight, R. (2018). Regulatory Status of Cannabidiol in the United States: A Perspective. *Cannabis Cannabinoid Res.* 3 (1), 190–194. doi:10.1089/can.2018.0030

Costiniuk, C. T., Saneei, Z., Routy, J. P., Margolese, S., Mandarino, E., Singer, J., et al. (2019). Oral Cannabinoids in People Living with HIV on Effective Antiretroviral Therapy: CTN PT028-Study Protocol for a Pilot Randomised Trial to Assess Safety, Tolerability and Effect on Immune Activation. *BMJ open* 9 (1), e024793. doi:10.1136/bmjopen-2018-024793

Crippa, J. A., Guimarães, F. S., Campos, A. C., and Zuardi, A. W. (2018). Translational Investigation of the Therapeutic Potential of Cannabidiol (CBD): Toward a New Age. *Front. Immunol.* 9, 2009. doi:10.3389/fimmu.2018.02009

Crippa, J. A. S., Pacheco, J. C., Zuardi, A. W., Guimarães, F. S., Campos, A. C., Osório, F. d. L., et al. (2021). Cannabidiol for COVID-19 Patients with Mild to Moderate Symptoms (CANDIDATE Study): A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. *Cannabis Cannabinoid Res. Adv. online Publ.* [Epub ahead of print] doi:10.1089/can.2021.0093

Danish Medicines Agency (2020). Cannabis-containing Products. Available at: https://laegemiddelstyrelsen.dk/en/special/medicinal-cannabis/ (Accessed February 15th, 2022).

Department of Health and Social Care (2018). Cannabis-based Products for Medicinal Use. Available at: https://assets.publishing.service.gov.uk/government/

uploads/system/uploads/attachment_data/file/753444/letter-with-guidance-on-cannabis-based-products-for-medicinal-use.pdf (Accessed February 15th, 2022).

Efron, D., Freeman, J. L., Cranswick, N., Payne, J. M., Mulraney, M., Prakash, C., et al. (2021). A Pilot Randomised Placebo-Controlled Trial of Cannabidiol to Reduce Severe Behavioural Problems in Children and Adolescents with Intellectual Disability. *Br. J. Clin. Pharmacol.* 87 (2), 436–446. doi:10.1111/bcp.14399

Efron, D., Taylor, K., Payne, J. M., Freeman, J. L., Cranswick, N., Mulraney, M., et al. (2020). Does Cannabidiol Reduce Severe Behavioural Problems in Children with Intellectual Disability? Study Protocol for a Pilot Single-Site Phase I/II Randomised Placebo Controlled Trial. *BMJ open* 10 (3), e034362. doi:10.1136/bmjopen-2019-034362

Farokhnia, M., McDiarmid, G. R., Newmeyer, M. N., Munjal, V., Abulseoud, O. A., Huestis, M. A., et al. (2020). Effects of Oral, Smoked, and Vaporized Cannabis on Endocrine Pathways Related to Appetite and Metabolism: a Randomized, Double-Blind, Placebo-Controlled, Human Laboratory Study. *Transl. Psychiatry* 10 (1), 71. doi:10.1038/s41398-020-0756-3

Federal Institute for Drugs and Medical Devices (2018). Annual Report 2017/18. Available at: https://www.bfarm.de/SharedDocs/Downloads/EN/BfArM/Publikationen/AnnualReport2017-18.pdf?blob=publicationFile (Accessed February 15th, 2022).

Federal Institute for Drugs and Medical Devices (2022a). BfArM Starts Selling Cannabis for Medical Purposes to Pharmacies. Available at: https://www.bfarm.de/SharedDocs/Pressemitteilungen/DE/2021/pm6-2021.html?nn=471310 (Accessed February 15th, 2022).

Federal Institute for Drugs and Medical Devices (2022b). Cannabis Agency. Available at: https://www.bfarm.de/DE/Bundesopiumstelle/Cannabis-als-Medizin/Cannabisagentur/_artikel.html (Accessed February 15th, 2022).

Federal Institute for Drugs and Medical Devices (2022c). Instructions for Pharmacists. Available at: https://www.bfarm.de/DE/Bundesopiumstelle/Cannabis-als-Medizin/Hinweise-fuer-Apotheker/_node.html (Accessed February 15th, 2022).

Federal Institute for Drugs and Medical Devices (2022d). Notes for Medical Doctors. Available at: https://www.bfarm.de/DE/Bundesopiumstelle/Cannabis-als-Medizin/Hinweise-fuer-Aerzte/_node.html (Accessed February 15th, 2022).

Fischer, B., Kuganesan, S., and Room, R. (2015). Medical Marijuana Programs: Implications for Cannabis Control Policy-Oobservations from Canada. *Int. J. Drug Policy* 26 (1), 15–19. doi:10.1016/j.drugpo.2014.09.007

Freeman, T. P., Hindocha, C., Baio, G., Shaban, N. D. C., Thomas, E. M., Astbury, D., et al. (2020). Cannabidiol for the Treatment of Cannabis Use Disorder: a Phase 2a, Double-Blind, Placebo-Controlled, Randomised, Adaptive Bayesian Trial. *Lancet Psychiatry* 7 (10), 865–874. doi:10.1016/S2215-0366(20)30290-X

General Medical Council (2022). Information for Doctors on Cannabis-Based Products for Medicinal Use. Available at: https://www.gmc-uk.org/ethical-guidance/learning-materials/information-for-doctors-on-cannabis-based-products-for-medicinal-use (Accessed February 15th, 2022).

German Institute for Medical Cannabis (2022). Mmedicinal Cannabis. Available at: https://difmc.de/medizinalcannabis/ (Accessed February 15th, 2022).

Gould, J. (2015). The Cannabis Crop. *Nature* 525 (7570), S2–S3. doi:10.1038/525S2a

Government of Canada (2019). Cannabis for Medical Purposes under the Cannabis Act: Information and Improvements. Available at: https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/medical-use-cannabis.html#_Access_to_cannabis (Accessed February 15th, 2022).

Grimison, P., Mersiades, A., Kirby, A., Lintzeris, N., Morton, R., Haber, P., et al. (2020). Oral THC:CBD Cannabis Extract for Refractory Chemotherapy-Induced Nausea and Vomiting: a Randomised, Placebo-Controlled, Phase II Crossover Trial. *Ann. Oncol.* 31 (11), 1553–1560. doi:10.1016/j.annonc.2020.07.020

Grotenhermen, F., and Müller-Vahl, K. (2012). The Therapeutic Potential of Cannabis and Cannabinoids. *Dtsch. Arztebl Int.* 109 (29-30), 495–501. doi:10.3238/arztebl.2012.0495

Grotenhermen, F. (2003). Pharmacokinetics and Pharmacodynamics of Cannabinoids. *Clin. Pharmacokinet.* 42 (4), 327–360. doi:10.2165/00003088-200342040-00003

Hardy, J., Haywood, A., Gogna, G., Martin, J., Yates, P., Greer, R., et al. (2020). Oral Medicinal Cannabinoids to Relieve Symptom Burden in the Palliative Care of Patients with Advanced Cancer: a Double-Blind, Placebo-Controlled, Randomised Clinical Trial of Efficacy and Safety of 1:1 Delta-9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD). *Trials* 21 (1), 611. doi:10.1186/s13063-020-04541-6

Health Canada (2016). Consumer Information—Cannabis. Available at: https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/dhp-mps/alt_formats/pdf/marihuana/info/cons-eng.pdf (Accessed February 15th, 2022).

Health Canada (2022). Marijuana Laws. Available at: https://medicalmarijuana.ca/patients/marijuana-laws/ (Accessed February 15th, 2022).

Health Direct (2019). Medicinal Cannabis. Available at: https://www.healthdirect.gov.au/medicinal-cannabis (Accessed February 15th, 2022).

Hendricks, O., Andersen, T. E., Christiansen, A. A., Primdahl, J., Hauge, E. M., Ellingsen, T., et al. (2019). Efficacy and Safety of Cannabidiol Followed by an Open Label Add-On of Tetrahydrocannabinol for the Treatment of Chronic Pain in Patients with Rheumatoid Arthritis or Ankylosing Spondylitis: Protocol for a Multicentre, Randomised, Placebo-Controlled Study. *BMJ open* 9 (6), e028197. doi:10.1136/bmjopen-2018-028197

Hill, K. P. (2015). Medical Marijuana for Treatment of Chronic Pain and Other Medical and Psychiatric Problems: A Clinical Review. *JAMA* 313 (24), 2474–2483. doi:10.1001/jama.2015.6199

Hotz, J., Fehlmann, B., Papassotiropoulos, A., de Quervain, D. J., and Schicktanz, N. S. (2021). Cannabidiol Enhances Verbal Episodic Memory in Healthy Young Participants: A Randomized Clinical Trial. *J. Psychiatr. Res.* 143, 327–333. doi:10.1016/j.jpsychires.2021.09.007

Hurd, Y. L., Spriggs, S., Alishayev, J., Winkel, G., Gurgov, K., Kudrich, C., et al. (2019). Cannabidiol for the Reduction of Cue-Induced Craving and Anxiety in Drug-Abstinent Individuals with Heroin Use Disorder: A Double-Blind Randomized Placebo-Controlled Trial. *Am. J. Psychiatry* 176 (11), 911–922. doi:10.1176/appi.ajp.2019.18101191

Izgelov, D., Davidson, E., Barasch, D., Regev, A., Domb, A. J., and Hoffman, A. (2020). Pharmacokinetic Investigation of Synthetic Cannabidiol Oral Formulations in Healthy Volunteers. *Eur. J. Pharm. Biopharm.* 154, 108–115. doi:10.1016/j.ejpb.2020.06.021

Jakubovski, E., Pisarenko, A., Fremer, C., Haas, M., May, M., Schumacher, C., et al. (2020). The CANNA-TICS Study Protocol: A Randomized Multi-Center Double-Blind Placebo Controlled Trial to Demonstrate the Efficacy and Safety of Nabiximols in the Treatment of Adults with Chronic Tic Disorders. *Front. Psychiatry* 11, 575826. doi:10.3389/fpsyt.2020.575826

Johnson, J. K., Johnson, R. M., Hodgkin, D., Jones, A. A., Kritikos, A., Doonan, S. M., et al. (2021). Medical Marijuana Laws (MMLs) and Dispensary Provisions Not Associated with Higher Odds of Adolescent Marijuana or Heavy Marijuana Use: A 46 State Analysis, 1991-2015. *Subst. Abus* 42 (4), 471–475. doi:10.1080/08897077.2021.1900986

Kayser, R. R., Haney, M., Raskin, M., Arout, C., and Simpson, H. B. (2020). Acute Effects of Cannabinoids on Symptoms of Obsessive-Compulsive Disorder: A Human Laboratory Study. *Depress Anxiety* 37 (8), 801–811. doi:10.1002/da.23032

Krcevski-Skvarc, N., Wells, C., and Häuser, W. (2018). Availability and Approval of Cannabis-Based Medicines for Chronic Pain Management and Palliative/supportive Care in Europe: A Survey of the Status in the Chapters of the European Pain Federation. *Eur. J. Pain* 22 (3), 440–454. doi:10.1002/ejp.1147

Lafaye, G., Karila, L., Blecha, L., and Benyamina, A. (2017). Cannabis, Cannabinoids, and Health. *Dialogues Clin. Neurosci.* 19 (3), 309–316. doi:10.31887/DCNS.2017.19.3/glafaye

Lawn, W., Hill, J., Hindocha, C., Yim, J., Yamamori, Y., Jones, G., et al. (2020). The Acute Effects of Cannabidiol on the Neural Correlates of Reward Anticipation and Feedback in Healthy Volunteers. *J. Psychopharmacol.* 34 (9), 969–980. doi:10.1177/0269881120944148

Lintzeris, N., Mills, L., Dunlop, A., Copeland, J., Mcgregor, I., Bruno, R., et al. (2020). Amp; Agonist Replacement for Cannabis Dependence Study Group. (Arc-D)Cannabis Use in Patients 3 Months after Ceasing Nabiximols for the Treatment of Cannabis Dependence: Results from a Placebo-Controlled Randomised Trial. *Drug alcohol dependence* 215, 108220. doi:10.1016/j.drugalcdep.2020.108220

Liu, Z., Galettis, P., Broyd, S. J., van Hell, H., Greenwood, L. M., de Krey, P., et al. (2020). Model-based Analysis on Systemic Availability of Co-administered Cannabinoids after Controlled Vaporised Administration. *Intern Med. J.* 50 (7), 846–853. doi:10.1111/imj.14415

Lopez, H. L., Cesareo, K. R., Raub, B., Kedia, A. W., Sandrock, J. E., Kerksick, C. M., et al. (2020). Effects of Hemp Extract on Markers of Wellness, Stress Resilience, Recovery and Clinical Biomarkers of Safety in Overweight, but Otherwise Healthy Subjects. *J. Diet. Suppl.* 17 (5), 561–586. doi:10.1080/19390211.2020.1765941

Marketrealist (2019). Medical Cannabis in Brazil Is Coming. Available at: https://marketrealist.com/2019/12/medical-cannabis-in-brazil-is-coming/ (Accessed February 15th, 2022).

Mead, A. (2017). The Legal Status of Cannabis (Marijuana) and Cannabidiol (CBD) under U.S. Law. *Epilepsy Behav.* 70 (Pt B), 288–291. doi:10.1016/j.yebeh.2016.11.021

Medicines and healthcare products Regulatory Agency (2020). The Supply, Manufacture, Importation and Distribution of Unlicensed Cannabis-Based Products for Medicinal Use in Humans 'specials'. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/869284/Cannabis_Guidance__unlicensed_CBPMs__updated_2020.pdf (Accessed February 15th, 2022).

Mersiades, A. J., Stockler, M. R., Olver, I. N., and Grimison, P. (2019). Medicinal Cannabis for Chemotherapy-Induced Nausea and Vomiting: Prescribing with Limited Evidence. *Med. J. Aust.* 210 (1), 11. doi:10.5694/mja17.01099

Ministério da Saúde (2019). Resolução da Diretoria Colegiada n 327. Available at: https://www.in.gov.br/en/web/dou/-/resolucao-da-diretoria-colegiada-rdc-n-327-de-9-de-dezembro-de-2019-232669072 (Accessed February 15th, 2022).

Mitelpunkt, A., Kramer, U., Hausman Kedem, M., Zilbershot Fink, E., Orbach, R., Chernuha, V., et al. (2019). The Safety, Tolerability, and Effectiveness of PTL-101, an Oral Cannabidiol Formulation, in Pediatric Intractable Epilepsy: A Phase II, Open-Label, Single-Center Study. *Epilepsy Behav.* 98 (Pt A), 233–237. doi:10.1016/j.yebeh.2019.07.007

MS Society (2021). Sativex (Nabiximols). Available at: https://www.mssociety.org.uk/about-ms/treatments-and-therapies/cannabis/sativex (Accessed February 15th, 2022).

Naftali, T., Bar-Lev Schleider, L., Scklerovsky Benjaminov, F., Konikoff, F. M., Matalon, S. T., and Ringel, Y. (2021b). Cannabis Is Associated with Clinical but Not Endoscopic Remission in Ulcerative Colitis: A Randomized Controlled Trial. *PloS one* 16 (2), e0246871. doi:10.1371/journal.pone.0246871

Naftali, T., Bar-Lev Schleider, L., Almog, S., Meiri, D., and Konikoff, F. M. (2021a). Oral CBD-Rich Cannabis Induces Clinical but Not Endoscopic Response in Patients with Crohn's Disease, a Randomised Controlled Trial. *J. Crohn's colitis* 15 (11), 1799–1806. doi:10.1093/ecco-jcc/jjab069

National Conferences of State Legislatures (2022). State Medical Cannabis Laws. Available at: https://www.ncsl.org/research/health/state-medical-marijuana-laws.aspx (Accessed February 15th, 2022).

National Health Service (2022). Cannabis-based Products for Medicinal Use: Frequently Asked Questions. Available at: https://www.england.nhs.uk/medicines-2/support-for-prescribers/cannabis-based-products-for-medicinal-use/cannabis-based-products-for-medicinal-use-frequently-asked-questions/ (Accessed February 15th, 2022).

National Health Service (2021). Medical Cannabis (And Cannabis Oils). Available at: https://www.nhs.uk/conditions/medical-cannabis/ (Accessed February 15th, 2022).

O'Neill, A., Wilson, R., Blest-Hopley, G., Annibale, L., Colizzi, M., Brammer, M., et al. (2021). Normalization of Mediotemporal and Prefrontal Activity, and Mediotemporal-Striatal Connectivity, May Underlie Antipsychotic Effects of Cannabidiol in Psychosis. *Psychol. Med.* 51 (4), 596–606. doi:10.1017/S0033291719003519

Office of Medicinal Cannabis (2022). Products and Services. Available at: https://english.cannabisbureau.nl/ (Accessed February 15th, 2022).

Perkins, D., Butler, J., Ong, K., Nguyen, T. H., Cox, S., Francis, B., et al. (2020). A Phase 1, Randomised, Placebo-Controlled, Dose Escalation Study to Investigate the Safety, Tolerability and Pharmacokinetics of Cannabidiol in Fed Healthy Volunteers. *Eur. J. Drug Metab. Pharmacokinet.* 45 (5), 575–586. doi:10.1007/s13318-020-00624-6

Pisanti, S., and Bifulco, M. (2019). Medical Cannabis: A Plurimillennial History of an Evergreen. *J. Cell Physiol.* 234 (6), 8342–8351. doi:10.1002/jcp.27725

Pretzsch, C. M., Freyberg, J., Voinescu, B., Lythgoe, D., Horder, J., Mendez, M. A., et al. (2019a). Effects of Cannabidiol on Brain Excitation and Inhibition Systems; a Randomised Placebo-Controlled Single Dose Trial during Magnetic Resonance Spectroscopy in Adults with and without Autism Spectrum Disorder. *Neuropsychopharmacol. official Publ. Am. Coll. Neuropsychopharmacol.* 44 (8), 1398–1405. doi:10.1038/s41386-019-0333-8

Pretzsch, C. M., Voinescu, B., Lythgoe, D., Horder, J., Mendez, M. A., Wichers, R., et al. (2019b). Effects of Cannabidivarin (CBDV) on Brain Excitation and Inhibition Systems in Adults with and without Autism Spectrum Disorder (ASD): a Single Dose Trial during Magnetic Resonance Spectroscopy. *Transl. Psychiatry* 9 (1), 313. doi:10.1038/s41398-019-0654-8

Pretzsch, C. M., Voinescu, B., Mendez, M. A., Wichers, R., Ajram, L., Ivin, G., et al. (2019c). The Effect of Cannabidiol (CBD) on Low-Frequency Activity and Functional Connectivity in the Brain of Adults with and without Autism Spectrum Disorder (ASD). *J. Psychopharmacol.* 33 (9), 1141–1148. doi:10.1177/0269881119858306

ProCon (2022). Legal Medical Marijuana States and DC. Available at: https://medicalmarijuana.procon.org/legal-medical-marijuana-states-and-dc/ (Accessed February 15th, 2022).

Rasche, T., Emmert, D., Radbruch, L., Conrad, R., and Mücke, M. (2019). Cannabis and Cannabinoids in Palliative Care. *Bundesgesundheitsblatt Gesundheitsforsch. Gesundheitsschutz* 62 (7), 830–835. doi:10.1007/s00103-019-02967-1

Reuters (2019). Brazil Approves Medical Marijuana Rules, Blocks Cannabis Cultivation. Available at: https://www.reuters.com/article/us-brazil-marijuana/brazil-approves-medical-marijuana-rules-blocks-cannabis-cultivation-idUSKBN1Y726W (Accessed February 15th, 2022).

Romano, L., and Hazekamp, A. (2019). An Overview of Galenic Preparation Methods for Medicinal Cannabis. *Cbc* 15 (2), 174–195. doi:10.2174/1573407214666180612080412

Ryan, J. E., McCabe, S. E., and Boyd, C. J. (2021). Medicinal Cannabis: Policy, Patients, and Providers. *Policy, Polit. Nurs. Pract.* 22 (2), 126–133. doi:10.1177/1527154421989609

S, G., Hb, S., K, L., R, T., Bs, R., Ps, S., et al. (2021). Safety and Efficacy of Low-Dose Medical Cannabis Oils in Multiple Sclerosis. *Mult. Scler. Relat. Disord.* 48, 102708. doi:10.1016/j.msard.2020.102708

Solowij, N., Broyd, S., Greenwood, L. M., van Hell, H., Martelozzo, D., Rueb, K., et al. (2019). A Randomised Controlled Trial of Vaporised Δ9-tetrahydrocannabinol and Cannabidiol Alone and in Combination in Frequent and Infrequent Cannabis Users: Acute Intoxication Effects. *Eur. Arch. Psychiatry Clin. Neurosci.* 269 (1), 17–35. doi:10.1007/s00406-019-00978-2

State of Israel - Minister of Health (2022). Cannabis for Medical Use and for Research. Available at: https://health.gov.il/English/Topics/cannabis/Pages/default.aspx (Accessed February 15th, 2022).

State of Israel - Minister of Health (2017). The First Course for Doctors to Prescribe Medical Cannabis approval Was Completed. 81 Doctors Will Begin to Authorize the Use of Medical Cannabis. Available at: https://health.gov.il/English/News_and_Events/Spokespersons_Messages/Pages/07092017_2.aspx (Accessed February 15th, 2022).

Stella, B., Baratta, F., Della Pepa, C., Arpicco, S., Gastaldi, D., and Dosio, F. (2021). Cannabinoid Formulations and Delivery Systems: Current and Future Options to Treat Pain. *Drugs* 81 (13), 1513–1557. doi:10.1007/s40265-021-01579-x

Swiss Confederation, Federal Office of Public Health (2021a). Cannabidiol (CBD) Containing Products - Overview and Implementation Guide. Available at: https://www.swissmedic.ch/swissmedic/it/home/news/mitteilungen/prodotti-contenenti-cbd–cannabidiol—panoramica.html (Accessed February 15th, 2022).

Swiss Confederation, Federal Office of Public Health (2020). Fact Sheet: Hemp-Based Medicines. Available at: https://www.bag.admin.ch/bag/it/home/medizin-und-forschung/heilmittel/med-anwend-cannabis/gesetzesaenderung-cannabisarzneimittel.html (Accessed February 15th, 2022).

Swiss Confederation, Federal Office of Public Health (2021c). Medical Application of Hemp. Available at: https://www.bag.admin.ch/bag/it/home/medizin-und-forschung/heilmittel/med-anwend-cannabis.html (Accessed February 15th, 2022).

Swiss Confederation, Federal Office of Public Health. (2021b). Hemp-based Medicines: Amendment of the Law. Available at: https://www.bag.admin.ch/bag/it/home/medizin-und-forschung/heilmittel/med-anwend-cannabis/gesetzesaenderung-cannabisarzneimittel.html (Accessed February 15th, 2022).

The Health Products Regulatory Authority (2017). Cannabis for Medical Use - A Scientific Review. Available at: https://www.hpra.ie/docs/default-source/publications-forms/newsletters/cannabis-for-medical-use—a-scientific-review.pdf?sfvrsn=7 (Accessed February 15th, 2022).

The Office of Drug Control (2021). Manufacturers and Suppliers of Medicinal Cannabis Products. https://www.odc.gov.au/manufacturers-and-suppliers-medicinal-cannabis-products (Accessed February 15th, 2022).

Thompson, M. D., Martin, R. C., Grayson, L. P., Ampah, S. B., Cutter, G., Szaflarski, J. P., et al. (2020). Cognitive Function and Adaptive Skills after a One-Year Trial of Cannabidiol (CBD) in a Pediatric Sample with Treatment-Resistant Epilepsy. *Epilepsy Behav.* 111, 107299. doi:10.1016/j.yebeh.2020.107299

Timler, A., Bulsara, C., Bulsara, M., Vickery, A., Smith, J., and Codde, J. (2020). Use of Cannabinoid-Based Medicine Among Older Residential Care Recipients Diagnosed with Dementia: Study Protocol for a Double-Blind Randomised Crossover Trial. *Trials* 21 (1), 188. doi:10.1186/s13063-020-4085-x

UK Government (2022). Medicinal Cannabis: Information and Resources. Available at: https://www.gov.uk/government/collections/medicinal-cannabis-information-and-resources (Accessed February 15th, 2022).

Urbi, B., Broadley, S., Bedlack, R., Russo, E., and Sabet, A. (2019). Study Protocol for a Randomised, Double-Blind, Placebo-Controlled Study Evaluating the Efficacy of Cannabis-Based Medicine Extract in Slowing the Disease pRogression of Amyotrophic Lateral Sclerosis or Motor Neurone Disease: the EMERALD Trial. *BMJ open* 9 (11), e029449. doi:10.1136/bmjopen-2019-029449

Van de Donk, T., Niesters, M., Kowal, M. A., Olofsen, E., Dahan, A., and van Velzen, M. (2019). An Experimental Randomized Study on the Analgesic Effects of Pharmaceutical-Grade Cannabis in Chronic Pain Patients with Fibromyalgia. *Pain* 160 (4), 860–869. doi:10.1097/j.pain.0000000000001464

Van der Flier, F. E., Kwee, C. M. B., Cath, D. C., Batelaan, N. M., Groenink, L., Duits, P., et al. (2019). Cannabidiol Enhancement of Exposure Therapy in Treatment Refractory Patients with Phobias: Study Protocol of a Randomized Controlled Trial. *BMC psychiatry* 19 (1), 69. doi:10.1186/s12888-019-2022-x

Wall, M. B., Pope, R., Freeman, T. P., Kowalczyk, O. S., Demetriou, L., Mokrysz, C., et al. (2019). Dissociable Effects of Cannabis with and without Cannabidiol on the Human Brain's Resting-State Functional Connectivity. *J. Psychopharmacol.* 33 (7), 822–830. doi:10.1177/0269881119841568

Whiting, P. F., Wolff, R. F., Deshpande, S., Di Nisio, M., Duffy, S., Hernandez, A. V., et al. (2015). Cannabinoids for Medical Use: A Systematic Review and Meta-Analysis. *JAMA* 313 (24), 2456–2473. doi:10.1001/jama.2015.6358

Xu, D. H., Cullen, B. D., Tang, M., and Fang, Y. (2020). The Effectiveness of Topical Cannabidiol Oil in Symptomatic Relief of Peripheral Neuropathy of the Lower Extremities. *Curr. Pharm. Biotechnol.* 21 (5), 390–402. doi:10.2174/1389201020666191202111534

Yassin, M., Oron, A., and Robinson, D. (2019). Effect of Adding Medical Cannabis to Analgesic Treatment in Patients with Low Back Pain Related to Fibromyalgia: an Observational Cross-Over Single Centre Study. *Clin. Exp. Rheumatol.* 37, 13–20.

Zylla, D. M., Eklund, J., Gilmore, G., Gavenda, A., Guggisberg, J., VazquezBenitez, G., et al. (2021). A Randomized Trial of Medical Cannabis in Patients with Stage IV Cancers to Assess Feasibility, Dose Requirements, Impact on Pain and Opioid Use, Safety, and Overall Patient Satisfaction. *Support Care Cancer* 29 (12), 7471–7478. doi:10.1007/s00520-021-06301-x

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Pharmacology

ORIGINAL RESEARCH
published: 06 June 2022
doi: 10.3389/fphar.2022.921493

Potency and Therapeutic THC and CBD Ratios: U.S. Cannabis Markets Overshoot

Sarah D. Pennypacker[1], *Katharine Cunnane*[1], *Mary Catherine Cash*[1,2] *and E. Alfonso Romero-Sandoval*[1]*

[1]Department of Anesthesiology, Pain Mechanisms Laboratory, Wake Forest University School of Medicine, Winston-Salem, NC, United States, [2]Department of Pharmacy, Wake Forest University School of Medicine, Winston-Salem, NC, United States

Background and aims: The effects exuded by cannabis are a result of the cannabinoids trans-Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), and is dependent upon their pharmacological interaction and linked to the two cannabinoids' concentrations and ratios. Based on current literature and trends of increasing cannabis potency, we postulate that most medical cannabis products with THC and CBD have ratios capable of producing significant acute intoxication and are similar to recreational products. We will test this by organizing products into clinically distinct categories according to TCH:CBD ratios, evaluating the data in terms of therapeutic potential, and comparing the data obtained from medical and recreational programs and from states with differing market policies.

Methods: We utilized data encompassing online herbal dispensary product offerings from nine U.S. states. The products were analyzed after being divided into four clinically significant THC: CBD ratio categories identified based on the literature: CBD can enhance THC effects (THC: CBD ratios ≥1:1), CBD has no significant effect on THC effects (ratios ~ 1:2), CBD can either have no effect or can mitigate THC effects (ratios 1:>2 < 6), or CBD is protective against THC effects (ratios ≤1:6).

Results: A significant number of products (58.5%) did not contain any information on CBD content. Across all states sampled, the majority (72–100%) of both medical and recreational products with CBD (>0%) fall into the most intoxicating ratio category (≥1:1 THC:CBD), with CBD likely enhancing THC's acute effects. The least intoxicating categories (1:>2 < 6 and ≤1:6 THC:CBD) provided the smallest number of products. Similarly, the majority of products without CBD (0%) contained highly potent amounts of THC (>15%). These results were consistent, regardless of differing market policies in place.

Conclusions: Despite the distinct goals of medical and recreational cannabis users, medical and recreational program product offerings are nearly identical. Patients seeking therapeutic benefits from herbal cannabis products are therefore at a substantial risk of unwanted side effects, regardless of whether they obtain products from medical or recreational programs. Efforts are needed to better inform patients of the risks associated with high potency cannabis and the interaction between THC and CBD, and to help shape policies that promote more therapeutic options.

OPEN ACCESS

Edited by:
Francesca Baratta, University of Turin, Italy

Reviewed by:
Vanessa Minervini, Creighton University, United States
Paolo Tucci, University of Foggia, Italy

***Correspondence:**
E. Alfonso Romero-Sandoval
earomero.sandoval@gmail.com

Specialty section:
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

Received: *15 April 2022*
Accepted: *20 May 2022*
Published: *06 June 2022*

Citation:
Pennypacker SD, Cunnane K, Cash MC and Romero-Sandoval EA (2022) Potency and Therapeutic THC and CBD Ratios: U.S. Cannabis Markets Overshoot. Front. Pharmacol. 13:921493. doi: 10.3389/fphar.2022.921493

Keywords: cannabidiol, tetrahydrocannabinol, marijuana, medical marijuana, herbal cannabis, cannabis market, potency, intoxication

INTRODUCTION

Trans-Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the two most prominent cannabinoids that comprise cannabis (Elsohly et al., 2014). The pharmacologic effects they each exude are quite distinct. For instance, CBD does not produce acute intoxication, has been proven to treat refractory epileptic syndromes in children, and may have anti-inflammatory, anxiolytic, and antipsychotic indications (Zuardi et al., 1993; Bergamaschi et al., 2011a; Bergamaschi et al., 2011b; Leweke et al., 2012; Iseger and Bossong, 2015; Devinsky et al., 2017). Yet, there is currently no substantial evidence that CBD alone has analgesic efficacy in humans—the primary indication for which patients seek out cannabis in the United States (U.S.) (Boehnke et al., 2019). On the other hand, THC produces the acute intoxication associated with cannabis and has been linked to multiple undesirable effects, such as paranoia, memory impairment, increased risk for psychotic illness, and cannabis dependency and the development of cannabis use disorder (CUD) (Di Forti et al., 2009; Izzo et al., 2009; Freeman et al., 2014).

Notably though, THC has shown promising analgesic efficacy (Abrams et al., 2007; Ellis et al., 2009; Ware et al., 2010; Wilsey et al., 2013; Wallace et al., 2015; Wilsey et al., 2016; van de Donk et al., 2019). This analgesic effect of THC is still under investigation (Boehnke and Clauw, 2019) but likely mirrors THC's concentration and thus cannabis' intoxication potential (Wilsey et al., 2013; Andreae et al., 2015; Wallace et al., 2015; van de Donk et al., 2019). In clinical trials studying the analgesic efficacy for cannabis, the THC concentrations utilized are consistently <10% (Abrams et al., 2007; Ellis et al., 2009; Ware et al., 2010; Wilsey et al., 2013; Wallace et al., 2015; Wilsey et al., 2016). In fact, significantly lower THC concentrations (1–3%) were used in several of the studies and resulted in sufficient clinical efficacy to manage pain (Wilsey et al., 2013; Wallace et al., 2015; Wilsey et al., 2016). Furthermore, adverse event potential and subsequent treatment discontinuation seems to increase at higher THC concentrations utilized in these studies. This parallel between THC concentration and intoxication and adverse event potential is increasingly becoming an issue as the potency of cannabis available rises (ElSohly et al., 2016; Chandra et al., 2019) despite patients often wishing to experience therapeutic benefits of THC without the associated subjective side effects (Joy et al., 1999; Grotenhermen, 2004; Hall, 2015). As a result, a difficult balancing act between analgesia and acute intoxication ensues.

Still, cannabis with high concentrations of THC (>15%) and greater intoxication potential is often favored in the recreational realm (Romero-Sandoval et al., 2018) and is associated with worse chronic pain in regular users (Boehnke et al., 2020). This discrepancy between the goals of medical and recreational products presumably should be reflected in the potency of the products each type of market offers. However, our previous findings demonstrated that average THC concentrations advertised online in medical programs are similar to those in recreational programs (Cash et al., 2020). Moreover, frequent medical cannabis users prefer inhaled cannabis with high levels of THC (Boehnke et al., 2020). The accessibility of high potency products could create a misconception about the safety of cannabis and downplay the risks and side effects associated with products containing high THC concentrations. It also leaves patients looking to use cannabis for medical purposes with mostly products outside the realm of what is considered potentially suitable for therapeutic purposes (Romero-Sandoval et al., 2018). It is important to note that while there may be some patients who enjoy the "high" or are willing to assume the risk of high THC consumption (as it may happen with opioids), this is not recommended from a medical standpoint. This sentiment is strongly supported by the International Association for the Study of Pain (IASP), which recently released a report which concluded that much more research is needed to determine the benefits and risks of cannabis for the treatment of pain before there is a chance cannabis can be endorsed for such usage (IASP Presidential Task Force on Cannabis and Cannabinoid Analgesia, 2021).

While these previous findings are certainly alarming, they only show a partial picture of the cannabis products offered in legal U.S. markets. CBD has long been proven to alter cannabis' effects, and while CBD data was presented alongside THC concentrations categories in our previous study, the data was not thoroughly analyzed in relation to THC:CBD ratios and concentrations (Cash et al., 2020). Literature suggests that different concentrations of THC and CBD and different ratios of THC:CBD induce variances in experienced subjective effects (Pennypacker and Romero-Sandoval, 2020). In fact, it appears that certain lower ratios of THC:CBD are more apt to produce an attenuation of THC induced effects (Dalton et al., 1976; Englund et al., 2013; van de Donk et al., 2019) while higher ratios are more likely to enhance THC induced effects (Arkell et al., 2019; Solowij et al., 2019; van de Donk et al., 2019). For instance, one study found that inhaled cannabis at a 2:1 THC:CBD ratio (8 mg THC (1.6%)/4 mg CBD (0.8%)) enhanced the subjects' intoxication when compared with THC alone (8 mg), but a 1:20 THC:CBD ratio (8 mg THC (1.6%)/400 mg CBD (80%)) reduced the subjects' intoxication when compared with THC alone (8 mg) (van de Donk et al., 2019). Notably, these findings are counterintuitive to the popular idea that CBD is simply protective against the intoxicating effects of THC, that CBD is the yin to THC's yang.

It is therefore important to determine whether the products available in dispensaries are pharmacologically safe for patients (medicinal) or the general public (adult use or recreational), not only by means of THC concentrations, but also CBD concentrations and the ratio of THC:CBD. Our previous findings clearly show that when analyzing the types of products offered in legal cannabis markets based solely on

THC, the majority of products contain levels not recommended (i.e., >15% THC) since they are associated with strong intoxicating effects (Cash et al., 2020). However, we wonder whether the combination of these high THC levels with certain CBD concentrations and/or the ratio of THC:CBD could result in a pharmacological interaction that reduces the risk of high levels of THC. We identified some products that are more pharmacologically amenable to medical purposes, based on their THC levels (i.e., <10%) (Cash et al., 2020); but it is also possible that these products will contain THC:CBD ratios that lead to a pharmacologic interaction that enhances THC intoxicating effects (Pennypacker and Romero-Sandoval, 2020). In other words, it is clinically relevant to garner whether or not the existing products contain these two cannabinoids at concentrations and ratios that are suitable for patients. Specifically, it is necessary to determine if CBD at the levels available in dispensaries will exude pharmacologic protective/beneficial effects or detrimental effects to established THC liabilities (e.g., stronger intoxication, withdrawal, tolerance, dependence, addiction, psychiatric issues, etc.).

Since medical cannabis programs mimic recreational programs, we hypothesize that ratios and concentrations of CBD in products available in medical cannabis programs are similar to that in recreational cannabis programs, with the majority at levels which will likely enhance THC's subjective effects. This study subsequently will test this hypothesis following these aims: 1) identify and categorize the THC:CBD ratios associated with different clinically meaningful pharmacologic effects when administered in conjunction via inhalation, 2) characterize the cannabis products available online within the determined ratio categories, 3) evaluate whether the probable pharmacologic effects of products labeled as recreational differ from the probable effects of products labeled as medical, and 4) determine if varying types of market structures (e.g., medical and recreational products offered in same facility or in separate facilities) provide clinically different cannabis offerings based on THC:CBD ratios.

MATERIALS AND METHODS

Data Collection

We utilized the publicly available data set from our previously published study (Cash et al., 2020). To summarize, states with legalized medical and/or recreational programs that have legalized cannabis for pain management were identified. The data sampling included online dispensary product offerings from nine U.S. states and spanned two distinct geographical locations: the Northeast region [Maine (ME), Massachusetts (MA), New Hampshire (NH), Rhode Island (RI) and Vermont (VT)] and the Western region [California (CA), Colorado (CO), New Mexico (NM), Washington (WA)] of the United States (U.S.). At the time of sampling, all of the Northeastern states as well as NM had legalized only the medical use of cannabis, and CA, CO, and WA had legalized cannabis for both medical and recreational use. Additionally, medical and recreational products were offered in separate facilities in WA, while both medical and recreational products were allowed to be offered in the same building in CO, and products were not differentiated medical or recreational in CA. Inhaled cannabis has a more favorable pharmacokinetic profile than other routes of administration and has shown analgesic efficacy for various chronic pain conditions, the most common reason cited for seeking out medical marijuana in the U.S. (Wilsey et al., 2013; Andreae et al., 2015; Wallace et al., 2015; Romero-Sandoval et al., 2018). Herbal products (flowers and pre-rolls) were therefore the focus of the sampling. Individual product cannabinoid data (THC and CBD content) was recorded.

Ratio Categorization

In order to carry out the first study aim, and based on our previous observations (Pennypacker and Romero-Sandoval, 2020), we identified four clinically significant THC:CBD ratio categories: CBD can enhance THC effects (THC:CBD ratios ≥1:1), CBD has no significant effect on THC effects (ratios ~1:2), CBD can either have no effect or is protective against THC effects (ratios 1:>2 < 6), or CBD is protective against THC effects (ratios ≤1:6). Products with THC:CBD ratios >0.7 were considered to fall into the first category, ≥1:1. Products with THC:CBD ratios ≥0.4 and <0.7 were considered to fall into the second category, ~ 1:2. Products with THC:CBD ratios <0.4 and >0.167 were considered to fall into the third category, 1:>2 < 6. And finally, products with THC:CBD ratios ≤0.167 were considered to fall into the fourth category, ≤1:6. While further investigation into concomitant administration of THC and CBD, their pharmacological interaction, and the resulting effects is certainly needed, this theme remained consistent throughout a thorough review of the literature (Pennypacker and Romero-Sandoval, 2020).

Statistical Analysis

Mean and standard deviation analysis was performed for each state and for distinct medical and recreational program comparisons. The four clinically significant THC:CBD ratio categories, ≥1:1, ~1:2, 1:>2 < 6, and ≤1:6, were analyzed for each state and program type. Either Student's T test or One-way ANOVA and Turkey's multiple comparison test were used, and a $p < 0.05$ was considered statistically significant. Relevant data is presented as (mean ± SD; median 25% percentile, 75% percentile).

RESULTS

Our results come from 8,534 herbal cannabis products (we did not exclude any product based on THC concentration) and their THC and CBD concentration information (Cash et al., 2020). These products were obtained from 653 dispensaries' websites from nine states; CA (n = 606 total products), CO (n = 545 for medical, n = 707 for recreational), ME (n = 37), MA (n = 332), NH (n = 106), NM (n = 668), RI (n = 49), VT (n = 21), WA (n = 2,834 for medical, n = 2,629 for recreational). We found that most of these products, 58.5%, do not have any CBD content information. Of the 3,545 products with CBD content information (41.5% of all surveyed products), 839 (26.5% of products with CBD information) reported 0% content and 2,606 (73.5% of products with CBD information) reported >0% CBD concentration . The proportion of products with no CBD content, with 0% CBD content, and with

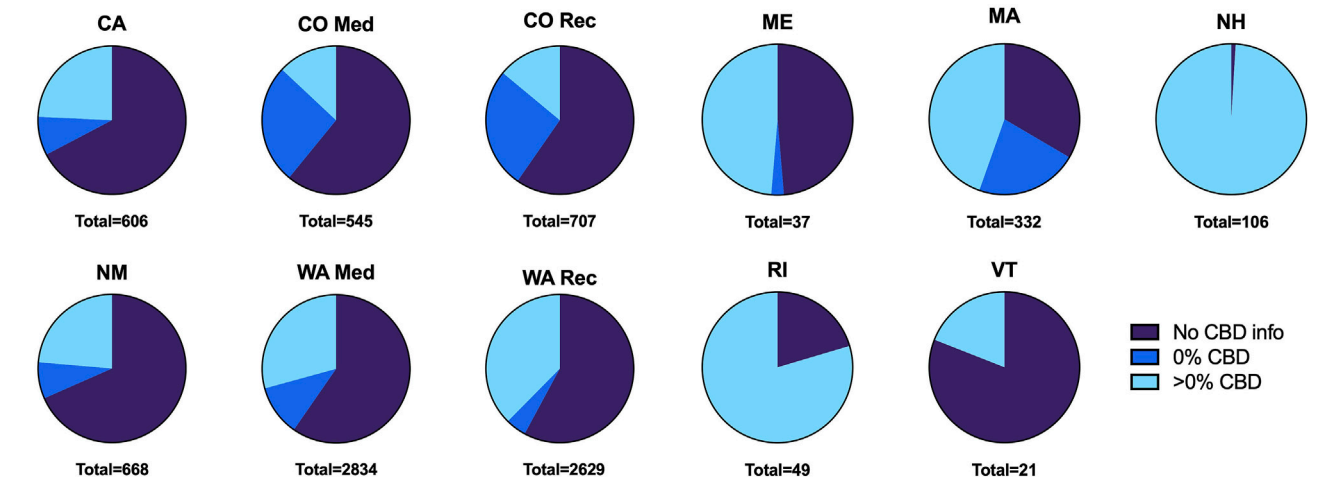


FIGURE 1 | Proportion of products by CBD content information provided per state. Total products sampled per state listed below each graph.

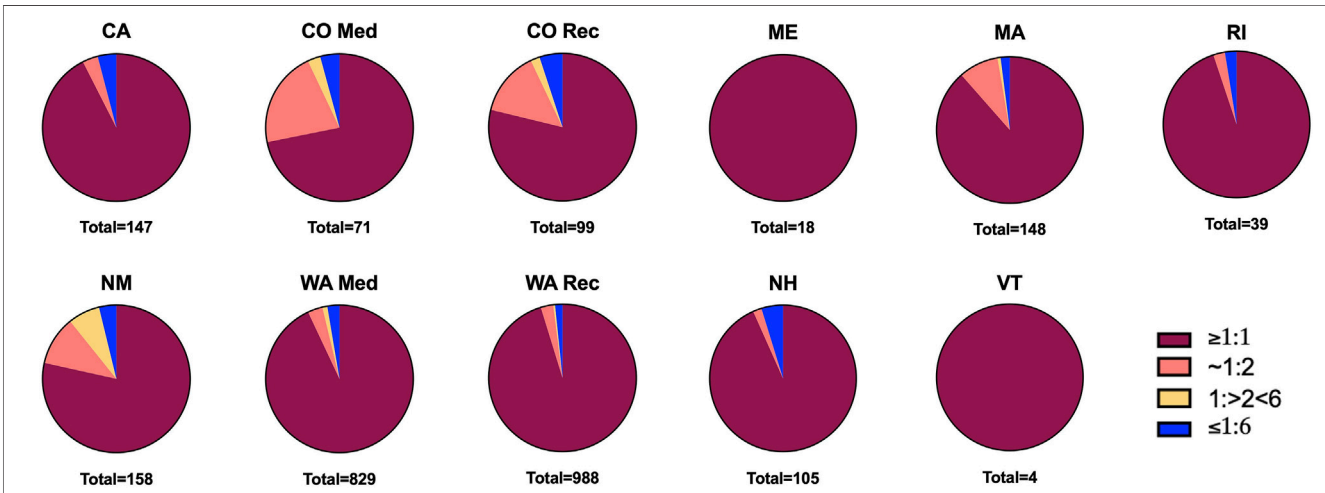


FIGURE 2 | Proportion of products within THC:CBD ratio categories per state. Total products per state listed below each graph.

>0% CBD content information varies widely among states (**Figure 1**).

For subsequent analysis, we used only products with >0% CBD content, unless otherwise indicated. We noticed that not all evaluated states offered products belonging to all four THC:CBD categories we consider clinically meaningful. However, all the states offer products from the THC:CBD ratios ≥1:1 category (CBD enhances THC effects, **Figure 2**). In fact, and in line with our hypothesis, the majority of both medical and recreational products analyzed (72–100%) fall into the foremost listed category, with CBD likely potentiating THC effects; and products likely to provide CBD mitigation of THC effects (THC:CBD ratios ≤1:6) make up the smallest category (0–5%, **Figure 2**).

Intriguingly, the majority of products within the THC:CBD ratios ≥1:1 category have >15% THC, a concentration that is highly intoxicating, in all states, with the exception of VT where products contain <10% THC (**Figure 3**). All other THC:CBD ratio categories are comprised of products with <10% THC in all studied states (**Figure 3**).

We observed that products with CBD information containing 0% CBD (839 products), have in average > 15% THC in all states with these products (NH, RI, and VT did not have this type of product); with NM, CA, CO (Medical and Recreational), and WA (Medical and Recreational) containing >20% and ME and MA containing 16.5 and 19.3% THC in average respectively (**Figure 4**). When products with CBD information containing <15% THC were examined (417 products deemed more suitable for medical purposes), we observed that the majority of products fall into the ~ 1:2, 1:>2 < 6, and ≤1:6 ratio categories in all states except in ME where ≥1:1 products dominate; the THC average ranges from 6–9% and CBD averages from 6–11%, except in ME where THC and CBD averages were 11.7 and 1.4% respectively (**Figure 4**). Potent products

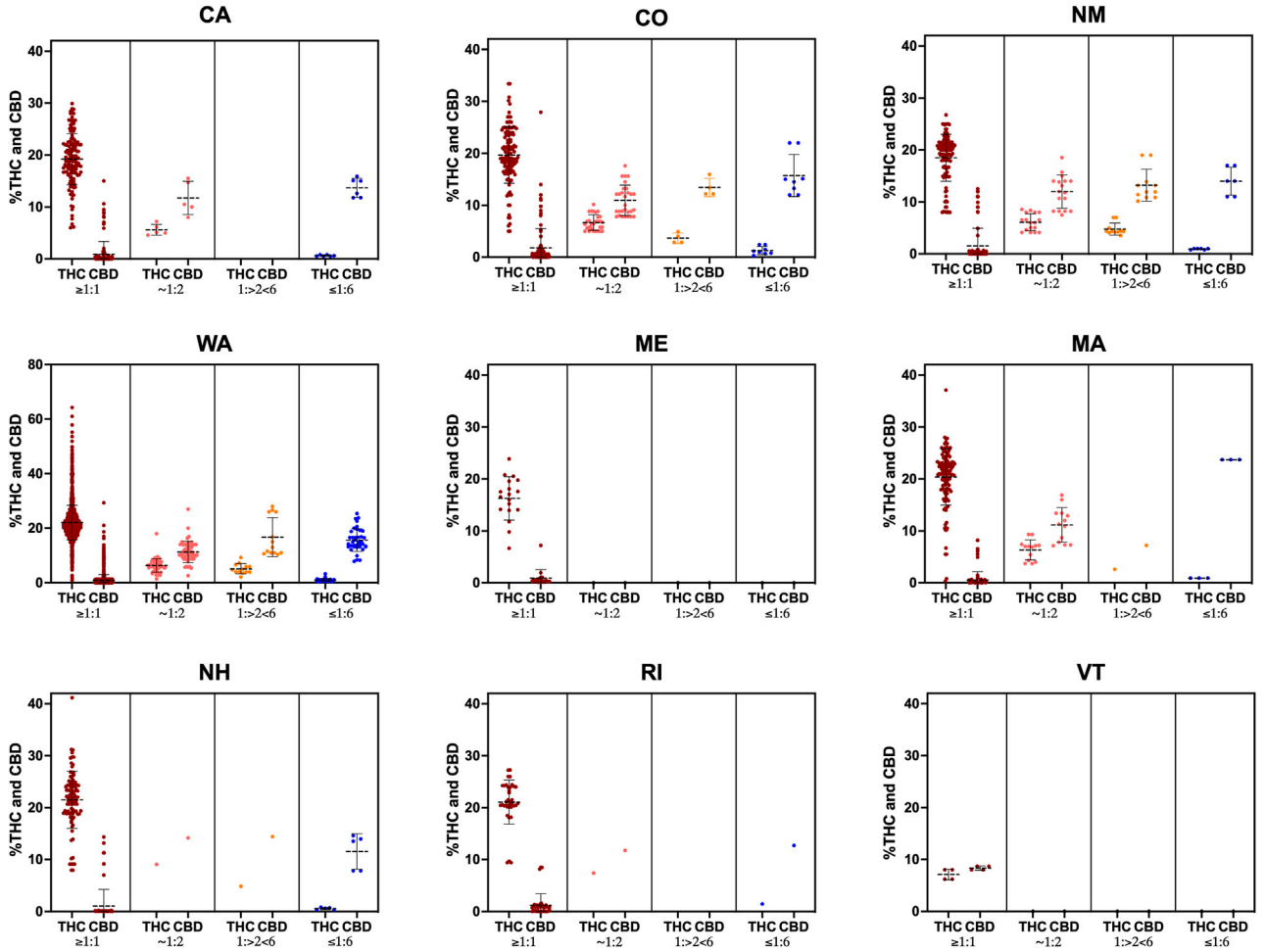


FIGURE 3 | THC and CBD percent distribution by ratio category for all products with >0% CBD in each state. Data shown as mean ± SD.

with >15% THC and CBD information were very similar to products with 0% CBD, namely containing in average > 20% THC in all cases, except in ME (18% THC average), and VT (did not have products in this category), and <1% CBD levels in all states, except in CO Medical (1.5% CBD average; **Figure 4**).

DISCUSSION

Overall, this study's results are alarming. They reveal current product offerings do not reflect scientific evidence regarding what concentrations of THC and CBD could be potentially therapeutic. Combined with holes in popular knowledge and misconceptions about THC and CBD, the current market can lead to problematic patient dosing as they try to maximize therapeutic benefits, such as analgesia, while subjecting themselves to THC's acute intoxicating effects. For instance, across all states, the vast majority of both medical and recreational products with CBD (>0%) fall into the THC:CBD ratio category ≥1:1, with CBD likely enhancing THC subjective effects. The THC:CBD categories ≤1:6 and 1:>2 < 6, those with the lowest intoxication potential, provide the least amount of products. It is notable that products with lower THC, considered suitable for medical purposes, might in fact not have significant analgesic value (Dalton et al., 1976; van de Donk et al., 2019), since they have THC: CBD ratios of ≤1:6 or 1:>2 < 6, where CBD would likely reduce THC effects. More potent products that may be suitable for regular users or patients who have developed tolerance, those with 10–15% THC and ratios ≥1:1 and ~ 1:2, are difficult to find in two major medical programs (CO and WA) when compared to >15% THC products. This leaves patients with mostly highly intoxicating options. Moreover, these findings are consistent across both medicinal and recreational programs, and in markets that offer both medical and recreational products (e.g., CA), or where all products are considered medical (e.g., NM). These findings also remain true regardless of whether they coexist in the same building (e.g., CO), or if they are in separated facilities (e.g., WA).

As shown, despite CBD having long been proven to pharmacologically alter cannabis' overall effects, a large portion of products did not provide information on CBD content. This could potentially lead to unwanted side effects as patients do not have all the information on the drug they are taking. The results reveal that

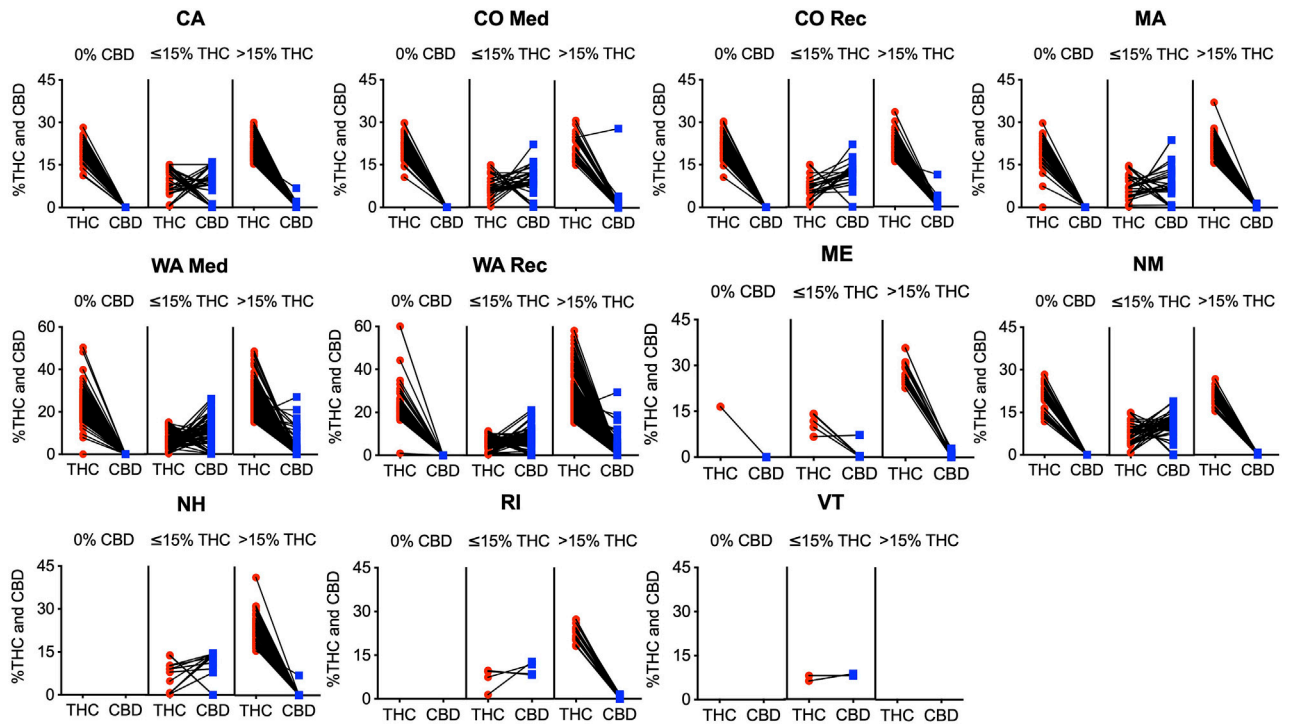


FIGURE 4 | All individual products with CBD content information with corresponding THC and CBD percentage in each state. The first column for each graph contains all products with 0% CBD and their corresponding THC content; the second and third columns contain products with >0% CBD, with the second column containing products with ≤15% THC, and the third column containing products with >15% THC.

products with 0% CBD are very potent (**Figure 4**), with most products containing >15% THC, and virtually all containing close to or >10% THC. These products, especially those with >15% THC are counter indicated clinically and are therefore not recommended or safe to be marketed as medical cannabis (Romero-Sandoval et al., 2018; Boehnke et al., 2020; Cash et al., 2020). Similarly, products with CBD and >15% THC overwhelmingly behave similarly to those without any CBD. Virtually all of these high potency products contain <1% CBD, with mean values close to 0%. Consequently, products with high THC are likely to have little CBD. This theme can be helpful to note, especially for the significant number of products that do not offer information on CBD content. There are a few product exceptions in Washington medical and Colorado medical programs where there is more variation in CBD content, even in the high potency products. While there are certainly not enough of these outlying products to change the overall market makeup, this variation seems to indicate that medical programs recognize a demand for products different than those in the recreational market. Still, based on the literature, these products with high potency THC and high CBD concentrations likely produce significant unwanted psychotropic effects and can be harmful to patients seeking chronic pain relief (Wilsey et al., 2013; Andreae et al., 2015; Wallace et al., 2015; Boehnke et al., 2020; Pennypacker and Romero-Sandoval, 2020).

Beyond recent research demonstrating the effects of cannabis constituents, the momentum of current policy trends elicits a pressing need to understand the clinical therapeutic value of the cannabis available in the emerging market. As of February 2022, 37 states have legalized the medical usage of cannabis, and 18 states and Washington, D.C. have fully legalized cannabis (for both medical and recreational usage). Meanwhile, the rise of the opioid epidemic in U.S. has placed pain management under scrutiny and jumpstarted the search for treatment options with less adverse effects. Cannabis is advantageously place to be, and is often cited as an one of these alternatives (Caldera, 2020). In fact, presence of medical cannabis programs may be associated with a reduced opioid usage (Lucas, 2017). In the midst of the U.S. cannabis markets' rapid evolution and the changing attitudes towards traditional pain management, fully understanding what cannabis products are offered from a pharmacologic perspective could both better inform patients and providers, and potentially shape usage and the future of the U.S. cannabis market.

We understand that our data show advertised products rather than consumer acquired products. However, our data matches the natural supply and demand dynamic of any commodity, for which cannabis is not an exception. Thus, the frequency of products identified in our study in terms of THC and CBD concentrations encompasses the frequency of product sales describe by others (Smart et al., 2017; Davenport, 2021). Furthermore, our data (frequency of potent herbal products) align with data on cannabis exposure from the National Poison Data System which shows that exposures more often involves plant material than other processed forms of cannabis products (e.g., edibles, concentrates, etc.) and this happens more often in states where adult cannabis use

(recreational) is legal (Dilley et al., 2021). Similarly, it is important to note that the data used in this study was collected in 2018 (Cash et al., 2020). This is a limitation of the study as some of the data may have changed. However, the trends on market behavior this paper highlights are still relevant. If there are any pertinent changes, they are likely detrimental as the potency of cannabis has continually been increasing over the past several decades (ElSohly et al., 2016; Chandra et al., 2019; ElSohly et al., 2021). These themes are not limited to just the herbal market, but have been reflected in the edible cannabis market as well (Steigerwald et al., 2018). It is also relevant to highlight the expansion of the CBD product market. We do not know the extent to which CBD shops are influencing the presence of CBD in herbal cannabis products that have THC. According to trends found in illicit herbal cannabis products seized by the Drug Enforcement Administration, THC's average potency (~ 4% in 1995, 9.75% in 2009, and 13.88% in 2019) continues to rise and outpace CBD content (~ 0.28% in 2001, 0.39% in 2009, and 0.56% 2019) (ElSohly et al., 2016; ElSohly et al., 2021). There was a substantial increase in the average THC:CBD ratio from 2009 to 2017 (24.81–103.48 respectively) which reversed in 2018 (54.39) and 2019 (24.58) (ElSohly et al., 2021). This reversal is potentially a result of the expanding legalization of marijuana and CBD product market, both of which should be reflected in this study's data based on the timeframe. We therefore believe that this data is still highly relevant and reflective of the current market overall.

Furthermore, it is important to recognize that while this study's results are concerning, they can also be seen as promising. In addition to the decreasing ratio recently noted, clinically meaningful options—those that can likely prove beneficial to patients—are offered in all states; they are just in the minority and need to be teased out. The hurdles ahead to salvage the medical cannabis market seem to be in two categories. First, changing public misconceptions about THC and CBD's interplay and perceptions of what THC and CBD percentages clinically correlate too. Specifically, there is a need for education emphasizing that different concentrations of THC and CBD correlate to different pharmacologic effects, that adding high concentrations of CBD does not negate the psychotropic effects of THC, and that high potency cannabis (>15% THC) is in fact counter indicated for medical use. This will result in a more informed patient population. It can also help sway the demand away from high potency products and reduce incentives for the cannabis market to continually increase the potency of their offerings. Secondly, adequate policies regarding medical cannabis should also reflect the pharmacology and clinical correlates. This can be achieved through several means. By enforcing that products advertised for medical purposes actually have efficacy based on scientific literature, new policies can help expose the medically relevant products and segregate them from the recreational products. This could prove extremely meaningful, as it has been shown that patients regard the information provided by dispensaries as safe and reliable (Capler et al., 2017). Policies can also highlight the various clinically relevant ratios, fleshing out and offering substantial options in the therapeutically relevant categories. Lastly, policies can recommend dispensing medical products in a stepwise fashion, with the more potent products offered on a more stringent basis, such as after lower potency options proved ineffective for a patient. This can ensure an overall safer market for patients looking to achieve therapeutic benefits from cannabis without the risk of amplifying THC acute effects.

CONCLUSION

In summary, medical cannabis programs' mirror recreational cannabis programs' herbal product offerings in terms of pharmacological profile, and do so regardless of facility type. An evaluation of these products' ratios and concentrations revealed that the majority are highly potent (>15% THC) and contain THC:CBD ratios that will likely produce an additive effect on THC's effects (≥1:1). And while analgesic effects likely parallel THC's potency, so does intoxication and the frequency of adverse events (Wilsey et al., 2013; Andreae et al., 2015; Wallace et al., 2015; van de Donk et al., 2019). Therefore, many of the products marketed for medical purposes are counter indicated pharmacologically and potentially harmful (Romero-Sandoval et al., 2018; Boehnke et al., 2020). On the other hand, options that are likely the most suitable for therapeutic use are limited, even in medical programs. Ultimately, these results can be used to better inform patient populations and relevant policies and help steer the herbal medical cannabis market to be more reflective of clinical evidence.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://doi.org/10.1371/journal.pone.0230167.s018.

AUTHOR CONTRIBUTIONS

Conceptualization: SP and ER-S. Data curation: KC, MC, and ER-S. Formal analysis: SP and ER-S. Funding acquisition: ER-S. Investigation: SP and ER-S. Methodology: ER-S. Project administration: ER-S. Resources: ER-S. Supervision: ER-S. Writing—original draft: SP and ER-S. Writing—review and editing: SP and ER-S.

FUNDING

Department of Anesthesiology and Pilot Research Award by the Center for Addiction Research, Wake Forest University School of Medicine.

ACKNOWLEDGMENTS

Department of Anesthesiology, Department of Biostatistics and Data Science, Department of Social Sciences and Health Policy, and Center for Addiction Research at Wake Forest University School of Medicine.

REFERENCES

Abrams, D. I., Jay, C. A., Shade, S. B., Vizoso, H., Reda, H., Press, S., et al. (2007). Cannabis in Painful HIV-Associated Sensory Neuropathy: A Randomized Placebo-Controlled Trial. *Neurology* 68 (7), 515–521. doi:10.1212/01.wnl.0000253187.66183.9c

Andreae, M. H., Carter, G. M., Shaparin, N., Suslov, K., Ellis, R. J., Ware, M. A., et al. (2015). Inhaled Cannabis for Chronic Neuropathic Pain: A Meta-Analysis of Individual Patient Data. *J. Pain* 16 (12), 1221–1232. doi:10.1016/j.jpain.2015.07.009

Arkell, T. R., Lintzeris, N., Kevin, R. C., Ramaekers, J. G., Vandrey, R., Irwin, C., et al. (2019). Cannabidiol (CBD) Content in Vaporized Cannabis Does Not Prevent Tetrahydrocannabinol (THC)-induced Impairment of Driving and Cognition. *Psychopharmacol. Berl.* 236 (9), 2713–2724. doi:10.1007/s00213-019-05246-8

Bergamaschi, M. M., Queiroz, R. H., Chagas, M. H., de Oliveira, D. C., De Martinis, B. S., Kapczinski, F., et al. (2011a). Cannabidiol Reduces the Anxiety Induced by Simulated Public Speaking in Treatment-Naïve Social Phobia Patients. *Neuropsychopharmacology* 36 (6), 1219–1226. doi:10.1038/npp.2011.6

Bergamaschi, M. M., Queiroz, R. H., Zuardi, A. W., and Crippa, J. A. (2011b). Safety and Side Effects of Cannabidiol, a Cannabis Sativa Constituent. *Curr. Drug Saf.* 6 (4), 237–249. doi:10.2174/157488611798280924

Boehnke, K. F., and Clauw, D. J. (2019). Brief Commentary: Cannabinoid Dosing for Chronic Pain Management. *Ann. Intern Med.* 170 (2), 118. doi:10.7326/M18-2972

Boehnke, K. F., Gangopadhyay, S., Clauw, D. J., and Haffajee, R. L. (2019). Qualifying Conditions of Medical Cannabis License Holders in the United States. *Health Aff. (Millwood)* 38 (2), 295–302. doi:10.1377/hlthaff.2018.05266

Boehnke, K. F., Scott, J. R., Litinas, E., Sisley, S., Williams, D. A., and Clauw, D. J. (2020). High-Frequency Medical Cannabis Use is Associated with Worse Pain Among Individuals with Chronic Pain. *J. Pain* 21 (5-6), 570–581. doi:10.1016/j.jpain.2019.09.006

Caldera, F. E. (2020). Medical Cannibus as an Alternative for Opioids for Chronic Pain: A Case Report. *SAGE Open Med. Case Rep.* 8, 2050313X20907015. doi:10.1177/2050313X20907015

Capler, R., Walsh, Z., Crosby, K., Belle-Isle, L., Holtzman, S., Lucas, P., et al. (2017). Are Dispensaries Indispensable? Patient Experiences of Access to Cannabis from Medical Cannabis Dispensaries in Canada. *Int. J. Drug Policy* 47, 1–8. doi:10.1016/j.drugpo.2017.05.046

Cash, M. C., Cunnane, K., Fan, C., and Romero-Sandoval, E. A. (2020). Mapping Cannabis Potency in Medical and Recreational Programs in the United States. *PLoS One* 15 (3), e0230167. doi:10.1371/journal.pone.0230167

Chandra, S., Radwan, M. M., Majumdar, C. G., Church, J. C., Freeman, T. P., and ElSohly, M. A. (2019). New Trends in Cannabis Potency in USA and Europe during the Last Decade (2008-2017). *Eur. Arch. Psychiatry Clin. Neurosci.* 269 (1), 5–15. doi:10.1007/s00406-019-00983-5

Dalton, W. S., Martz, R., Lemberger, L., Rodda, B. E., and Forney, R. B. (1976). Influence of Cannabidiol on Delta-9-Tetrahydrocannabinol Effects. *Clin. Pharmacol. Ther.* 19 (3), 300–309. doi:10.1002/cpt1976193300

Davenport, S. (2021). Price and Product Variation in Washington's Recreational Cannabis Market. *Int. J. Drug Policy* 91, 102547. doi:10.1016/j.drugpo.2019.08.004

Devinsky, O., Cross, J. H., Laux, L., Marsh, E., Miller, I., Nabbout, R., et al. (2017). Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. *N. Engl. J. Med.* 376 (21), 2011–2020. doi:10.1056/NEJMoa1611618

Di Forti, M., Morgan, C., Dazzan, P., Pariante, C., Mondelli, V., Marques, T. R., et al. (2009). High-potency Cannabis and the Risk of Psychosis. *Br. J. Psychiatry* 195 (6), 488–491. doi:10.1192/bjp.bp.109.064220

Dilley, J. A., Graves, J. M., Brooks-Russell, A., Whitehill, J. M., and Liebelt, E. L. (2021). Trends and Characteristics of Manufactured Cannabis Product and Cannabis Plant Product Exposures Reported to US Poison Control Centers, 2017-2019. *JAMA Netw. Open* 4 (5), e2110925. doi:10.1001/jamanetworkopen.2021.10925

Ellis, R. J., Toperoff, W., Vaida, F., van den Brande, G., Gonzales, J., Gouaux, B., et al. (2009). Smoked Medicinal Cannabis for Neuropathic Pain in HIV: A Randomized, Crossover Clinical Trial. *Neuropsychopharmacology* 34 (3), 672–680. doi:10.1038/npp.2008.120

Elsohly, M. A., Gul, W., Wanas, A. S., and Radwan, M. M. (2014). Synthetic Cannabinoids: Analysis and Metabolites. *Life Sci.* 97 (1), 78–90. doi:10.1016/j.lfs.2013.12.212

ElSohly, M. A., Mehmedic, Z., Foster, S., Gon, C., Chandra, S., and Church, J. C. (2016). Changes in Cannabis Potency over the Last 2 Decades (1995-2014): Analysis of Current Data in the United States. *Biol. Psychiatry* 79 (7), 613–619. doi:10.1016/j.biopsych.2016.01.004

ElSohly, M. A., Chandra, S., Radwan, M., Majumdar, C. G., and Church, J. C. (2021). A Comprehensive Review of Cannabis Potency in the United States in the Last Decade. *Biol. Psychiatry Cognitive Neurosci. Neuroimaging* 6 (6), 603–606. doi:10.1016/j.bpsc.2020.12.016

Englund, A., Morrison, P. D., Nottage, J., Hague, D., Kane, F., Bonaccorso, S., et al. (2013). Cannabidiol Inhibits THC-Elicited Paranoid Symptoms and Hippocampal-dependent Memory Impairment. *J. Psychopharmacol.* 27 (1), 19–27. doi:10.1177/0269881112460109

Freeman, T. P., Morgan, C. J., Hindocha, C., Schafer, G., Das, R. K., and Curran, H. V. (2014). Just Say 'know': How Do Cannabinoid Concentrations Influence Users' Estimates of Cannabis Potency and the Amount They Roll in Joints? *Addiction* 109 (10), 1686–1694. doi:10.1111/add.12634

Grotenhermen, F. (2004). Cannabinoids for Therapeutic Use. *Am. J. Drug Deliv.* 2 (4), 229–240. doi:10.2165/00137696-200402040-00003

Hall, W. (2015). U.S. Policy Responses to Calls for the Medical Use of Cannabis. *Yale J. Biol. Med.* 88 (3), 257–264.

IASP Presidential Task Force on Cannabis and Cannabinoid Analgesia (2021). International Association for the Study of Pain Presidential Task Force on Cannabis and Cannabinoid Analgesia Position Statement. *PAIN* 162, S1–S2. doi:10.1097/j.pain.0000000000002265

Iseger, T. A., and Bossong, M. G. (2015). A Systematic Review of the Antipsychotic Properties of Cannabidiol in Humans. *Schizophr. Res.* 162 (1-3), 153–161. doi:10.1016/j.schres.2015.01.033

Izzo, A. A., Borrelli, F., Capasso, R., Di Marzo, V., and Mechoulam, R. (2009). Non-psychotropic Plant Cannabinoids: New Therapeutic Opportunities from an Ancient Herb. *Trends Pharmacol. Sci.* 30 (10), 515–527. doi:10.1016/j.tips.2009.07.006

Joy, J. E., Watson, S. J., and Benson, J. A. (1999). *Marijuana and Medicine: Assessing the Science Base*. Washington, DC: National Academies Press. doi:10.17226/6376

Leweke, F. M., Piomelli, D., Pahlisch, F., Muhl, D., Gerth, C. W., Hoyer, C., et al. (2012). Cannabidiol Enhances Anandamide Signaling and Alleviates Psychotic Symptoms of Schizophrenia. *Transl. Psychiatry* 2, e94. doi:10.1038/tp.2012.15

Lucas, P. (2017). Rationale for Cannabis-Based Interventions in the Opioid Overdose Crisis. *Harm Reduct. J.* 14 (1), 58. doi:10.1186/s12954-017-0183-9

Pennypacker, S. D., and Romero-Sandoval, E. A. (2020). CBD and THC: Do They Complement Each Other like Yin and Yang? *Pharmacotherapy* 40 (11), 1152–1165. doi:10.1002/phar.2469

Romero-Sandoval, E. A., Fincham, J. E., Kolano, A. L., Sharpe, B. N., and Alvarado-Vázquez, P. A. (2018). Cannabis for Chronic Pain: Challenges and Considerations. *Pharmacotherapy* 38 (6), 651–662. doi:10.1002/phar.2115

Smart, R., Caulkins, J. P., Kilmer, B., Davenport, S., and Midgette, G. (2017). Variation in Cannabis Potency and Prices in a Newly Legal Market: Evidence from 30 Million Cannabis Sales in Washington State. *Addiction* 112 (12), 2167–2177. doi:10.1111/add.13886

Solowij, N., Broyd, S., Greenwood, L. M., van Hell, H., Martelozzo, D., Rueb, K., et al. (2019). A Randomised Controlled Trial of Vaporised Δ9-tetrahydrocannabinol and Cannabidiol Alone and in Combination in Frequent and Infrequent Cannabis Users: Acute Intoxication Effects. *Eur. Arch. Psychiatry Clin. Neurosci.* 269 (1), 17–35. doi:10.1007/s00406-019-00978-2

Steigerwald, S., Wong, P. O., Khorasani, A., and Keyhani, S. (2018). The Form and Content of Cannabis Products in the United States. *J. Gen. Intern Med.* 33 (9), 1426–1428. doi:10.1007/s11606-018-4480-0

van de Donk, T., Niesters, M., Kowal, M. A., Olofsen, E., Dahan, A., and van Velzen, M. (2019). An Experimental Randomized Study on the Analgesic Effects of Pharmaceutical-Grade Cannabis in Chronic Pain Patients with Fibromyalgia. *Pain* 160 (4), 860–869. doi:10.1097/j.pain.0000000000001464

Wallace, M. S., Marcotte, T. D., Umlauf, A., Gouaux, B., and Atkinson, J. H. (2015). Efficacy of Inhaled Cannabis on Painful Diabetic Neuropathy. *J. Pain* 16 (7), 616–627. doi:10.1016/j.jpain.2015.03.008

Ware, M. A., Wang, T., Shapiro, S., Robinson, A., Ducruet, T., Huynh, T., et al. (2010). Smoked Cannabis for Chronic Neuropathic Pain: A Randomized Controlled Trial. *CMAJ* 182 (14), E694–E701. doi:10.1503/cmaj.091414

Wilsey, B., Marcotte, T., Deutsch, R., Gouaux, B., Sakai, S., and Donaghe, H. (2013). Low-Dose Vaporized Cannabis Significantly Improves Neuropathic Pain. *J. Pain* 14 (2), 136–148. doi:10.1016/j.jpain.2012.10.009

Wilsey, B., Marcotte, T. D., Deutsch, R., Zhao, H., Prasad, H., and Phan, A. (2016). An Exploratory Human Laboratory Experiment Evaluating Vaporized Cannabis in the Treatment of Neuropathic Pain from Spinal Cord Injury and Disease. *J. Pain* 17 (9), 982–1000. doi:10.1016/j.jpain.2016.05.010

Zuardi, A. W., Cosme, R. A., Graeff, F. G., and Guimarães, F. S. (1993). Effects of Ipsapirone and Cannabidiol on Human Experimental Anxiety. *J. Psychopharmacol.* 7 (1 Suppl. l), 82–88. doi:10.1177/026988119300700112

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.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership