THE PHARMACOLOGY OF KRATOM AND ITS ALKALOIDS

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THE PHARMACOLOGY OF KRATOM AND ITS ALKALOIDS

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EDITORIAL
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Editorial: The Pharmacology of Kratom and Its Alkaloids

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Editorial on the Research Topic

The Pharmacology of Kratom and Its Alkaloids

Kratom (*Mitragyna speciosa* Korth.) is an ethnomedicinal tree native to Southeast Asia with a long history of traditional use. During the last two decades, all indicators and metrics we have accessed suggest that kratom consumption in the United States has increased. Although there are now many self-reported motivations for kratom use, the most prominent is the self-treatment of pain, which is mediated at least in part through opioid receptors as detailed in this special issue's comprehensive review of mitragynine and its diastereomers "The Chemical and Pharmacological Properties of Mitragynine and Its Diastereomers: An Insight Review" (Karunakaran et al.), along with new research that indicates the involvement of adrenergic and, potentially, cannabinoid receptors, as described in "Methadone, Buprenorphine, and Clonidine Attenuate Mitragynine Withdrawal in Rats" (Hassan et al.) and "Mitragynine (Kratom)-Induced Cognitive Impairments in Mice Resemble Δ9-THC and Morphine Effects: Reversal by Cannabinoid CB1 Receptor Antagonism" (Iman et al.). Many individuals are reporting success using kratom to reduce opioid use despite kratom's own, generally milder, withdrawal syndrome. Also in this issue, data on kratom's potential for treating other highly-prevalent substance use disorders is being examined, as shown in "Evaluation of Kratom Opioid Derivatives as Potential Treatment Option for Alcohol Use Disorder" (Gutridge et al.).

It is noteworthy that kratom remains unregulated in the United States at the Federal level while research on its medicinal, abuse liability, and toxicity profile accrues. Such research is exemplified in "Kratom abuse potential, 2021: An updated eight-factor analysis" (Henningfield et al.), which provides a critical and rigorous evaluation of kratom pharmacology and its public health relevance. The toxicity of kratom and its over 40 known alkaloids, of which mitragynine is reported to be the most abundant, require further investigation. Another review in this issue, "Kratom Alkaloids: Interactions With Enzymes, Receptors, and Cellular Barriers" (Hanapi et al.), summarizes present knowledge regarding potential interactions of kratom and its alkaloids with enzymes and receptors that may contribute to adverse effects and affect cell barrier function. Developmental toxicity and teratogenicity appear to be distinctly different from classical opioids such as morphine, as detailed in "Comparative Toxicity Assessment of Kratom Decoction, Mitragynine and Speciociliatine Versus Morphine on Zebrafish (Danio rerio) Embryos" (Damodaran et al.). There have also been isolated cases of cardiovascular toxicity linked to kratom use, a topic further explored in "Assessment of Cardiovascular Functioning Among Regular Kratom (Mitragyna speciosa Korth.) Users: A Case Series" (Leong Bin Abdullah and Singh). Relatedly, another review, "The Adverse Cardiovascular Effects and Cardiotoxicity of Kratom (Mitragyna speciosa Korth: A Comprehensive Review" (Leong Bin Abdullah and Singh) provides insights into the currently available literature on kratom cardiotoxicity.

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The use of kratom to mitigate withdrawal symptoms from an opioid use disorder (OUD) appears to correlate with its increased availability and in recognition that such use has been well characterized in Southeast Asia, as described in "Kratom Use Within the Context of the Evolving Opioid Crisis and the COVID-19 Pandemic in the United States" (Prozialeck et al.). Another prominent applicability of kratom reported by users is in the self-treatment of mood and anxiety problems. Interestingly, some of the pathways involved in antidepressant effects are also involved in addiction, most notably the expression of ΔFosB. However, while kratom did show antidepressant and analgesic activity in rats in a new research study, it did not affect ΔFosB, suggesting a differential antidepressant and analgesic mechanism (see "The Antidepressant-Like and Analgesic Effects of Kratom Alkaloids are accompanied by Changes in Low Frequency Oscillations but not ΔFosB Accumulation") (Buckhalter et al.).

Much of the current clinical knowledge about kratom comes from either case reports or large surveys conducted among its regular users. In this issue, "Kratom Use in the United States: Both a Regional Phenomenon and a White Middle-Class Phenomenon? Evidence From NSDUH 2019 and an Online Convenience Sample" (Rogers et al.) presents survey findings from two national convenience samples with kratom-use histories that describe subgroups of users and speculates about demographic and psychosocial factors associated with use that may be changing as kratom products, media, and marketing strategies diversify. Results indicate that kratom is primarily used by White, younger, employed, and middle-class consumers, but without clear regional trends yet established. Another survey comprised of current and former kratom users, "Searching for a signal: Self-reported kratom dose-effect relationships among a sample of United States adults with regular kratom use histories" Smith et al. corroborates prior work indicating that kratom's withdrawal severity has a link with kratom intake (dose). The survey found that kratom acute effects typically begin within minutes, but last for hours, and that effects were reported as largely compatible with and even helpful in meeting daily roles and obligations.

Finally, the increasing use of kratom in the United States and globally requires healthcare professionals to gain sufficient knowledge of this plant and products derived from it in order to facilitate productive clinical conversations. Many healthcare professionals who may encounter patients or clients using kratom do not have extensive training or expertise in medicinal chemistry or toxicology. As there are presently no published human experimental studies of kratom or its alkaloids, treatment providers have few resources with which to scientifically-inform their clinical understanding of kratom or their interactions with kratom-using people. Many available case reports lack essential context; information readily available on the internet may be inaccurate or decontextualized. This special issue is therefore particularly distinguished by the manuscript "Understanding Kratom Use: A Guide for Healthcare Providers" (Swogger et al.) which provides information about kratom to clinicians in easily understandable terms. This paper is a starting point for helping clinicians develop best practices until more human data on kratom are available.

We hope that this special issue provides readers with an updated overview of kratom, its alkaloids, and the people who use it and that it sparks interest among researchers and clinicians alike into this diverse and complex plant that we are only just beginning to understand.

AUTHOR CONTRIBUTIONS

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

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Methadone, Buprenorphine, and Clonidine Attenuate Mitragynine Withdrawal in Rats

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Background: Kratom or *Mitragyna speciosa* Korth has been widely used to relieve the severity of opioid withdrawal in natural settings. However, several studies have reported that kratom may by itself cause dependence following chronic consumption. Yet, there is currently no formal treatment for kratom dependence. Mitragynine, is the major psychoactive alkaloid in kratom. Chronic mitragynine treatment can cause addiction-like symptoms in rodent models including withdrawal behaviour. In this study we assessed whether the prescription drugs, methadone, buprenorphine and clonidine, could mitigate mitragynine withdrawal effects. In order to assess treatment safety, we also evaluated hematological, biochemical and histopathological treatment effects.

Methods: We induced mitragynine withdrawal behaviour in a chronic treatment paradigm in rats. Methadone (1.0 mg/kg), buprenorphine (0.8 mg/kg) and clonidine (0.1 mg/kg) were i.p. administered over four days during mitragynine withdrawal. These treatments were stopped and withdrawal sign assessment continued. Thereafter, toxicological profiles of the treatments were evaluated in the blood and in organs.

Results: Chronic mitragynine treatment caused significant withdrawal behaviour lasting at least 5 days. Methadone, buprenorphine, as well as clonidine treatments significantly attenuated these withdrawal signs. No major effects on blood or organ toxicity were observed.

Conclusion: These data suggest that the already available prescription medications methadone, buprenorphine, and clonidine are capable to alleviate mitragynine withdrawal signs rats. This may suggest them as treatment options also for problematic mitragynine/kratom use in humans.

Keywords: mitragynine, kratom, withdrawal, replacement, methadone, burprenorphine, clonidine

INTRODUCTION

Mitragyna speciosa Korth or kratom is traditionally used in South-East Asia, particularly in Thailand and Malaysia, for its psychoactive effects. Kratom leaves have been claimed to have both psychostimulant- and opium-like narcotic effects. At low dose it acts as a stimulant, while being sedative at high doses (Jansen and Prast, 1988). Locals historically use kratom to combat exhaustion and survive working under bright sunlight through its psychostimulant-like effect.

Furthermore, kratom is also used to self-medicate for opioid withdrawal symptoms and as a replacement for heroin and morphine (Beckett et al., 1965; Grundmann, 2017). Currently, kratom emerged in the self-management of pain and opioid withdrawal, especially in the United States (Prozialeck, 2016; Grundmann, 2017). Kratom can be easily purchased on the internet. It has cheap prices and being marketed in many forms, from tablet to extract, in leaf form, as topical creams, balms or tinctures (Stanciu et al., 2019). In the United States, kratom is marketed and regulated as a dietary or herbal supplement. Individuals apply it for management of anxiety, pain, opioid use disorder, and depression (Boyer et al., 2008; Grundmann, 2017; Coe et al., 2019). Nevertheless, complications have arisen from this. The poorly regulated botanical and dietary supplement market which is also made up of adulterated products and where kratom products are sold, may partially account for the fatal cases (Obeng et al., 2020) that arise from their consumption. Indeed, death from kratom ingestion is exceedingly uncommon (Eastlack et al., 2020). Nonetheless, it can occur as a result of poly-substance abuse, which can contribute to an increased mortality risk (Neerman et al., 2013). Additionally, the Center for Disease Control and Prevention (CDC) reported 152 kratom-related deaths between the period of July 2016 and December 2017, all of which contained polydrug (Kuehn, 2019). Furthermore, 156 deaths have been linked to kratom use, with 87% being linked to polydrug use (Corkery et al., 2019).

Whilst kratom has benefits, it also has been reported that they can cause dependence and addiction-like symptoms after long-term consumption in humans (Vicknasingam et al., 2010; Ahmad and Aziz, 2012; Singh et al., 2014; Singh et al., 2018a; Müller et al., 2020; Anand and Hosanagar, 2021). Kratom withdrawal symptoms include, jerky movement, muscle ache, aggression, wet nose, and hostility in natural settings (Suwanlert, 1975). Furthermore, kratom users have been reported to have difficulties in combating kratom withdrawal while trying to stop its consumption (Ahmad and Aziz, 2012; Singh et al., 2014).

Kratom leaves contain over 40 alkaloids where mitragynine is the main indole alkaloid (Adkins et al., 2011; Yearsley, 2016). For this reason, we believed that mitragynine might be one of the alkaloids that modulate the effects in kratom. Currently, there is no specific treatment implemented in managing kratom dependence and withdrawal symptoms. Since no standardized treatment for kratom dependence is currently applied, therefore, the present study aims to investigate whether the available prescription drugs for opioid management; methadone, buprenorphine and clonidine, would mitigate the withdrawal symptoms caused by chronic mitragynine exposure.

MATERIALS AND METHODS

Animals

All animal experiments were performed in accordance with approved guidelines and regulations of the Universiti Sains Malaysia (USM) Institutional Animal Care and Use Committee (USM IACUC) [Reference number: USM/Animal Ethics Approval/2016/ (Santiago and Edelman, 1985) (736)]. Animals were purchased from Animal Research and Service Centre (ARASC), Universiti Sains Malaysia, Penang, Malaysia. All 30 tested animals were male Sprague-Dawley rats (200–300 g). They were naive and used in a single experiment only. Animals were socially housed in groups of six per cage under standard laboratory conditions, with temperature-controlled environment (24 ± 1°C) during habituation and were then placed individually prior to the treatment. The room was maintained on a 12 h light/12 h dark normal cycle (lights on from 07:00 to 19:00 h). Animals were handled for one week prior to commencement of the experiments. Food and water were available *ad libitum*.

Drugs Preparation

Methadone hydrochloride, buprenorphine hydrochloride and clonidine hydrochloride were purchased from Sigma Chemicals Co. (United States). Mitragynine was extracted, isolated and verified from fresh leaves of *Mitragyna speciosa* at the Centre for Drug Research, Universiti Sains Malaysia as described previously (Utar et al., 2011). Purified mitragynine was confirmed by high performance liquid chromatography (HPLC) and proton nuclear magnetic resonance (^{1}H-NMR) (400 MHz) analysis (Jamil et al., 2013). Mitragynine obtained by this procedure was approximately 98% pure (Hassan et al., 2019). Fresh stocks of methadone, buprenorphine, mitragynine and clonidine were prepared daily according to the weight of animals in the experimental design. They were dissolved in vehicle (20% (v/v) Tween 80 which was diluted with physiological saline (0.9% NaCl); Sigma Aldrich, United Kingdom) and injected intraperitoneally (i.p.).

Experimental Design for Replacement Treatment in Mitragynine Withdrawal Model

The previously established mitragynine withdrawal model was used (Hassan et al., 2021). Mitragynine (30 mg/kg, i.p.) was injected once per day over a period of 14 days. The vehicle group received 20% Tween 80 also once daily for 14 days. For both the vehicle- and mitragynine groups, withdrawal symptoms were assessed on day 15, twenty-four hours after abstinence from the drug. In this model, the effectiveness of the available prescription medications, methadone, buprenorphine and clonidine, were accessed. All the replacement treatments were applied for four days and then abruptly stopped on day 5 in order to determine whether or not the mitragynine withdrawal signs will resurface. This design followed the replacement routine described by Hassan et al. (2020). In a subsequent test, we examined hematological, biochemical and histopathological effects of the mitragynine and the replacement treatments.

TABLE 1 | The counted signs and checked signs with the respective weighing factors for the evaluation of mitragynine-withdrawal severity in rats.

Counted signs	Weighing factors	Checked signs (Checked every 10 min)	Weighing factors
Chewing	2	Squeaking on touch	1
Head shakes	2	Hostility on handling	1
Exploring	1	Diarrhoea	1
Digging	2	—	—
Yawning	2	—	—
Teeth chattering	2	—	—
Wet dog shakes	2	—	—
Writhing	2	—	—

Experiment I: Methadone, Buprenorphine and Clonidine Replacement Treatments in Mitragynine Withdrawn Rats.

The methadone dose used in the present study (1.0 mg/kg, i. p.) was selected to be in the pharmacological range and below the LD_{50} value to reduce fatality risk (McCormick et al., 1984; Chevillard et al., 2010). Methadone was dissolved in vehicle (20% Tween 80; Sigma Aldrich, United Kingdom) and injected i. p. 10 min before withdrawal testing, i.e., 23 h 50 min after the last mitragynine dose (Chevillard et al., 2010). Thereafter, methadone was repeatedly administered every 8 h (Chevillard et al., 2010) for four days of replacement treatment.

The buprenorphine dose used in the present study (0.8 mg/kg, i.p.) was selected to be in the pharmacological range of previous studies (Cowan et al., 1977; McLaughlin and Dewey, 1994; Morgan et al., 1999). Buprenorphine was dissolved in vehicle (20% Tween 80; Sigma Aldrich, United Kingdom) and injected i.p. 30 min before withdrawal testing, i.e., 23.5 h after the last mitragynine dose (Cowan et al., 1977). Buprenorphine was then administered every 12 h (Schaap et al., 2012) for four days.

The clonidine dose used in the present study (0.1 mg/kg, i.p.) was based on the previous study by Tierney et al. (1988). In the present study, clonidine was dissolved in vehicle (20% Tween 80; Sigma Aldrich, United Kingdom) and injected i.p. 10 min before withdrawal testing, i.e., 23 h 50 min after the last mitragynine dose (Tierney et al., 1988; Sireesha et al., 2015). Thereafter, clonidine was administered every 12 h for four days (Feily and Abbasi, 2009; Moayeri et al., 2018).

Assessment of Withdrawal Behaviour

Trained observers who were blind to treatment and time points, scored all behaviours from video and counted the frequency of the signs of spontaneous withdrawal: chewing, head shakes, exploring, digging, yawning, teeth chattering, wet dog shakes, and writhing as well as checked the signs of squeaking on touch, hostility on handling, and diarrhoea. The observers showed an inter-rater reliability for this scoring of r = 0.99. The recording was started once the animals were placed in an open field test box for 30 min and the withdrawal behaviour were scored. The test was performed daily for 4 days of replacement therapy and on day 5 when the treatment had stopped. The withdrawal behaviours were distinguished as "counted signs", including chewing, head shakes, exploring, digging, yawning, teeth chattering, wet dog shakes, writhing, and as "checked signs", including squeaking on touch, hostility on handling and diarrhoea. Thereby, counted signs and checked signs were further processed by multiplying with the respective weighting factors for evaluation of the severity of withdrawal signs using the previously described scoring method by Hassan et al. (2020), Rahman et al. (2002), and Sabetghadam et al. (2013) (**Table 1**).

Experiment II

Hematological Analysis

After behavioural studies ended on day 5, all the treated rats were euthanized with sodium pentobarbital (100 mg/kg) intraperitoneally. Blood samples were collected via cardiac puncture and transferred into tubes containing ethylenediamine tetraacetic acid (EDTA). Blood samples were analysed for several hematological parameters such as red blood cell count (Total RBC), haemoglobin, percentage of packed cell volume (PCV%), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), percentage of red cell distribution width (RDW%), total of white blood cell count (WBC), percentage of lymphocyte, monocytes, eosinophils, basophils, and platelet counts (PLT).

Biochemical Analysis

The collected blood was transferred into serum-separating tubes for biochemical analysis. The biochemical parameters analysed were total bilirubin, aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, sodium, potassium, chloride, urea, creatinine, total cholesterol, triglycerides, calcium, phosphorus, total protein, albumin, globulin and albumin/globulin ratio (A/G ratio).

Histopathological Analysis

On day 5, animal tissue samples of targeted organs (heart, lung, kidney, liver) were harvested after behavioural testing. The tissues were stained using Hematoxylin and Eosin. Then, the slides were viewed under light microscope equipped with a digital camera. The sections were analysed for structural changes, degenerative alterations, necrosis and signs of inflammation.

Statistical Analysis

All data were expressed as mean ± standard error of the mean (SEM). The substitution treatments were analysed by two-way

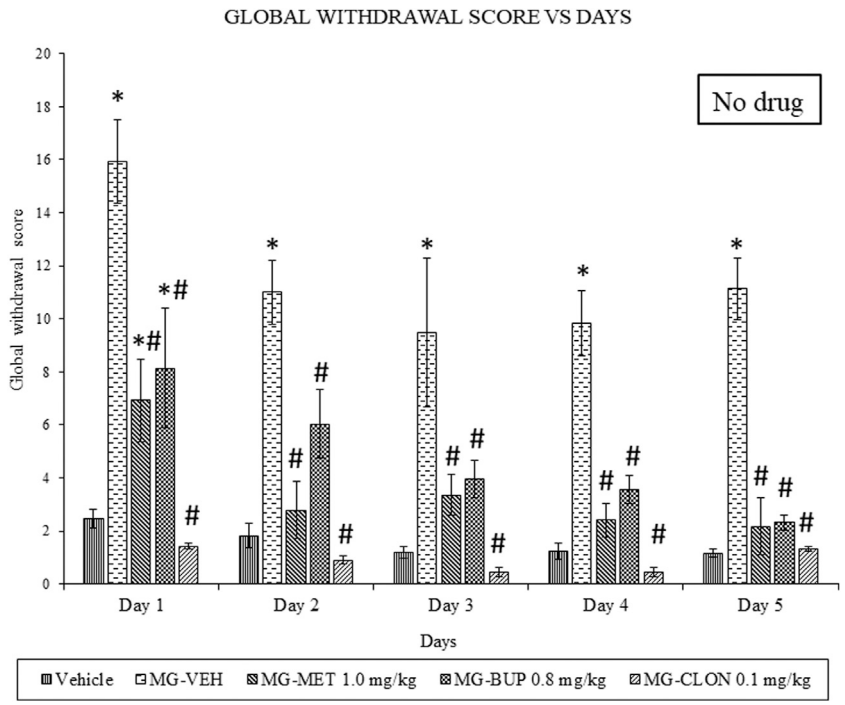


FIGURE 1 | Methadone (MET), Buprenorphine (BUP) and Clonidine (CLON) reduced behavioural signs of mitragynine withdrawal in rats. Data represent means (±SEM) of global withdrawal signs (n = 6/group; *p < 0.05, vs. Vehicle, #p < 0.05 vs. mitragynine-vehicle, MG-VEH).

ANOVA for repeated measures with test "day" as within factor and treatment combination as a "treatment" between factors. In order to analyse single group differences on each treatment day, pre-planned comparisons were calculated using Bonferroni test (Ramsey and Edwards, 1993). Hematological and biochemical parameters were analysed by one-way repeated measures ANOVA and Bonferroni post-hoc tests. Each of the hematological and biochemical parameters as fixed factor or within factor, whereas treatment combination is between factors. A significance level of $p < 0.05$ was used to test for statistical significance. GraphPad Prism 8.0 software (GraphPad Software Inc., La Jolla, CA, United States) was used to perform the statistics.

RESULT

Experiment I: Methadone, Buprenorphine and Clonidine Replacement Treatments in Attenuating Withdrawal Effects Due to Mitragynine

We found that chronic mitragynine treatment induced significant withdrawal behaviour on all 5 days after cessation of administration (**Figure 1**). Methadone, buprenorphine, as well as clonidine significantly attenuated the mitragynine withdrawal effects on all 4 days of the replacement treatment, and on day 5 when no treatment was given. A two-way ANOVA showed significant treatment ($F_{4,75}$ = 77.69, $p < 0.0001$) and day effects ($F_{4,75}$ = 9.327, $p < 0.0001$), but no significant treatment × day interaction ($F_{16,75}$ = 1.222, p = 0.2721). On day 1, methadone significantly reduced withdrawal signs as compared to vehicle and mitragynine groups ($p < 0.05$) (**Figure 1**). From day 2 to day 4 as well as on day 5 on which no replacement treatment was given, there was a significant difference compared to the mitragynine group ($p < 0.05$), but not to the vehicle group ($p > 0.05$). Buprenorphine also significantly alleviated the withdrawal signs as compared to vehicle and mitragynine groups on day 1 ($p < 0.05$) (**Figure 1**). A significant mitigation of withdrawal signs was also observed from day 2 to day 5, as compared to the mitragynine group ($p < 0.05$), but not compared to the vehicle group ($p > 0.05$). The strongest suppression of mitragynine withdrawal effects was observed after clonidine treatment (day 1–5; vs. mitragynine-vehicle: $p < 0.05$). From day 1 to day 5, no significant difference was shown between the mitragynine-clonidine treated group compared to the vehicle group ($p > 0.05$) and significant result has been revealed as compared to mitragynine group ($p < 0.05$).

Experiment II

Hematological and Biochemical Analysis

Hematological and biochemical analyses of the blood samples were taken on day 5 and results are displayed in **Tables 2, 3**, respectively. The references range value of both hematological and biochemical analyses were based on these following studies Nugraheni et al. (2017), He et al. (2017), Houtmeyers et al. (2016) and Petterino and Argentino-Storino (2006). The hematological analysis revealed a significant increase in mean corpuscular

TABLE 2 | Hematological analysis on day 5 in mitragynine replacement treatments.

Replacement groups	Vehicle	Mitragynine – Vehicle	Mitragynine 1 mg/kg Methadone	Mitragynine 0.8 mg/kg Buprenorphine	Mitragynine 1.1 mg/kg Clonidine	References range value
Total RBC (x 10^12/L)	6.97 ± 0.24	8.23 ± 0.6	7.93 ± 0.35	8 ± 0.17	7.93 ± 0.37	6.39–8.01
Hemoglobin (gm/L)	155.67 ± 8.19	168 ± 12.7	168 ± 4.36	159 ± 8.02	156.67 ± 9.61	135–159
PCV (%)	49.33 ± 1.86	47 ± 0.04	45 ± 1.73	46.33 ± 1.76	43.67 ± 0.02	42–49
MCV (fL)	58.33 ± 2.03	57 ± 1.73	58 ± 0.58	59 ± 2.08	55 ± 1.15	58.01–67.00
MCH (pg)	20.67 ± 0.33	20.33 ± 0.33	21 ± 0.58	19 ± 1.53	19.67 ± 0.33	18.70–21.20
MCHC (g/L)	316.67 ± 8.82	360 ± 5.77	376.67 ± 8.82*	320 ± 20	360 ± 5.77	310–336
RDW (%)	15.17 ± 0.42	14.93 ± 0.94	14.93 ± 0.12	15.4 ± 0.58	14.93 ± 0.26	13.03–16.57
Total WBC (x 109/L)	4.2 ± 1.18	4.8 ± 1.29	4.4 ± 0.71	6.47 ± 0.18	5.13 ± 0.96	3.00–9.22
Lymphocytes (%)	76.33 ± 2.03	66 ± 5.86	65.67 ± 6.64	76.33 ± 1.45	69 ± 1.53	51.8–89.7
Monocytes (%)	2.67 ± 0.67	0.33 ± 0.33	3 ± 0.58	2.67 ± 0.67	0.67 ± 0.33	1.3–6.0
Eosinophils (%)	0 ± 0	0.33 ± 0.33	0.33 ± 0.33	0.58 ± 0.01	0 ± 0	0.5–7.2
Basophils (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0–0.6
Platelet count (x 10^9/L)	682 ± 112.65	529 ± 62.69	702.33 ± 18.89	946.33 ± 3.53#	483.33 ± 39.54	529.0–1,383.0

Total RBC, red blood cell count; PCV%, percentage of packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW%, percentage of red cell distribution width; WBC, total of white blood cell count, Lymphocytes (%), Percentage of lymphocyte; Monocytes (%), Percentage of monocytes; Eosinophils (%), Percentage of eosinophils; Basophils (%), Percentage of basophils; PLT, Platelet counts.
*Data represent means ± SEM of n = 6 rats/group *p < 0.05 vs. Vehicle, #p < 0.05 vs. Mitragynine-Vehicle, analysed by one-way repeated measures ANOVA and Bonferroni post-hoc test.*

TABLE 3 | Biological analysis on day 5 in mitragynine replacement treatments.

Replacement groups	Vehicle	Mitragynine – Vehicle	Mitragynine 1 mg/kg Methadone	Mitragynine 0.8 mg/kg Buprenorphine	Mitragynine 0.1 mg/kg Clonidine	References range value
Total bilirubin (umol/L)	1.71 ± 0	2.28 ± 0.57	1.71 ± 0	2.28 ± 0.57	2.28 ± 0.57	0.0–5.1
Aspartate aminotransferase, AST (U/L)	117 ± 4.58	205.33 ± 36.08	169 ± 20.03	147 ± 1	220.33 ± 35.57	56.1–201.8
Alanine aminotransferase, ALT (U/L)	59 ± 6.11	74 ± 2.08	53 ± 2.31	58.33 ± 3.38	55.33 ± 6.69	34.9–218.1
Alkaline phosphatase (U/L)	299.67 ± 28.01	381.67 ± 119.7	337.33 ± 48.55	421 ± 86.97	253.33 ± 32.69	131.6–459.0
Sodium (mmol/L)	141.67 ± 1.45	139.33 ± 0.67	141 ± 1.73	143.67 ± 0.67	140.33 ± 0.88	121.9–162.6
Potassium (mmol/L)	6.7 ± 1.26	7.67 ± 0.12	7.8 ± 0.85	6.77 ± 0.24	7.1 ± 0.15	4.0–8.0
Chloride (mmol/L)	102.33 ± 0.88	102.33 ± 0.33	103 ± 0.58	104 ± 0	104.67 ± 1.2	81.5–104.0
Urea (mmol/L)	9.80 ± 0.3	8.33 ± 0.45	8.05 ± 0.11	14.45 ± 6.04	10.03 ± 0.45	4.32–34.4
Creatinine (umol/L)	49.87 ± 2.35	46.93 ± 0.59	46.35 ± 1.63	73.92 ± 33.93	43.71 ± 1.28	35.4–79.6
Total cholesterol (mmol/L)	1.70 ± 0.08	2.1 ± 0.11	1.59 ± 0.09#	1.47 ± 0.07#	1.74 ± 0.11	0.68–1.77
Triglycerides (mmol/L)	0.93 ± 0.19	0.82 ± 0.19	0.98 ± 0.09	0.99 ± 0.14	0.65 ± 0.15	0.23–0.99
Calcium (mmol/L)	2.41 ± 0.11	2.33 ± 0.11	2.48 ± 0.01	2.38 ± 0.03	2.28 ± 0.06	2.1–2.9
Phosphorus (mmol/L)	2 ± 0.30	2.36 ± 0.28	2.13 ± 0.25	2.22 ± 0.09	2.35 ± 0.17	1–3.94
Total protein (g/L)	70 ± 4.36	73.67 ± 1.76	65.67 ± 0.88	67.67 ± 1.86	69.67 ± 3.38	52–71
Albumin (g/L)	27 ± 2.08	28 ± 0.58	25.67 ± 0.88	26 ± 1.53	26.33 ± 1.33	26.85–34.55
Globulin (g/L)	43.33 ± 2.33	45.67 ± 2.19	40 ± 1.53	41.67 ± 1.2	43.33 ± 3.18	13–48
Albumin/Globulin ratio (g/L)	0.6 ± 0.06	0.63 ± 0.07	0.63 ± 0.03	0.63 ± 0.03	0.63 ± 0.07	0.6–1.21

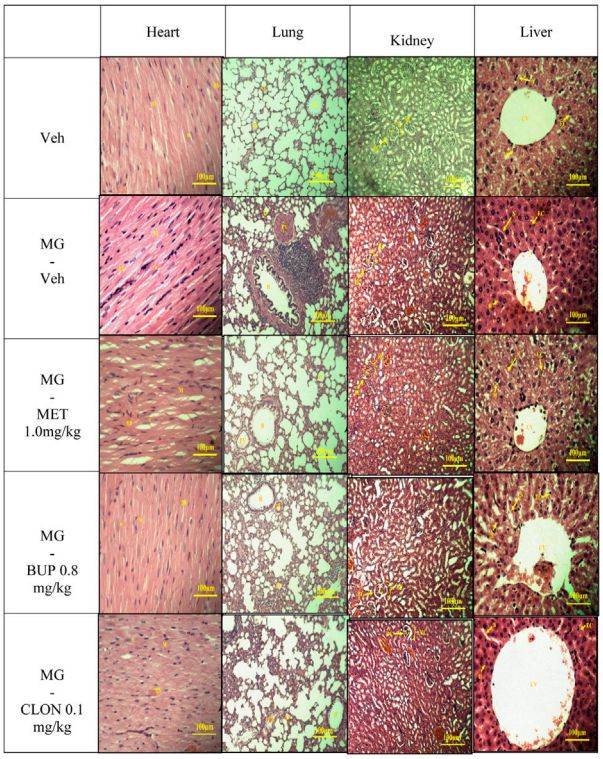
*Data represent means ± SEM of n = 6 rats/group *p < 0.05 vs. Vehicle, #p < 0.05 vs. Mitragynine-Vehicle, analysed by one-way repeated measures ANOVA and Bonferroni post-hoc test.*

hemoglobin concentration (MCHC). This was higher than the reference range in the mitragynine-methadone group and significantly increased compared to the vehicle control group ($p < 0.05$). A significant increase in platelet count was found in the mitragynine-buprenorphine group ($p < 0.05$ vs vehicle; **Table 2**). The biochemical analysis revealed a significant reduction of total cholesterol in the mitragynine-methadone and mitragynine-buprenorphine groups ($p < 0.05$) compared to vehicle control (**Table 3**).

Histopathology Analysis

The results of histopathological examination of the transverse sections of heart, lung, kidney, and liver are shown in **Table 4**. In general, there were no differences between the vehicle and the treatment groups by cross-examination of the microscopic structures of the heart, kidney and liver. However, the lung structure revealed a slight difference effect in the alveoli size in all replacement treated groups when compared to the vehicle group (**Table 4**).

TABLE 4 | The microscopic structures of the organs in mitragynine replacement treatments.



DISCUSSION

When a person is compelled to take a drug on a regular or continuous basis in order to achieve psychic effects on mental state, such as euphoria, and to avoid physical discomfort (withdrawal), this is referred to as drug dependence (Gupta and Gupta, 2018; Müller, 2020). Dependence in kratom is well documented in human (Suwanlert, 1975; Vicknasingam et al., 2010; Ahmad and Aziz, 2012; Singh et al., 2014). Nevertheless, although kratom has been reported to cause dependence and withdrawal signs, these symptoms are usually milder than after opiate withdrawal (Prozialeck, 2016; Saingam et al., 2016; Grundmann, 2017; Singh et al., 2018b; Swogger and Walsh, 2018). Singh et al. (2018b) reported that kratom depended patients did not seek for medication as kratom withdrawal symptoms were mostly rather mild and only lasted between one to three days. A widely held view is that kratom is not as risky as opioid drugs and that the danger can be outweighed by the potential benefits (Prozialeck, 2016). However, aggressive kratom consumption pattern may cause escalation of consumption and more severe withdrawal signs (Müller et al., 2021). Indeed, withdrawal periods are highly aversive, which makes it hard for an individual to maintain abstinence (Swogger and Walsh, 2018).

At the moment, there is no particular treatment for kratom dependence and withdrawal symptoms. Nonetheless, several studies have been conducted in which buprenorphine was substituted for kratom (Buresh, 2018; Bowe and Kerr, 2020; Lei et al., 2021). The recent study by Weiss and Douglas (Weiss and Douglas, 2021) demonstrated that patients who used less than 20 g of kratom per day could begin opioid agonist therapy with 4/1 mg-8/2 mg buprenorphine-naloxone/day, whereas patients who used more than 40 g of kratom per day could begin with 12/3 mg-16/4 mg buprenorphine-naloxone/day. Kratom leaves do contain more than 40 alkaloids, with mitragynine being the most common indole alkaloid (Adkins et al., 2011; Yearsley, 2016). As a result, we presumed that mitragynine was one of the alkaloids that modulated the effects of kratom. However, one feature that should be noted is that the alkaloid contents vary according to geographical regions and seasons (Hassan et al., 2013). Stages in leaf maturity too are a contributing factor (Raffa, 2015). Studies have shown that mitragynine content is much more abundant in Thai kratom as compared to the Malaysian species, amounting to 66.2 and 12%, respectively (Takayama et al., 1998; Takayama, 2004). This would suggest that kratom substitution treatment is difficult to be evaluated due to differences in mitragynine content. Therefore, the current study will focus on mitragynine rather than kratom dependence. Mitragynine is a psychoactive substance which also can produce dependence for which no pharmacotreatment is available yet. Mitragynine has been reported to cause physical dependence after spontaneously mitragynine withdrawal (Hassan et al., 2021) and after naloxone-precipitated withdrawal (Harun et al., 2020) in animal studies. In addition, Yusoff et al. (2016) also reported that chronic mitragynine withdrawal triggers anxiety-like behaviour in rats. Because there is presently no established treatment for kratom dependence, the present study investigated the effectiveness of methadone, buprenorphine, and clonidine, in attenuating the withdrawal symptoms induced by persistent mitragynine exposure. Buprenorphine, methadone, and clonidine have been identified as the most effective opioid detoxification medications (Meader, 2010). Thus, that is why we used those treatments in the current study. In addition, the opioid and non-opioid mitragynine receptors that may be involved in mitragynine withdrawal were also being considered, and the drug used in the current study was influenced by them. This research is, to the best of our knowledge, the first study on the effects of methadone, buprenorphine and clonidine on mitragynine withdrawal symptoms.

We recently published a rat mitragynine withdrawal model (Hassan et al., 2021). Thus therefore, we used this model in the following experiments in this present work. Overall, this study showed a marked reduction in withdrawal symptoms in mitragynine withdrawn rats receiving methadone, buprenorphine and clonidine. In addition, no resurface of the withdrawal symptoms was seen on day 5 of the cessation (**Figure 1**). This suggests that methadone, buprenorphine and clonidine are capable in alleviating mitragynine withdrawal signs during and after the replacement therapy. This may also imply that mitragynine withdrawal modulates the same receptors as the mechanisms of action in methadone, buprenorphine and clonidine.

Mitragynine has been proved to bind at opioid receptor, which has been demonstrated by several researchers via *in vivo and in vitro* studies. Matsumoto et al. (Matsumoto et al. (1996a) has reported that involvement of opioid receptors in the action of mitragynine. Mitragynine acting on opioid systems can also be

observed in the studies of ileum motility inhibition (Watanabe et al., 1997) and inhibition of gastric acid secretion (Tsuchiya et al., 2002) that has been reversed by naloxone. Furthermore, Shamima et al. (2012) also reported the antinociceptive effects of mitragynine when it showed a significant decrease in the latency time compared to mitragynine alone after intraperitoneally administered of naloxone (non-selective opioid antagonist) and naltrindole (delta-opioid antagonist). Naloxonazine, a μ1-receptor antagonist did reduce the antinociceptive effect of mitragynine, but it is not statistically significant, which indicates that mitragynine may not only act specifically on μ1-receptor. However, norbinaltorpimine (norBNI) (i.p.) partially blocked the effect of mitragynine and significantly decreased the latency time when compared with mitragynine alone from 30 to 60 min, but not up to 120 min time, indicate that mitragynine may partially act via kappa opioid receptor (Shamima et al., 2012). In addition, Kruegel et al. (2016) has demonstrated that mitragynine acts as a partial agonist at the human mu-opioid receptor (MOP) and competitive antagonists at kappa-(KOP) and delta- (DOP) opioid receptors in *in intro* study. Indeed, this matter is still in question with the facts about whether mitragynine fully or partially works on the mu-opioid receptor remaining uncertain. Nonetheless, Yusoff et al. (2017) revealed that mitragynine-induced CPP establishment, but not expression, is mediated by an opioid receptor mechanism. Moreover, recently, the effect of naloxone on precipitated of mitragynine withdrawal effects was described by Harun et al. (2020), suggesting that the mu-opioid receptor is responsible for the development of mitragynine dependence.

However, Hiranita et al. (2019) suggested that mitragynine is not mediated through opioid receptor, as naltrexone did not antagonize the effects of mitragynine. It was proposed that activation of serotonergic and noradrenergic pathways along the spinal contributed to the antinociceptive activity of mitragynine (Matsumoto et al., 1996b). It also reported to bind at non-opioid receptors includes alpha-$_2$ adrenergic receptors, adenosine A_2a receptors, dopamine D_2 receptors, and the serotonin receptors 5-HT_{2C} and 5-HT_7 (Boyer et al., 2008; Prozialeck et al., 2012; Kruegel and Grundmann, 2018). Currently, an *in vitro* study evaluated the adrenergic effects by mitragynine using human monoclonal receptors expressed in Chinese hamster ovary (CHO) cells and revealed that mitragynine is a partial agonist at alpha $_{1A}$ and $_D$ adrenergic receptors (Obeng et al., 2020).

Methadone and buprenorphine are both opioids, acting fully and partially on mu-opioid receptor, respectively (Kleber, 2007). Methadone and buprenorphine have been approved by the FDA in opioid replacement therapy (Bart, 2012). Methadone safety is well documented and proven (Kreek, 1973). However, if taken beyond the tolerance of the person, methadone could cause respiratory depression (Bart, 2012). The respiratory depression could also be fatal in the event of overdose since there is no ceiling level to it (Mattick et al., 2008). Moreover, unknown drug-drug interaction can also lead to death. Records indicate that methadone patients who use other controlled drugs in conjunction with methadone commonly face serious adverse effects (Mattick et al., 2008). Since the 1970s, buprenorphine has been accepted as an alternative treatment of opioid dependence (Mattick et al., 2008). Buprenorphine is also used as an analgesic in acute pain management. Buprenorphine has ceiling effects on respiratory depression (Ling and Compton, 2005). In contrast, a full agonist for respiratory depression which caused a robust decrease in respiratory ventilation following intracerebroventricular buprenorphine administration has been reported by Kuo et al. (2015). Moreover, buprenorphine also does not appear to exhibit a ceiling effect for analgesia (Dahan et al., 2006). At high dose, it antagonizes the analgesic effects of other opioids thereby complicating management of pain in patients maintained on high-dose buprenorphine (Heinzerling et al., 2019). Its antagonist properties can also cause a precipitation of acute opiate withdrawal effects if administered to an individual who is physically dependent on opioids.

Clonidine is a non-opioid drug, that is a partial agonist of alpha$_2$-adrenergic receptors (Giovannitti et al., 2015; Arora and Vohora, 2016). It was used in opioid substitution treatment over the years (Jamadarkhana and Gopal, 2010). It has analgesic effects (Kariya et al., 1998) and has been reported to decrease opioid dosage without affecting the quality of analgesia (Rostaing et al., 1991). It can reduce opiate withdrawal signs in inpatient and outpatient settings (Washton and Resnick, 1981; Tierney et al., 1988), via binding to α_2-autoreceptors in the locus coeruleus and suppressing hyperactivity during withdrawal (Kleber, 2007). However, clonidine has adverse effects, particularly hypotension, which can restrict optimal clonidine dose for opioid withdrawal. It was reported to cause rebound hypertensive episodes in long-term clonidine therapy but proved as safe in short-term use (Kariya et al., 1998). Kleber (2007) also showed that clonidine can be used to treat residual mild withdrawal symptoms for a few days to a week as long as the patient does not become hypotensive. Nevertheless, clonidine has been reported to be less effective compared to methadone during early opioid detoxification phase when withdrawal symptoms were more pronounced and patients more likely to drop out (Mattick and Hall, 1996). On the other hand, clonidine showed in clinical trial a similar efficacy as buprenorphine in the reduction of withdrawal symptoms (Cheskin et al., 1994; Ziaaddini et al., 2012). Clonidine treated patients however suffered from lower blood pressure compared to buprenorphine treated patients (Cheskin et al., 1994).

Hematological, Biochemical and Histopathological Changes in Mitragynine and the Replacement Drugs

Biochemical and histopathological evaluations following mitragynine and/or the replacement drugs in blood and selected vital organs have also been conducted in the present study. In the hematological analysis (**Table 2**), MCHC increases in mitragynine-vehicle, mitragynine-0.1 mg/kg clonidine and mitragynine-1.0 mg/kg methadone. This might be due to the withdrawal effect that might be still exist in the body of the rats. Moreover, a substantial rise in MCHC level had also been reported in heroin and opium dependent and withdrawal groups (Haghpanah et al., 2010). In addition, the mitragynine-0.1 mg/kg clonidine treatment lowered platelet count level below the normal reference range without affecting other hematological parameters. This indicates

that the rats might be affected with biological variations namely variability between individual rats and temporal variation (Peng et al., 2004), which warrants further details investigations.

In biochemical analysis (**Table 3**), mitragynine- 1.0 mg/kg methadone and mitragynine-0.8 mg/kg buprenorphine significantly reduced the total cholesterol level as compared to mitragynine-vehicle within the normal reference range value. A high level of total cholesterol has also been reported among kratom users who had a high daily mean frequency of kratom use (Leong Bin Abdullah et al., 2020).

Overall, a healthy set of organ cells except for lung can be observed in light microscopy examination of histopathology of all mitragynine replacement groups (**Table 4**). Previously, Macko et al. (1972) performed a subchronic study in rats and dogs in which no adverse effects were seen after oral administration of 5 or 50 mg/kg/day of mitragynine for six weeks, five days a week. Another subchronic study conducted by Sabetghadam et al. (2013) reported that mitragynine was relatively safe at lower subchronic doses (1–10 mg/kg, oral), but showed toxicity at the highest dose (subchronic 28 days: 100 mg/kg, oral) in the liver, kidney and brain. This dose caused also hematological and biochemical changes. All these toxicity data were reported after oral application though, which differs from the present study, which used i.p. applications. After i.p. administration, the primary route of absorption is through the mesenteric vessels, which drain into the portal vein and pass through the liver (Lukas et al., 1971). Substances administered i.p. can, therefore, undergo hepatic metabolism before reaching the systemic circulation (Turner et al., 2011). Moreover, in all treated groups, particularly the mitragynine-vehicle group, mild lung histopathological changes were observed compared with vehicle control group (**Table 4**), suggesting that the selected doses of drugs administration at determined duration, were too small to cause histopathological damage but sufficient to show signs of drug intoxication.

Opiates, stimulants, and cannabinoids are three classes of drugs that can cause respiratory manifestation (Glassroth et al., 1987). There are pulmonary patho-histological findings that have the direct effect on lung; edema, pulmonary hemorrhage and appear of siderophages, pulmonary artery medial hypertrophy, panacinar emphysema, bronchiolitis obliterans, interstitial pneumonia or fibrosis (Karch and Stephens, 2000). In addition, Awadalla and Salah-Eldin (2016) reported that opioids can cause a drop in plasma antioxidant levels, possibly indicating that the antioxidant defence mechanism against oxidative damage has failed. Indeed, oxidative stress during mitragynine withdrawal has been reported by Hassan et al. (2021). Therefore, the mild lung histopathological changes might be related to oxidative stress.

Opioid usage is inextricably connected to respiratory depression, or hypoventilation. However, it has an indirect effect on the lungs (Radke et al., 2014). Respiratory depression occurs when the body is unable to efficiently eliminate carbon dioxide. This can result in the lungs' inefficient utilisation of oxygen. As a result, the body produces more carbon dioxide and has insufficient oxygen. Basically, mu-opioid receptors are mostly found in the brainstem and are expressed on neurons that govern breathing (Boom et al., 2012). The activation of mu-opioid receptors causes opioid-induced analgesia as well as respiratory depression (Boom et al., 2012). Opioids exert their respiratory depressant effect via two distinct mechanisms: decreased chemoreceptor sensitivity and decreased activity in the central respiratory centres (White and Irvine, 1999). The carotid and aortic bodies, as well as the lungs, contain peripheral chemoreceptors. They boost signal transduction in response to decreased partial pressure of oxygen (pO_2) or increased partial pressure of carbon dioxide (pCO_2). The central receptors, which are located in the medulla but separate from the main respiratory centre, respond only to elevated pCO_2 (Radke et al., 2014). Mu- and delta opioid receptors are found in the central respiratory regions of the medulla (Radke et al., 2014). The respiratory centre's opioid activity induces a decrease in respiratory rate and tidal volume, both of which can contribute to a decrease in minute ventilation (White and Irvine, 1999). These effects are also dose-dependent where, at low dosages of opiates appear to decrease tidal volume, whereas larger doses appear to decrease both tidal volume and respiratory rate (Santiago and Edelman, 1985). In addition, in kratom itself, no single case can be solely attributed to respiratory failure, a sharp contrast to other opioids where respiratory depression is the most common cause of death (Kruegel and Grundmann, 2018). Plus, even though mitragynine activated the G-protein–mediated signaling pathway much like traditional opioids, it did not "recruit" β-arrestin-2 (Váradi et al., 2016), suggested that mitragynine has less side effect in terms of respiratory depression while it remains as a potent analgesic.

CONCLUSION

In conclusion, four-day replacement therapy with available prescription drugs, methadone, buprenorphine, and clonidine, significantly attenuated mitragynine withdrawal signs in rats. This is the first study that reports these possible treatments for mitragynine withdrawal. As the present mitragynine substitution treatment was a preliminary study, further investigations would be required to confirm these findings in future.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by Universiti Sains Malaysia (USM) Institutional Animal Care and Use Committee. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

ZH conceptualized the idea. ZH and RH designed, performed the experiments and analysed data. RH, SS, CM and ZH wrote the paper. All authors read and approved the manuscript.

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The Antidepressant-Like and Analgesic Effects of Kratom Alkaloids are accompanied by Changes in Low Frequency Oscillations but not ΔFosB Accumulation

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Mitragyna speciosa ("kratom"), employed as a traditional medicine to improve mood and relieve pain, has shown increased use in Europe and North America. Here, the dose-dependent effects of a purified alkaloid kratom extract on neuronal oscillatory systems function, analgesia, and antidepressant-like behaviour were evaluated and kratom-induced changes in ΔFosB expression determined. Male rats were administered a low or high dose of kratom (containing 0.5 or 1 mg/kg of mitragynine, respectively) for seven days. Acute or repeated low dose kratom suppressed ventral tegmental area (VTA) theta oscillatory power whereas acute or repeated high dose kratom increased delta power, and reduced theta power, in the nucleus accumbens (NAc), prefrontal cortex (PFC), cingulate cortex (Cg) and VTA. The repeated administration of low dose kratom additionally elevated delta power in PFC, decreased theta power in NAc and PFC, and suppressed beta and low gamma power in Cg. Suppressed high gamma power in NAc and PFC was seen selectively following repeated high dose kratom. Both doses of kratom elevated NAc-PFC, VTA-NAc, and VTA-Cg coherence. Low dose kratom had antidepressant-like properties whereas both doses produced analgesia. No kratom-induced changes in ΔFosB expression were evident. These results support a role for kratom as having both antidepressant and analgesic properties that are accompanied by specific changes in neuronal circuit function. However, the absence of drug-induced changes in ΔFosB expression suggest that the drug may circumvent this cellular signaling pathway, a pathway known for its significant role in addiction.

Keywords: kratom, neuronal oscillations, depression, analgesia, mitragynine, ΔFosB, *Mitragyna speciosa* 3

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INTRODUCTION

Mitragyna speciosa, commonly referred to as kratom, is a tree species that is native to Southeast Asia and it has been used by individuals for centuries both recreationally and medicinally to improve mood and manage acute and chronic pain (Singh et al., 2016; Ismail et al., 2019). However, the increase of kratom sales across Europe and North America have resulted in growing concerns over its safety, with several European countries and states within the United States banning the plant or instituting age restrictions in its use (Cinosi et al., 2015). Despite these restrictions, it is estimated there are several million users of kratom (Henningfield et al., 2018). Consumption of kratom leaves has been reported to have dose-dependent effects, in that lower doses have been found to induce mild stimulant-like effects and higher doses have been found to induce opioid like analgesic effects (Kruegel and Grundmann, 2018). These outcomes of ingesting the plant material have historically been attributed to only two alkaloids that are typically present within the kratom leaf material, namely mitragynine and its derivative 7-hydroxymitragynine (7-HMG), however it is well established that there are at least 40 other alkaloids that accumulate within the plant, albeit in various amounts (Prozialeck et al., 2012; Eastlack et al., 2020). Strikingly, almost nothing is known about the biological properties of these other alkaloids, or of the combined biological effects of the plant as a whole.

The alkaloid profile observed within kratom is dominated by the class of compounds known as the monoterpenoid indole alkaloids, of which mitragynine and 7-HMG are the most studied examples. Both alkaloids have been found to activate the mu-opioid receptor (MOR), however unlike many other MOR agonists, they are ß-arrestin sparing (Kruegel et al., 2016; Ismail et al., 2019). For this reason these alkaloids have been termed "atypical opioids" (Adkins et al., 2011; Kruegel et al., 2019), and as ß-arrestin signaling has been shown to mediate opioid-induced tolerance and side effects such as respiratory depression, it is believed that kratom may offer an analgesic alternative (Takayama et al., 2002; Kruegel et al., 2016; Ismail et al., 2019).

Behaviours are highly coupled to neuronal oscillations (Buzsáki and Draguhn, 2004; Buzsáki et al., 2013), rhythmic neuronal population activity that is critical to regional communication (Fries, 2005; Barardi et al., 2014). These oscillations have been linked to neuropsychiatric disorders such as addiction and depression (Fitzgerald and Watson, 2018; Theriault and Perreault, 2019; Zhu et al., 2019; Thériault et al., 2021), as well as drug responses (Reakkamnuan et al., 2017; Manduca et al., 2020), and thus may serve as useful biomarkers of disease states or establishing the therapeutic efficacy of novel drugs. Although there is less known about the role of neuronal oscillations in the context of analgesia, high frequency cortical oscillations are thought to be involved in the perception of pain (Whittington et al., 1998; Croft et al., 2002). For instance, morphine-induced frequency-specific alterations in oscillatory activity in brain regions such as the NAc and VTA are well known (Reakkamnuan et al., 2017; Ahmadi Soleimani et al., 2018), as well as in the cortex (Liu et al., 2005; Zuo et al., 2007). It is therefore intriguing that although there is some evidence for analgesic and antidepressant properties of kratom alkaloids (Matsumoto et al., 2004; Takayama, 2004; Kumarnsit et al., 2007a; Kumarnsit et al., 2007b; Sabetghadam et al., 2010; Idayu et al., 2011; Grundmann, 2017), the impact of these alkaloids on the neuronal oscillatory activity in the brain is lacking. Two studies examining the neurophysiological effects of mitragynine in rats did, however, demonstrate frequency-specific changes in cortical oscillatory power (Yusoff et al., 2016; Thériault et al., 2020) with no effects in other regions including the VTA, NAc, thalamus, amygdala, or hippocampus (Thériault et al., 2020).

These findings suggest that kratom-induced behavioural changes will be accompanied by region-specific alterations in neurophysiological circuit function. Therefore, to better understand this link, this study evaluated the dose-dependent effects of a purified alkaloid isolate from kratom on neuronal oscillatory activity in various brain regions in rats following acute and 7 days of administration. The antidepressant and analgesic effects of the extract were also determined at both time points, as well as the ability of the isolate to induce ΔFosB expression, a putative molecular switch for addiction.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (Charles River, QC) weighing approximately 300–350 g at the start of the experiment were used. Animals were housed in a temperature-controlled colony room, maintained on a 12-h reverse light/dark cycle (0700 h lights off; 1900 h lights on) with unrestricted access to food and water available ad libitum. Animals were handled daily for a minimum of 7 consecutive days prior to the beginning of the experiment. Electrophysiological and behavioural testing was always conducted during the dark phase of the day/night cycle. An extra cohort of animals were added to increase sample size for the behavioural studies. All animals underwent identical behavioural procedures. No group differences in animals that underwent the same treatments was evident between the two cohorts and so animals were pooled. All procedures were approved by the Animal Care Committee of the University of Guelph and followed the guidelines of the Canadian Council on Animal Care.

Plant Material and Growth Conditions

Mitragyna speciosa (Korth.) Havil were obtained from Dad's Greenhouse, Ohio, United States, and imported to the University of Guelph as 6–18″ saplings. Trees were maintained in growth chambers with a 16-h photoperiod (175 μmol $m^{-2}s^{-1}$; mixed cool white and incandescent bulbs) and a day/night temperature regime of 28°C/26°C, with a constant relative humidity of 80%. Plants were grown for a minimum of 4 months in a 2:1:1 (v/v) mixture of coco coir (Millennium soils Coir):perlite (Therm-o-rock East Inc.):turface (Turface Athletics) before harvesting material. The plant material was identified and authenticated by Dr. Carole Ann Lacroix and a voucher specimen

(No. 102033) was deposited at the Ontario Agricultural College Herbarium in Guelph, Ontario, Canada.

Alkaloid Extraction and Preparation

Mature leaf tissue from *Mitragyna speciosa* plants were dried at 50°C for 48 h and 100 g of this material was extracted with 2 L of an acetic acid solution (0.5 M) at 80°C for 30 min. The crude extract was filtered (0.22 µm PTFE) and then passed through a 60 ml column containing polyvinylpyrrolidone (PVPP, 110 µm particle size, Sigma-Aldrich) to remove any polyphenolic compounds. The crude Kratom extract was sequentially chromatographed over 50 ml of Diaion HP-20 resin (Supelco) equilibrated with distilled water and the reversed-phase column was then washed with 20% (v/v) methanol before elution with 100% methanol followed by methanol/ethyl acetate (50:50 v/v). The recovered alkaloid fractions were pooled and reduced to a volume of 200 ml on a Rotary Evaporator (RE-200AA) at 70°C. The isolate was then loaded onto an ion exchange resin (AmberChrom 50WX2, 200–400, Sigma Aldrich) and washed with 500 ml of acetic acid in ethanol (0.025 M), followed by 250 ml of 100% ethanol. Alkaloids were eluted with 340 ml of 2.8 M ammonium hydroxide in ethanol and then brought to final volume of 150 ml. This purified alkaloid extract was subjected to phase separation with chloroform (300 ml). The organic layer was extracted and reduced to dryness, *in vacuo*, and resuspended in 10 ml of hydrochloric acid (0.2 M). After complete resuspension of the alkaloid extract, the pH was brought to 5.0 with NaOH and adjusted to 1.0 mg/ml of mitragyine equivalents, accordingly.

Instrumentation and Alkaloid Analysis

Ajmalicine (Sigma) and mitragynine, 7-hydroxymitragynine, paynantheine, speciogynine, mitraphylline, speciociliatine (Cayman Chemicals) were used as external standards for quantification on the basis of peak area revealed by HPLC analysis as described below. Alkaloids fractions were analyzed using an Agilent 1,260 Infinity liquid chromatography system equipped with a reversed-phase Kinetex EVO C18 100Å column (150 × 4.6 mm, 5 µm). Chromatographic separation of kratom alkaloids were achieved using a binary gradient with ammonium bicarbonate buffer (5 mM pH 9.5; A) and acetonitrile (B), starting with 70% solvent A transitioning to 70% solvent B over the course of 17 min at a flow rate of 1.5 ml/min. Alkaloids were quantified at 226 nm. The alkaloids fractions 3 (3-isoajmalicine) and 10 (corynantheidine) eluted at 8.12 and 14.67 min, respectively, and were subsequently collected. Approximately 0.3 mg of each compound were evaporated to dryness, resuspended in deuterated chloroform, and analyzed using ^{1}H NMR. NMR spectra were collected on a Bruker AVANCE III 600 MHz spectrometer equipped with a 5 mm TCI cryoprobe. The sample temperature was regulated at 298 ± 1 K.

Drugs

Rats were intraperitoneally (i.p.) injected daily with the kratom isolate at a dose of 0.5 or 1.0 mg/kg of mitragynine equivalents (low and high dose, respectively) or with a saline for a period of seven days. As kratom is normally ingested orally, we used the i.p. route since it also has an important first pass effect but is not as stressful as oral gavage which would likely alter brain wave patterns. Moreover, since the metabolites of kratom alkaloids have been shown to have biological effects, the i.p. route would ensure that they are metabolized in a manner similar to oral intake in humans.

These doses were selected based on preliminary dose response studies and were 5 and 10 times less than calculated LD50 of tested animals (a mitragynine equivalent of 5 mg/kg). At the doses employed in the present study, animals showed no adverse effects with acute or repeated administration. We have previously characterized isolated mitragynine effects (10 mg/kg i.p., a standard dose used in the literature (Foss et al., 2020; Japarin et al., 2021; Suhaimi et al., 2021)) on neuronal oscillatory activity where we showed moderate frequency-specific changes in cortical regions only (Thériault et al., 2020). Further, our preliminary behavioural findings showed no effects of the same 10 mg/kg dose of mitragynine on behavioural responses in the tail-flick test (**Supplementary Figure S1**). As the 10 mg/kg dose is 10–20 times higher than the doses used in the present study, we therefore chose not to include a mitragynine group as neurophysiological and behavioural effects would likely be minimal or absent.

Electrode Implantation Surgery

Electrode implantation surgeries were performed as previously described (Foute Nelong et al., 2019). Custom electrode microarrays were built using prefabricated Delrin templates and polyimide-insulated stainless-steel wires (A-M Systems: 791600, 0.008″) that were inserted through polyimide cannula. All arrays used had an electrode impedance of less than 2 MΩ. Isoflurane was used to anesthetize the rats at 5% induction and 2.5% maintenance and body temperature maintained at 37°C using a thermostat-regulated heating pad. Animals were injected subcutaneously with 0.9% saline (3 ml) to ensure adequate hydration during surgeries, and 5 mg/ml carprofen (0.4 ml, s.c.) as well as a lidocaine/bupivacaine injection at the incision site. Electrodes were implanted bilaterally into the medial PFC (AP: +3.24 mm, ML: ± 0.6 mm, DV: −3.8 mm), Cg (AP: +1.9 mm, ML: ± 0.5 mm, DV: −2.8 mm), NAc (AP: +1.92, ML: ± 1.2 mm, DV: −6.6 mm) and the VTA (AP: −4.8 mm, ML: ± 0.7, DV: −8.5 mm). A ground/reference screw was secured in the skull behind lambda and additional anchor screws were attached to the skull.

Local Field Potential Recordings

Animals were habituated to the recording boxes (18″ × 18″ × 18″) for 15 min/day for 2 days. Local field potential (LFP) recordings (Wireless 2100-system, Multichannel Systems) were performed in awake and freely moving animals on days 1 and 7 with a sampling frequency of 1 kHz. On each day of testing baseline LFP recordings were collected for 15 min prior to animals receiving their assigned kratom dose (0, 0.5, 1.0 mg/kg i.p.). Rats were then placed back into the boxes and recordings were collected for an additional 30 min. Routines from the Chronux software package for MATLAB (MathWorks) were used to analyze LFP spectral power and coherence between brain regions. Recordings were segmented, detrended, de-noised and low-pass filtered to remove frequencies greater

than 100 Hz. Continuous multitaper spectral power for the normalized data (to total spectral power) and coherence was calculated for delta (1–4 Hz), theta (>4–12 Hz), beta (>12–30 Hz), low gamma (30–60 Hz), and high gamma (>60–100 Hz).

Forced Swim Test

The forced swim test (FST) is a test used to evaluate behavioural despair and to determine the antidepressant properties of drugs, and was performed as previously described (Thériault et al., 2021) immediately following LFP recordings. The pre-test was carried out twenty-four hours prior to drug administration in which animals were placed in a plexiglass cylinder with water (24 ± 1°C) filled to a height of 30 cm for 15 min. Animals were then dried with a towel and put back into their home cage. Twenty-four hours later, following LFP recordings, animals were once again placed in the water-filled cylinder for a testing period of 5 min. For subsequent testing on day 7, the pre-test was not conducted. The following behavioural parameters were assessed at 5-s intervals: immobility (floating without active movements, other than those that are needed to keep nose above water), climbing (attempting to escape the cylinder with front paws breaking the surface of the water) and swimming (paddling of limbs across the surface of the water).

Tail-Flick Test

To assess the acute and chronic analgesic properties of kratom, the tail-flick test was performed as previously described (Tu et al., 2016) at 40 min post-drug administration. This test evaluates pain responses in animals and is used to measure the effectiveness of analgesics through heat exposure to the animals' tails. Animals were gently restrained using a towel and the middle third of the tail was placed in the groove on the automated tail-flick apparatus (Columbus Instruments, Columbus, OH). Radiant heat from a light was applied to the underside of the tail and the time it took (in seconds) for rats to withdraw their tail from the heat source was measured as their tail-flick latency. The intensity of the radiant heat was pre-set at 15 (approximately 60°C) throughout the experiment. An average of two baseline tail-flick latencies in all animals were recorded prior to drug administration. To prevent tissue damage, a cut-off time of 10°s was used.

ΔFosB Immunohistochemistry

Following behavioural testing on the final day animals were perfused with 4% paraformaldehyde (PFA). Brains were extracted, flash frozen and stored at −80°C. Fluorescence immunohistochemistry was performed as previously described (Perreault et al., 2012) on PFA-fixed floating coronal brain sections (30 μm). Free-floating sections from the PFC, Cg, NAc and VTA were washed in TBS (60.5 mM Tris, 87.6 mM NaCl pH 7.6), then blocked (10% goat serum, 1% BSA, 0.2% Triton-X, 1X TBS), and incubated with primary ΔFosB antibody (Cell Signalling, Catalogue #14695, 1:200) in buffer (2% goat serum, 0.01% Triton-X and 1X TBS) for 60 h at 4°C. Following incubation, the brain sections were washed in TBS, blocked (5% goat serum, 0.5% BSA, 0.01% Triton-X, 1X TBS) and incubated for 2 h at room temperature with a secondary anti-rabbit Alexa Fluor 488. After three washes in TBS, brain sections were mounted on slides with Prolong Gold (Thermo Fisher Scientific). Images were acquired by fluorescence microscopy (Etaluma Lumascope) with a 20X objective lens, and cell counting was performed to quantify the mean number of ΔFosB positive cells in two sections of each brain region.

Statistical Analysis

LFP power analysis was performed on 30 s epochs and is reported as means ± sem. taken every 5 min. For the coherence 30s epochs were analyzed at 30 min post-injection. Quantification of the data at each frequency measure and time point is reported as mean ± sem with spectral power curves presented as normalized data (to total power) with jackknife estimates of sem. For the power time courses a repeated measures ANOVA was performed for each frequency with Time as the within subject variable and Treatment as the between-subjects variable. In case of significant interactions or main effects, group comparisons at each time point were performed using a one-way ANOVA with Treatment as the between subjects variable and was followed by Tukey's *post-hoc* test. The Games-Howell post-hoc test was used to determine group mean differences if the data did not pass Levene's test for homogeneity of variance. Data were removed only if the signal quality was poor. No data were removed as a result of electrode misplacement. For the FST, the data are expressed as percent change from controls whereas the tail-flick data are expressed as percent change from baseline measures taken on each day (averaged between two readings). Data on each day were analyzed using a one-way ANOVA with Treatment as the between subjects factor followed by Tukey's post-hoc test for group comparisons. Paired t-tests were used to compare means on day 1 and day 7. For the ΔFosB data, group comparisons were performed using Student's t-test. Prior to all analyses, normality was assessed using the Shapiro-Wilk test.

RESULTS

To elucidate the dose-dependent antidepressant and analgesic effects of kratom, an alkaloid isolate was first prepared from mature kratom leaf material. The final Kratom alkaloid preparation (**Figure 1**) contained at least nine main annotated alkaloid species, of which seven were identified by comparison to commercially available standards: 1, mitraphylline; 2, 7-hydroxymitragynine; 4, ajmalicine; 5, speciociliatine; 6, paynantheine; 7, speciogynine, 8, mitragynine (**Figure 1**, **Supplementary Figure S2**). Alkaloids 3 and 10 eluted at 8.12 and 14.67 min, respectively, and were subsequently determined by ^{1}H NMR. The ^{1}H NMR spectra obtained for fractions 3 and 10 matched with those reported previously in the literature which were identified as 3-isoajmalicine and corynantheidine, respectively (**Figure 1**, **Supplementary Figure S3,S4**). This alkaloid isolate, *in toto*, was administered to rats and LFP recordings were taken to evaluate associated impacts on neural systems function, followed by assessments in the FST and tail-flick tests. These measures were evaluated following an acute

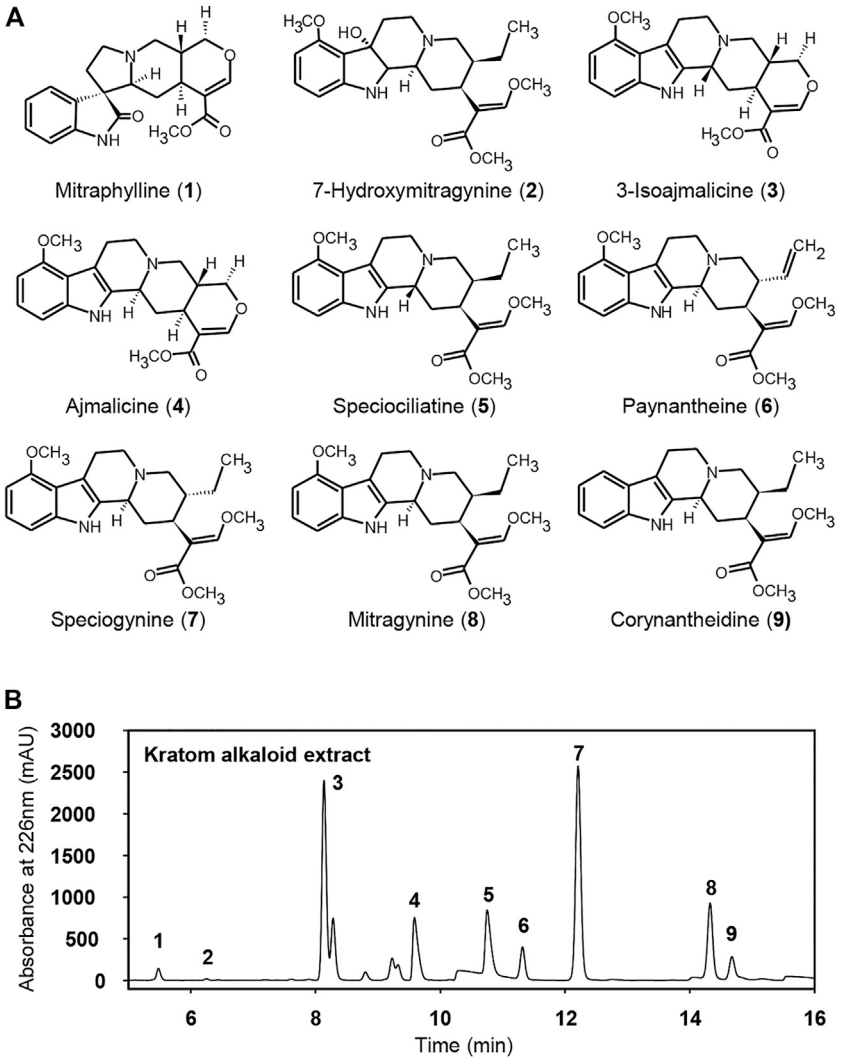

FIGURE 1 | Constituent alkaloids in whole kratom extract. **(A)** Compound structures and **(B)** HPLC chromatogram of the alkaloid profile of the kratom leaves extract at 226 nm. The peaks represent respectively: 1, mitraphylline; 2, 7-hydroxymitragynine; 3, 3-isoajmalicine; 4, ajmalicine; 5, speciociliatine; 6, paynantheine; 7, speciogynine, 8, mitragynine, and 9, corynantheidine.

injection of kratom and again following the repeated administration of the kratom isolate for 7 days. The experimental timeline is shown in **Figure 2A**.

Spectral Power

Nucleus Accumbens

Brain rhythms, or neuronal oscillations, are highly conserved across species, are coupled to specific behavioural states, and are a key indicator of the communication status between neurons (Buzsaki and Draguhn, 2004; Buzsáki et al., 2013). The low frequency bands, delta (0.5–4 Hz), theta (>4–8 Hz), and alpha (>8–12 Hz), are slow waves and are critical in long-distance or between region communication, whereas the high frequency bands, beta (>12–30 Hz) and gamma (>30 Hz), are fast waves that play a role in short-distance or within region communication (Buzsáki and Draguhn, 2004). Power spectra depicting changes in NAc oscillatory power 30 min post-injection on day 1 and day 7 are shown (**Figures 2B,C**) with quantification of the spectra at 5 min time points also depicted (**Figures 2D–H**). There were no baseline group differences in spectral power at any frequencies (**Figures 2D–H**, left panels). Administration of low dose (0.5 mg/kg) kratom had no effect on delta power on day 1 or day 7. However, acute administration of high dose (1 mg/kg) kratom induced a significant increase in delta power, compared to both the low dose group ($p < 0.001$) and saline controls ($p < 0.001$), across the 30 min time period (**Figure 2D**, left panel) [Time: $F(5,100) = 2.9$, $p = 0.016$; Treatment × Time: $F(10,100) = 2.4$, $p = 0.012$; Treatment: $F(2,20) = 29.4$, $p < 0.001$]. On day 7, prior to the last kratom injection baseline delta power was elevated in those rats that received high dose kratom ($p = 0.013$) indicating potentially lingering drug effects from the day 6 injection. Following the final administration of high

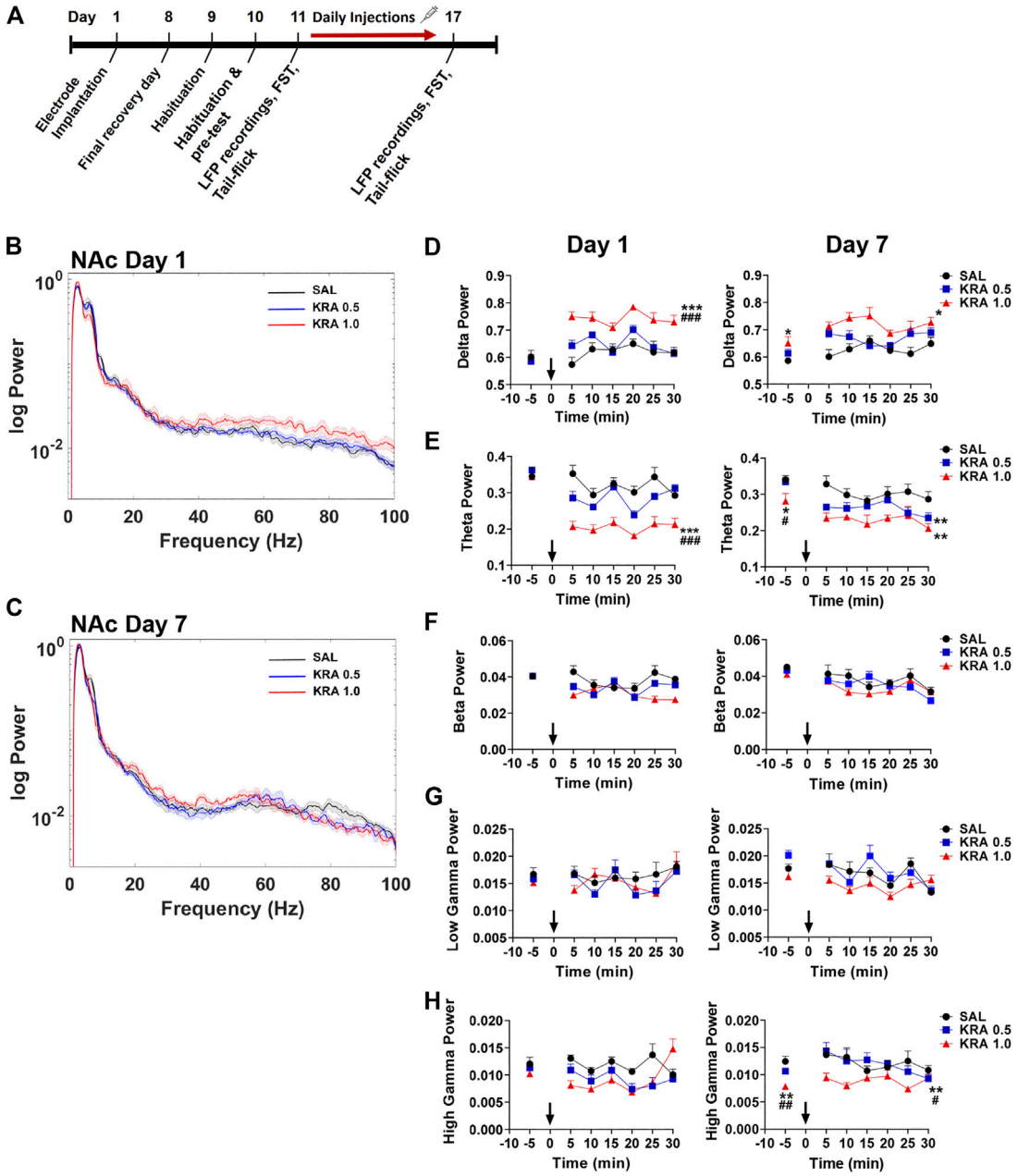

FIGURE 2 | Time course of dose-dependent effects of kratom on NAc spectral power. **(A)** Experimental timeline is shown. **(B,C)** Representative power spectra showing dose-dependent (0, 0.5, 1.0 mg/kg) effects of kratom 30 min post-injection on day 1 and day 7. **(D)** High dose (1.0 mg/kg) kratom increased delta power across 30 min on both day 1 and day 7 with lasting changes observed prior to the final injection of high dose kratom. **(E)** Only high dose kratom decreased theta power on day 1 whereas both doses of kratom reduced theta power on day 7. Prior to the final injection a baseline suppression in theta power was evident in response to the high dose of kratom. **(F,G)** No effects of kratom administration on beta or low gamma power were observed on day 1 or day 7. **(H)** On day 1, no kratom-induced changes in high gamma power were observed. On day 7, high dose kratom decreased baseline high gamma power. This decrease in response to high dose kratom persisted following the day 7 injection. $N = 7–10$ rats per group, with 2 electrodes/rat. Curves are represented as means with jackknife estimates of sem depicted by the shaded areas. Bars represent mean ± sem. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to saline control rats. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.01$ compared to low dose kratom treated rats.

dose kratom this increase was maintained across the recording period (**Figure 2D**, right panel) [Time: $F(5,150) = 4.1$, $p = 0.001$; Treatment × Time: $F(10,150) = 2.2$, $p = 0.020$; Treatment: $F(2,30) = 4.7$, $p = 0.016$].

Opposite to that observed with delta power, on day 1 only high dose kratom significantly decreased theta power across the 30-min testing period when compared to low dose kratom or saline controls ($p < 0.001$, **Figure 2E**, left panel). On day 7, a baseline

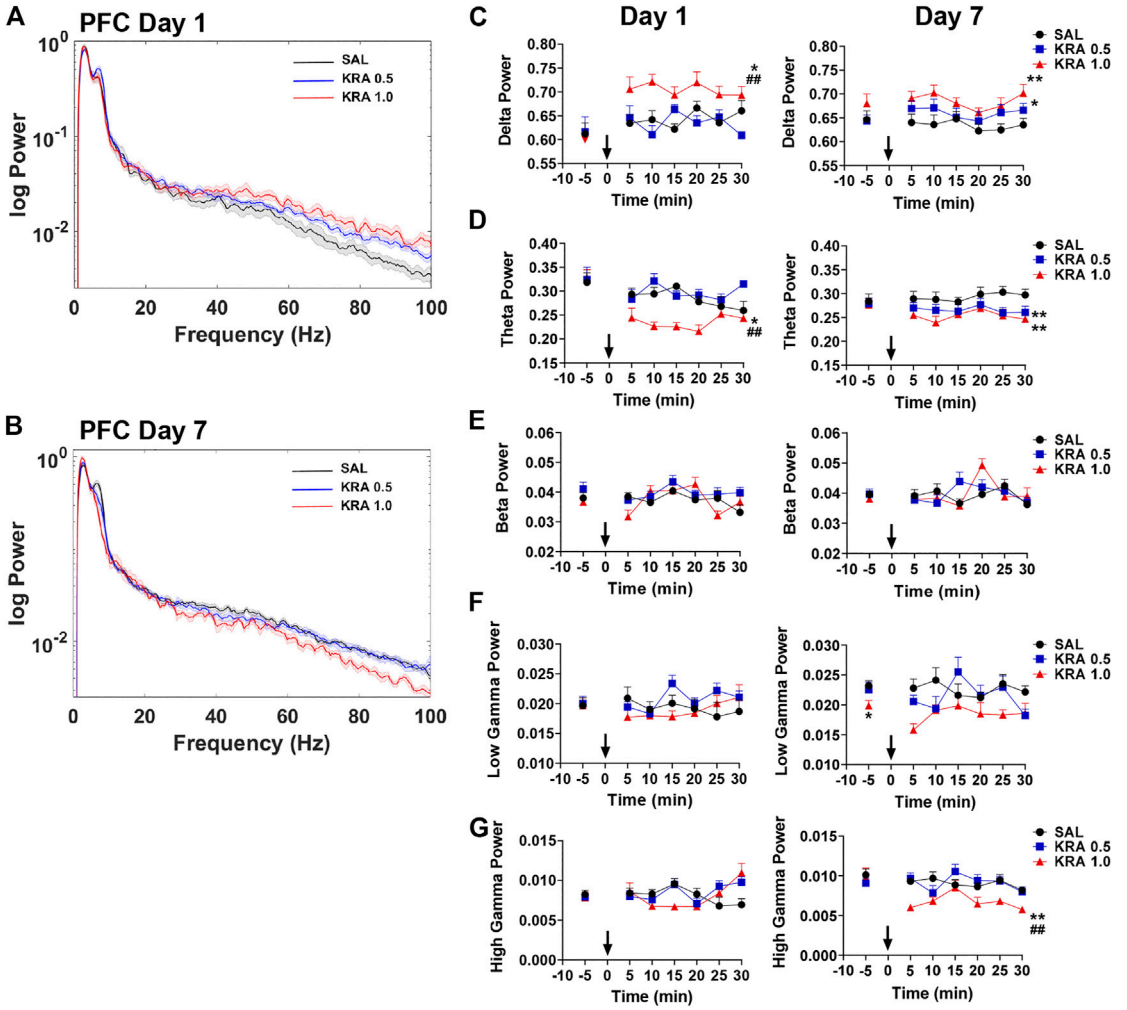

FIGURE 3 | Time course of dose-dependent effects of kratom on PFC spectral power. **(A,B)** Representative power spectra showing dose-dependent (0, 0.5, 1.0 mg/kg) effects of kratom 30 min post-injection on day 1 and day 7. **(C)** High dose kratom increased delta power on day 1 and day 7 whereas low dose kratom increased delta power on day 7. **(D)** Reduced theta power in response to only high dose kratom on day 1. On day 7, decreased theta power was shown in response to both doses of kratom. **(E,F)** No drug-induced changes were observed in beta or low gamma power on day 1 or day 7. **(G)** On day 1, kratom had no effects on high gamma power. Reduced high gamma power was, however, evident on day 7 in response to high dose kratom. $N = 7–10$ rats per group, with 2 electrodes/rat. Curves are represented as means with jackknife estimates of sem depicted by the shaded areas. Bars represent mean ± sem. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to saline control rats. $^{\#\#}p < 0.01$ compared to low dose kratom treated rats.

suppression in theta power was evident in the high dose kratom group compared to both the low dose kratom ($p = 0.038$) and control group ($p = 0.013$), an effect strengthened after the final injection of high dose kratom that was maintained ($p = 0.003$ versus controls, **Figure 2E**, right panel). Reduced theta power was also evident in the low dose group, however this effect was short-lived, only evident at 5 min post-injection ($p = 0.001$, compared to saline controls) [Time: $F(5,105) = 3.6$, $p = 0.005$; Time × Treatment: $F(10,105) = 3.8$, $p < 0.001$; Treatment: $F(2,21) = 10.4$, $p = 0.001$]. In the NAc on day 1 and day 7, there were no significant effects of treatment in the beta and low gamma frequency bands in response to either dose of kratom (**Figures 2F,G**). Similarly, there were no observed effects in the high gamma frequency band on day 1 (**Figure 2H**, left panel). However, on day 7, baseline recordings showed that animals that had received repeated administration of high dose kratom had lower baseline high gamma power compared to the low dose group ($p = 0.006$) or saline controls ($p = 0.002$) (**Figure 2H**, right panel). Post-injection, this decrease in high gamma power was maintained throughout the recording period such that high dose kratom reduced high gamma power compared to both the lower dose ($p = 0.03$) of kratom and saline controls ($p = 0.006$) [Treatment: $F(2,22) = 6.4$, $p = 0.007$]. Together these findings indicate significant effects of acute and chronic high dose kratom on NAc low frequency power, with an additional suppression of high gamma power selectively with repeated administration.

Prefrontal Cortex

In the PFC, representative power spectra showing the effects of kratom 30 min post-injection on day 1 and day 7 are shown

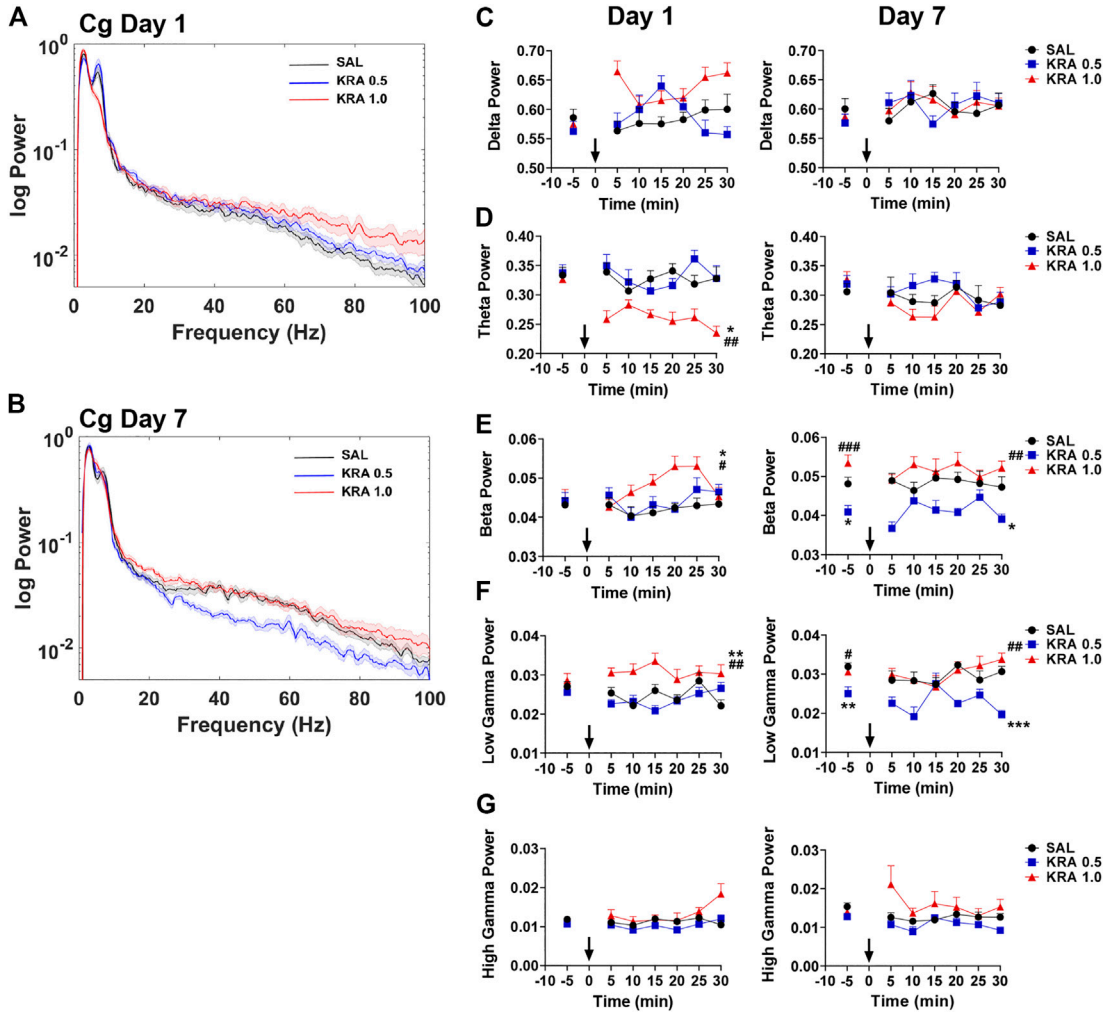

FIGURE 4 | Time course of dose-dependent effects of kratom on Cg spectral power. **(A,B)** Representative power spectra showing dose-dependent (0, 0.5, 1.0 mg/kg) effects of kratom 30 min post-injection on day 1 and day 7. **(C)** No kratom-induced changes in delta power were evident on day 1 or day 7 in the Cg. **(D)** High dose kratom supressed theta power in on day 1 with no changes evident on day 7. **(E)** High dose kratom increased beta power on day 1. On day 7, reduced baseline beta power was observed in response to low dose kratom, and this change persisted following the final injection. **(F)** High dose kratom elevated low gamma power on day 1. Low dose kratom decreased baseline low gamma power on day 7 and this effect was maintained after the last injection. **(G)** No effects of kratom treatment were observed in the high gamma frequency band. N = 7–10 rats per group, with 2 electrodes/rat. Curves are represented as means with jackknife estimates of sem depicted by the shaded areas. Bars represent mean ± sem. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to saline control rats. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.01$ compared to low dose kratom treated rats.

(**Figures 3A,B**). In this region, the repeated measures ANOVA revealed a significant effect of Treatment on delta power on both day 1 and day 7 (**Figure 3C**) [Day 1: Treatment: $F(2,26) = 10.0$, $p = 0.001$; Day 7: Treatment: $F(2,20) = 8.0$, $p = 0.003$]. Overall, only the high dose kratom increased delta power on day 1, relative to the low dose kratom ($p = 0.001$) and saline controls ($p = 0.012$) (**Figure 3C**, left panel). However, on day 7, both low and high dose kratom were found to significantly increase delta power across 30 min ($p = 0.019$ and $p = 0.003$, respectively) (**Figure 3C**, right panel). Opposite to the observed delta power changes, a significant decrease overall in theta power was induced by high dose kratom on day 1 in the PFC compared to low dose kratom ($p < 0.001$) and controls ($p = 0.014$), with no effects of low dose kratom (**Figure 3D**, left panel) [Time × Treatment: $F(10,140) = 2.0$, $p = 0.036$; Treatment: $F(2,28) = 12.6$, $p < 0.001$]. On day 7, however, both low ($p < 0.001$) and high ($p = 0.002$) dose kratom suppressed theta power across the recording period (**Figure 3D**, right panel) [Treatment: $F(2,23) = 11.4$, $p < 0.001$]. There were no effects of kratom administration on spectral power in the beta frequency band on either day (**Figure 3E**). Similarly, no observed drug effects in low or high gamma power were evident on day 1 (**Figures 3F,G**, left panels). However, baseline data taken prior to the day 7 injection showed that animals had received prior treatment with high dose kratom had suppressed low gamma power ($p = 0.029$) compared to saline controls (**Figure 3F**, right panel). Following the day 7 injection, high dose kratom suppressed high gamma power compared to both low dose kratom and control groups ($p < 0.01$) (**Figure 3G**, right panel)

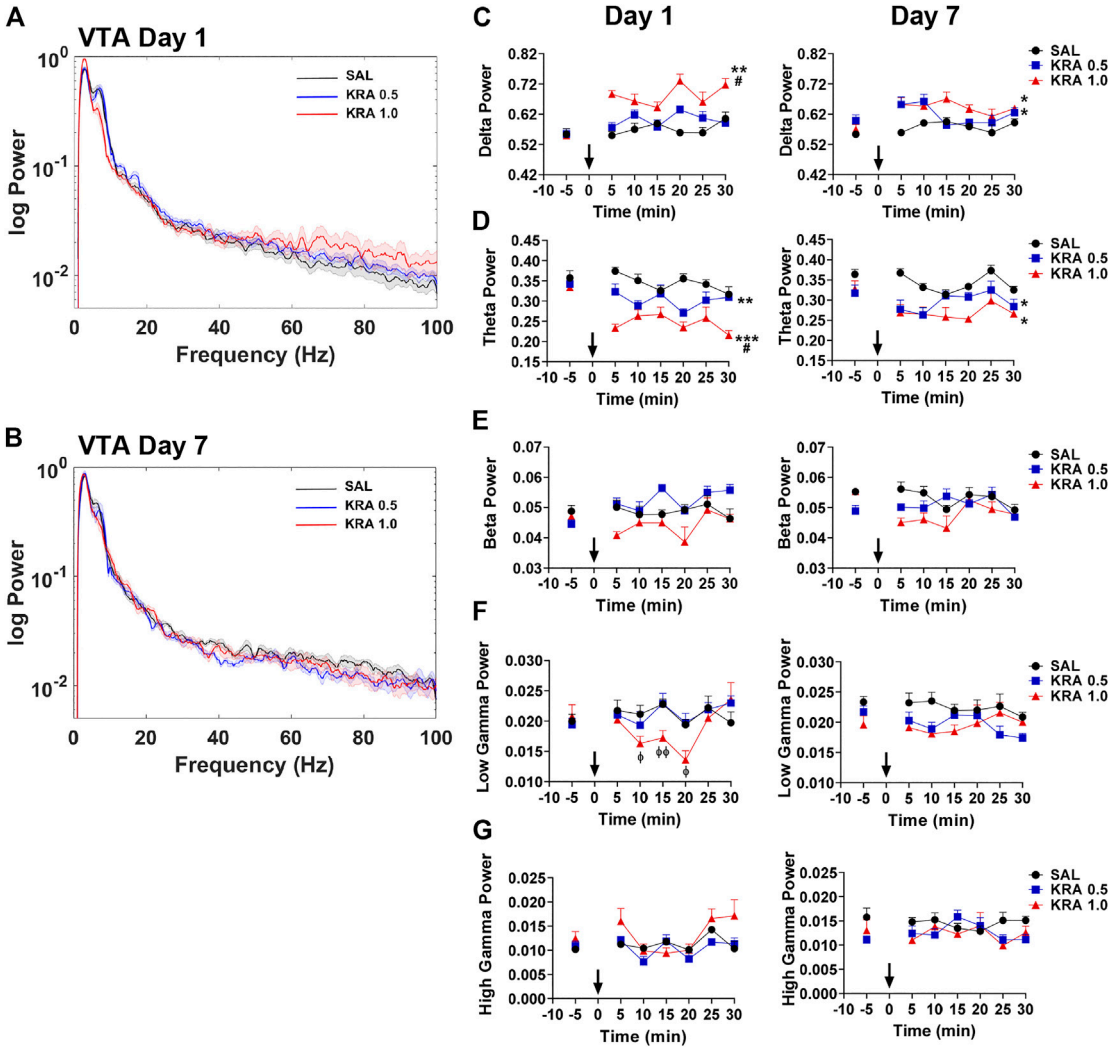

FIGURE 5 | Time course of dose-dependent effects of kratom on VTA spectral power. **(A,B)** Representative power spectra showing dose-dependent (0, 0.5, 1.0 mg/kg) effects of kratom 30 min post-injection on day 1 and day 7. **(C)** On day 1, high dose kratom increased delta power whereas on day 7, an elevation in power was induced by both doses of kratom. **(D)** On both day 1 and day 7, both doses of kratom resulted in a reduction in theta power. **(E)** No drug-induced changes in beta power were evident. **(F)** A transient decrease in the low gamma power in response to only high dose kratom on day 1 with no effects on day 7 **(G)** No effects of kratom treatment on high gamma power on day 1 or day 7. $N = 7$–10 rats per group, with 2 electrodes/rat. Curves are represented as means with jackknife estimates of sem depicted by the shaded areas. Bars represent mean ± sem. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to saline control rats. $^{\#}p < 0.05$ compared to low dose kratom treated rats.

[Treatment: F(2,25) = 7.3, p = 0.003]. Overall, similar to that observed in NAc, these findings demonstrate prominent effects of kratom in the low frequency range in the PFC, with additional effects to suppress high gamma power.

Cingulate Cortex

Kratom-induced changes in neural oscillatory power in the Cg were next evaluated (**Figure 4**), with representative power spectra at 30 min displayed in **Figures 4A,B**. On the first day of testing, a repeated measures ANOVA revealed a significant interaction between Time and Treatment in delta power within the Cg [delta: F(10,115) = 2.2, p = 0.022]. Overall, no significant group differences were found on either day (**Figure 4C**). For the theta frequency band, high dose kratom induced a significant decrease in oscillatory power on day 1, compared to both low (p = 0.007) dose kratom and saline controls (p = 0.011) [Treatment: F(2,23) = 6.5, p = 0.006], but not on day 7 (**Figure 4D**, right panel). When beta power was examined, significant effects of Treatment were observed on both days [beta day 1: Treatment: F(2,23) = 5.2, p = 0.014; beta day 7: Treatment: F(2,20) = 10.9, p < 0.001]. On day 1, high dose kratom significantly increased beta power compared to low dose kratom (p = 0.012) and controls (p = 0.028) (**Figure 4E**, left panel). On day 7, baseline differences in beta power were observed, such that animals that received low dose kratom repeatedly prior to the final day of testing had a significantly reduced baseline beta power (p = 0.019). Following

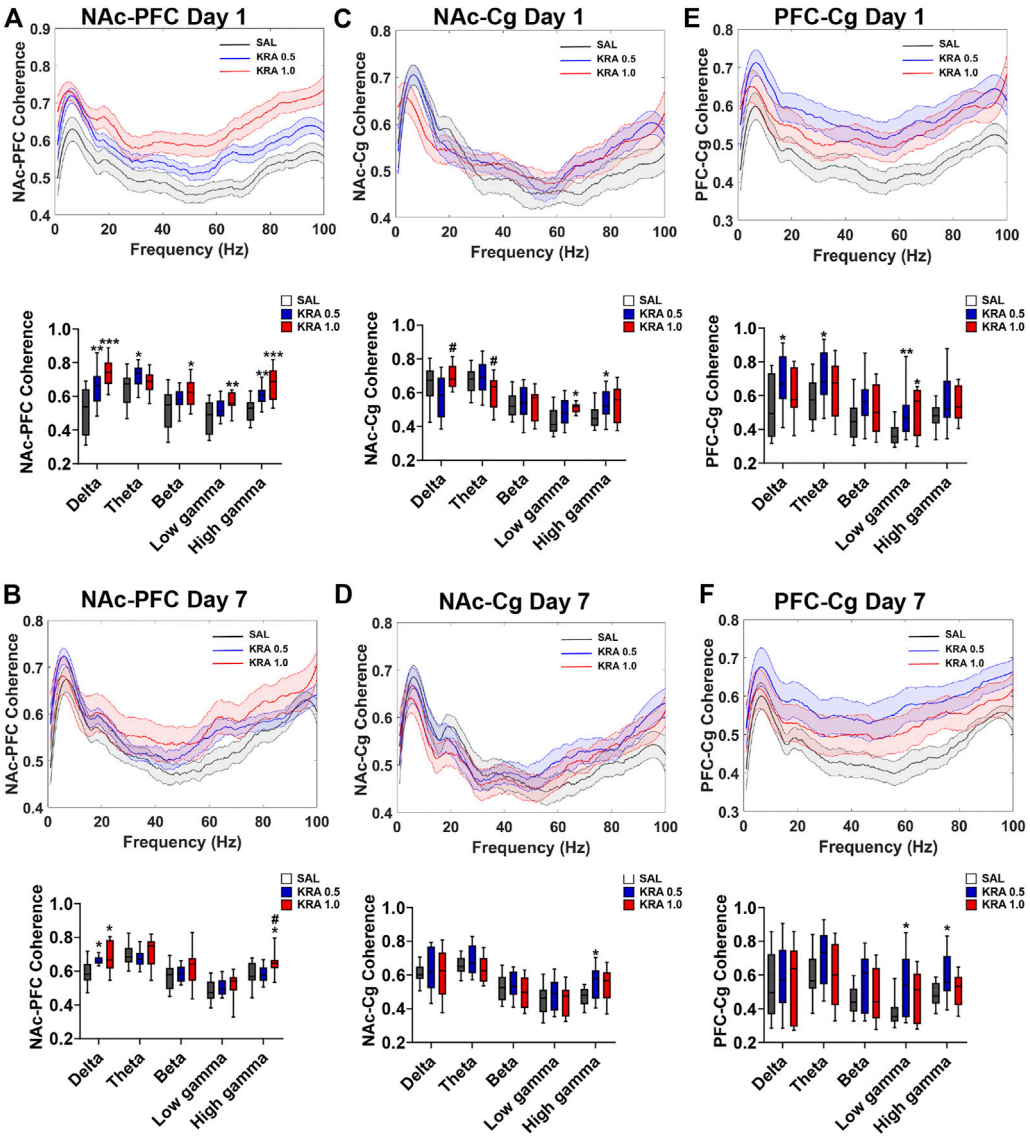

FIGURE 6 | Dose-dependent effects of acute and repeated kratom administration on NAc-PFC, NAc-Cg, and PFC-Cg coherence. Coherence across frequencies and quantification showing the effect of acute and repeated low (0.5 mg/kg) or high (1 mg/kg) dose kratom 30 min post-injection. Data shown were taken from 30 s epochs. **(A)** On day 1, both doses of kratom increased NAc-PFC delta and high gamma coherence. Low dose kratom increased theta coherence and high dose kratom increased beta and low gamma coherence. **(B)** On day 7, both doses of kratom increased delta coherence whereas only high dose kratom induced an increase in high gamma coherence. **(C)** On day 1, high dose kratom increased low gamma NAc-Cg coherence whereas low dose kratom increased high gamma coherence. **(D)** NAc-Cg coherence was increased in the high gamma frequency band only in response to low dose kratom on day 7. **(E)** Low dose kratom increased low frequency and low gamma coherence between the PFC-Cg. High dose kratom also increased coherence in the low gamma frequency band on day 1. **(F)** On day 7, only low dose kratom elevated PFC-Cg low and high gamma coherence. $N = 7–10$ rats per group, with 2 electrodes/rat. Curves are represented as means with jackknife estimates of sem depicted by the shaded areas. Quantification of coherence are represented as boxplots with min/max values. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to saline control rats. $^{\#}p < 0.05$ compared to low dose kratom treated rats.

the final injection, this decrease in beta power persisted across the 30 min testing period ($p = 0.017$) (**Figure 4E**, right panel). As well, in the Cg, following an acute injection of high dose kratom, a significant increase in low gamma power was observed (**Figure 4F**, left panel), relative to low dose kratom ($p = 0.001$) and saline control ($p = 0.004$) groups [Treatment: $F(2,21) = 10.6$, $p = 0.001$]. On day 7, low dose kratom decreased low gamma power baseline measures ($p = 0.008$) and, following the final injection, this effect was maintained ($p < 0.001$) (**Figure 4F**, right panel) [Treatment: $F(2,20) = 11.6$, $p < 0.001$]. Overall, in the high gamma frequency band no significant group differences were found on day 1 or day 7 (**Figure 4G** [Time × Treatment: $F(10,115) = 3.3$, $p = 0.001$]. These findings indicate that, unlike NAc and PFC, the effects of kratom in the Cg appear restricted to the theta and low gamma frequency bands.

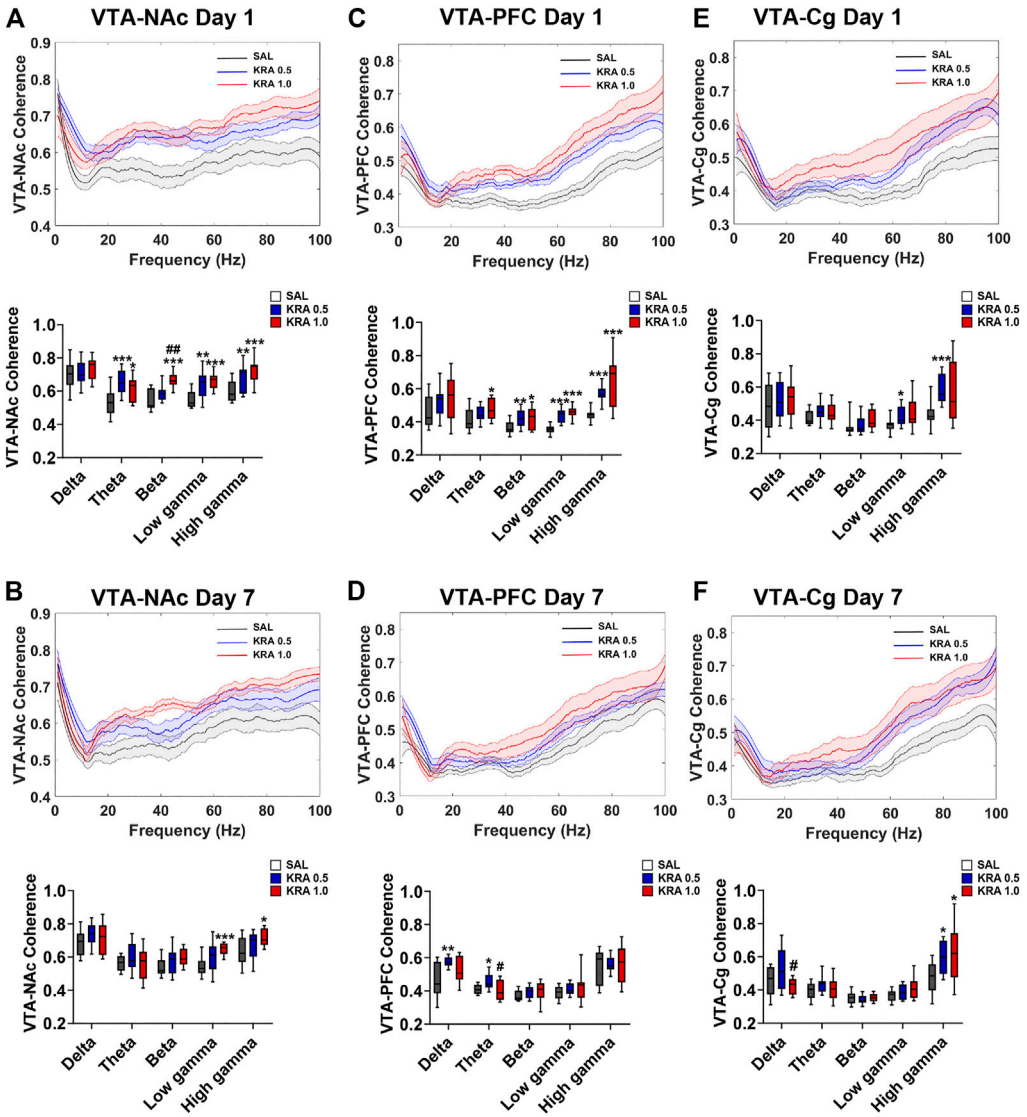

FIGURE 7 | Dose-dependent effects of acute and repeated kratom administration on VTA-NAc, VTA-PFC, and VTA-Cg coherence. Coherence across frequencies and quantification showing the effect of acute and repeated low (0.5 mg/kg) or high (1 mg/kg) dose kratom 30 min post-injection. Data shown were taken from 30 s epochs. **(A)** High dose kratom increased VTA-NAc beta coherence whereas theta, low gamma and high gamma coherence was increased by both doses of kratom. **(B)** On day 7, only high dose kratom increased low and high gamma coherence between VTA-NAc. **(C)** VTA-PFC coherence was increased in the high frequency bands in response to both doses of kratom. In the theta band, only high dose kratom increased coherence on day 1. **(D)** On day 7, repeated injections of low dose kratom increased VTA-PFC low frequency coherence. **(E)** An acute injection of low dose kratom elevated VTA-Cg low and high gamma coherence. **(F)** Repeated injections of both doses of kratom increased high gamma coherence between VTA-Cg. *N* = 7–10 rats per group, with 2 electrodes/rat. Curves are represented as means with jackknife estimates of sem depicted by the shaded areas. Quantification of coherence are represented as boxplots with min/max values. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to saline control rats. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to low dose kratom treated rats.

Ventral Tegmental Area

Alterations in VTA spectral power 30 min following acute or repeated kratom injections are depicted in **Figures 5A,B**. Only an acute injection of high dose kratom resulted in elevated delta power compared to low dose kratom ($p = 0.015$) and control ($p = 0.001$) groups (**Figure 2**, **Figure5C**, left panel) [Time x Treatment: $F(10,115) = 1.9$, $p = 0.045$; Treatment: $F(2,23) = 9.9$, $p = 0.001$]. On the final day of testing both high ($p = 0.018$) and low ($p = 0.041$) dose kratom elevated delta power (**Figure 5C**, right panel) [Time: $F(5,100) = 3.5$, $p = 0.006$; Time x Treatment: $F(10,100) = 2.0$, $p = 0.038$; Treatment: $F(2,20) = 5.0$, $p = 0.018$]. Similar to the observed effects of acute high dose kratom on theta power in the NAc, PFC and Cg, a significant decrease in VTA theta power was shown ($p < 0.001$) (**Figure 5D**, left panel). A similar, albeit less robust, effect was observed in response to low dose kratom a ($p = 0.006$) [Treatment: $F(2,26) = 16.4$, $p < 0.001$]. On day 7, these dose-dependent effects were maintained (**Figure 5D**, right panel) [Time: $F(5,105) = 5.2$, $p < 0.001$;

Treatment: $F(2,21) = 5.2$, $p = 0.014$]. There were no observed changes in beta power in response to kratom administration on either day of testing (**Figure 5E**). Repeated measures ANOVA revealed time-dependent changes in low gamma power in response to high dose kratom whereby there was a transient decrease in low gamma power that normalized by the end of the 30 min (**Figure 5F**, left panel) [Time: $F(5,115) = 3.7$, $p = 0.004$; Time x Treatment: $F(10,115) = 4.4$, $p < 0.001$]. There were no group differences in low gamma power on day 7. For high gamma power, no drug effects were evident on day 1 or 7, although a Treatment effect was evident (**Figure 5G**, right panel) [Treatment: $F(2,21) = 5.7$, $p = 0.011$]. Thus, the effects of kratom in the VTA were restricted to the low frequency bands delta and theta.

Coherence

To evaluate the effect of both acute and repeated kratom administration on inter-regional communication, coherence was assessed on the first and final day of testing, just prior to behavioural testing (**Figures 6**, **7**). On the first day of testing, a Main Effect of Treatment in the NAc-PFC was evident in all frequencies (**Figure 6A**) [delta: $F(2,41) = 13.2$, $p < 0.001$; theta: $F(2,40) = 4.3$, $p = 0.02$; beta: $F(2,43) = 4.1$, $p = 0.02$; low gamma: $F(2,42) = 5.6$, $p = 0.007$; high gamma: $F(2,44) = 15.0$, $p < 0.001$]. In the delta and high gamma bands, both low ($p = 0.005$) and high ($p < 0.001$) doses of kratom resulted in elevated coherence in comparison to the control animals. In the theta frequency, only low dose kratom ($p = 0.015$) increased coherence relative to controls. On day 7, this elevated NAc-PFC coherence was evident only in the delta and high gamma frequencies (**Figure 6B**) [delta: $F(2,32) = 6.0$, $p = 0.006$; high gamma: $F(2,37) = 4.3$, $p = 0.02$]. However, while both low ($p = 0.015$) and high ($p = 0.03$) doses of kratom increased coherence in the delta band, only the high dose increased high gamma coherence ($p = 0.04$).

Upon examination of NAc-Cg coherence, a significant Treatment Effect of kratom was found in the low frequencies and in the gamma frequencies on the first day of testing (**Figure 6C**). [delta: $F(2,39) = 4.9$, $p = 0.013$; theta: $F(2,43) = 3.4$, $p = 0.04$; low gamma: $F(2,39) = 4.0$, $p = 0.027$; high gamma: $F(2,41) = 3.4$, $p = 0.04$). On day 1, low dose ($p = 0.014$) and high dose ($p = 0.014$) kratom increased coherence in high and low gamma bands, respectively compared to controls. This increased coherence in the high gamma band following administration of low dose kratom was maintained on day 7 ($p = 0.02$) (**Figure 6D**) [Treatment Effect in high gamma on day 7; $F(2,39) = 3.5$,$p = 0.038$]. When PFC-Cg coherence was examined, a significant kratom Treatment effect on day 1 was found in the delta and theta bands, as well as the low gamma frequency (**Figure 6E**) [delta: $F(2,42) = 4.8$, $p = 0.014$; theta: $F(2,45) = 3.5$, $p = 0.04$; low gamma: $F(2,40) = 4.7$, $p = 0.015$]. In the low gamma frequency, both low ($p = 0.007$) and high ($p = 0.016$) doses of kratom increased coherence. On day 7, the increase in low gamma coherence as a result of kratom treatment persisted, but only for the low dose ($p = 0.014$) (**Figure 6F**) [Treatment Effect in low gamma on day 7: $F(2,38) = 3.8$, $p = 0.03$].

A significant effect of Treatment in both VTA-NAc coherence and VTA-PFC coherence on day 1 was found in the theta, beta, low gamma and high gamma frequencies [VTA-NAc: theta: $F(2,41) = 10.2$, $p < 0.001$; beta: $F(2,35) = 18.6$, $p < 0.001$; low gamma: $F(2,38) = 11.3$, $p < 0.001$; high gamma: $F(2,40) = 10.2$, $p < 0.001$][VTA-PFC: theta: $F(2,40) = 4.0$, $p = 0.025$; beta: $F(2,42) = 6.1$, $p = 0.005$; low gamma: $F(2,39) = 28.6$, $p < 0.001$; high gamma: $F(2,38) = 15.9$, $p < 0.001$]. Examining VTA-NAc coherence (**Figure 7A**) on day 1, both low ($p < 0.001$) and high ($p = 0.017$) doses of kratom increased theta coherence. In the beta band, there was a significant increase in coherence in response to the high dose kratom, relative to the low dose group ($p = 0.001$) and controls ($p < 0.001$). When low gamma coherence was assessed, both low and high dose kratom ($p = 0.002$, $p < 0.001$ respectively) increased coherence. Similarly, an increase in high gamma coherence was observed in response to both doses of kratom ($p = 0.009$ for low dose and $p < 0.001$ for high dose kratom). An effect of Treatment in VTA-NAc coherence persisted at day 7 in the gamma frequency bands (**Figure 7B**) [low gamma: $F(2,36) = 7.0$, $p = 0.003$; high gamma: $F(2,360) = 3.9$, $p = 0.028$]. Finally, when assessing VTA-PFC coherence (**Figure 7C**) on day 1 in the beta frequency, there was an increase in beta coherence in response to the low ($p = 0.002$) and high ($p = 0.039$) doses of kratom. In the low gamma band, both kratom doses increased coherence ($p < 0.001$ and $p < 0.001$ respectively). Similarly, a significant increase in high gamma coherence was observed in response to both doses of kratom ($p < 0.001$ for both doses). The kratom Treatment effects that were apparent on day 1 in VTA-PFC coherence were not maintained following the repeated administration of kratom (**Figure 7D**).

A Treatment effect in VTA-Cg coherence was evident in the low and high gamma frequency bands following an acute injection of kratom (**Figure 7E**) [low gamma: $F(2,42) = 3.6$, $p = 0.036$; high gamma: $F(2,39) = 5.5$, $p = 0.008$]. In the high gamma band specifically, only the low dose of kratom increased coherence ($p < 0.001$). On day 7, there was again an effect of Treatment in the high gamma band [$F(2,39) = 4.4$, $p = 0.019$] with increased coherence following administration of low dose kratom ($p = 0.012$) (**Figure 7F**).

Behaviour

To investigate the potential antidepressant-like and analgesic effects of kratom, dose-dependent drug effects were evaluated first in the FST, followed by the tail-flick test, immediately after the LFP recordings (**Figure 8A**). Following a single injection of kratom, we found that low dose kratom significantly reduced immobility in the FST compared with saline controls (88.8 ± 25.5 *versus* 176.8 ± 40.1, $p < 0.001$) ($F(2,30) = 16.3$, $p < 0.001$). This selective decrease in immobility was again apparent following daily administration of low dose kratom for 7 days (165.4 ± 31.3 *versus* 239.1 ± 35.6, $p < 0.001$) ($F(2,28) = 22.0$, $p < 0.001$). There were no effects of high dose kratom on immobility time in the FST. However, it should be noted that the variability of the high dose group on day 1 was much greater than that observed on day 7. Further, between day 1 and day 7, there was an overall increase in FST immobility across all groups (approximately 52%) that was likely representative of learned behaviour. However, the direction and magnitude of

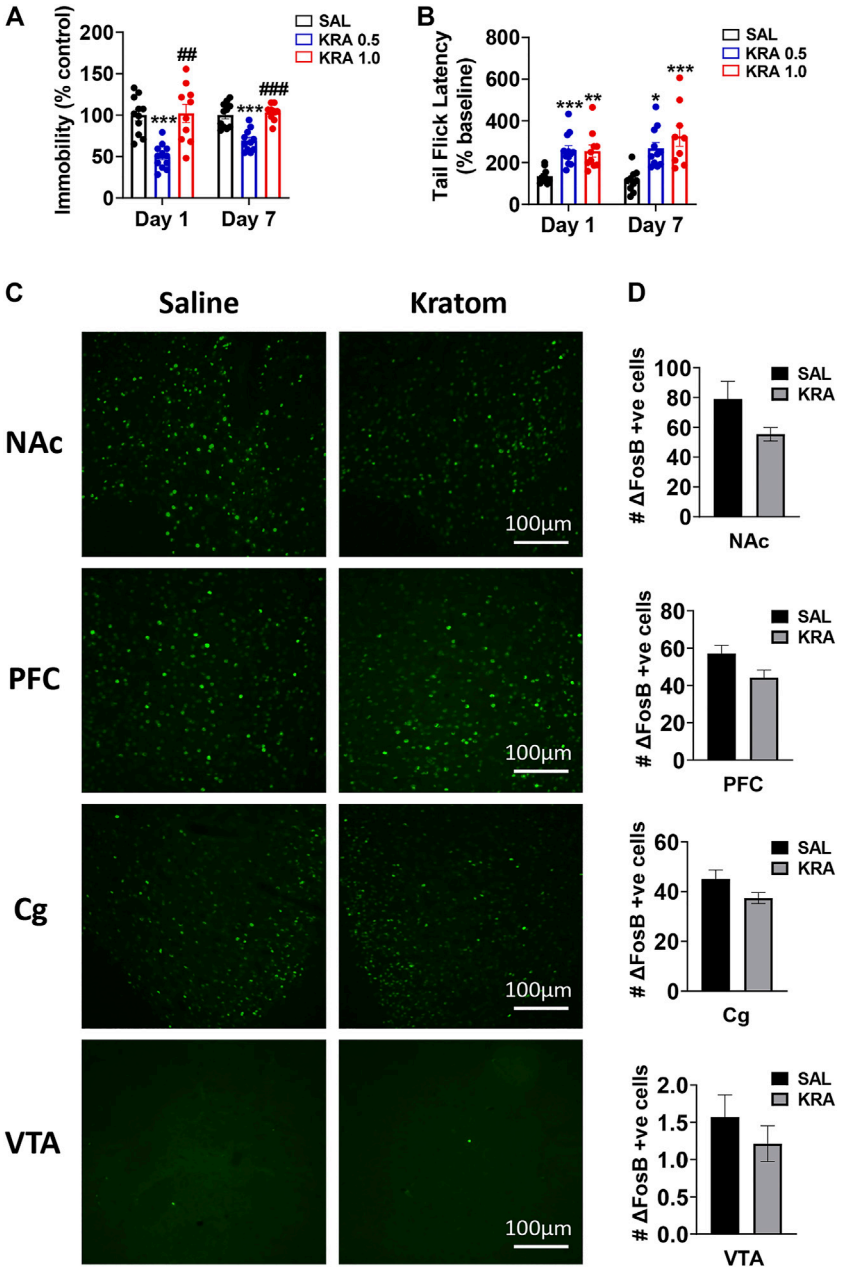

FIGURE 8 | Effects of kratom on behaviour and ΔFosB expression in rats. **(A)** Low (0.5 mg/kg) dose, but not high (1 mg/kg) dose kratom significantly reduced immobility time in the FST 30 min post-injection on both day 1 and day 7. **(B)** Both doses of kratom significantly increased tail-flick latencies compared to pre-drug baseline latencies 40 min post-acute and repeated injections. *N* = 10–13 rats per group. **(C,D)** Representative images and quantification depicting no change in ΔFosB expression in response to high dose kratom in any brain region. *N* = 7-8 rats per group. Bars represent mean ± sem. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to saline control rats. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to low dose kratom treated rats.

group differences were maintained between both days of testing. Next, we evaluated the analgesic efficacy of both doses of kratom using the tail-flick test at 40 min post-injection (**Figure 8B**). On both days of testing, animals treated with either dose of kratom showed increased tail-flick latencies, when compared with baseline values. On day 1, significant analgesic effects were observed for both low dose ($p < 0.001$) and high dose ($p = 0.003$) kratom ($F(2,30) = 10.5$, $p < 0.001$). Following repeated administration, both doses again displayed significant analgesic effects ($p = 0.011$ for low dose kratom, $p < 0.001$ for high dose kratom) ($F(2,28) = 10.5$, $p < 0.001$).

ΔFosB Expression

There has been some evidence to suggest that 7-HMG, one of the compounds found in kratom, has addictive properties (Hemby et al., 2019). As the transcription factor ΔFosB has been suggested

as a molecular switch for addiction (Nestler et al., 2001), the impact of repeated treatment with high dose kratom on expression of ΔFosB was next evaluated (**Figures 8C,D**). The high dose was chosen as the mean analgesic response was slightly higher in this group, with some animals showing the strongest analgesic responses (up to 600% increase in tail-flick latency from baseline). The ANOVA revealed a significant Main Effect of Treatment [$F(3,48) = 9.6$, $p = 0.003$]. Within each region there were no significant drug effects although trends towards reduced ΔFosB expression were evident that were strongest in the PFC ($p = 0.057$, **Figure 8D**).

DISCUSSION

In the present study, we sought to evaluate the dose-dependent analgesic and antidepressant-like effects induced by the kratom alkaloid extract in rats, and to elucidate changes in neural oscillatory patterns following acute and repeated drug administration. We found that only the low dose of kratom resulted in antidepressant-like effects, reducing the immobility time in the FST after both acute and repeated administration. In addition, both doses of kratom demonstrated analgesic properties in the tail-flick test upon initial administration as well as following repeated dosing. These behavioural effects were accompanied by a dose-and region-specific elevation in delta power and the suppression of theta and high gamma power. Further, enhanced coherence between all brain regions in response to both doses of kratom were shown on the first and final day of testing, with the most robust effects observed in the NAc-PFC, VTA-NAc, and VTA-Cg pathways. No significant changes in the expression of the addiction marker ΔFosB were evident in any of the brain regions following repeated administration of the high dose of kratom.

Taken together, the analgesic activity and antidepressant potential of kratom alkaloids that were observed in this study support anecdotal evidence surrounding the use of the plant among mainstream kratom enthusiasts—indeed, the consumption of kratom as a self-treatment strategy for the relief of acute and chronic pain are on the rise, especially in the United States (Kruegel and Grundmann, 2018; Veltri and Grundmann, 2019; Palamar, 2021). Our findings also underscore two additional important points, the first being that the alkaloid composition in kratom can vary depending on the growth conditions. For example, although mitragynine is often reported to be the most abundant alkaloid in kratom, our evidence shows that this is not always the case. Indeed, we showed that the alkaloids 3-isoajmalicine and speciogynine were twice as abundant than mitragynine in our samples. The second point is that the biological effects of kratom as a whole may be different then that of its innate chemical constituents, a noteworthy consideration as the pharmacology of kratom has traditionally focused on the individual alkaloids that typically accumulate within the plant. Accordingly, and with mixed results, these studies have often not considered how the suite of kratom alkaloids behave in a biological system. This latter point is especially relevant considering that dried kratom leaves or a decoction of the alkaloids therein is the primary mode of consumption among end users.

Oscillatory Changes Accompanying the Analgesic Effects of Kratom

To date, a limited number of experiments conducted thus far have demonstrated the analgesic efficacy of the entire kratom extract, with all of its alkaloids present. Therefore, we evaluated the analgesic effects of two doses of kratom in the tail-flick test, a behavioural test used to assess heat-evoked pain in animals. We found that both the low and high doses of kratom increased tail-flick latencies on the first and final day of injections, suggesting analgesic effects. These findings are consistent with studies that also have investigated the effect of kratom extracts on the behavioural output in the tail-flick test (Sabetghadam et al., 2010), as well as in the hot plate test, another behavioural measure of analgesia in rodents (Reanmongkol et al., 2007). It is important to note however, that one study reported analgesic effects in the hot plate test but not the tail-flick test in response to kratom (Reanmongkol et al., 2007). This may be due to differences in extract preparation and origin of the kratom leaf (Sabetghadam et al., 2010). Further, some authors have postulated that a reason for this difference could be due to the different components involved in both the hot plate and tail-flick test whereby supraspinal pathways and spinal pathways are involved, respectively (Matsumoto et al., 2004; Reanmongkol et al., 2007).

The present findings demonstrated oscillatory changes within the VTA and NAc in response to both doses of kratom, two regions of the mesolimbic dopamine system, a pathway that has been found to be activated in response to acute pain as well as pain relief (Borsook et al., 2016), and regions that play important roles in mediating the rewarding and analgesic effects of opioids (Wise, 1989; Harris and Peng, 2020). These regions in particular are suggested to be involved in pain processing, as the offset of pain has been found to be rewarding (Borsook et al., 2016; Harris and Peng, 2020). This idea is supported through clinical neuroimaging studies conducted in individuals suffering from chronic pain (Wood et al., 2007) as well as in individuals presented with noxious stimuli (Becerra et al., 2001; Becerra and Borsook, 2008) where activation of the mesolimbic network is observed. Therefore, this pathway is considered an essential target for the treatment of pain as its activation is believed to induce analgesic effects and may modulate the effectiveness of analgesic medications (Mitsi and Zachariou, 2016; Taylor et al., 2016; Kami et al., 2018).

Overall, in the NAc and VTA kratom administration induced an increase in delta power that was concomitant with a reduction in theta power. Interestingly, these findings are similar to another preclinical study using rats that found reduced theta power in the NAc and increased delta power in the VTA following morphine administration (Ahmadi Soleimani et al., 2018). Morphine is an established analgesic that is similar to the kratom alkaloids mitragynine and 7-HMG, in that it binds to the MOR to exert its effects (Ream and Michael, 2011; Kruegel et al., 2016). Therefore, it is possible that the similarities in the

drug-induced electrophysiological patterns may play a role in the reported analgesic properties of these compounds. Our observed changes in NAc, however, were not in agreement with a study conducted by Cheaha et al. (2015) that showed an absence of oscillatory changes in NAc of mice when administered 80 mg/kg of an alkaloid enriched kratom extract. Aside from the species used in the studies, this discrepancy likely results from differences in the extract used. Specifically, whereas the extract from Cheaha et al. (2015) was enriched with mitragynine, the extract used herein showed significant levels of other alkaloids, two of which that were in greater abundance than mitragynine. Indeed, we have previously shown that synthetic mitragynine has no effects on NAc oscillations (Thériault et al., 2020). Differences in the composition of the extract are also exemplified by the dose used, with their dose as much as 160 times higher than the one used in the present study. Together these findings do highlight, however, that differences in plant composition potentially produce discrete and significant differences on brain function.

Although we did see changes in delta and theta oscillations within the mesolimbic pathway, our observed effects were not always found with both doses on each assessment day, despite analgesia being evident upon acute and at the end of repeated administration of kratom. This suggests that while these oscillatory changes could reflect alterations in the activity of the mesolimbic pathway, it may be that analgesia is not specifically coupled to these region and frequency-specific oscillations. We also noted that there were long lasting drug-induced changes found in specific frequencies prior to the final injection that occurred in the NAc, Cg and PFC, but not in the VTA, reflecting region-dependent differences in response duration that lasted at least 24 h. Such long lasting functional changes are notable as, to our knowledge, such prolonged effects are not seen with morphine, which has a half-life of approximately 2 h in rodents (Emery et al., 2017). Of critical importance, there is conflicting evidence as to whether mitragynine has agonist activity at murine or rat MORs (Kruegel et al., 2016; Obeng et al., 2021) suggesting that the observed kratom-induced effects may be mediated by other receptors, by other alkaloids in the extract, or *via* mitragynine metabolites. Indeed, 7-HMG does have agonist properties at rodent MORs (Kruegel et al., 2016; Obeng et al., 2021). Furthermore, the extract used in the present study contained substantial quantities of speciogynine and 3-isoajmalicine. Although little is known about the pharmacological and physiological impacts of these compounds in brain, pharmacological activity may be MOR-independent as they do not appear to have pharmacological activity at MORs (Kruegel et al., 2016). Added to this speciogynine, as well as other alkaloids in the extract such as corynantheidine, speciocilliatine, and paynantheine, have been demonstrated to have moderate or potent inhibitory effects on CYP enzymes (Kamble et al., 2020), which may contribute to increased duration of effects due to reduced metabolism. With respect to VTA-NAc coherence, elevated activity was observed in the high frequency bands following an acute injection of either dose of kratom, with the effects also present after 7 days selectively with the high dose. This action appears similar to that of morphine, with a previous study in mice showing a morphine-induced increase in VTA-NAc gamma coherence (Reakkamnuan et al., 2017). Clinical electroencephalogram (EEG) studies have previously reported that the perception of pain may be associated with gamma rhythms, and that the disruption of these rhythms may contribute to analgesic effects (Whittington et al., 1998; Croft et al., 2002).

Perhaps one of the most interesting findings was the observation of increased delta coherence between the NAc-PFC in response to both doses of kratom following acute and repeated injections. The projection from the PFC to the NAc has been reported to be implicated in the regulation of pain (Baliki et al., 2010). Preclinical studies have also demonstrated the involvement of this pathway in the modulation of pain through the inactivation or activation of NAc-PFC connections (Lee et al., 2015; Martinez et al., 2017; Zhou et al., 2018). Specifically, one of these studies demonstrated that activation of NAc-PFC projections through optogenetics resulted in pain relief when animals were subjected to acute thermal stimulation in a behavioural test that is used to measure acute pain (Martinez et al., 2017). Together, these studies provide evidence that the NAc-PFC may be an important pathway to target for the relief of acute pain. Therefore, our findings may suggest that increased NAc-PFC coherence, a proxy measure of functional connectivity, induced by both doses of kratom possibly underlie the analgesic effects that we observed in the tail-flick test. It is important to note that while we observed changes in coherence with both doses, whether or not these underly the analgesic responses observed in the tail-flick test is unknown. Limited research has been conducted to link analgesic responses to neurophysiological changes. Nonetheless, these brain wave patterns provide a good measure for drug responses and may give insight into the addictive properties of novel compounds. Oscillations have been shown to be coupled to addictive states (Dejean et al., 2013; Zhu et al., 2019). Specifically, in a clinical study, opiate dependent patients exhibited significant reorganization of brain oscillations in all EEG oscillatory channels (Fingelkurts et al., 2006). These oscillatory adaptations are further evident in rodents upon administration of opioids (Reakkamnuan et al., 2017; Zhu et al., 2019). In particular, a recent study where rats were repeatedly administered the opioid heroin, enhanced theta band power and decreased gamma band power in the medial PFC were shown (Zhu et al., 2019).

Oscillatory Changes Accompanying the Antidepressant-like Effects of Kratom

It was demonstrated that only the low dose of kratom had antidepressant-like properties emphasizing the important relationship between kratom dose and behavioural outcome. To our knowledge, there are no other examples of antidepressants losing effectiveness at high doses, although the effective dose of typical antidepressants is highly dependent on the individual. It is quite possible that the higher dose of kratom had additional biological effects not captured in the present study that offset the antidepressant-like properties of the drug.

Ketamine, for example, while a highly effective antidepressant at low doses, induces a psychosis-like state at higher doses (Anticevic et al., 2015; Hoflich et al., 2015; Rivolta et al., 2015), and distinct dose- and time-dependent changes in neuronal oscillatory activity, particularly in the high freqency range, have been documented for this drug in both humans and animals (Rivolta et al., 2015; Manduca et al., 2020).

These findings are in line with a previous report that found that a single injection of a kratom alkaloid extract was sufficient to significantly reduce FST immobility time in mice (Kumarnsit et al., 2007b). The antidepressant-like activity of kratom was further demonstrated in a separate study conducted by Kumarnsit et al. (2007a), where intragastric administration of kratom reduced the amount of time rodents spent immobile in the tail suspension test (TST), another test commonly used to measure behavioural despair. Furthermore, a preclinical study looking solely at isolated mitragynine found that the alkaloid had dose-dependent antidepressant effects, with the higher dose (30 mg/kg) being of almost equal efficacy to that of a standard preclinical dose of fluoxetine or amitriptyline, two established antidepressants (Idayu et al., 2011). Specifically, mitragynine when administered to mice was found to significantly reduce immobility time in the FST and the TST, reductions that were comparable to mice who were administered the antidepressants (Idayu et al., 2011). These antidepressant-like effects were accompanied with a marked reduction in corticosterone concentrations signifying a role for the hypothalamic-pituitary-adrenal axis in mediating the observed effects (Idayu et al., 2011). Overall, these findings may suggest that a mechanism of action of commonly used antidepressants may be similar to that of mitragynine (Idayu et al., 2011).

Administration of high dose kratom suppressed theta power in PFC and Cg, a finding in line with another study reporting reduced theta power in cortical regions as measured by EEG (Cheaha et al., 2015). This study additionally compared those findings to that of the antidepressant fluoxetine and found the same reduction in cortical theta power (Cheaha et al., 2015). Here, we observed a reduction in theta power in response to only the high dose of kratom, and only the low dose exhibiting antidepressant properties. A reduction in Cg beta and low gamma power was also evident following repeated low dose kratom, yet the antidepressant effect was evident upon acute kratom administration, as well as following repeated dosing. Given that we were unable to demonstrate low dose-specific changes in oscillations that were present at both time points, it is possible that we simply did not capture the region-specific oscillatory changes coupled to the antidepressant effect of the drug. The hippocampus, for example, is a brain region involved in learning and memory and plays an important role in the pathophysiology of depression (Campbell and MacQueen, 2004). A recent study conducted by our group (Thériault et al., 2021) found that temporal changes in oscillations in response to chronic mild stress occurred first in the dorsal hippocampus with subsequent oscillatory changes in other brain regions that eventually culminated in the manifestation of depression-like behaviour. Other limbic brain regions implicated in the pathophysiology of depression include the amygdala and thalamus whereby functional and structural changes in these regions have been observed in depressed individuals (Pandya et al., 2012). As such, it is important to evaluate multiple different brain regions to capture relevant changes within the putative depression network.

Notably, elevated high gamma coherence between the NAc-Cg following acute and repeated injections of low dose kratom was evident. Thériault et al. (2021) similarly found an increase in NAc-Cg high gamma coherence that was evident in animals who were found to be resilient to stress and thus did not develop a depression-like phenotype following chronic daily stressors. In line with this, a low dose of ketamine administered to rats was also found to increase high gamma coherence in the NAc-Cg and these changes were postulated to be associated with a reduction of immobility time in the FST (Manduca et al., 2020). Further, alterations in the gamma frequency band have been reported to arise following pharmacological treatments that are successful in reversing symptoms of depression (Fitzgerald and Watson, 2018). Thus, it is possible that the enhancement in NAc-Cg gamma coherence observed in our study following acute and repeated low dose kratom may play a role, at least in part, in the observed antidepressant-like effects.

Effects of Kratom on ΔFosB Expression

The repeated administration of drugs of abuse, including analgesics such as morphine, have been found to induce accumulation of ΔFosB in several brain regions (Marttila et al., 2006; Núñez et al., 2010; Li et al., 2012; Perreault et al., 2016), a process suggested to represent a molecular switch for addiction (Nestler et al., 2001). Further, it has been previously shown that overexpression of ΔFosB in the NAc of mice results in behavioural changes similar to those induced by chronic morphine administration such as rapid analgesic tolerance and enhanced drug sensitivity (Zachariou et al., 2006). Thus, these findings provide evidence that ΔFosB likely plays an important role in mediating the effects of opiates on the brain (Zachariou et al., 2006). However, although there has been controversy as to the addictive potential of kratom (Harun et al., 2015; Yusoff et al., 2016; Yusoff et al., 2017; Negus and Freeman, 2018; Hemby et al., 2019), to our knowledge there have been no studies that have evaluated kratom- or alkaloid specific-induced changes in the expression of this marker. We found no effects of the high dose of kratom on ΔFosB accumulation in any of the regions examined, a finding suggesting a lack of addictive potential for the dose and extract used. However, in the current study addictive behaviours were not explicitly evaluated, and thus such a conclusion is premature. Preclinical rodent studies have demonstrated the addiction potential of mitragynine as it was found to induce locomotor sensitization (Ismail et al., 2017) and elicit conditioned place preference thereby demonstrating that the drug has rewarding effects (Yusoff et al., 2017). However, no studies to date have evaluated the addictive properties of the extract as a whole.

In addition, in the present study overall tolerance to the effects of kratom were not evident throughout our 7-day regimen, an effect commonly seen with repeated administration of opioid analgesics, including in such tests as the tail-flick test used herein (Listos et al., 2019). The mechanism of action of opioids such as morphine is well documented in the literature, whereby

morphine binds to and activates the MOR, a G protein-coupled receptor (Ream and Michael, 2011). Upon binding to the MOR, one of the induced intracellular signalling pathways results in the phosphorylation of the receptor and subsequent recruitment of the regulatory protein ß-arrestin 2 (Ream and Michael, 2011). The activation of ß-arrestin 2 has been found to contribute to morphine tolerance and mediates side effects such as respiratory depression (Caron et al., 2000; Váradi et al., 2017). Tolerance commonly arises following the repeated administration of drugs such as opioids whereby the original dose used to achieve analgesia is no longer found to be effective, therefore a larger dose must be administered to achieve the same pharmacological effects (Hurlé, 2001). Unfortunately, the exact mechanism of action of the whole kratom extract in the brain is unclear. However, the alkaloids mitragynine and 7-HMG have been identified as agonists at the MOR where they demonstrate biased activation and thus do not recruit ß-arrestin 2 (Takayama et al., 2002; Kruegel et al., 2016; Ismail et al., 2017). As such, it has been postulated that kratom may demonstrate analgesic efficacy, without bringing forth the typical life-threatening and adverse side effects of commonly prescribed opioids (Takayama et al., 2002; Kruegel et al., 2016; Ismail et al., 2017). In the present study it appears as though kratom has analgesic effects without inducing significant tolerance or ΔFosB expression, suggesting that the kratom extract may have therapeutic potential in the absence of unwanted side effects. However, further reward and addiction studies, such as those evaluating conditioned place preference or self-administration, are necessary to determine more conclusively the impact of kratom on these behaviours.

In conclusion, we showed that kratom exerted dose-dependent antidepressant-like and analgesic effects that were accompanied by frequency specific changes in neuronal oscillatory activity. In addition, the repeated administration of high dose kratom did not result in the accumulation of ΔFosB in any of the regions studied. Whereas this latter finding may indicate a lack of addictive potential, caution is warranted as only a single dose of one specific extract was evaluated. This study provides a promising direction to explore the untapped potential of kratom-based alkaloids for the management of mood and pain related disorders.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by University of Guelph Animal Care Committee.

AUTHOR CONTRIBUTIONS

SB, JK, TA, and MP planned and designed the experiments. SB, ES, SF, DR, JM, MSA-A-W, and JF performed the research. SB, ES, TA, MP analyzed the data and prepared figures. SB, ES, TA, JK, MP wrote and/or edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.696461/full#supplementary-material

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BRIEF RESEARCH REPORT
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Comparative Toxicity Assessment of Kratom Decoction, Mitragynine and Speciociliatine Versus Morphine on Zebrafish (*Danio rerio*) Embryos

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Background: Kratom (*Mitragyna speciosa* Korth), a popular opioid-like plant holds its therapeutic potential in pain management and opioid dependence. However, there are growing concerns about the safety or potential toxicity risk of kratom after prolonged use.

Aim of the study: The study aimed to assess the possible toxic effects of kratom decoction and its major alkaloids, mitragynine, and speciociliatine in comparison to morphine in an embryonic zebrafish model.

Methods: The zebrafish embryos were exposed to kratom decoction (1,000–62.5 µg/ml), mitragynine, speciociliatine, and morphine (100–3.125 µg/ml) for 96 h post-fertilization (hpf). The toxicity parameters, namely mortality, hatching rate, heart rate, and morphological malformations were examined at 24, 48, 72, and 96 hpf, respectively.

Results: Kratom decoction at a concentration range of ≥500 µg/ml caused 100% mortality of zebrafish embryos and decreased the hatching rate in a concentration-dependent manner. Meanwhile, mitragynine and speciociliatine exposure resulted in 100% mortality of zebrafish embryos at 100 µg/ml. Both alkaloids caused significant alterations in the morphological development of zebrafish embryos including hatching inhibition and spinal curvature (scoliosis) at the highest concentration. While exposure to morphine induced significant morphological malformations such as pericardial oedema, spinal curvature (lordosis), and yolk edema in zebrafish embryos.

Conclusion: Our findings provide evidence for embryonic developmental toxicity of kratom decoction and its alkaloids both mitragynine and speciociliatine at the highest concentration, hence suggesting that kratom consumption may have potential teratogenicity risk during pregnancy and thereby warrants further investigations.

Keywords: kratom, mitragynine, speciociliatine, morphine, zebrafish, embryotoxicity

INTRODUCTION

Mitragyna speciosa Korth. (*Rubiaceae*), is known as Ketum or Biak-biak in Malaysia and Kratom in Thailand. This plant holds various therapeutic potentials especially in pain management and opioid dependence (Adkins et al., 2011; Cinosi et al., 2015). People in the countryside often consumed kratom in the form of a decoction, where mature fresh leaves are harvested and brewed for several hours. . Kratom leaves can also be chewed, smoked, and ingested as a solution or taken with tea/coffee (Assanangkronchai et al., 2007; Singh et al., 2016). Given its curative properties, kratom leaves are traditionally used to treat pain, diabetes, and diarrhea (Hassan et al., 2013; Singh et al., 2016). In fact, it is also used to enhance mood and ameliorate opioid withdrawal among illicit opioid users (Vicknasingam et al., 2010; Saraf et al., 2019).

Kratom leaves contains more than 40 indole alkaloids and is reported to produce unique pharmacological effects through its complex synergistic or antagonistic interactions (Obeng et al., 2020; Chear et al., 2021). Among these, mitragynine is the major alkaloid found in kratom leaves. Other active alkaloids present in kratom leaves are 7-hydroxymitragynine, speciogynine, speciociliatine, and paynantheine (Sharma et al., 2019; Chear et al., 2021). Mitragynine is known to reduce pain in preclinical evaluations (Matsumoto et al., 1996; Carpenter et al., 2016). In a recent randomized, double-blind placebo clinical trial, kratom decoction is shown to have the potential to suppress pain, however further clinical studies are needed to support its utility (Vicknasingam et al., 2020). Lately, we reported that speciociliatine showed a better binding affinity (Ki = 54.5 nM) towards the human mu-opioid receptor compared to its diastereoisomer—mitragynine (Ki = 161 nM). This alkaloid constitutes 9% of the total alkaloid present in kratom leaves. Further to this, speciociliatine demonstrated a better antinociceptive effect in rats when compared to mitragynine (Obeng et al., 2020). Taken together, these findings indicate that mitragynine along with speciociliatine could be a potential drug candidate for opioid substitution therapy and pain treatment.

The Zebrafish (*Danio rerio*), a small aquatic vertebrate, is a valid translational model in the field of neuroscience research, toxicology, or translational medicine (Gut et al., 2017; Vaz et al., 2018; Cassar et al., 2019). Importantly, zebrafish shared about 70% of human genes, and about 84% of genes known to human diseases are also present in zebrafish (Howe et al., 2013). Additionally, zebrafish also shared physiological and anatomical similarities in cardiovascular, nervous, and digestive systems with mammals (Hsu et al., 2007). Besides that, zebrafish have become a preferred animal model for extensive drug discovery research due to their small size, high fecundity, optical transparency, and fast development (Strähle et al., 2012; Vaz et al., 2018). More importantly, the zebrafish embryo has served as a promising model for screening toxicants that affect early embryonic development because of its comparable cell structure (i.e. embryonic yolk sac), and development pathway with humans (Link and Megason, 2008; Sant and Timme-Laragy, 2018). In fact, the external development of zebrafish embryos offers a great advantage to overcome the limitation of observing minute changes caused by toxicants during the early embryonic development due to the involvement of the maternal system in humans (He et al., 2012). The outcomes of various toxicity studies indicate that zebrafish embryos is a valuable animal model to anticipate the acute toxicity and teratogenicity effects of natural products/drugs in mammals especially humans (Strähle et al., 2012; Chakraborty et al., 2016; Blahova et al., 2020; Mektrirat et al., 2020).

Previously we tested kratom decoction for its efficacy in mitigating pain in regular kratom users. Despite it being consumed widely as a decoction, the preclinical toxicity data on kratom decoction and its active alkaloids remains limited and urgently warrants further investigations to support propspective human trial studies. Given this, the present study aimed to investigate the toxic effects of kratom decoction and its two active alkaloids mitragynine and speciociliatine on zebrafish embryos. Since kratom is shown to have morphine-like effects, we also assess the toxic effects of morphine concurrently with mitragynine and speciociliatine for comparison purposes.

METHODS

Zebrafish Husbandry and Breeding

Zebrafish (*Danio rerio*), wild-type AB strain were used in this study. The zebrafish were maintained in the automated housing system (Tecniplast, Italy) that automatically regulates the pH (7.5 ± 0.5), temperature (28°C ±0.5), salinity, and water flow with 14 h light:10 h dark cycle (light onset: 8 am; light offset: 10 pm). They were fed daily with tetraMin® tropical flakes and live brine shrimp twice per day. Embryos were obtained from spawning sexually matured male and female adult zebrafish at a ratio of 2:2 through natural mating. According to European legislation (EU Directive, 2010/63/EU), no animal ethics permission was requested for zebrafish larvae below 120 h post-fertilization (hpf) (Strähle et al., 2012).

Plant Materials

Approximately 4 kg of fresh kratom (*Mitragyna speciosa* Korth.) leaves were collected from a local farm located at Permatang Rawa, Penang, Malaysia. The plant was authenticated by a botanist, Dr. Rosazlina binti Rusly from the School of Biological Sciences, Universiti Sains Malaysia. A voucher specimen [NEL-(K2)-2019(02)] was deposited at the Herbarium of School of Biological Sciences, Universiti Sains Malaysia.

Preparation of Kratom Decoction

The collected fresh kratom leaves (1 kg) were washed with tap water and ripped into small pieces before placing them into a boiling pot of water (4 L). The leaves were brewed for approximately 2 h at constant heat until the volume was reduced to approximately one-third of the initial volume. After that, the solution (1 L) was left to cool, filtered, and freeze-dried to yield a lyophilized kratom decoction extract. The lyophilized extract was kept at −80°C before high

performance liquid chromatography (HPLC) analysis and toxicity evaluation.

Extraction and Isolation of Mitragynine and Speciociliatine

Mitragynine (1) and speciociliatine (2) were extracted and purified from the fresh *M. speciosa* leaves according to the method described in our previous study (Chear et al., 2021). The detailed isolation procedures and spectroscopic data are provided in **Supplementary Methods** and **Supplementary Figures S1–6**.

HPLC Analysis

Chemicals and Reagents

The reference standards: mitragynine (1) and speciociliatine (2) (purity ≥97%) (**Supplementary Figures S7, S8**) were extracted according to the method described by Saref et al. (2019). Solvents—acetonitrile and methanol used for analysis were of LC grade (Merck, Germany). Formic acid (98–100%) was purchased from Merck (Germany). Deionized water (18.2 MΩ) was used for the HPLC analysis.

Analytical Method

The content of mitragynine (1) and speciociliatine (2) in the prepared kratom decoction sample were determined using a validated HPLC method as described in our previous study (Saref et al., 2019). The detailed HPLC analytical methodology is provided in **Supplementary Methods**.

Fish Embryo Acute Toxicity Test

The stock solutions of kratom decoction (2 mg/ml), mitragynine (200 μg/ml), speciociliatine (200 μg/ml), and morphine (200 μg/ml), were prepared in 0.1% dimethyl sulfoxide (DMSO). A series of working concentrations ranging from 1,000–62.5 μg/ml (kratom decoction) and 100 to 3.125 μg/ml (mitragynine, speciociliatine, and morphine) were prepared by serial dilution of the stock solution. System water was used as negative control and 0.1% DMSO as solvent control, whereas 20 μg/ml doxorubicin was used as a positive control. The FET test was performed according to the Organization for Economic Co-operation and Development (OECD) TG 236 guideline (OECD, 2013). Briefly, forty embryos (n = 40, <3 hpf) were pre-exposed to either solvent, negative or positive controls, or kratom decoction, mitragynine, speciociliatine, and morphine at various test concentrations in the petri dishes to optimize the exposure duration. Then, embryos were observed under the microscope (Olympus SZ61 Zoom Stereo Microscope), and fertilized embryos (n = 20) that reached the blastula stage with normal cleavage pattern were randomly transferred to 24-well plates, one embryo in each well with 1.5 ml of the test sample. Next, well plates were incubated at 26 ± 1°C under a 14 h light: 10 h dark cycle. The test samples were renewed on the daily basis (semi-static exposure). The tests were performed in triplicate. Lethality parameters, such as coagulation of embryos, lack of somite formation, non-detachment of the tail, and lack of heartbeat and sub-lethal parameters include pericardial oedema, yolk sac oedema, spinal curvature (kyphosis, lordosis or scoliosis), heartbeat and hatching rate at 24, 48, 72 and 96 hpf were examined under zoom stereo microscope. The heart rate of larvae (n = 5) was counted for 15 s using a stopwatch under the stereomicroscope when the larvae were immobile, and then multiply by 4 to obtain the beats per minute. The sub-lethal morphological effect was expressed as the percentage of embryos with malformation over total alive embryos at 24, 48, 72, and 96 hpf (Nagel, 2002; Blahova, 2020).

Statistical Analysis

Statistical analysis was performed using the software Graph Pad Prism. 5. Data were expressed as mean ± standard deviation (SD). Concentration-response curves were used to determine the lethal concentration, LC_{50} value. The mortality rate at 24, 48, 72, and 96 hpf were analyzed using a two-way repeated-measure ANOVA, followed by Bonferroni *post hoc* test. The hatching rate, heartbeat, and sub-lethal morphological effect data were analyzed using one-way ANOVA followed by Dunnett's *post hoc* test. Probability values of less than 5% ($p < 0.05$) are considered significant.

RESULTS

Mitragynine and Speciociliatine Content

Based on HPLC analysis, the amount of mitragynine (1) and speciociliatine (2) detected in the prepared kratom decoction (lyophilized extract, 1,000 μg/ml) were 37.63 ± 3.01 and 5.49 ± 0.37 μg/ml, respectively. The HPLC chromatograms of lyophilized kratom extracts and its detected mitragynine 1) and speciociliatine 2) are provided in **Supplementary Figure S9**.

Mortality

The test was validated according to OECD 236 guideline criteria, as shown by less than 5% mortality rate in system water (negative control) and 0.1% DMSO (solvent control), whereas 100% mortality rate in 20 μg/ml doxorubicin (positive control) at 96 hpf (**Supplementary Figure S11**).

As shown in **Figure 1**, kratom decoction, mitragynine, and speciociliatine caused mortality of zebrafish embryos in a time and concentration-dependent manner. Zebrafish embryos exposed to 1,000 μg/ml kratom decoction showed 100% mortality at 48 hpf, similar finding for 500 μg/ml at 72 hpf. At 250 μg/ml, the mortality rate was gradually increased from 48 hpf to 96 hpf ($p < 0.001$, versus negative control group). With regards to alkaloid compounds, both mitragynine and speciociliatine at the highest concentration (100 μg/ml) killed 100% of the embryos at 72 hpf. Besides that, the mortality rate of embryos in both mitragynine and speciociliatine groups at concentrations 50 and 25 μg/ml was significantly increased at 96 hpf ($p < 0.01$, versus negative control group). Mitragynine at concentrations of 50 and 25 μg/ml killed 93.33% and 21.67% of the embryos, respectively, whereas the mortality rate of speciociliatine at 50 and 25 μg/ml were 16.67% and 13.33%, respectively. These results indicate that speciociliatine is safer than mitragynine. In morphine-exposed embryos, the highest concentration (100 μg/ml) significantly increased the mortality rate at 96 hpf in comparison to the negative control group ($p < 0.001$).

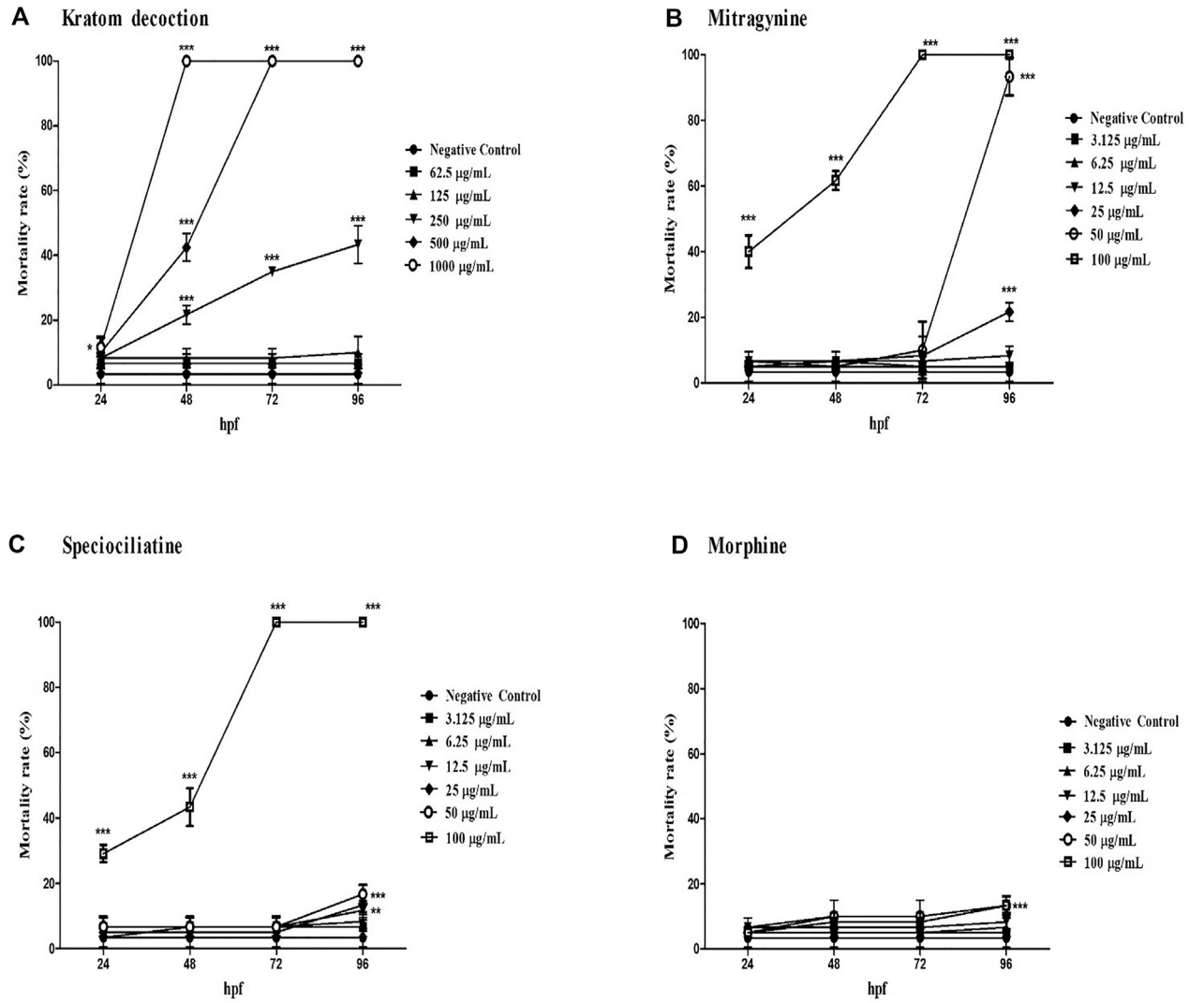

FIGURE 1 | The mortality rate of zebrafish embryos exposed to **(A)** kratom decoction, **(B)** mitragynine, **(C)** speciociliatine, and **(D)** morphine at 24, 48, 72, and 96 hpf. Data are expressed as the mean ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$ significantly different from negative control group.

The concentration-response curve of kratom decoction, mitragynine, speciociliatine, and morphine for mortality rate at 96 hpf are shown in **Supplementary Figure S12**. The LC_{50} of kratom decoction and mitragynine at 96 hpf were 260.68 µg/ml and 32.01 µg/ml, respectively. We were unable to calculate the actual LC_{50} value of speciociliatine since it showed an exponential increase of mortality rate at 100 µg/ml concentration, hence the estimated LC_{50} of speciociliatine at 96 hpf from the concentration-response curve was 79.86 µg/ml. For morphine, the highest mortality rate recorded was less than 20%, and it is not possible to calculate the LC_{50} value.

Hatching Rate

In general, zebrafish embryos started to hatch from 48–72 hpf (Kimmel et al., 1995). As shown in **Figure 2**, untreated embryos (negative control group) showed a 100% of hatching rate at 72 hpf. One-way ANOVA revealed that kratom decoction at 250, 125 and 62.5 µg/ml affect the hatching rate of zebrafish embryos, as shown by 0% hatching rate at 72 hpf (**Figure 2A**). At 96 hpf, the highest concentration of kratom decoction (250 µg/ml) resulted in a 0% hatching rate, indicating complete inhibition of hatching ($p < 0.001$, versus negative control group). In addition, the percentage of embryos hatched was only 9.06% and 28.36% in the group treated with 125 and 62.5 µg/ml of kratom decoction at 96 hpf, respectively ($p < 0.001$). These data indicated concentration-dependent delayed hatching in the kratom decoction-treated groups in comparison to the control group. Hatching inhibition was also found in higher concentrations of mitragynine and speciociliatine (50 and 25 µg/ml) treated groups at 72 and 96 hpf, respectively ($p < 0.01$, versus negative control group). Morphine did not affect the hatching rate of zebrafish embryos at 72 and 96 hpf.

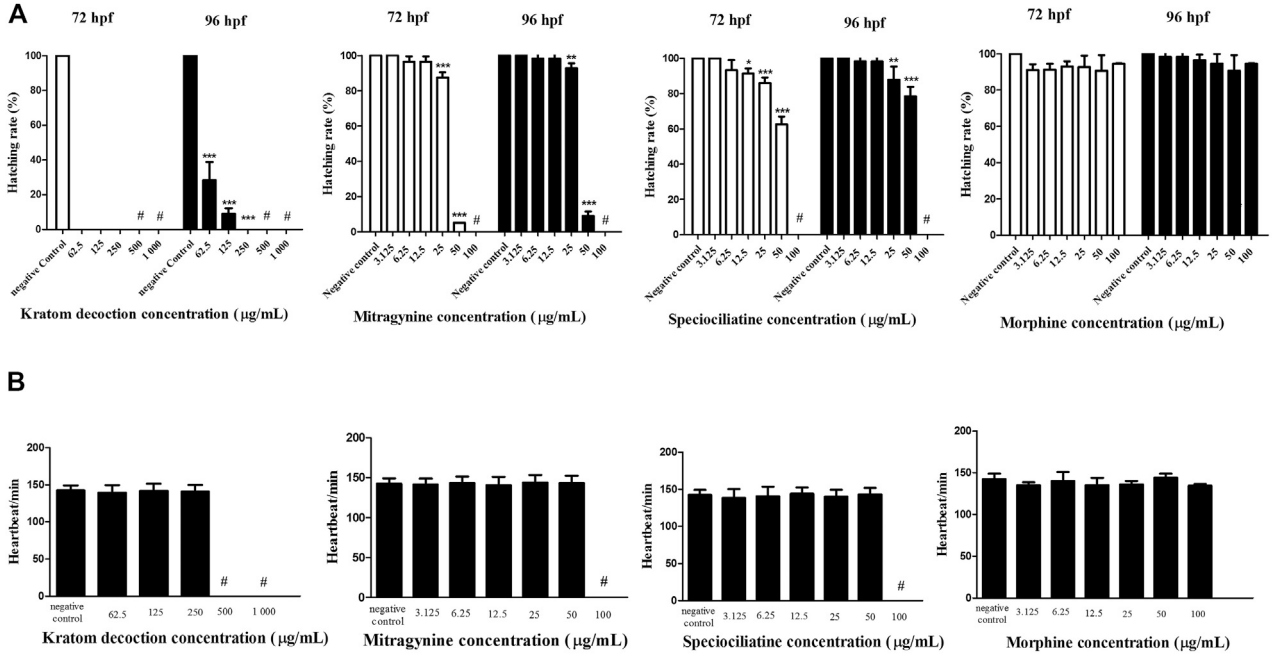

FIGURE 2 | Zebrafish embryos exposed to kratom decoction, mitragynine, speciociliatine, and morphine. **(A)** hatching rate at 72 and 96 hpf and **(B)** heart rate per minute. Data are expressed as the mean ± SD $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ significantly different from the negative control group. # indicate not determined.

Heart Rate

The heart rate per minute of zebrafish embryos at 96 hpf is shown in **Figure 2B**. One-way ANOVA showed that exposure of kratom decoction, mitragynine, speciociliatine, and morphine until 96 h did not affect the heart rate of exposed zebrafish embryos ($p > 0.05$). The heart rate of kratom decoction (1,000 and 500 μg/ml), mitragynine, and speciociliatine (100 μg/ml) was not determined, as there were no surviving embryos at 96 hpf.

Morphological Malformations

Table 1; **Figure 3** show the sub-lethal morphological alterations in zebrafish embryos exposed to kratom decoction, mitragynine, speciociliatine, and morphine for 96 h, respectively. Zebrafish embryos exposed to system water or 0.1% DMSO showed normal morphology features with normal body shape, straight spine, pigmented body, and round yolk sac (**Figure 3A**). Meanwhile, embryos exposed to 20 μg/ml doxorubicin displayed pericardial oedema, spinal curvature (lordosis), and small eyes (**Figure 3B**). Kratom decoction at concentrations ≤250 μg/ml did not show any morphological malformations, except for hatching inhibition compared to the negative control group ($p > 0.05$, **Figure 3C**). As shown in **Figure 3D**, mitragynine at 50 μg/ml caused spinal curvature (scoliosis) in zebrafish embryos ($p < 0.01$, versus negative control group). Meanwhile, the embryos exposed to speciociliatine at the concentration ≥25 μg/ml exhibited signs of scoliosis ($p < 0.01$, versus negative control group, **Figure 3E**). Morphine at the concentration ≥6.25 μg/ml showed pericardial oedema, meanwhile, 100 μg/ml exhibited spinal curvature (lordosis) and yolk oedema as compared to the negative control group ($p < 0.05$, versus negative control group, **Figure 3F**).

DISCUSSION

With kratom having many medicinal applicability, it is important to ensure that the plant and its alkaloids are safe for human consumption. To date, there is no pre-clinical toxicity data on kratom decoction, despite it being used pervasively in the community. So far, only one study have managed to previously determine kratom extract effects in zebrafish embryos (Ramli et al., 2020). To the best of our knowledge, this study is among the first to investigate the embryotoxicity of kratom alkaloids both mitragynine and speciociliatine in comparison to morphine in zebrafish embryos.

This study demonstrates that acute embryonic exposure to kratom decoction, mitragynine, and speciociliatine affected survival, hatching, and body morphology of zebrafish embryos in a concentration and time-dependent manner, indicating that higher extract/compound concentrations and longer exposure times affect the zebrafish embryo development. Herein, kratom decoction at a concentration of ≥500 μg/ml caused 100% mortality of zebrafish embryos at 96 hpf. A previously published acute toxicity study reported that methanolic extract of kratom up to 1,000 mg/kg did not cause any mortality in mice (Harizal et al., 2010). This discrepancy is probably due to the high sensitivity of zebrafish embryos in their early development stages to external stimuli/exposure (Hill et al., 2005). Thus, there is a possibility that a high concentration of kratom decoction may be toxic to zebrafish embryos, but not to other

TABLE 1 | Morphological malformations in zebrafish embryos at 96 hpf.

Concentration (μg/ml)	Morphological abnormalities (%)		
	Pericardial oedema	**Yolk oedema**	**Spinal curvature**
Negative Control	0	0	0
Solvent Control	0	0	0
Positive control	100 ± 0.00	100 ± 0.00	67.18 ± 20.59
Kratom decoction			
31.25	0	0	0
62.5	0	0	0
125	0	0	0
250	0	0	0
500	[a]	[a]	[a]
1000	[a]	[a]	[a]
Mitragynine			
3.125	0	0	0
6.25	0	0	0
12.5	0	0	0
25	0	0	3.92 ± 3.40
50	0	0	7.34 ± 2.80**
100	[a]	[a]	[a]
Speciociliatine			
3.125	0	0	0
6.25	0	0	0
12.5	0	0	0
25	0	0	12.48 ± 2.88***
50	0	0	25.05 ± 3.54***
100	[a]	[a]	[a]
Morphine			
3.125	5.36 ± 0.17	1.75 ± 3.04	5.26 ± 5.26
6.25	7.02 ± 3.04*	3.51 ± 3.04	5.46 ± 5.56
12.5	7.21 ± 3.38*	3.61 ± 3.13	5.36 ± 0.17
25	7.42 ± 3.21*	3.81 ± 3.31	7.42 ± 3.21
50	9.26 ± 3.21**	5.56 ± 1.09	7.41 ± 3.21
100	9.06 ± 3.05**	7.21 ± 2.87*	9.16 ± 3.38*

*Data are expressed as the mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001 significantly different from the negative control group.*
[a]*indicate not determined.*

species, and the toxicity level of the extract might also rely on the development stage of animals.

With regards to alkaloids, the LC_{50} value of mitragynine was 32.01 μg/ml at 96 hpf, while the estimated LC_{50} value of speciociliatine was 79.86 μg/ml, indicating that speciociliatine is relatively safer than mitragynine. However, kratom decoction showed lower embryotoxicity compared to its major active alkaloids with an LC_{50} value of 260.68 μg/ml. HPLC analysis reveals that the concentration of mitragynine and speciociliatine detected in the lyophilized kratom decoction was relatively low which was approximately 3.76 and 0.55% w/w of lyophilized powder, respectively. This suggests that the embryotoxicity observed in kratom decoction treatment might not be link to mitragynine and speciociliatine per se since their respective LC_{50} values (as a single agent) are far higher compared to kratom decoction (as a mixture). The embryotoxicity observed in kratom decoction might be due to the presence of other phytochemicals such as other indole and oxindole alkaloids, terpenes, flavonoids, phenolics, plant peptides, polysaccharides, etc. The single or synergic effect of these compounds in the overall embryotoxicity of kratom decoction cannot be ruled out as well, therefore this warrants further investigation. For morphine, we could not determine the LC_{50} value because the highest mortality rate recorded was less than 20%, indicating that morphine was relatively safe even at a higher concentration range up to 100 μg/ml. Altogether, kratom decoction, mitragynine, and speciociliatine at the highest dose display more toxic effects in terms of embryo survival, when compared to morphine.

Hatching is the most important process in the development stage of zebrafish and its retardation following exposure to chemical/drugs like environmental pollutants, nanomaterials, or natural product is a sign of sub-lethal toxicological effects on zebrafish embryos (Liu et al., 2014; De la Paz et al., 2017). In the present study, we found that exposure to kratom decoction significantly reduced the hatching rate of the zebrafish embryos. Notably, mitragynine and speciociliatine at concentrations of 25 and 50 μg/ml appeared to be associated with delayed hatching process. However, morphine did not affect the

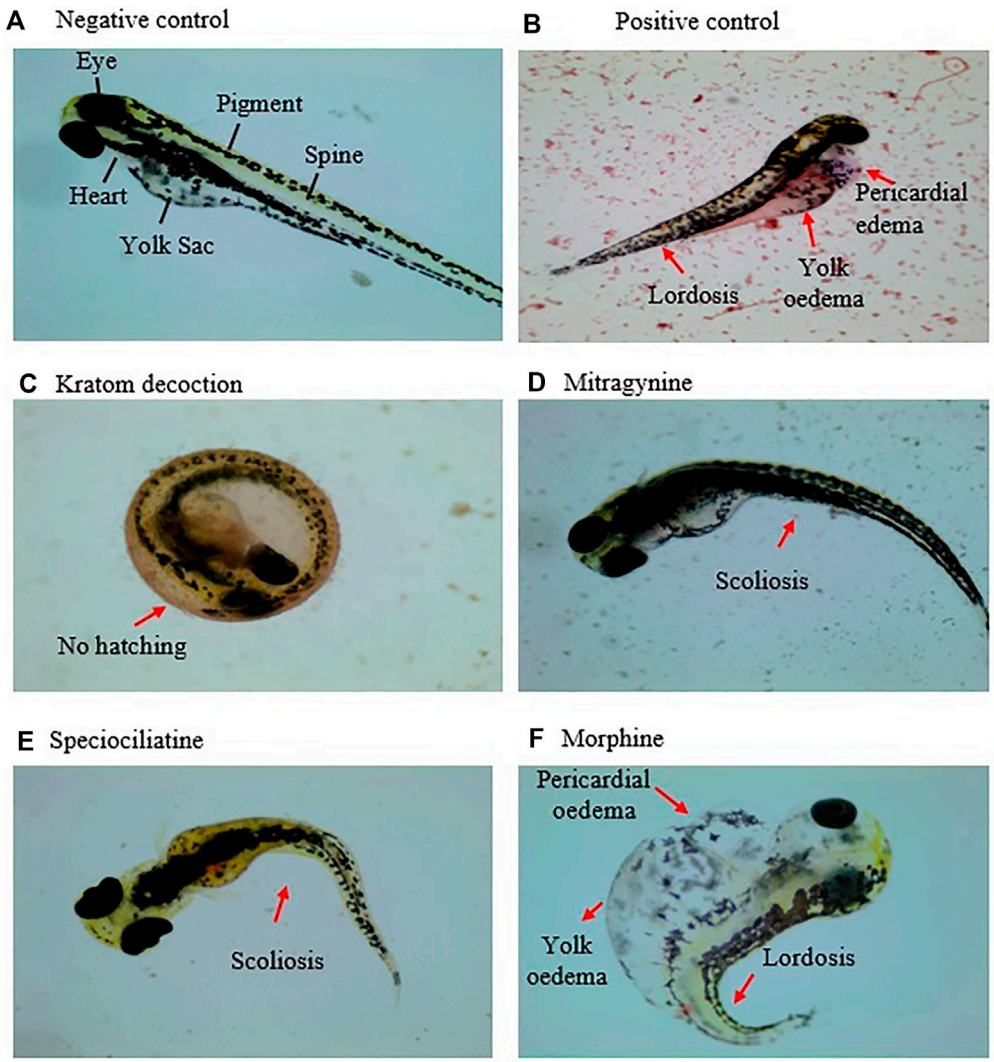

FIGURE 3 | Morphology of the zebrafish embryo at 96 hpf exposed to **(A)** system water (normal morphology), **(B)** doxorubicin, 20 µg/ml, **(C)** kratom decoction, **(D)** mitragynine, **(E)** speciociliatine, and **(F)** morphine. Morphological malformations are denoted with red arrows.

hatching of zebrafish embryos. In zebrafish, hatching enzyme 1 (HE1) is secreted from hatching gland cells (HGCs) to digest the outer chorion layer for the natural hatching process to occur (Zhou et al., 2009). The delayed hatching process observed in this study might be due to delayed HE1 secretion. A study by De la Paz et al. (2017) showed that triazole, fungicides inhibit the hatching process via reduction of HGCs secretion and administration of dopamine type 2 (D2) receptors antagonist able to reverse the effect of triazole. Their results suggest that the dopaminergic system regulates the secretion of HGCs in zebrafish embryos. Since mitragynine has been revealed to bind to the D2 receptor (Boyer et al., 2008), it is possible that kratom indirectly affects the HGCs secretion by regulating the dopaminergic system via D2 receptor-mediated signaling pathways. This notion warrants further investigation.

Morphological malformations such as spinal curvature, yolk oedema, and pericardial oedema are important sub-lethal parameters observed in zebrafish embryos when exposed to toxic chemicals (Chahardehi et al., 2020). An example, drugs such as alcohol and nicotine that are known to affect human fetal development have been reported to induce morphological defects in the zebrafish embryos (Lantz-McPeak et al., 2015). Spinal curvature can be further specified into three types: lordosis (spine curved inward), kyphosis (spine curved outward), and scoliosis (spine curved sideways) (von Hellfeld et al., 2020). In this study, spinal curvature (scoliosis) was observed in mitragynine (50 µg/ml) and speciociliatine (25 and 50 µg/ml) exposed groups. It is possible that mitragynine or speciociliatine may trigger neuroinflammation pathways in cerebrospinal fluid by activating pro-inflammation signals that in turn could lead to spinal deformities (Van Gennip

et al., 2018). On the other hand, zebrafish embryos exposed to morphine had morphological malformations, including yolk oedema, pericardial oedema, and spinal curvature (lordosis) in a concentration-dependent manner. Correspondingly, Cadena et al. (2021) also observed the morphological malformations (yolk oedema, spine deformation, and tail deformation) in zebrafish embryos following exposure to 10 μg/ml morphine. The result indicates that morphine is more prone to cause abnormal embryonic development than kratom and its alkaloids mitragynine and speciociliatine in zebrafish. Taken together, it is plausible that the differences seen in the types of vertebral changes (i.e. demineralization, increased density, and alteration in intervertebral spacing) induced by kratom alkaloids and morphine could lead to different spinal curvature morphology (Eissa et al., 2009; Yashwanth et al., 2016). Overall, it is also apparent that kratom and morphine may act on a different pathway to induce toxicity during embryogenesis in zebrafish. However, the exact mechanisms involved is yet to be elucidated.

Overall, we have demonstrated that kratom (≥500 μg/ml) and its alkaloids mitragynine and speciociliatine (≥50 μg/ml) have certain undesirable effects on embryonic development by affecting survival, hatching, and body morphology of zebrafish embryos. This finding suggests that the potential risk of kratom intake during pregnancy on the development of the fetus is based on the fact that the early embryo developmental process of zebrafish is similar to humans. However, this notion should be interpreted with caution, and warrants further investigation in other animal models such as rodent.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because According to European legislation (EU Directive, 2010/63/EU), no animal ethics permission was requested for zebrafish larvae below 120 h post-fertilization (hpf).

AUTHOR CONTRIBUTIONS

TD, NC, and SR designed the experiment. TD and NC performed the research and analyzed the data. TD wrote the first draft of the manuscript. SR, VM, and MM reviewed and edited the manuscript.

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Assessment of Cardiovascular Functioning Among Regular Kratom (*Mitragyna speciosa* Korth) Users: A Case Series

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Multiorgan toxicities have been extensively reported in kratom (*Mitragyna speciosa* Korth) users in Western countries but not in Southeast Asia. Existing literature argued that this discrepancy may be due to underreporting of kratom-related toxicity cases in Southeast Asia. Hence, this case series filled the research gap by clinically assessing the cardiovascular functioning and serum mitragynine level of regular kratom users in its traditional settings in Malaysia. Nine regular kratom users without history of polysubstance use were recruited from the same community via snowball sampling and were subjected to electrocardiogram (ECG) and echocardiogram assessments. Serum mitragynine analysis was also performed by solid-phase extraction and liquid chromatography-tandem mass spectrometry. The mean serum mitragynine level was 10.3 mg/L (SD = 6.9) and ranged from 2.5 mg/L to 22.4 mg/L. Those who consumed an average daily quantity of four or more glasses of brewed kratom juice (p = 0.045) and those who had prolonged QTc intervals (p = 0.017) had significantly higher serum mitragynine level. Echocardiographic findings of all the respondents were normal except one reported left ventricular hypertrophy and another had trivial tricuspid regurgitation with pulmonary artery systolic pressure (PASP) of 10 + 5 mmHg. Regular kratom use without concomitant use of other illicit substances may not provoke any risk of cardiovascular impairment or toxicity except for prolonged QTc interval, which appeared to be dose dependent. However, as this study was limited by a small sample size, future studies with larger sample size are warranted to confirm our findings.

Keywords: cardiovascular functioning, electrocardiogram, echocardiogram, serum mitragynine, regular kratom use

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INTRODUCTION

The leaves of *Mitragyna speciosa* (Korth) or better known as kratom, a subtropical plant native to the region of Southeast Asia, exhibits psychotropic properties and has been used as a traditional remedy for symptomatic relief of various illnesses. It has been used in Thailand and Malaysia for centuries but for the past decade, its use in Western countries, such as United States and European nations, as a self-prescribe medication for depression, anxiety disorders, chronic pain and as a substitute to illicit and prescription opioids soared exponentially (Grundmann, 2017). Despite its therapeutic potential, kratom was listed as "drug of concern" by the U.S. Drug Enforcement Administration (DEA) in

response to multiple reports of toxicity and mortality cases possibly related to kratom use (Fluyau and Revadigar, 2017).

Reports of individual cases of multiorgan toxicities in U.S. and Europe have been published. Several case reports of kratom induced hepatitis, intrahepatic cholestasis, hepatomegaly, and acute liver failure have been documented (Dorman et al., 2015; Griffiths et al., 2018; Waters et al., 2018; Antony and Lee, 2019; Fernandes et al., 2019; Osborne et al., 2019). Post-mortem findings alleged to be associated with accidental deaths have linked kratom use with hepatomegaly, congested liver, fatty liver, liver steatosis, and liver fibrosis (Corkery et al., 2019). The renal toxidrome reportedly link to kratom use are congested kidney, distended bladder, urinary retention, kidney stones, and nephritis (Corkery et al., 2019). Despite various toxidrome reported in the West, toxicity related to kratom use has not been documented in Southeast Asia (Singh et al., 2016).

Cases of cardiotoxicity have also been documented, such as ventricular arrythmia, ventricular tachycardia, ventricular fibrillation, cardiomegaly, cardiomyopathy, coronary atherosclerosis, focal band necrosis in myocardium, myocardial infarction, hypertensive cardiovascular disease, left ventricular hypertrophy, myocardial ischemia, and myocarditis (Aggarwal et al., 2018; Corkery et al., 2019; ELJack et al., 2020; Eastlack et al., 2020; Sheikh et al., 2021). Again, cardiotoxicity has only been reported in the West, but not in Southeast Asia. The current literature has ostensibly suggested that this discrepancy may be caused by the underreporting of kratom-related toxicity cases in Southeast Asia (Corkery et al., 2019). In addition, *in vitro* studies on human induced pluripotent stem cell-derived cardiomyocytes indicated that mitragynine, the most abundant psychoactive alkaloid in kratom extract, is capable of prolonging the action potential duration of cardiomyocytes and increased the risk of prolonged QTc interval and torsades de pointes (Lu et al., 2014). This was followed by a recent study of Electrocardiogram (ECG) in Malaysian subjects which highlighted that regular kratom use may increase the risk of borderline QTc interval [431–450 ms; (Leong Abdullah et al., 2021)]. Given the cardiovascular risk, Leong Abdullah et al. (2021) study was limited by the absence of the serum mitragynine analysis in regular kratom users and cardiac pathology was not examined with echocardiogram. Hence, we conducted this case series to fill the research gap by examining the cardiovascular functioning with electrocardiogram and echocardiogram, and serum mitragynine analysis was performed among regular kratom users in its traditional settings in Malaysia. To the best of our knowledge, to date this case series was the first to examine the echocardiogram and serum mitragynine level in addition to electrocardiogram analysis to assess cardiovascular functioning among regular kratom users without concomitant use of other illicit substances.

METHODS

Respondent Recruitment

All the nine regular kratom users were recruited from a targeted community located in the state of Penang in Peninsular Malaysia which has a high prevalence of kratom use. Snowball sampling was employed in which an informant who was a regular kratom user who resided in the targeted community was briefed on the purpose and procedures of the case series and assisted in the recruitment drive. The eligibility criteria for the study were: 1) self-reported as a regular kratom user who consumed kratom on a daily basis in the last 12 months, and 2) have no significant history of medical illness, illicit drug and alcohol consumption, psychiatric disorder and had not consumed any medications on regular basis. The eligible subjects were then asked to provide their written informed consent, before they were enrolled in the assessments which were carried out at the Advanced Medical and Dental Institute, Universiti Sains Malaysia. This study has received approval from the Human Ethics Committee of Universiti Sains Malaysia (code: USM/JEPeM/19010054). Respondents were also screened with rapid urine test-kits for opioids, methamphetamine/amphetamine, ketamine, benzodiazepine, cannabis, methadone, and phencyclidine.

Study Procedures

Data on demographic characteristics, clinical data, and kratom use history were elicited. Then, the resting electrocardiogram (ECG) and transthoracic echocardiogram assessments of all the respondents were carried out. Blood sample was also collected from each respondent for serum mitragynine analysis and to evaluate the physical health status of the respondents. **Figure 1** summarizes the schematic presentation of the study design.

Demographic and Clinical Characteristics

In the context of demographic characteristics, the data collected include age, marital status, education, employment, and monthly income. Responses for age were reported as continuous variable. Responses for marital status were recorded as either married, single, or divorce/widower. Responses for education were reported as studied up to primary education, studied up to secondary education, or studied up to tertiary education. Responses for employment were grouped into either employed or unemployed. Finally, responses for monthly income were recorded as continuous variable.

As for clinical characteristics of the respondents, the data recorded include history of medical illness, family history of heart disease, history of cigarette smoking, blood pressure and resting pulse rate. These data were assessed and recorded by the medical doctor in the research team. History of medical illness and family history of heart disease were reported as either presence or absence. Since all the respondents had history of cigarette smoking on a daily basis, this variable was assessed based on the question, "On average, how many sticks of cigarette do you smoke in a day?" Response categories ranged from 1 to 30 sticks. Blood pressure was measured in mmHg and as the average of two readings taken for each respondent. In addition, the resting pulse rate of the respondents were measured in beats per minute.

Kratom Use History

Kratom use history elicited from the respondents included age of first kratom use, duration of kratom use, average daily quantity of kratom use, and time of last kratom consumption prior to the

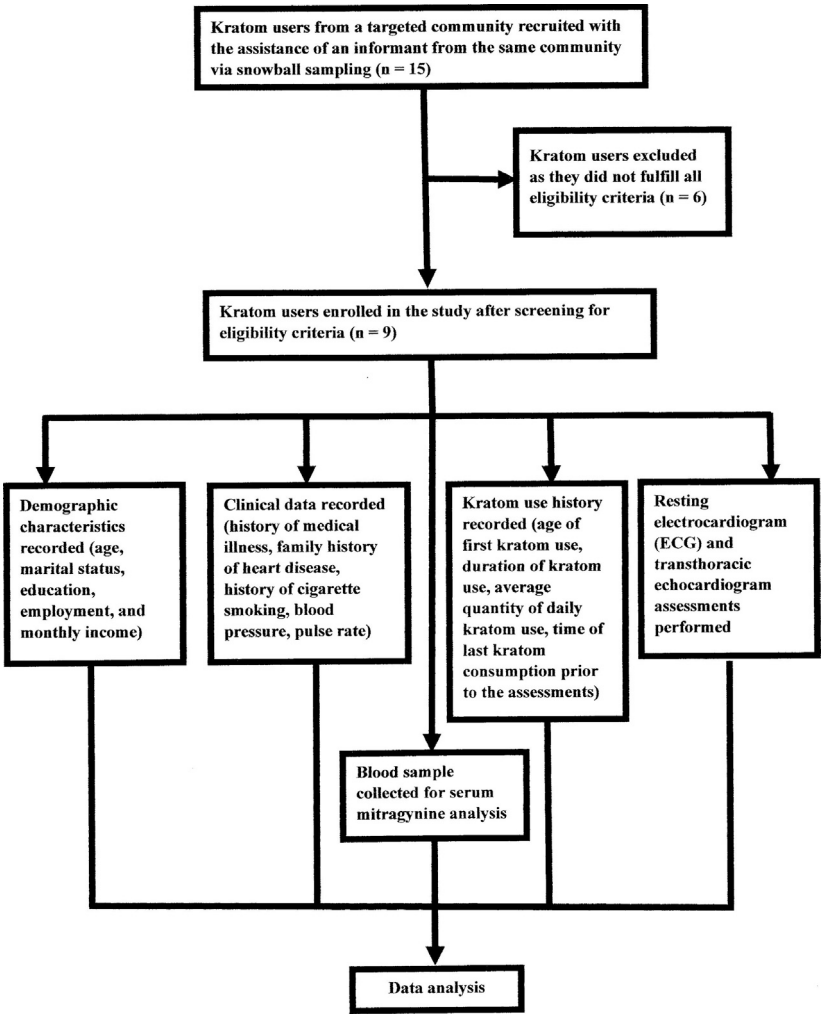

FIGURE 1 | Schematic presentation of the study design.

assessments (blood collection, ECG, and echocardiogram). History of illicit drugs consumption was also recorded. The age of first kratom use was evaluated through the question, "What was your age when you first started to consume kratom?." Response categories ranged from 6 years old to 40 years old. The duration of kratom use was assessed through the question, "How many years have you been consuming kratom juice?." Response categories ranged from 1 year to 40 years. Since all the respondents consumed kratom on a daily basis, the average daily quantity of kratom use was elicited through the question, "On average, how many glasses of kratom juice do you consumed in a day?." The response categories ranged from 1 to 30 glasses per day. The time of last kratom use prior to the assessments was evaluated through the question, "How many hours ago did you last consumed kratom prior to blood collection, ECG and echocardiogram assessments?." Response categories ranged from 1 to 72 h. History of illicit drugs consumption was assessed through the question, "Do you currently consumed or in the past had consumed any illicit drugs such as heroin, morphine, methamphetamine or Ice/Syabu, Ecstasy, benzodiazepine or Erimin 5, cannabis or ganja, ketamine, LSD, phencyclidine, inhalant, and/or alcohol?." The responses were reported as either presence or absence.

Resting ECG and Echocardiogram Assessments

The resting ECG and echocardiogram assessments, and the interpretation of the findings were carried out by a trained cardiologist. The measurements of the ECG parameters performed in this study are as follow:

1) PR interval was measured from the beginning of the upslope of the P wave to the beginning of the QRS wave. The normal range was from 0.12 to 0.20 s (Rosenthal, 2020).
2) QRS interval was measured from the beginning of the Q wave (at the end of PR interval) to the end of the S wave. The normal range was from 0.08 to 0.10 s (Rosenthal, 2020).
3) QT interval was measured from the beginning of the QRS complex to the end of the T wave (Rosenthal, 2020).

4) RR interval is the distance between the peaks of two consecutive QRS complex. RR interval was measured as 60/heart rate in this study. The normal range was from 0.6 to 1.2 s (Vandenberk et al., 2016; Rosenthal, 2020).

The definitions of abnormal ECG in this study are as follow:

1) Left axis deviation was defined when lead I was positive but lead aVF was negative (0° to −90°) according to the quadrant or two-lead approach (Surawicz et al., 2009).
2) Sinus tachycardia was defined if the heart rate was greater than 100 beats per minute with regular heart rhythm and a normal P wave [upright, normal morphology and consistent; (Crawford, 2017)].
3) A normal corrected QT (QTc) interval in male subjects was defined as up to 430 ms, while a QTc interval between 431 and 450 ms was considered as borderline QTc interval, and QTc of above 450 ms was considered abnormal or prolonged (Straus et al., 2006). The QTc interval was calculated using Framingham formula [QTc = QT + 0.154 (1-RR)], which has been reported to give the best rate of correction for QT interval (Vandenberk et al., 2016).

The definitions of abnormal echocardiogram in this study are as follow:

1) Left ventricular ejection fraction (LVEF):
   The modified Simpson method was used to measure LVEF. LVEF for males is evaluated in the following way (American College of Cardiology, 2020):
   a) Normal: 50–70%, midpoint 60%;
   b) Mild dysfunction: 40–49%, midpoint 45%;
   c) Moderate dysfunction: 30–39%, midpoint 35%;
   d) Severe dysfunction: <30%;
   e) Hyperdynamic EF: >70%
2) LVH was reported if left ventricular mass index threshold was >115 g/m^2 (Barbieri et al., 2012).
3) Mild tricuspid regurgitation was identified if the Doppler echocardiogram showed the following (Hahn et al., 2019):
   a) A small, narrow, central jet;
   b) Soft or incomplete jet by CW Doppler;
   c) PISA radius of ≤0.5 cm at Nyquist 28 cm/s; and
   d) Right ventricle and atrium of normal size

Blood Sample Collection

A total of 10 ml of blood sample was collected from each respondent for serum mitragynine analysis. Blood investigations such as complete blood count, renal profile, serum electrolytes, liver function test, thyroid function test, fasting blood sugar, and fasting lipid profile were also performed; in addition to the history and physical examination elicited by the medical doctor in the research team, to rule out the presence of any medical illnesses or abnormal blood parameters. All assessments (history taking, blood investigations, ECG, transthoracic echocardiogram, and serum mitragynine) were performed on the same assessment day.

Serum Mitragynine Analysis

Serum mitragynine level of all the respondents were analyzed using the solid-phase extraction and liquid chromatography-tandem mass spectrometry. The description of the serum mitragynine analysis is illustrated in **Supplementary Appendix S1** in the Supplementary material.

Data Analysis

All data were analyzed with Statistical Package for Social Sciences (SPSS) version 26 (SPSS 26; SPSS Inc., Chicago, Illinois, United States). The mean serum mitragynine of the regular kratom users were reported as the serum mitragynine level was normally distributed (normality evaluated with Shapiro-Wilk test which p-value of >0.05). The differences in serum mitrgynine level between kratom users who consumed four glasses or more kratom juice/day and less than four glasses of kratom juice/day as well as between those with prolonged QTc interval and normal QTc was assessed with independent t-test [as the dependent variable of serum mitragynine was normally distributed and t-test is valid for small sample size up to between 2 and 5 subjects per group; (de Winter 2013)]. Statistical significance was set at $p < 0.05$.

RESULTS

Initially, 15 regular kratom users were identified by the informant from the targeted community. However, only 9 kratom users were enrolled in the study as 6 kratom users did not fulfilled all the eligibility criteria (4 users had history of polysubstance use and 2 users had history of medical illnesses). The details of the respondent's characteristics (such as demographic and clinical characteristics, kratom use history, the ECG and echocardiogram findings, and serum mitragynine level), as well as the association between average quantity of daily kratom consumption, QTc intervals, and serum mitragynine levels of the respondents are presented below.

Respondent Characteristics

The details of the demographics and clinical characteristics, vital signs, history of illicit drug and alcohol use, and kratom use characteristics of all the respondents are presented in **Table 1**. The details of the main ECG findings, other ECG parameters, transthoracic echocardiogram findings, and serum mitragynine level of all the respondents are summarized in **Table 2**. While the full blood investigation findings of the kratom users are presented in **Supplementary Table S1** in the Supplementary material. All the blood investigations (complete blood count, renal profile, serum electrolytes, liver function test, thyroid function test, and fasting blood sugar) of the cases were normal except cases 1, 4, and 7 exhibited high serum triglyceride and cases 5 and 6 had high serum LDL. The selected characteristics of the cases are summarized below:

1) Case 1 was a 19-years old male, who had been using kratom for the past 3 years with an average daily kratom consumption of four glasses of kratom juice and his last kratom consumption was 2 h prior to time of assessment. His ECG indicated sinus tachycardia and prolonged QTc interval (468 ms) with a normal

TABLE 1 | Detailed demographic and clinical data, and kratom use characteristics of the respondents.

Variables	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9
Age	19 years	35 years	23 years	43 years	18 years	21 years	18 years	22 years	18 years
Marital status	Single	Single	Single	Married	Single	Single	Single	Single	Single
Education	Up to secondary education	Up to secondary education	Up to secondary education	Up to secondary education	Up to secondary education	Up to tertiary education	Up to secondary education	Up to secondary education	Up to secondary education
Employment	Unemployed	Employed	Employed	Employed	Employed	Unemployed	Employed	Unemployed	Unemployed
Monthly income	<RM 1000	RM 1800	<RM 1000	RM 2500	RM 1100	<RM 1000	RM 1200	<RM 1000	RM 1400
History of medical illness	No	No	No	No	No	No	No	No	No
Family history of heart diseases	No	No	No	No	No	No	No	No	No
Average quantity of daily cigarette smoking (sticks/day)	20	20	20	20	3	10	20	20	5
Blood pressure (mmHg)	133/91	116/68	136/63	157/94	127/82	133/74	110/65	128/77	135/76
Pulse rate (beats/minute)	120	73	117	89	117	81	74	96	86
Age started using kratom	16 years old	21 years old	13 years old	30 years old	13 years old	21 years old	14 years old	14 years old	16 years old
Duration of kratom use	3 years	14 years	8 years	13 years	5 years	14 years	4 years	8 years	2 years
Average daily quantity of kratom use (glasses of kratom juice per day)	4	2	4	4	3	2	4	6	3
Time of last kratom consumption prior to the assessments	2 h prior	2 h prior	3 h prior	2 h prior	2 h prior	2 h prior	3 h prior	2 h prior	2 h prior
History of intake of other illicit drugs and alcohol	No	No	No	No	No	No	No	No	No

QRS interval (80 ms), while the other ECG parameters were normal. His echocardiogram findings were normal with a LVEF of 65%. He recorded a serum mitragynine level of 9.6 mg/L.

2) Case 2 was a 35-years old male, who had been using kratom for the past 14 years with an average daily kratom consumption of two glasses of kratom juice and his last kratom consumption was 2 h prior to time of assessment. His ECG findings were unremarkable where the ECG parameters were all normal. His echocardiogram findings were also normal with a LVEF of 64%. He recorded a serum mitragynine level of 3.6 mg/L.
3) Case 3 was a 23-years old male, who had been using kratom for the past 8 years with an average daily kratom consumption of four glasses of kratom juice and his last kratom consumption was 3 h prior to time of assessment. His ECG indicated prolonged QTc interval (471 ms) with a normal QRS interval (92 ms), while the other ECG parameters were normal. His echocardiogram indicated presence of trivial tricuspid regurgitation with pulmonary artery systolic pressure (PASP) of 10 + 5 mmHg and his LVEF was 61%. He recorded a serum mitragynine level of 11.3 mg/L.
4) Case 4 was a 43-years old male, who had been using kratom for the past 13 years with an average daily kratom consumption of four glasses of kratom juice and his last kratom consumption was 2 h prior to time of assessment. His ECG findings revealed presence of left axis deviation and prolonged QTc interval (466 ms) with a normal QRS interval (84 ms), while the other ECG parameters were normal. His echocardiogram indicated left ventricular hypertrophy with a LVEF of 63%. He recorded a serum mitragynine level of 22.4 mg/L.
5) Case 5 was an 18-years old male, who had been using kratom for the past 5 years with an average daily kratom consumption

TABLE 2 | Cardiovascular findings and serum mitragynine level of the respondents.

case	Main ECG findings	Other ECG parameters	Echocardiogram findings	Serum mitragynine (mg/L)
Case 1	-Sinus tachycardia -Normal axis -No ischemic changes -No heart block -QTc = 468 ms	-PR interval = 120 ms - QRS interval = 80 ms -QT interval = 393 ms -RR interval = 512 ms	-All chamber size normal -LVEF = 65% -No MR by CFM -No AR/AS by CFM -No TR by CFM -No PR by CFM -No pericardial effusion -No RWMA -No intracardiac shunt	9.6
Case 2	-Sinus rhythm -Normal axis -No ischemic changes -No heart block -QTc = 428 ms	-PR interval = 180 ms - QRS interval = 100 ms -QT interval = 426 ms -RR interval = 984 ms	-All chamber size normal -LVEF = 64% -No MR by CFM -No AR/AS by CFM -No TR by CFM -No PR by CFM -No pericardial effusion -No RWMA -No intracardiac shunt	3.6
Case 3	-Sinus rhythm -Normal axis -No ischemic changes -No heart block -QTc = 472 ms	-PR interval = 170 ms -QRS interval = 92 ms -QT interval = 432 ms -RR interval = 741 ms	-All chamber size normal -LVEF = 61% -No MR by CFM -No AR/AS by CFM -Trivial TR by CFM with PASP 10 + 5 mmHg -No PR by CFM -No pericardial effusion -No RWMA -No intracardiac shunt	11.3
Case 4	-Sinus rhythm -Left axis deviation -No ischemic changes -No heart block -QTc = 466 ms	-PR interval = 170 ms -QRS interval = 84 ms -QT interval = 415 ms -RR interval = 667 ms	-Left ventricular hypertrophy -All chamber size normal -LVEF = 63% -No MR by CFM -No AR/AS by CFM -No TR by CFM -No PR by CFM -No pericardial effusion -No RWMA -No intracardiac shunt	22.4
Case 5	-Sinus tachycardia -Normal axis -No ischemic changes -No heart block -QTc = 411 ms	-PR interval = 166 ms -QRS interval = 81 ms -QT interval = 350 ms -RR interval = 600 ms	-All chamber size normal -LVEF = 67% -No MR by CFM -No AR/AS by CFM -No TR by CFM -No PR by CFM -No pericardial effusion -No RWMA -No intracardiac shunt	6.8
Case 6	-Sinus rhythm -Normal axis -No ischemic changes -No heart block -QTc = 411 ms	-PR interval = 137 ms -QRS interval = 88 ms -QT interval = 384 ms -RR interval = 822 ms	-All chamber size normal -LVEF = 63% -No MR by CFM -No AR/AS by CFM -No TR by CFM -No PR by CFM -No pericardial effusion -No RWMA -No intracardiac shunt	8.0
Case 7	-Sinus rhythm -Normal axis -T inversion over inferior leads (III, aVF) -No heart block	-PR interval = 148 ms -QRS interval = 100 ms	-All chamber size normal -LVEF = 68% -No MR by CFM -No AR/AS by CFM	20.4

(Continued on following page)

TABLE 2 | (*Continued*) Cardiovascular findings and serum mitragynine level of the respondents.

case	Main ECG findings	Other ECG parameters	Echocardiogram findings	Serum mitragynine (mg/L)
	-QTc = 467 ms	-QT interval = 426 ms -RR interval = 731 ms	-No TR by CFM -No PR by CFM -No pericardial effusion -No RWMA -No intracardiac shunt	
Case 8	-Sinus rhythm -Normal axis -No ischemic changes -No heart block -QTc = 424 ms	-PR interval = 150 ms -QRS interval = 100 ms -QT interval = 374 ms -RR interval = 674 ms	-All chamber size normal -LVEF = 65% -No MR by CFM -No AR/AS by CFM -No TR by CFM -No PR by CFM -No pericardial effusion -No RWMA -No intracardiac shunt	8.5
Case 9	-Sinus rhythm -Normal axis -No ischemic changes -No heart block -QTc = 414 ms	-PR interval = 150 ms -QRS interval = 90 ms -QT interval = 352 ms -RR interval = 600 ms	-All chamber size normal -LVEF = 69% -No MR by CFM -No AR/AS by CFM -No TR by CFM -No PR by CFM -No pericardial effusion -No RWMA -No intracardiac shunt	2.5

LVEF, left ventricular ejection fraction; CFM, colour flow mapping; RWMA, regional wall motion abnormalities; MR, mitral regurgitation; AR, aortic regurgitation; AS, aortic stenosis; TR, tricuspid regurgitation; PR, pulmonary regurgitation.

TABLE 3 | The association between average daily quantity of kratom use, QTc intervals, and serum level of mitragynine among the regular kratom users.

Variables	Mean serum mitragynine (SD)	Mean difference	t	p-value
Average daily quantity of kratom use				
<4 glasses (*n*= 3)	4.300 (2.234)	–9.067	–3.129	0.017[a]
≥4 glasses (*n* = 6)	13.367 (6.356)	—	—	—
QTc intervals				
Normal (<430 ms) (*n* = 5)	5.880 (2.685)	–10.045	–2.934	0.045[a]
Prolonged (>50 ms) (*n* = 4)	15.925 (6.412)	—	—	—

[a]*Statistical significance at* $p < 0.05$, *SD, standard deviation.*

of three glasses of kratom juice and his last kratom consumption was 2 h prior to time of assessment. His ECG findings were unremarkable except for presence of sinus tachycardia. His echocardiogram was normal with a LVEF of 67%. He recorded a serum mitragynine level of 6.8 mg/L.

6) Case 6 was a 21-years old male, who had been using kratom for the past 14 years with an average daily kratom consumption of four glasses of kratom juice and his last kratom consumption was 2 h prior to time of assessment. His ECG findings were unremarkable where the ECG parameters were all normal. His echocardiogram findings were also normal with a LVEF of 63%. He recorded a serum mitragynine level of 8.0 mg/L.
7) Case 7 was an 18-years old male, who had been using kratom for the past 4 years with an average daily kratom consumption of four glasses of kratom juice and his last kratom consumption was 3 h prior to time of assessment. His ECG findings revealed presence of prolonged QTc interval (467 ms) with a normal QRS interval of 100 ms, while the other ECG parameters were normal. His echocardiogram indicated left ventricular hypertrophy with a LVEF of 68%. He recorded a serum mitragynine level of 20.4 mg/L.
8) Case 8 was a 22-years old male, who had been using kratom for the past 8 years with an average daily kratom consumption of 6 glasses of kratom juice and his last kratom consumption was 2 h prior to time of assessment. His ECG findings were unremarkable where the ECG parameters were all normal. His echocardiogram findings were also normal with a LVEF of 65%. He recorded a serum mitragynine level of 8.5 mg/L.
9) Case 9 was an 18-years old male, who had been using kratom for the past 2 years with an average daily kratom consumption of three glasses of kratom juice and his last

kratom consumption was 2 h prior to time of assessment. His ECG findings were unremarkable where all the ECG parameters were all normal. His echocardiogram findings were also normal with a LVEF of 69%. He recorded a serum mitragynine level of 2.5 mg/L.

The Association Between Average Quantity of Daily Kratom Use, QTc Intervals, and Serum Level of Mitragynine Among the Regular Kratom Users

The association between average daily quantity of kratom use, QTc intervals, and serum mitragynine levels of the respondents are summarized in **Table 3**. Regular kratom users who consumed an average daily quantity of four or more glasses of kratom juice (freshly brewed kratom juice) registered a significantly higher serum mitragynine level compared with those who consumed less than four glasses of kratom juice [mean serum mitragynine $_{(< 4 \text{ glasses})}$ = 4.300, standard deviation (SD) = 2.234; mean serum mitragynine $_{(\geq 4 \text{ glasses})}$ = 13.367, SD = 6.356; $p = 0.045$]. Similarly, respondents who recorded prolonged QTc interval corresponded to significantly higher serum mitragynine level compared with those who had normal QTc interval [mean serum mitragynine $_{(\text{normal QTc})}$ = 5.880, SD = 2.685; mean serum mitragynine $_{(\text{prolonged QTc})}$ = 15.925, SD = 6.412; $p = 0.017$].

DISCUSSION

This case series examined the cardiovascular functioning and serum mitragynine level of regular kratom users who ingested brewed kratom juice. Our findings pinpointed to a few salient points among the case series of regular kratom users: 1) the mean serum mitragynine level of all the kratom users was 10.3 mg/L (SD = 6.9) and ranged from 2.5 mg/L to 22.4 mg/L; 2) Higher average daily quantity of kratom use (more than four glasses of kratom juice) was associated with higher serum mitragynine level; 3) 4 cases with serum mitragynine level of ≥9.6 mg/L exhibited prolonged QTc intervals; 4) kratom users with prolonged QTc intervals reported significantly higher serum mitragynine levels compared with those with normal QTc intervals; and 5) echocardiogram and other ECG findings (including the PR interval, QRS interval, and RR interval) were normal for the respondents except left ventricular hypertrophy was reported in one user, T wave inversion in inferior leads (III, aVF) in one user, and trivial tricuspid regurgitation with PASP 10 + 5 mmHg in another user were reported; with serum mitragynine of 22.4, 20.4 and 11.3 mg/L, respectively.

In the context of regular kratom consumption on cardiovascular function, a higher serum mitragynine level was associated with prolonged QTc interval. Our finding is in agreement with a former *in vitro* study of mitragynine effect on human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs; Lu et al., 2014). The QTc interval prolongation was most likely caused by prolonged repolarization and not depolarization since no changes in the QRS complex was found. Mitragynine and its analogues suppressed rapid delayed rectifier potassium current (I_{Kr}) by 67–84%, and significantly prolonged action potential duration (APD) in hiPSC-CMs in a dose-dependent manner without exerting changes in the L-type Ca^{2+} current ($I_{Ca,L}$). Hence, mitragynine could induced prolonged QTc interval at increasing serum level (Lu et al., 2014). The human ether-a-go-go-related gene (hERG) encode for a pore forming subunit of the I_{Kr} channel and hence, it is involved in the channel trafficking of ions across the cell membrane of cardiomyocytes. A more recent *in vitro* study on the mechanism of mitragynine-induced inhibition on the human ether-a-go-go-related gene 1a/1b (hERG1a/1b) confirmed that mitragynine suppressed the I_{Kr} current at a half-maximal inhibitory concentration (IC_{50}) value of 332.70 nM and induced significant decreased of the fully glycosylated (fg) hERG1a protein expression at lower dose, indicating that mitragynine directly block channel trafficking at lower dosage. Contrastingly, mitragynine at high dose upregulates the core-glycosylated (cg) hERG1a protein expression and hERG1a-Hsp90 complexes, revealing that mitragynine may induced hERG1a channel misfolding and triggered the unfolded protein response (UPR) and endoplasmic reticulum-associated protein degradation (ERAD) system as part of the compensatory mechanism of increasing ER stress (Tay et al., 2019). This possibility warrants further investigation in the future.

A summary of 156 cases of kratom-related deaths in the West and its post-mortem findings revealed that left ventricular hypertrophy was observed in six cases, while myocardial ischemia and infarction were detected in three cases (Corkery et al., 2019). Despite one respondent presented with left ventricular hypertrophy, one respondent had T wave inversion in inferior leads III and aVF (possibly indicative of myocardial ischemia), and another had trivial tricuspid regurgitation. Unfortunately, the small sample size of this case series precludes firm conclusions about the clinical relevance of these findings but does stress the need for further research in larger sample of kratom users to compare with control subjects.

As for the serum mitragynine level which was associated with kratom-related deaths, post-mortem reports of mortality cases affirmed that the mean serum mitragynine level for mortality cases which involved co-administration of kratom with other substances was at 0.890 mg/L (range = 0.000089–16.00 mg/L), while the mean serum mitragynine level for death cases related to kratom use as the sole substance was at 2.128 mg/L (range = 0.016–16.000; Corkery et al., 2019). Interestingly, the mean serum mitragynine reported in this case series was much higher (10.3 mg/L, range = from 2.5 to 22.4 mg/L); but the respondents were only using kratom without the use of other illicit substances, had no history of medical illness, alcohol consumption and psychiatric disorder, and had not consumed any medications on regular basis. As pointed out by Corkery et al. (2019), kratom toxicity in the West may arise from the potentiation effect of mitragynine and its metabolite 7-hydroxymitragynine on other co-administered substances, increasing the latter toxic effects on different organ systems. Mitragynine may also act as CYP2D6 inhibitor, which inhibit the metabolism of co-administered substances in the liver, increasing their toxic potential (Hanapi et al., 2013; Hughes, 2019). Moreover, it is unclear whether the mortality cases reported by Corkery et al. (2019) were caused by kratom use

per se or have been compounded partially by underlying medical disorders as the health background of the reported death cases were not assessed thoroughly. Besides, kratom users in the United States consists of naïve users and they may not be using kratom on daily basis and experienced tolerance. Hence, they may be more prone to kratom toxicity.

Our findings must be interpreted with caution considering several limitations. First, the sample size of this study was small. Besides, the association between serum mitragynine level and QTc intervals among the respondents which was assessed by univariate analysis may not indicate the causative effect of serum mitragynine level on the QTc intervals. Hence, prospective study with larger sample size and use of more robust statistical analysis is needed to confirm our findings. Second, the case series design may limit the reliability of this study as there is no control group for comparison. Assessing the dose response relationship with the average number of glasses of kratom juice consumed daily may not be optimal as those who consumed higher average daily quantity of kratom may represent two different populations of kratom users, such as those who consumed higher dose of kratom with the absence of tolerance on one hand and those who consumed higher dose of kratom due to extensive tolerance on the other hand. Third, the kratom users were recruited only from one state in Peninsular Malaysia (Penang) and this affects the generalizability of our findings. Besides Penang, kratom use is also common in the states of Perlis and Kedah in Peninsular Malaysia. Fourth, we failed to recruit female kratom users for this case series. Nevertheless, regular female kratom users are rare in Malaysia as most consumed it for its medicinal properties in relieving diarrhea, cough, myalgia, and abdominal discomfort (Singh et al., 2016). Finally, all the respondents in this study consumed kratom on daily basis for longer than 1 year in duration. Hence, the effect of initial or periodic kratom use on the cardiovascular functioning could not be determined in this study. Moreover, since this study excluded kratom users with chronic diseases, the effect of regular kratom use on the cardiovascular functioning of users with comorbid chronic illnesses could not be evaluated.

CONCLUSION

To conclude, this was the first case series which investigated cardiovascular functioning and its association with serum mitragynine level among regular kratom users who ingest freshly brewed kratom solution on a daily basis. Our findings add to the paucity of information on kratom side-effects and serves as a guideline to facilitate clinicians to understand that: 1) higher quantity of daily kratom consumption did increase the serum mitragynine level and 2) regular kratom use without concomitant use of other substances (even if the serum mitragynine level of as high as 22.4 mg/L) may not lead to any risk of cardiotoxicity except for prolonged QTc interval, which was dose dependent. However, torsades de pointes was not observed in all the regular kratom users in this study. Hence, kratom users who visited the emergency department suspected of kratom overdose or toxicity warrant an ECG examination, and perhaps Holter monitoring should also be considered. Based on our study findings, in order to delineate kratom's safety profile, there is an urgent need for studies to assess the serum cardiac markers, echocardiogram, Holter monitoring, serum mitragynine and 7-hydroxymitragynine to fully determine the potential cardiotoxicity risk of regular kratom consumption.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Research Ethics Committee USM, Division of Research & Innovation (R&I), USM Health Campus, 16150, Kubang Kerian, Kelantan. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ML and DS conceptualized and design the study. ML and DS involved in data collection. DS involved in data and statistical analysis. ML wrote the first draft of the manuscript. All authors involved in the revision of the manuscript and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.723567/full#supplementary-material

Supplementary Appendix S1 | Analysis of Mitragynine in Blood Samples using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS).

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Kratom Use Within the Context of the Evolving Opioid Crisis and the COVID-19 Pandemic in the United States

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Kratom (*Mitragyna speciosa*, Korth.) is an evergreen tree that is indigenous to Southeast Asia. When ingested, kratom leaves or decoctions from the leaves have been reported to produce complex stimulant and opioid-like effects. For generations, native populations in Southeast Asia have used kratom products to stave off fatigue, improve mood, alleviate pain and manage symptoms of opioid withdrawal. Despite the long history of kratom use in Asia, it is only within the past 10–20 years that kratom has emerged as an important herbal agent in the United States, where it is being used for the self-treatment of pain, opioid withdrawal symptoms, and mood disorders. The increase in the use of kratom in the United States has coincided with the serious epidemic of opioid abuse and dependence. Since 2015, efforts to restrict access to prescription opioids have resulted in a marked increase in the use of "street" opioids such as heroin and illicit fentanyl. At the same time, many patients with chronic pain conditions or opioid use disorder have been denied access to appropriate medical help. The lack of access to care for patients with chronic pain and opioid use disorder has been magnified by the emergence of the COVID-19 pandemic. In this report, we highlight how these converging factors have led to a surge in interest in kratom as a potential harm reduction agent in the treatment of pain and opioid use disorder.

Keywords: kratom, opioid crisis, COVID-19 pandemic, drug abuse, opioid use disorder

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INTRODUCTION

Kratom (also known as ketum) is a tree-like plant (*Mitragyna speciosa*, Korth) that is native to Thailand, Malaysia, Indonesia and other regions of Southeast Asia (Adkins et al., 2011; Prozialeck et al., 2012; Cinosi et al., 2015). For generations, indigenous peoples in Southeast Asia have used fresh kratom leaves (either unprocessed or brewed into teas or other decoctions) as a mild stimulant to stave off fatigue, or as an opioid substitute to treat pain or opioid use disorder (Vicknasingam et al., 2010; Singh et al., 2016). Pharmacologic studies have shown that kratom leaves contain over 40 active alkaloids with two of the best characterized being mitragynine and 7-hydroxymitragynine (Adkins et al., 2011; Prozialeck et al., 2012; Kruegel and Grundmann, 2018; Raffa et al., 2018; Prozialeck et al., 2019). Mitragynine has partial biased activity at mu-type opioid receptors, mixed activities at delta opioid receptors, and a variety of effects on other

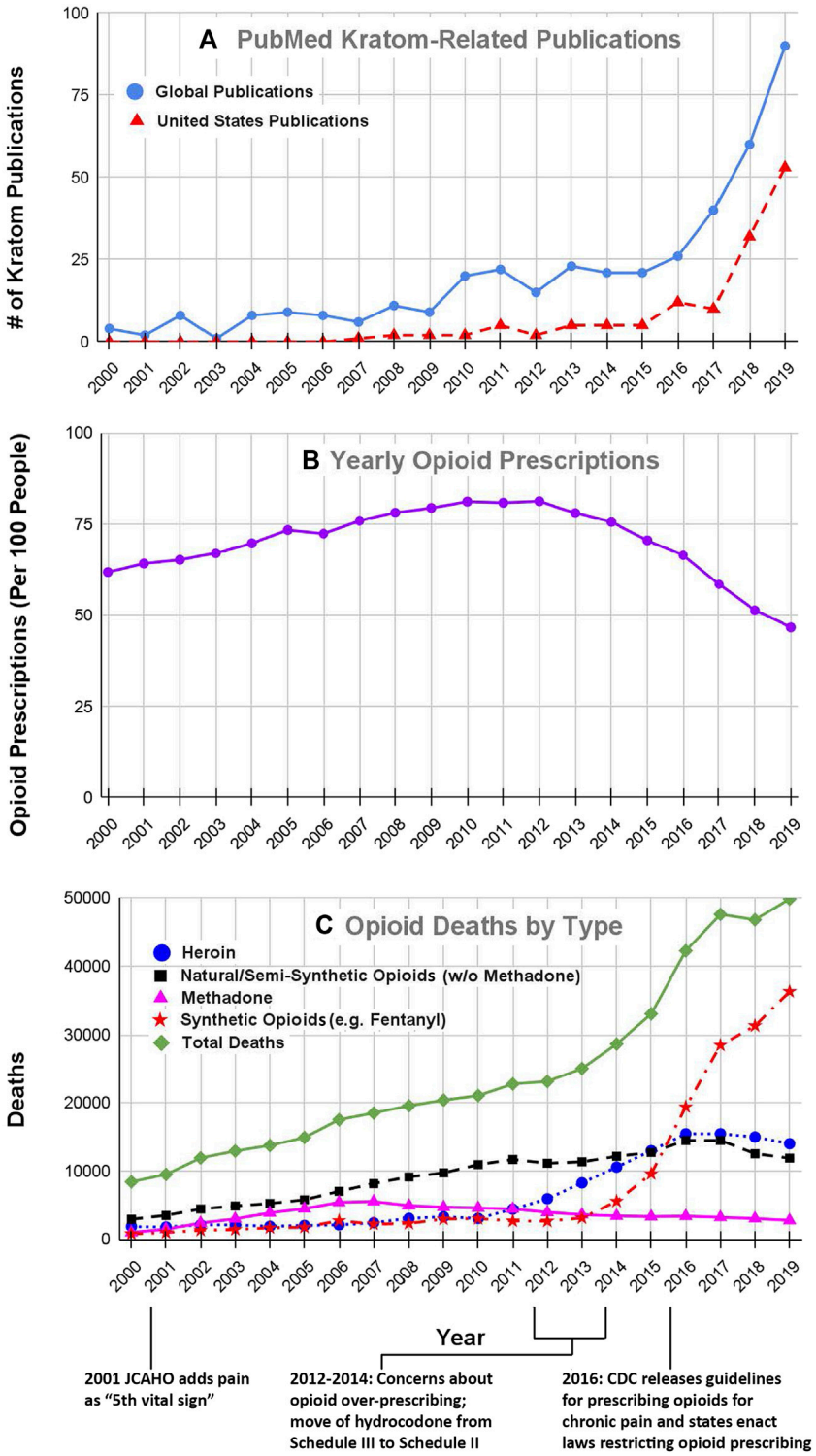

FIGURE 1 | Kratom-related publications **(A)**, opioid prescribing rates **(B)** and number of opioid overdose deaths **(C)** for years 2000–2019. The number of Kratom-related publications (**Figure 1A**) was obtained from a search of the United States National Library of Medicine's PubMed database, using the search term "kratom" on april 26, 2021. The solid black line shows the total number of publications for each year, whereas the broken line shows the number of publications in which at least one author was based in the United States. The data for opioid prescribing rates for the years 2000–2006 were obtained from (Kenan et al., 2012); data for the years 2006–2019 were obtained from the CDC database at https://www.cdc.gov/drugoverdose/maps/rxrate-maps.html. Each point represents the number of prescriptions per 100 people for each year. Please note that only 10–20% of people received *any* prescriptions which indicates that people received multiple prescriptions within the same year. Data for the number of opioid-related overdose deaths by drug type were obtained from both the CDC and NIDA data bases at https://www.cdc.gov/nchs/data/databriefs/db394-tables-508.pdf#page=3 and http://www.drugabuse.gov/drug-topics/trends-statistics/overdose-death-rates. All of these databases are in the public domain. The data that were extracted and used for these analyses are included as a supplemental table.

neurotransmitter systems in the central nervous system (Kruegel et al., 2016; Varadi et al., 2016; Obeng et al., 2020; Todd et al., 2020).

Even though kratom has been used in Southeast Asia for generations, it is only over the past 10–20 years that kratom use has expanded to Europe and North America (Prozialeck et al., 2012; Grundmann, 2017). In the United States, kratom products are used extensively for the self-management of pain, opioid use disorder and depression (Swogger et al., 2015; Grundmann, 2017; Swogger and Walsh, 2018; Schimmel et al., 2021). It has been estimated that there may be as many as 1–3 million kratom users in the United States (Prozialeck et al., 2019; Palamar, 2021; Schimmel et al., 2021). The most widely used products include chopped or powdered, dried leaf material (either bulk or in capsule or tablet form) or concentrated extracts that are formulated as teas or capsules (Prozialeck et al., 2012; Grundmann, 2017; Prozialeck et al., 2019; Wilson et al., 2020). These products are widely available from internet vendors or in specialty stores commonly known as "head shops" or "smoke shops", although some products are now being sold through chain stores that specialize in the sale of herbal supplements.

Kratom is regarded as a new dietary ingredient under the United States Food and Drug Administration (FDA) Dietary Supplement Health and Education Act (for reviews see: (Henningfield et al., 2018; Prozialeck et al., 2019)). Although it remains legal in most of the United States, at the time of writing, several states, such as Alabama, Florida, Indiana, Arkansas, Wisconsin and Tennessee, have passed legislation banning the local sale and possession of kratom (Prozialeck et al., 2019; AKA, 2020). At the same time, several states are in the process of adopting so called "kratom consumer protection acts", which allow for the sale and use of kratom, but also include standards for the quality control of kratom products (AKA, 2020).

Evidence for Increased Interest in Kratom

The emergence of kratom as a product or drug of interest in the United States is evident from the results of our literature searches. Our search of the US National Library of Medicine's PubMed database in April 2021 using the keyword "kratom" yielded a total of 517 articles and reviews. **Figure 1A** shows the number of kratom articles that were published each year in the period from 2000–2019 which was just before the emergence of the COVID-19 pandemic. As may be seen in the solid blue line in **Figure 1**, the total number of articles increased steadily from an average of fewer than five per year in the early 2000s to over 90 per year in 2019. Since 2019, this trend has continued, with 91 publications in 2020 and 42 from January-April of 2021 (data not shown in graph). In conjunction with this literature review, we also searched the list of authors for each article to identify papers in which at least some of the work originated in the United States. The broken red line in **Figure 1A** shows the number of kratom-related articles in which at least one of the authors was based in the United States. We identified a total of 218 articles that were published between 2000 and 2019. Interestingly, we found no papers with American-based authors before 2007. However, since then, the number of articles with American authors has increased markedly. This trend has continued to the present day. In 2020, there were 57 such articles and from January-April of 2021 there were 25 such articles. These results clearly show that the interest in kratom among American researchers has increased markedly over the past decade.

A second line of evidence showing the increased interest in kratom involves the mentions of kratom in reports to poison control centers in the United States. In 2016 Anwar and co-workers analyzed data from the United States National Poison Data System and found that from 2010 to 2015 the number of reports of kratom toxicity increased from about 20 per year to over 250 per year. Subsequent studies showed this trend was also evident for the time period from 2011 to 2018 (Davidson et al., 2021; Eggleston et al., 2019; O'Neill-Dee et al., 2019; Olsen et al., 2019). Anwar et al. (2016) had also reported that as many as 11 deaths in the 2010–2015 time frame may have been at least partly attributable to kratom, although the exact role of kratom in the deaths is unclear (Cumpston et al., 2018; Wing, 2018; Corkery et al., 2019; Hicks, 2019). The issues of kratom toxicity and kratom-related deaths are considered in more detail later.

One additional factor that may have facilitated interest in kratom in the early 2000s was the rapid development of internet communication that became available to increasing numbers of people around the world in the early part of the 21st century (Prozialeck et al., 2012; Williams and Nikitin, 2020). With the increased ease of internet communication, information about kratom, which had been little-known outside of Southeast Asia, could be rapidly disseminated globally.

Various investigators have noted that the increased interest in kratom seemed to coincide with several aspects of the evolving opioid crisis in the United States (Boyer et al., 2008; Prozialeck et al., 2012; Prozialeck, 2016; Bestha, 2018; Coe et al., 2019; Prozialeck et al., 2019) and that recent restrictions on access to prescription opioids for pain management may have further increased demand for kratom (Prozialeck, 2016; Prozialeck et al., 2019). With the emergence of COVID-19 in 2019, and the evolution of the COVID-19 pandemic in 2020, many patients may have faced even further reductions in access to prescription opioids, which could have contributed to an increase in the use of illicit "street" opioids such as heroin, fentanyl and new fentanyl analogs (Manchikanti et al., 2021; Nguyen and Buxton, 2021). It seems likely that this increase in the use of street opioids and the concomitant problems of opioid dependence may have further increased demand for kratom. In this report, we trace the evolution of kratom use in the United states and highlight the likely associations among the development of the ongoing opioid crisis, the unintended consequences of efforts to restrict access to prescription opioids for pain management, and the possible impact of the COVID-19 pandemic on demand for kratom.

Origins and Evolution of the Ongoing Opioid Crisis in the United States

Opium and drugs derived from its analogs (both natural and synthetic) have been used throughout human history for the management of pain and other conditions such as cough and diarrhea (for review see (Hanson et al., 2006)). In addition, these opioid substances have long been used and abused for their euphoric effects. Attitudes among medical professionals and the American public regarding the use of opioids for pain management have changed and fluctuated over the years (for reviews see, (Ray, 1996; Hanson et al., 2006)).

In the 19th century, opioids were not regulated and were widely available for use without medical guidance (Ray, 1996; Hanson et al., 2006). This led to widespread opioid abuse and dependence that resulted in the passage of federal laws restricting access to opioids. Throughout the first half of the 20th century, the resulting regulations reduced opioid use. However, in the 1960s the use of both medical and recreational opioids soared (Ray, 1996; Hanson et al., 2006). This surge resulted in changes in regulations and attitudes that further restricted access to opioids, a situation that persisted until the mid-1990s and the early 2000s, when the aggressive marketing of opioid products such as Oxycontin® and major changes in regulatory policies resulted in a marked increase in the prescribing of prescription opioids for pain management (Rummans et al., 2018). **Figure 1B** shows the number of opioid prescriptions/100 people in the United States for each year from 2000 to 2019. Note that the number of opioid prescriptions increased steadily from 2000 to 2012 at which point numbers plateaued and then by 2015 began to decline. We suggest that the increase in opioid prescribing in the early 2000s can be traced to two factors; the policy changes that were instituted by the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) in the year 2000–2001 and the promotion by pharmaceutical companies of opioid products such as Oxycontin® that were touted as safe for use in chronic pain.

In 2000, JCAHO was the primary organization of associations involved in the accreditation of hospitals and other healthcare organizations in the US. A special JCAHO task force had found that many patients throughout the US were suffering from serious pain that was not being treated adequately by their healthcare providers. In response to this situation, JCAHO incorporated specific requirements regarding proper pain assessment and pain management into their accreditation standards, which required that pain be assessed as if it were a "vital sign" (Berry and Dahl, 2000; Phillips, 2000). In cases where the patient reported significant levels of pain, the standards mandated appropriate pain management interventions, which often included various drugs, particularly opioid analgesics. Over the years, JCAHO was reorganized and renamed as the "Joint Commission" and their pain management standards were modified. It is also important to note the initial changes in JCAHO/Joint Commission policies occurred in 2001, a few years after the company Purdue Pharma, had released OxyContin®, a sustained release form of the opioid oxycodone and aggressively marketed the product as safe for treatment of chronic pain (Manchikanti et al., 2012; Thompson et al., 2012; Rummans et al., 2018).

In this environment, physicians and other healthcare providers were under increased pressure to prescribe analgesic drugs (Manchikanti et al., 2012; Rummans et al., 2018), and as may be seen in **Figure 1B**, the number of prescriptions for opioid analgesics rose markedly from 2000 to 2012.

Recognition of the Opioid Crisis and Unintended Consequences of Drug Policy Decisions

This increase in opioid prescribing resulted in concomitant increases in opioid abuse and deaths involving both prescription opioids (mainly morphine, codeine, hydrocodone and oxycodone) and "street drugs", such as heroin and fentanyl. **Figure 1C** summarizes data for opioid overdose deaths for the period from 2000 to 2019. The rate of overdose deaths from prescription opioids, both natural and semisynthetic (represented by the broken black line in **Figure 1B**) increased from 2,917 in the year 2000 to over 11,000 in 2011, while deaths from street drugs such as heroin (blue circles in **Figure 1B**) increased only slightly during the same time period.

By the year 2012, it was readily apparent from the soaring rate of opioid-related overdose deaths that the opioid problem in the US had grown to alarming proportions. This drew the attention of many investigators in the biomedical research community and caused federal regulatory agencies such as the US Centers for disease Control and Prevention (CDC), FDA and US Drug Enforcement Administration (DEA) to begin interventions that were intended to reduce the use of prescription opioids (CDC, 2012; Manchikanti et al., 2012; Nelson & Perrone, 2012; Stayner and Copenhaver, 2012; Rummans et al., 2018). For example, pharmaceutical companies began to develop abuse-deterrent formulations of opioids such as oxycodone (Schaeffer, 2012). In 2014, the DEA moved hydrocodone and products containing it from Schedule III to Schedule II under the Controlled Substance Act (DEA, 2014). Two years later, the CDC followed suit and developed new guidelines to discourage the use of opioids for the management of chronic non-cancer pain (Dowell et al., 2016). Even though the CDC actions were intended to serve as "guidelines", many clinicians, practice groups, healthcare systems, and even legal authorities, interpreted the "guidelines" as absolute requirements, and some local jurisdictions led by the state of Washington enacted laws to restrict the prescribing of opioids for many types of pain (Franklin et al., 2012; Stolbach and Nelson, 2016; Brookes, 2019). These interventions appeared to achieve the intended goal as evidenced by a marked decrease in the number of opioid prescriptions beginning in 2012 and continuing through 2019 (see **Figure 1B**).

Unfortunately, these efforts to restrict access to prescription opioids also may have had several unintended consequences, the most notable being a marked increase in the use of street opioids, such as heroin and fentanyl, and an alarming increase in the number of opioid-related overdose deaths. It should be noted that the surge in abuse of street opioids was also driven by a surge in the supply of heroin followed by fentanyl and its analogs being smuggled into the country. The recent surge in deaths from fentanyl and its analogs (broken red line in **Figure 1C**) is poses a major public health challenge.

Impact of the COVID-19 Pandemic on Supply and Demand for Kratom

The current COVID-19 pandemic first emerged from China in the late fall to early winter of 2019 and has had a major impact on almost all aspects of healthcare delivery around the world (WHO, 2021). The impact of COVID-19 on supply and demand for kratom has been complex, variable and somewhat unpredictable (Singh et al., 2020). In light of the many case reports and kratom

user summaries claiming that kratom has beneficial analgesic and mood-enhancing effects (Prevete et al., 2021), some COVID-19 patients have turned to kratom as a means of treating the pain, lethargy and depression that are commonly associated with COVID-19 infections (Metastasio et al., 2020; Singh et al., 2020). While we are aware of no direct studies showing that kratom can slow transmission and progression of COVID-19 infection, there is one case report suggesting that kratom can alleviate the pain, lethargy and lack of energy that are often experienced by COVID-19 patients (Metastasio et al., 2020). Further studies are needed to clarify this issue.

Besides its direct impact on public health in the US, the COVID-19 pandemic also has had many subtle and less direct effects. For example, many people with non-COVID health issues have encountered difficulties in obtaining appropriate care for their non-COVID problems, as national health leaders called for hospitals to forgo routine visits as well as screening and elective procedures (Puntillo et al., 2020; Caton et al., 2021; Jacka et al., 2021; Kedia et al., 2021; Linas et al., 2021; Mun et al., 2021; Peckham et al., 2021). The lack of access to care has been especially acute for patients with chronic pain problems and/or substance use disorders (Jacka et al., 2021; Kedia et al., 2021; Narayan and Balkrishnan, 2021). The lack of access to medically-assisted care for patients with opioid use disorder during the COVID-19 pandemic has been particularly severe (Jacka et al., 2021; Joudrey et al., 2021). These factors have probably resulted in a well-documented surge in the abuse of street opioids and their many attendant problems (CDC, 2021; Manchikanti et al., 2021). At the same time, it would be expected that the demand for kratom products would increase concomitantly (Singh et al., 2020).

Problems in Estimating Levels of Kratom Usage in the United States

In preparing this report, we attempted to determine directly how estimates of the levels of kratom usage in the US may have changed over the years. Unfortunately, we were unable to perform such analyses. Data for kratom usage in the late 20th and the early part of the 21st century are not available, and data for use over the past 10 years are quite variable. Results of national survey-based analyses have indicated that there are an estimated 2–3 million kratom users in the United States (Palamar, 2021; Schimmel et al., 2021). However, as Palamar noted (2021), such surveys can often under-estimate usage within the general population. Other estimates of kratom usage based on import data from Indonesia and average kratom consumption may actually be much higher, perhaps as many as 10–20 million (AKA, 2019; Henningfield et al., 2019). Unfortunately, data on levels of kratom imports over the years are not reliable and are skewed by import alerts by the FDA and seizures of kratom shipments in recent years (FDA, 2021). The issue is further complicated by the fact that kratom is currently banned in six states. As a result of these uncertainties, we focused our analyses on better-defined measures of kratom use such as the number of scientific publications and reports of toxicities associated with the use of purported kratom products.

Kratom Safety Concerns

Over the past decade federal agencies including the CDC, FDA and DEA have raised concerns about kratom toxicity and claimed that there is no evidence that kratom is effective in the treatment of any clinical condition. In 2016, the DEA proposed that kratom's alkaloid constituents mitragynine and 7-hydroxymitragynine be classified as Schedule I controlled substances, which would have effectively banned the use of kratom in the US (DEA, 2016a; 2016b). The announcement of these plans sparked vigorous opposition from many patients and patient advocacy groups who claim that kratom had helped them manage opioid withdrawal or chronic pain (Anson, 2016; Prozialeck, 2016; Wing, 2016; Prozialeck et al., 2019). The advocates' responses included a march and demonstration at the White House on September 13, 2016, and a petition was sent to President Obama. In addition, several leading kratom researchers noted that many of the reports of kratom-related deaths may have involved extremely high doses of kratom, the use of kratom products that were adulterated with other drugs, confounding health conditions or the concomitant use of other drugs (Henningfield et al., 2019; Prozialeck et al., 2019; Ramanathan and McCurdy, 2020). In response to these challenges, the director of the DEA announced that the kratom ban would be temporarily placed on hold (DEA, 2016c), a situation that persists to the present day. It should be noted that in February of 2021, it was revealed that the Department of Health and Human Services had actually rescinded the request to move kratom to Schedule I status in 2018, but that information was not released to the public (AKA, 2021).

In evaluating the safety of kratom products it is important to consider kratom within the context of the opioid crisis. In their proposal to schedule kratom, the DEA cited about 44 deaths that may have involved kratom from 2010 to 2016. In that same time frame, over 217,000 people died of opioid poisoning. Overwhelming evidence now indicates that, unlike opioids, kratom does not depress respiratory function to the same degree and is far less dangerous in overdose situations than classic opioids (Henningfield et al., 2019; Prozialeck et al., 2019). In addition, kratom has been shown to reduce craving for opioids in subjects with opioid use disorder (for reviews see (Prozialeck et al., 2019; Sharma and McCurdy, 2021; Singh et al., 2021). In this regard, kratom may have potential as a harm-reduction agent in the treatment of opioid use disorder, similar to cannabis (Ding et al., 2020; Lucas et al., 2021; Socias et al., 2021).

Kratom clearly contains pharmacologically-active compounds and, as such, does have potential for causing toxic effects. Reported toxic effects are actually quite different from those of classic opioids and commonly include: agitation, seizures, arrhythmias and hepatic injury (Eastlack et al., 2020; Kerrigan and Basiliere, 2021; Prozialeck et al., 2012; Schimmel and Dart, 2020). It is important to note, however, that almost all of the reports of toxicity involved the use of kratom products in the West (Davidson et al., 2021; Prozialeck et al., 2019). By contrast, there are few reports of serious adverse effects when kratom products are used in their traditional manner in Southeast Asia (Davidson et al., 2021; Prozialeck et al., 2019; Ramanathan and

McCurdy, 2020). This discrepancy suggests that the problem might not be related to the toxicity of kratom per se, but rather the poor quality of some kratom products being sold in the West, including the United States. Various studies indicate that many kratom products may be adulterated with other drugs or be contaminated with toxic metals and infectious microbes (Prozialeck et al., 2019; Prozialeck et al., 2020). In addition to the potential for acute toxicity, kratom can produce a state of physical dependence for which the term "kratom use disorder" has been coined. Dependence on kratom can lead to compulsive use and the appearance of withdrawal symptoms when kratom use is stopped. The symptoms of kratom withdrawal commonly include drug craving, anxiety, insomnia, irritability, and diarrhea (Prozialeck et al., 2012; Singh et al., 2018b). However, the symptoms of kratom withdrawal are quantitatively different, and generally less severe, than those of opioid withdrawal (Singh et al., 2018a; Singh et al., 2021; Stanciu et al., 2021; Vento et al., 2021). Numerous studies have shown that kratom has lower abuse potential than classical opioids (Singh et al., 2018a; Henningfield et al., 2018; Wilson et al., 2021).

Caveats and Limitations

There are several limitations to the present analyses. As noted previously, hard data on the number of kratom users are not available. As a result, we focused our analyses and discussion on more quantifiable measure of kratom use, such as the number of scientific publications and reports of kratom overdoses. In addition, data on opioid prescriptions and overdose deaths for the year 2020 have not yet been finalized by any federal agencies. To date, the CDC has only issued a report on "Provisional Drug Overdose Death Counts" for the year (CDC, 2021). However, the preliminary data in that report clearly show that the opioid overdose crisis has worsened during 2020, at the same time that the COVID-19 pandemic was evolving. Unfortunately, the data do not necessarily show a cause and effect relationship between the two events. This issue is further complicated by the lack of hard data on the impact of the COVID-19 pandemic on supply and demand for kratom. Based on the available data, we think it is highly likely that the COVID-19 pandemic may have triggered an increase in kratom usage, but additional studies are needed to either confirm or refute this possibility.

General Perspective and Conclusions

In this discussion, we have postulated that the increased interest in kratom in the US is mainly the result of changing patterns in the use of opioids for pain management and a marked surge in the use of street opioids, such as heroin, fentanyl and emerging fentanyl analogs. In considering this issue, we must also consider the possibility that the increased use of kratom itself may be one of the factors driving the current opioid epidemic. While most researchers are of the opinion that kratom may be useful as a "harm reduction" agent in the treatment of opioid use disorder (McMahon et al., 2019; Prozialeck et al., 2019; Grundmann et al., 2021; Sharma and McCurdy, 2021; Wilson et al., 2021), some have suggested that kratom may be a possible "gateway drug" that can lead users to try harder, more addictive drugs such as street opioids (Tayabali et al., 2018; Schimmel et al., 2021). While this may be the case for a small number of users, it does not seem to be a problem for the vast majority of users (Henningfield et al., 2018; Prozialeck et al., 2019). In fact, there is little or no evidence indicating that kratom is a "gateway" drug for most users (Henningfield et al., 2018; Prozialeck et al., 2019; Garcia-Romeu et al., 2020; Grundmann et al., 2021).

Even though legitimate questions regarding the safety and quality control of kratom products remain to be resolved, the therapeutic potential of kratom and its constituent compounds merit further study. There are numerous active compounds within kratom that appear to have multiple physiologic and psychologic effects beyond analgesia. Online and in-person studies have indicated there may be potential for kratom to produce antidepressant, anxiolytic and antipsychotic effects (Swogger et al., 2015; Grundmann et al., 2018; Coe et al., 2019; Ramanathan and McCurdy, 2020; Grundmann et al., 2021; Sharma and McCurdy, 2021; Smith et al., 2021). In light of these findings, there is clearly a need for further research on safety and efficacy of kratom and its active compounds. With regard to COVID-19, it is well documented that the pandemic has decreased access to medically-assisted treatment for patients with opioid use disorder (Joudrey et al., 2021; Narayan and Balkrishnan, 2021), a situation that leads to an increase in the use of kratom (Prevete et al., 2021). Even though COVID-19 is primarily viewed as a pulmonary disease, infected patients often exhibit symptoms of pain, lethargy and depression. Published analyses of content on kratom user discussion websites indicate that many individuals use kratom to treat such complaints (Smith et al., 2021). Therefore, it seems highly likely that COVID-19 patients may be using kratom for self-medication. There is an urgent need for studies on the impact of the COVID-19 pandemic on levels of kratom usage along with clinical trials of the potential benefits and toxicities of kratom in patients infected with COVID-19.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.cdc.gov/drugoverdose/maps/rxrate-maps.html https://www.cdc.gov/nchs/data/databriefs/db394-tables-508. pdf#page = 3 http://www.drugabuse.gov/drug-topics/trends-statistics/overdose-death-rates.

AUTHOR CONTRIBUTIONS

WP conceived the idea for the manuscript and oversaw the literature searches and writing of the manuscript. PL assisted with literature search and compilation of data. OG contributed to the development of concepts, the evaluation of the literature reviews and the actual writing of the manuscript. MK and MM assisted with the literature reviews, the analysis of the data and the writing of the manuscript. LP assisted in the analysis of the data and preparing the graphs.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.729220/full#supplementary-material

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Mitragynine (Kratom)-Induced Cognitive Impairments in Mice Resemble Δ9-THC and Morphine Effects: Reversal by Cannabinoid CB_1 Receptor Antagonism

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Kratom is a widely abused plant-based drug preparation with a global interest in recent years, well beyond its native grounds in Southeast Asia. Mitragynine, its major psychoactive constituent is known to exhibit opioid-like behavioral effects with resultant neuroplasticity in the brain reward system. Its chronic administration is associated with cognitive impairments in animal studies. However, the underlying molecular mechanism for such a deficit remains elusive. In this study, the involvement of cannabinoid type-1 (CB_1) receptors in cognitive deficits after chronic mitragynine exposures was investigated for 28 days (with incremental dose sensitization from 1 to 25 mg/kg) in adult male Swiss albino mice using the IntelliCage® system. Chronic high-dose mitragynine exposure (5–25 mg/kg, intraperitoneal [i.p.]), but not low-dose exposure (1–4 mg/kg, i.p.), induced hyperlocomotion, potentiated the preference for sucrose reward, increased resistance to punishment, and impaired place learning and its reversal. Comparable deficits were also observed after chronic treatments with Δ-9-tetrahydrocannabinol (THC, 2 mg/kg, i.p.) or morphine (5 mg/kg, subcutaneous). Mitragynine-, morphine-, and THC-induced learning and memory deficits were reversed by co-treatment with the CB_1 receptor antagonist, NIDA-41020 (10 mg/kg, i.p.). A significant upregulation of CB_1 receptor expression was found in the hippocampal CA1 region and ventral tegmental area after chronic high-dose mitragynine and morphine, whereas a downregulation was observed after chronic THC. In conclusion, the present study suggests a plausible role of the CB_1 receptor in mediating the dose-dependent cognitive deficits after chronic high-dose mitragynine exposure. This also highlights the potential of CB_1 receptor antagonism in ameliorating the cognitive deficits associated with long-term kratom/mitragynine consumption in humans.

Keywords: kratom, mitragynine, morphine, Δ9-tetrahydrocannabinol (THC), cannabinoid receptor 1 (CB1), cognition

INTRODUCTION

Kratom (*Mitragyna speciosa Korth*) is a native plant to the Philippine islands, New Guinea, and Southeast Asia, predominantly Malaysia, Thailand, and Indonesia. It is recognized as a local medicinal plant chiefly because of its antinociceptive and psychostimulant effects (Jansen and Prast, 1988; Hassan et al., 2013; Raffa, 2014). Since 2004, due to the growing concerns over the plant's narcotic properties and abuse liabilities, the Malaysian government has criminalized kratom's major alkaloid, mitragynine, under the Third Schedule of Poisons (Psychotropic Substances) Regulations, Poison Act 1952. Kratom is also currently regulated under the respective Narcotics Act in Thailand, Australia, and Myanmar (Bergen-Cico and MacClurg, 2016; Singh et al., 2017). Despite legal restrictions in such countries, the recreational use and abuse of kratom remains prevalent throughout Malaysia and Thailand (Ahmad and Aziz, 2012; Singh et al., 2017; Singh et al., 2019). In fact, claims and reports over the internet regarding its potential as a cheap opioid substitute have attracted the Western users to use kratom to self-medicate for opioid withdrawal and chronic pain, apart from being sold as a dietary supplement in recent decades in the United States and Europe (Boyer et al., 2008; Grundmann, 2017; Coe et al., 2019; Müller et al., 2020). The majority of long-term kratom users (over three-quarters) report developing dependence and an inability to cease its use, mainly due to its unpleasant withdrawal symptoms (Suwanlert, 1975; Boyer et al., 2008; Vicknasingam et al., 2010; Ahmad and Aziz, 2012; Saingam et al., 2013; Grundmann, 2017; Singh et al., 2019). Kratom and mitragynine are marketed for Western users either as a pure preparation (Cornara et al., 2013; Forrester, 2013; Coe et al., 2019) or as one herbal ingredient of "legal" or "herbal high" preparations, which are distributed in the form of powders, pills, and capsules under various names such as Krypton, K2, or Spice (Dresen et al., 2010; Arndt et al., 2011; Singh et al., 2014; Tavakoli et al., 2017). The emergence of reports on the serious adverse effects associated with kratom/mitragynine abuse has prompted a ban on kratom in several states in the United States (McWhirter and Morris, 2010; Nelsen et al., 2010; Holler et al., 2011; Kapp et al., 2011; Kronstrand et al., 2011; Forrester, 2013; Neerman et al., 2013; Trakulsrichai et al., 2013; Eggleston et al., 2019). Currently, the United States Drug and Enforcement Administration and Food and Drug Administration remain vigilant in considering to place kratom into Schedule I of the Controlled Substances Act (Henningfield et al., 2018).

Numerous studies on the major alkaloid, mitragynine, and the less abundant constituent, 7-hydroxymitragynine, of kratom have demonstrated the high binding affinity for supraspinal μ- and δ-opioid receptors governing the antinociceptive and antitussive actions of these constituents and their rewarding properties (Matsumoto et al., 1996; Thongpradichote et al., 1998; Takayama, 2004; Matsumoto et al., 2006; Yusoff et al., 2017). Several studies have demonstrated that acute and chronic administrations of mitragynine caused significant cognitive and emotional impairments in animals (Apryani et al., 2010; Hazim et al., 2011; Harizal et al., 2012; Yusoff et al., 2016; Iman et al., 2017; Hassan et al., 2019). Recent studies also reported that high doses of mitragynine (5 and 10 mg/kg) cause spatial/place learning deficit, accompanied by a disruption to synaptic transmission and long-term potentiation (LTP) at the CA1 field of the rat hippocampus, and electroencephalogram deficits (Hassan et al., 2019; Suhaimi et al., 2021). Thus far, the exact neural mechanisms that underlie these adverse effects on cognition remain elusive. In this context, little is known about low-dose mitragynine (1–4 mg/kg) despite the reported dose-dependent pharmacological effects of kratom/mitragynine—psychostimulants at low doses and opioid-like depressant effects at high doses (>5 mg/kg) (Suwanlert, 1975; Hassan et al., 2013; Saingam et al., 2013; Singh et al., 2019). Therefore, this study explores the low- and high-dose mitragynine range to reflect the spectrum of potential adverse effects on cognition and links the role of the endocannabinoid system for the first time to the best of the author's knowledge.

The endocannabinoid system and in particular the cannabinoid type-1 (CB_1) receptors are well known for their role in the reinforcing effects of addictive substances and long-term behavioral sensitization after chronic use. This receptor system has been recognized for its reciprocal interaction with the opioid system and brain reward circuitry (Justinova et al., 2009; Katona, 2009; Scavone et al., 2013; Parsons and Hurd, 2015; Laredo et al., 2017; Manzanares et al., 2018). CB_1 and opioid receptors co-localization at the brain areas governing motivation, learning and memory, and behavioral control lends support to this reciprocity (Pickel et al., 2004; Justinova et al., 2009; Wilson-Poe et al., 2012; Scavone et al., 2013; Manzanares et al., 2018). Changes in the expression level of CB_1 receptors in response to drugs of abuse were observed in both animal and human studies, suggesting its contribution to long-term plasticity associated with drug administration (Justinova et al., 2009; Maldonado et al., 2013; Jin et al., 2014; Vlachou and Panagis, 2014; Manzanares et al., 2018). This study investigated the potential role of CB_1 receptors in mediating the cognitive deficits induced by a chronic mitragynine sensitization.

MATERIALS AND METHODS

Animals

A total of one hundred adult male Swiss albino mice (n = 100; weight: 30–35 g) were purchased from the breeding colony of the Animal Research and Service Centre of Universiti Sains Malaysia (Health Campus) in Kelantan, Malaysia. All mice were approximately 8–9 weeks old at the beginning of the experiment and naive. The mice were initially housed in groups of five per cage, for a minimum of 5 days before behavioral testing. These polypropylene cages had free access to standard commercial food pellets and tap water *ad libitum*. They were maintained under controlled environmental conditions (temperature, 22 ± 2°C; 50 ± 5% humidity; 12:12-h light/dark schedule). The physical behaviors of each mouse were observed throughout habituation and experimentation. Mice showing any signs of aggression (i.e., fighting/attacks and

biting) that may affect their social behaviors were excluded from the analyses.

All the animals were maintained according to the specified duration of pre- and post-drug exposure. The experimental protocols for care and use of laboratory animals were approved by the Universiti Sains Malaysia (USM) Institutional Animal Care and Use Committee (USM IACUC) [Approval No: USM/AEA/2016/(101)(755)].

Drugs and Chemicals

Mitragynine was supplied by the Centre for Drug Research, USM. Fresh *M. speciosa* leaves were harvested from Perlis, Malaysia, and authenticated by the Herbarium of the School of Biological Sciences, Universiti Sains Malaysia (Voucher No: USM1707-2017). Mitragynine, the active principal alkaloid of *M. speciosa* was isolated by the method reported by Beng et al. (2011) and Jamil et al. (2013). Purified mitragynine was confirmed by high-performance liquid chromatography and proton nuclear magnetic resonance (400 MHz) analyses (Jamil et al., 2013). Mitragynine obtained by this procedure was approximately 98% pure, with high stability at 4 to −20°C for 6 months. The dried mitragynine extract was sealed in a bottle and stored at 4°C until use with a prior inspection and written approval obtained from the Division of Pharmacy, Ministry of Health, Malaysia. Morphine sulfate (Pharmaniaga, Malaysia) and Δ-9-tetrahydrocannabinol [THC, Lipomed AG, Switzerland] were used as reference drugs. 20% Tween 20 (Bio-Rad Laboratories, United States) was used as a vehicle. NIDA-41020 (Sigma, United States) was used as the CB_1 receptor antagonist.

IntelliCage Apparatus

The IntelliCage® system (TSE Systems GmbH, Bad Homburg, Germany) is a fully automated behavioral platform designed for short- and long-term cognitive monitoring of individual radio frequency–tagged (RFID) mice living in social groups, as described in detail in earlier studies (Galsworthy et al., 2005; Lipp et al., 2005; Endo et al., 2011; Kiryk et al., 2020). Briefly, the IntelliCage is a standard polycarbonate cage (55 cm width × 38 cm depth × 21 cm height), which accommodates up to 16 mice at a time. A triangular operant test chambers (15 × 15 × 21 cm) are fitted at each of the corners. Entry (or visit) into each operant chamber is identified by a circular RFID antenna that detects the animal's unique ID tags and records their visits. Two round apertures, equipped with motorized doors, on each chamber wall permit free access to water bottles. Small motorized doors at the apertures can be programmed to close to limit water access and are able to detect mouse nose poke patterns (individual pokes at each doors). Animals can be trained to perform fixed or progressive ratio nose pokes at the door to allow access to water. Mounted above each door is a motorized valve for delivery of air-puffs as a form of negative reinforcement or punishment.

Behavioral Design in the IntelliCage System

All animals were subcutaneously (s.c.) implanted with sterile glass-covered microtransponders (12 × 2 mm; Datamars, Switzerland) for individual recognition in the IntelliCage using supplied disposable syringe and an injector (Datamars, Switzerland). After 48 h of implantation, all animals were checked for microtransponder retention with a handheld electronic reader (Datamars, Switzerland) before being released into the IntelliCage.

Drug Sensitization

The incentive-sensitization theory of addiction posits the progressive increase in the neurological and behavioral stimulatory effects of a drug following repeated intermittent administration. The drug-induced hypersensitization of the brain reward circuits is hypothesized to cause a pathological transition from drug "liking" to "wanting" that underlies compulsive substance use as previously demonstrated with chronic challenges of morphine, cocaine, ethanol, nicotine, and cannabinoids (Berridge and Robinson, 2011; Marinho et al., 2015; Grigutsch et al., 2019; Lopes et al., 2020).

All mice were taken out from the IntelliCage once daily (in the morning) to receive their assigned 28-day drug sensitization regimen. In the drug + NIDA-41020 groups, mice were sensitized with mitragynine, morphine, or THC for the first 14 days (Richards et al., 1999; Paine et al., 2003; Olmstead, 2006; Yusoff et al., 2016), whereas NIDA-41020 (without coadministration of mitragynine, morphine, or THC) was administered starting from Day 15 onward. The interventions for the receptor antagonist groups were designed to eliminate any mitragynine, morphine, or THC cross-interaction with opioid and/or other receptor systems, thus avoiding any interference with the study objectives. All drug sensitizations were performed at a volume of 1 ml/kg body weight. Mice were returned to IntelliCage immediately after injection handling. All mice were randomly assigned to 10 groups (n = 10/group). Treatment details are shown in **Table 1**.

The incremental dosage of mitragynine (low and high) were selected to mimic human kratom consumption, which often develops into dependency and tolerance (i.e., increase number of kratom leaves and frequency of intake) after prolonged consumption (Saingam et al., 2013; Singh et al., 2014; Singh et al., 2019). The selected low-dose mitragynine (from 1 to 4 mg/kg; in increments of 1 mg/kg) has been shown to produce light stimulant effects (Yusoff et al., 2016). The selected dose and route of high-dose mitragynine administration (from 5 to 25 mg/kg; in increments of 5 mg/kg) have previously been shown to affect locomotion, cognition, and memory functions in mice (Apryani et al., 2010; Yusoff et al., 2016; Iman et al., 2017; Hassan et al., 2019). The selected doses and routes of administration of morphine sulfate (5 mg/kg, s.c.) and THC (2 mg/kg, intraperitoneal [i.p.]) have been shown to develop profound morphine- (Abdel-Zaher et al., 2013) and THC-induced (Vlachou et al., 2007; Zuardi et al., 2012; Iman et al., 2017) tolerance and dependence in mice, respectively, without any confounding toxic effects. The dose of CB_1 receptor antagonist NIDA-41020 (10 mg/kg, i.p.) was chosen because it effectively attenuated behavioral effects of morphine in a previous study (Bdeer et al., 2014).

TABLE 1 | Description of experimental groups and drug sensitization.

	Intervention groups	Description
1	Untreated	The untreated group that served as a negative control
2	Vehicle control	Daily i.p. injection of 20% Tween-20 (1 ml/kg) for 28 days
3	Morphine	Daily s.c. injection of morphine sulfate (5 mg/kg) for 28 days
4	Morphine + NIDA-41020	Daily s.c. injection of morphine sulfate (5 mg/kg) from Day 1 to Day 14, followed by daily i.p. administration of NIDA-41020 (10 mg/kg) from Day 15 to Day 28
5	THC	Daily i.p. injection of THC (2 mg/kg) for 28 days
6	THC + NIDA-41020	Daily i.p. injection of THC (2 mg/kg) from Day 1 to Day 14, followed by daily i.p. administration of NIDA-41020 (10 mg/kg) from Day 15 to Day 28
7	Mit high	Daily i.p. injection of mitragynine (from 5 to 25 mg/kg; in increments of 5 mg/kg) for 28 days Day 1–3 = 5 mg/kg of mitragynine Day 4–6 = 10 mg/kg of mitragynine Day 7–9 = 15 mg/kg of mitragynine Day 10–12 = 20 mg/kg of mitragynine Day 13–28 = 25 mg/kg of mitragynine
8	Mit high + NIDA-41020	Daily i.p. injection of mitragynine (from 5 to 25 mg/kg; in increments of 5 mg/kg) from Day 1 to Day 14, followed by daily i.p. administration of NIDA-41020 (10 mg/kg) from Day 15 to Day 28 Day 1–3 = 5 mg/kg of mitragynine Day 4–6 = 10 mg/kg of mitragynine Day 7–9 = 15 mg/kg of mitragynine Day 10–12 = 20 mg/kg of mitragynine Day 13–14 = 25 mg/kg of mitragynine Day 15–28 = 10 mg/kg of NIDA-41020
9	Mit low	Daily i.p. injection of mitragynine (from 1 to 4 mg/kg; in increments of 1 mg/kg) for 28 days Day 1–3 = 1 mg/kg of mitragynine Day 4–6 = 2 mg/kg of mitragynine Day 7–9 = 3 mg/kg of mitragynine Day 10–28 = 4 mg/kg of mitragynine
10	Mit low + NIDA-41020	Daily i.p. injection of mitragynine (from 1 to 4 mg/kg; in increments of 1 mg/kg) from Day 1 to Day 14, followed by daily i.p. administration of NIDA-41020 (10 mg/kg) from Day 15 to Day 28 Day 1–3 = 1 mg/kg of mitragynine Day 4–6 = 2 mg/kg of mitragynine Day 7–9 = 3 mg/kg of mitragynine Day 10–14 = 4 mg/kg of mitragynine Day 15–28 = 10 mg/kg of NIDA-41020

NIDA-41020, Sigma, United States, is the CB_1 receptor antagonist.
i.p., intraperitoneal; Mit high, high-dose mitragynine; Mit low, low-dose mitragynine; s.c., subcutaneous; THC, Δ-9-tetrahydrocannabinol.

Behavioral Parameters in the IntelliCage System

Following their introduction to the IntelliCage, the mice were allowed free access to all IntelliCage corners for 4 days to measure their baseline exploratory behaviors before drug intervention (see Baseline Phase section). Subsequently, the mice were subjected to daily sensitization for 28 days (see Sensitization Phase section) according to their assigned drug intervention. The following parameters (**Figure 1**) have been adapted with modifications from Radwanska and Kaczmarek (2012), as also described previously (Iman et al., 2017), and applied through appropriately designed learning protocols in the IntelliCage software as has been given below.

Baseline Phase

During the pre-intervention days, mice were given free access to all cage areas for 4 days without receiving any drug intervention. All water-access doors were opened. Animals were tested for exploratory activity in the novel cage/environment, measured as the number of visits during the first hour in the IntelliCage, and in the familiar cage/environment, measured as the number of visits per day during the following 3 consecutive days.

Sensitization Phase

Activity in the familiar environment (post-intervention, Days 1–7): the IntelliCage setup was identical to the baseline phase. Data collected comprised of the number of visits per day for 3 consecutive days following drug sensitization (Day 5–7). As established in previous studies, the mean number of corner visits was used as a proxy for general exploratory activities (Galsworthy et al., 2005; Radwanska and Kaczmarek, 2012) for comparison with baseline exploratory activities.

Sucrose reward preference (Day 8 and 9): mice had access to normal tap water at one active corner and 10% sucrose solution at the other active corner. The doors of the two remaining inactive corners were closed throughout this protocol.

Persistence in sucrose-seeking (Day 10–12): mice were subjected to air-puff punishment (0.4 bar, 2-s duration) following sucrose-reward drinking. The doors of the two remaining inactive corners were closed throughout this protocol.

Sucrose extinction (Day 13 and 14): the IntelliCage setup identical to the baseline phase was used to prepare the mice for the subsequent protocol.

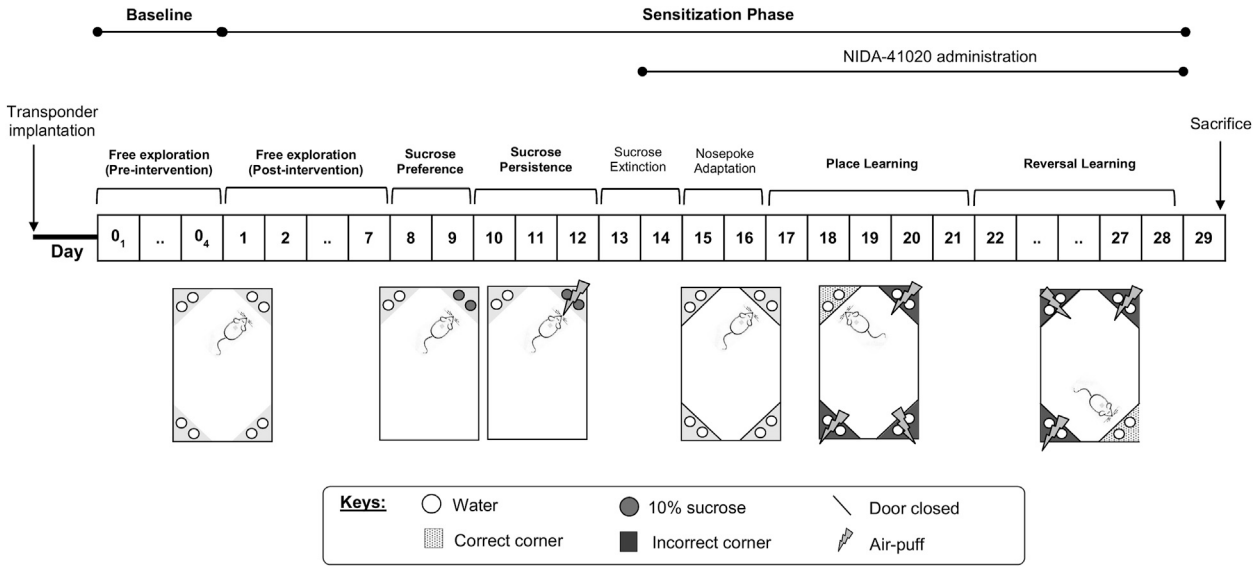


FIGURE 1 | Summary of the IntelliCage experimental protocol.

Nose poke adaptation (Day 15 and 16): water-access doors were closed at all corners. Mice had to perform one nose poke, which opened the respective door, in order to drink. Opened doors were automatically closed after 5 s. The least preferred corners of the individual mouse were determined for programming the subsequent place learning protocol.

Place learning (Day 17–21): all doors remained closed, but access to water was restricted to one water-rewarded corner (the individual least preferred corner during nose poke adaptation) termed the "correct corner." Three successive nose pokes at the individual correct corner opened the respective door for 5 s. The percentage of visits with nose pokes to the correct corner were determined as an indicator for learning.

Reversal learning (Day 22–28): the same procedure as for place learning was followed, but the correct water-rewarded corner was reversed to the diagonal opposite corner. The percentage of visits with nose pokes in the newly placed correct corner was measured as an indicator for reversal learning.

Brain Sample Collection

Following 24 h after the end of the IntelliCage study (Day 29), all mice were euthanized with pentobarbital (10 mg/kg, i.p.). Mice for an immunohistochemistry study were transcardially perfused with phosphate-buffered saline, followed by 10% (v/v) neutral buffered formalin (NBF), with a steady flow rate of 10.0 ml/min using an IPC Digital Peristaltic Pump (Ismatec, Germany). The whole brain of each mouse was isolated and cut along the hemispheric fissure with a sharp blade, dividing the brain into halves, and post-fixed in 10% (v/v) NBF at room temperature overnight before tissue processing. Mice for the western blot and quantitative real-time polymerase chain reaction (qPCR) studies were decapitated under pentobarbital euthanasia, followed by rapid removal of their brain on ice and dividing it into the two hemispheres. The cerebral hemisphere tissues collected for the western blot study were immediately stored at −80°C, while the tissues for the qPCR study were immersed in RNAlater® solution and stored at −80°C until further use.

Immunohistochemistry

Immunohistochemistry staining was performed to investigate CB_1 receptor expression in the mouse hippocampus and ventral tegmental area (VTA) brain regions after chronic drug treatment. For assessment of the specificity of the reaction, positive controls (mouse cerebellum) (Ashton et al., 2004) and negative controls (incubation without primary antibodies) were routinely included.

The processed mice brain tissues blocked in paraffin were cut into a series of 4-μm-thick sagittal sections using a microtome. The hippocampus and VTA brain regions were determined using the Allen Mouse Brain Atlas© online portal (Allen Institute for Brain Science, 2004) and visualized under a light microscope. Systemic random sampling technique was used to select one in every five sections for a total of four sections per brain. The selected ribbons of sections were mounted on poly-L-lysine–coated slides, air-dried overnight, deparaffinized with xylene, cleared, and rehydrated in graded ethanol.

Following reduction of endogenous peroxides through pre-incubation with 1% hydrogen peroxide (Merck, Germany), the sections were microwaved for antigen retrieval in 1X Tris-EDTA (pH 9.0) for 20 min. The sections were then blocked for nonspecific background staining with 5% BSA (Sigma, United States; 15 min RT) and incubated overnight at 4°C with rabbit polyclonal primary antibody against cannabinoid receptor type-1 (Anti-CB_1 receptor; Cat No: ab23703; Abcam, United Kingdom; dilution 1:200). Sections were then incubated with horseradish peroxidase (HRP)–conjugated secondary antibody (goat anti-rabbit IgG H+L HRP; Cat No: ab205718; Abcam, United Kingdom; dilution 1:1,000; 1 h RT). Sections were

stained using the 3,3′-diaminobenzidine (DAB) Enhanced Liquid Substrate System (Sigma, United States) for chromogenic detection and counterstained with hematoxylin. Each step was followed by an appropriate wash per triplicate in Tris-buffered saline and 0.5% Tween-20 (Bio-Rad, United States. The sections were then dehydrated in ascending concentrations of ethanol, cleared in xylene, and mounted. The staining pattern was assessed using a light microscope according to the DAB chromogen reaction uptake.

Digital images of immunohistochemical staining were observed and captured using an Olympus BX41 microscope with Olympus cellSens Standard software (Version 1.16; Tokyo, Japan). CB_1 receptor immunoreactivity was quantified from the optical density (OD) of DAB signal using color deconvolution paradigm in NIH Image J software (Ruifrok and Johnston, 2001). The systematic random sampling technique was used to select the area of interest of the CA1 hippocampal and VTA regions for OD analysis. The measured OD was automatically corrected against the white background value. The immunoreactive density profile from at least 15 sections per group was then averaged to determine the mean OD value. A histogram of the OD values was plotted for further statistical analyses.

Western Blot

The frozen brain tissues were homogenized using syringe-based technique in 20 volumes of T-PER™ Tissue Protein Extraction Reagent and Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo Scientific, United States). Brain homogenates were centrifuged at 10,000 × *g* for 10 min at 4°C. The supernatant was aspirated, and the protein lysate obtained was kept at −20°C until further analysis. All procedures were performed using prechilled reagents on ice. Total protein concentrations were determined using Quick Start™ Bradford Protein Assay kit (Bio-Rad, United States) and NanoDrop™ 2000/2000c Spectrophotometer (Thermo Scientific, United States).

Protein lysates (30 µg) were individually heated at 95°C for 10 min in 2X Laemmli sample buffer (1:1 ratio) and resolved by electrophoresis in 10% sodium dodecyl sulfate (SDS)–polyacrylamide gel using Mini-PROTEAN® electrophoresis tank (Bio-Rad, United States) at 100 V for 90 min in 1X Running Buffer (Tris-glycine SDS, pH 8.3, Bio-Rad, United States). The gel was transferred onto a polyvinylidene fluoride (PVDF) membrane using the iBlot™ 2 Dry Blotting System using preprogramed voltage combinations (i.e., 20 V for 1 min, 23 V for 4 min, and 25 V for 2 min). Blotted PVDF membranes were incubated in 10 ml 1X iBind™ Solution for 10 min at RT to block nonspecific binding. The blocked membranes were then assembled onto the iBind Western System and sequentially incubated with anti–CB_1 receptor primary antibody (dilution 1:1,000) and HRP-conjugated secondary antibody (dilution 1:2000).

Immunoreactive protein bands were visualized using Pierce™ ECL Western Blotting Substrate (Thermo Scientific, United States) and placed onto the Fusion© FX7 Molecular Imager platform (Vilber Lourmat, France) for resulting signal image acquisition. Anti-β-actin antibody (dilution 1:2000) was used as a loading control for the western blot study. The CB_1 receptor immunoreactive band intensities were quantified using Image J software (NIH Image, United States) and normalized to β-actin control. A band normalization–arbitrary value histogram was plotted for further statistical analysis.

Quantitative Real-Time PCR

Total RNAs from brain tissue homogenates were extracted using GeneJET™ RNA Purification Kit (Thermo Scientific, United States) following the manufacturer's instructions. Extracted RNA samples were measured for total RNA concentration and purity using NanoDrop™ 2000/2000c Spectrophotometer and checked for RNA integrity using 1% agarose gel electrophoresis. Subsequently, 500 ng of the total RNA was converted to complementary DNA (cDNA) in 20-µl reactions using AffinityScript QPCR cDNA Synthesis Kit (Agilent, United States), consisting of 2X First-Strand Master Mix, AffinityScript RT/RNase Block Enzyme Mixture, 0.1 µg/µl Oligo (dT) and random primers, following the manufacturer's instructions. Each cDNA template reaction was then adjusted to 50 µl using RNase-free water and kept at −20°C until use.

Each qPCR assay was prepared with 50-ng cDNA template in a 20-µl reaction in triplicate using 2X Brilliant III Ultra-Fast QPCR Master Mix with ROX reference dye (Agilent, United States) on Stratagene Mx3005P Real-time PCR Machine (Agilent, United States). The primer-probe sets used were predesigned PrimeTime qPCR Assays (Integrated DNA Technologies, United States), as given below:

Cnr1 (GenBank® Accession No. NM_007726; 25 bp):
GCAAATTTCCTTGTAGCAGAGAG (forward),
TGAGAAAGAGGTGCCAGGA (reverse) and
/56FAM/ACAGGTGCC/ZEN/GAGGGAGCTTC/3IABkFQ/
(probe);
β-*actin* housekeeping gene (GenBank Accession No. NM_007393; 25 bp):
GATTACTGCTCTGGCTCCTAG (forward),
GACTCATCGTACTCCTGCTTG (reverse) and
/56-FAM/CTGGCCTCA/ZEN/CTGTCCACCTTCC/
3IABkFQ/(probe).

Each qPCR assay was validated using 5-point serial dilutions of the first-strand cDNA template with PCR efficiency rates between 96 and 102% with $R^2 > 0.990$. The thermal cycling incubation conditions for qPCR analysis were activation at 95°C for 3 min, denaturation at 95°C for 15 s, and annealing at 55°C for 20 s for 40 cycles. Relative mRNA expression of the *Cnr1* gene was determined using Relative Expression Software Tool (REST©) software and normalized to the β-actin reference gene. Subsequently, gene normalization–expression ratio histograms were plotted for further statistical analysis.

Statistical Analyses

Results were analyzed as mean ± standard error of mean (SEM). In the IntelliCage study, comparison between groups was performed using one-way or two-way repeated measures of ANOVA, followed by *post-hoc* Tukey test. One-way ANOVA with *post-hoc* Tukey test was used to analyze the CB_1 receptor expression in immunohistochemistry and the western blot

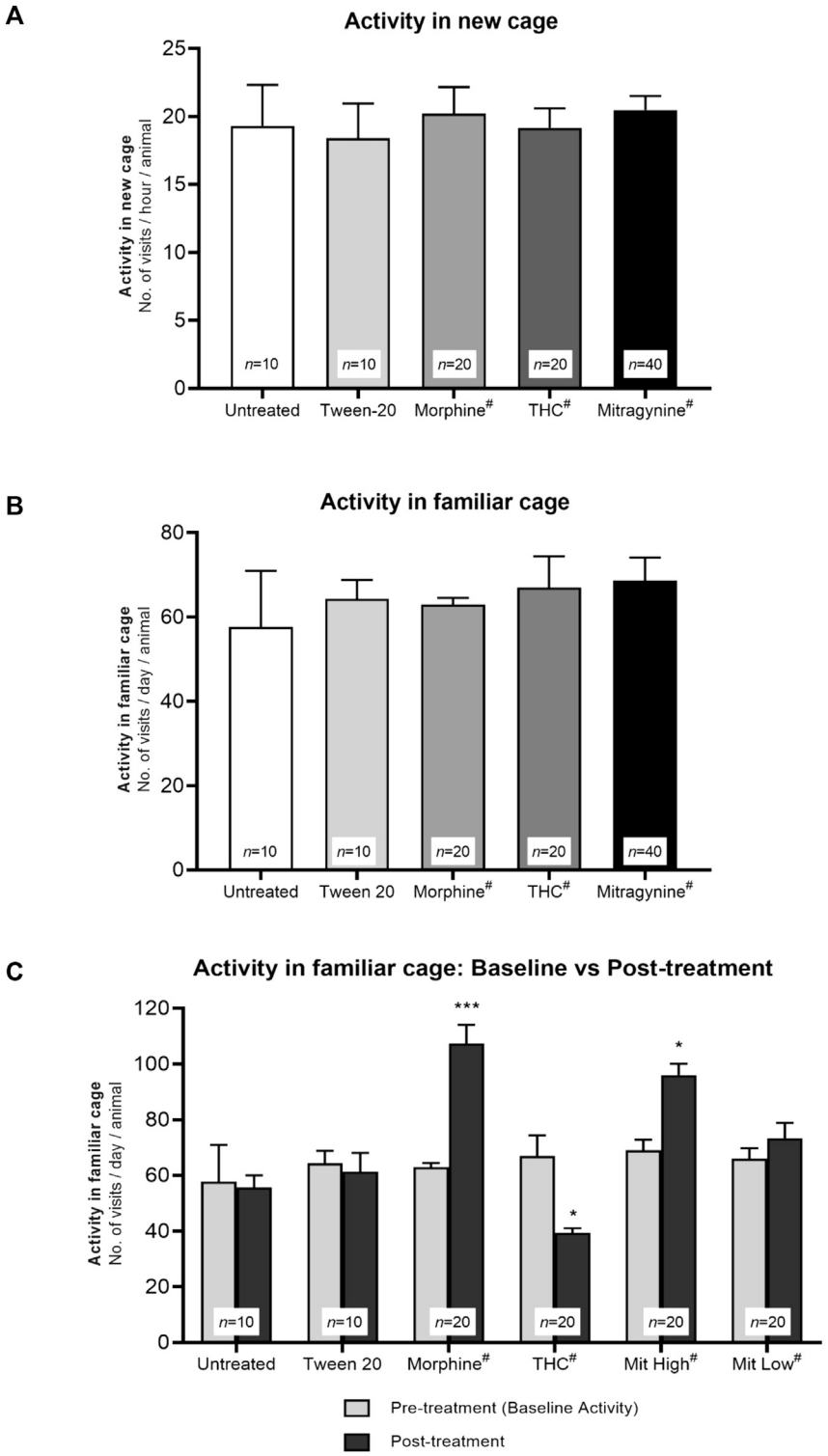


FIGURE 2 | Effects of drug intervention on exploratory activities. Baseline exploratory activity in **(A)** novel and **(B)** familiar environment. The mean number of visits to all corners per test group during the first hour in the novel IntelliCage and 3 consecutive days in the familiar IntelliCage environment throughout the baseline phase. Without any drug intervention, all groups in **(A)** and **(B)** showed no significant differences in novelty-induced exploration and baseline daily activity ($p > 0.05$; one-way ANOVA). **(C)** Effects of drug sensitization to daily activity in the familiar IntelliCage environment. The mean number of visits to all corners during 3 days of baseline phase vs 3 days of sensitization phase (Day 5–7). Morphine and high-dose mitragynine–treated mice showed a significant increment in their general familiar vs baseline activities, in which the increments are comparable (not significant) between mitragynine vs morphine. By contrast, THC-treated mice showed a significant decrease in general activity. *$p < 0.05$ vs baseline; **$p < 0.01$ vs baseline (two-way repeated measures ANOVA followed by *post-hoc* Tukey test). #includes animals from drug only and drug + receptor antagonist groups (i.e., each group $n = 10$).

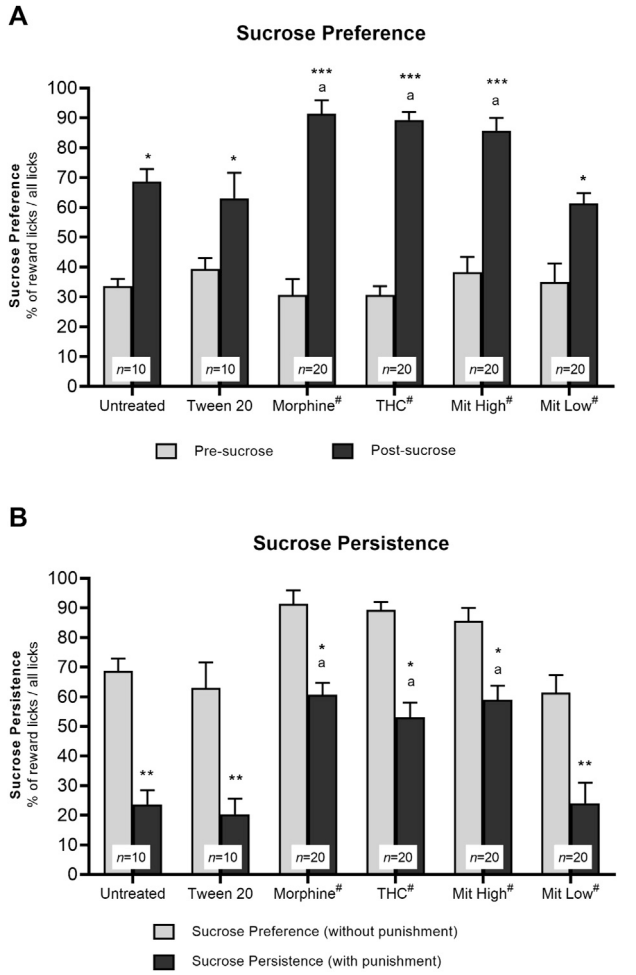


FIGURE 3 | Effects of drug intervention on preference for natural reward (sucrose) and persistence for sucrose seeking. **(A)** Preference for sucrose reward. The percentage of licks at the natural reward (10% sucrose) corner during the 2-day sucrose phase vs pre-sucrose (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs pre-sucrose; a: $p < 0.05$ vs Tween 20; ns = $p > 0.05$ vs morphine; two-way repeated measures ANOVA with *post-hoc* Tukey test). **(B)** Persistence of sucrose seeking. The percentage of reward licks when paired with air-puff punishment measured during the 3-day phase vs without air-puffs. *$p < 0.05$; **$p < 0.01$ vs reward licks without punishment; a: $p < 0.05$ vs Tween 20 (two-way repeated measures ANOVA with *post-hoc* Tukey). #includes animals from drug only and drug + receptor antagonist groups (i.e., each group $n = 10$).

studies, as well as *Cnr1* gene expression in the qPCR study. All statistical procedures were performed using GraphPad Prism version 8.0. For all analyses, $p < 0.05$ was accepted to be statistically significant.

RESULTS

Mitragynine Enhances Exploratory Activity in the IntelliCage

Novelty-induced exploration of mice was measured by the mean number of visits to all corners during the first hours in the novel IntelliCage, while daily exploratory behaviors were measured by the mean number of visits to all corners during three consecutive days in familiar IntelliCage environment. No mice received any drug and/or receptor antagonist intervention during the course of the baseline exploratory phase. Therefore, as expected from the predrug baseline phase, no significant difference between the groups was displayed with regard to the number of corner visits performed in the novel environment (**Figure 2A**; $F_{4, 95} = 0.883$, $p = 0.919$) or familiar environment (**Figure 2B**; $F_{4, 95} = 1.161$, $p = 0.859$).

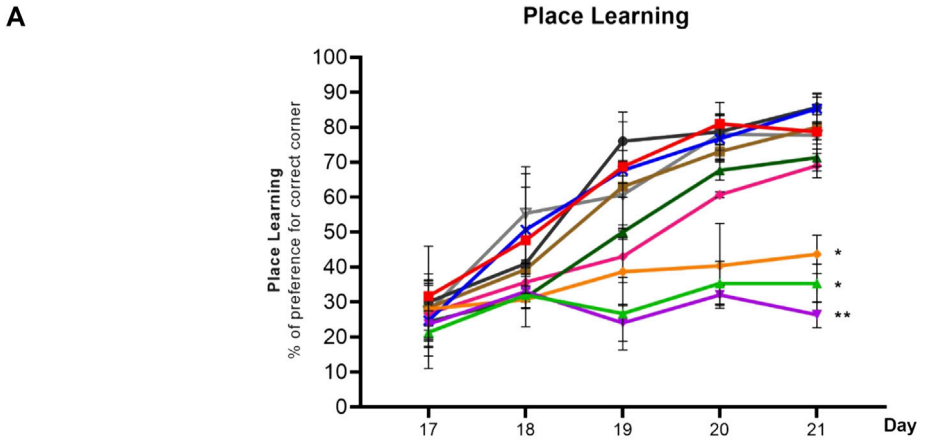
The number of visits post–drug sensitization was measured by the mean number of daily corner visits during Day 5–7 of sensitization, as compared with baseline visits. A two-way repeated measures ANOVA found statistically significant difference in exploratory activities between the baseline and sensitization phases across groups (**Figure 2C**; effect of treatment: $F_{5, 94} = 7.96$, $p = 0.0016$; effect of day: $F_{1, 94} = 6.03$, $p = 0.032$; treatment × day interaction: $F_{5, 94} = 10.86$, $p = 0.0004$). The morphine and high-dose mitragynine (5–25 mg/kg) groups performed significantly more corner visits following drug sensitization than the baseline group (morphine: $p < 0.001$, high-dose mitragynine: $p = 0.015$), with no significant difference between both groups (mitragynine vs morphine: $p > 0.5804$). By contrast, THC-sensitized mice performed significantly fewer corner visits than their baseline visits ($p = 0.011$). The untreated, Tween 20 vehicle, and low-dose mitragynine (1–4 mg/kg) groups did not differ significantly between their baseline and post-intervention exploratory activities in the familiar IntelliCage environment (untreated: $p = 0.9998$; Tween 20: $p = 0.9983$; low-dose mitragynine: $p = 0.929$).

Mitragynine Enhances Sucrose Reward Preference in the IntelliCage

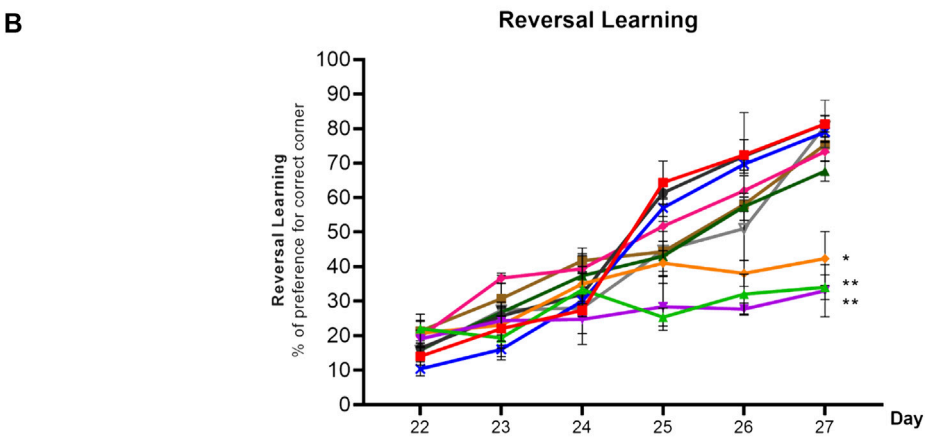
In the sucrose preference test, all mice showed strong preference for sucrose over water as demonstrated by the overall marked increase in lick preference for the corner once it was associated with sucrose as opposed to pre-sucrose (**Figure 3A**; effect of time: $F_{1, 94} = 259.3$, $p < 0.001$; effect of treatment: $F_{5, 94} = 2.925$, $p = 0.403$; treatment × time interaction: $F_{5, 94} = 6.315$, $p = 0.004$; in untreated: $p = 0.008$; Tween-20: $p = 0.018$; morphine: $p < 0.001$; THC: $p < 0.001$; high-dose mitragynine: $p < 0.001$; low-dose mitragynine: $p = 0.0084$ vs pre-sucrose). Morphine-, THC-, and high-dose mitragynine-sensitized groups elicited stronger preference for sucrose reward than untreated and vehicle control groups (morphine group: $p = 0.0214$; THC group: $p = 0.0340$; mitragynine group: $p = 0.0463$ vs untreated). Interestingly, the mice in the high-dose mitragynine group showed a similar preference for sucrose reward as those in the morphine and THC groups ($p = 0.9857$ vs morphine, $p = 0.9979$ vs THC), which may suggest comparable reward-seeking traits associated with prolonged high-dose mitragynine administration. The low-dose mitragynine group, however, showed comparable sucrose preference as the control group ($p = 0.9963$ vs untreated).

Mitragynine Enhances Resistance to Punishment in Sucrose Seeking in the IntelliCage

Two-way repeated measures ANOVA showed a statistically significant reduction in sucrose consumption in all mice,



No. of trials: Mean no. of all visits with nosepokes	17	18	19	20	21
Untreated	72	57	59	47	61
Tween 20	50	42	48	66	70
Morphine	92	97	85	105	108
Morphine + NIDA-41020	59	56	76	74	90
THC	38	44	33	35	39
THC + NIDA-41020	43	38	51	68	55
Mit High	93	79	75	63	62
Mit High + NIDA-41020	105	88	75	64	75
Mit Low	70	73	62	47	48
Mit Low + NIDA-41020	48	36	50	42	66



No. of trials: Mean no. of all visits with nosepokes	22	23	24	25	26	27
Untreated	63	46	58	62	64	71
Tween 20	72	59	54	62	61	52
Morphine	109	111	96	82	93	87
Morphine + NIDA-41020	51	54	49	68	65	61
THC	32	29	49	31	37	29
THC + NIDA-41020	54	45	64	63	52	56
Mit High	97	77	89	99	103	101
Mit High + NIDA-41020	69	62	65	59	72	54
Mit Low	62	67	63	59	51	61
Mit Low + NIDA-41020	60	52	71	49	54	54

FIGURE 4 | Effects of drug intervention on place learning and reversal learning, and NIDA-41020 antagonism. Data are shown as the percentage of preference for the correct (water-reinforced) corner and number of trials (the mean number of all visits with nose pokes are shown in the table below the graph) **(A)** during the 5 days of place learning phase, as well as **(B)** 6 consecutive days of the reversal learning phase. Data revealed highly significant learning and reversal learning in untreated and Tween 20 control groups and the low-dose mitragynine group. The morphine-, THC-, and high-dose mitragynine-treated groups failed to attain place learning and reversal learning. The cannabinoid CB_1 receptor antagonist NIDA-41020 significantly reversed morphine-, THC-, and mitragynine-induced place learning and reversal learning impairment in mice. *$p < 0.05$; **$p < 0.01$ vs Tween 20 (two-way ANOVA with *post-hoc* Tukey; n = 10/group).

irrespective of the intervention groups, following air-puff punishment (**Figure 3B**; effect of time: $F_{1,\ 94} = 118.3$, $p < 0.001$; treatment × time interaction: $F_{5,\ 94} = 6.315$, $p = 0.004$), with statistically significance effect observed between groups ($F_{5,\ 94} = 40.27$, $p < 0.0001$). *Post-hoc* comparisons indicated that sensitized mice were significantly more resistant to punishment when reward licks were associated with air-puff punishment (morphine: $p = 0.0226$; THC: $p = 0.0177$; high-dose mitragynine: $p = 0.0495$ vs reward licks without air-puff punishment) as compared with those in the control groups (untreated: $p = 0.0016$; Tween-20: $p = 0.0025$). Furthermore, all three sensitized groups showed a similar ability to withstand and resist air-puff punishment to obtain sucrose reward (high-dose mitragynine: $p = 0.9968$ vs morphine; THC: $p = 0.9880$ vs morphine). Meanwhile, the low-dose mitragynine group showed comparable resistance to punishment as the control group ($p = 0.9996$ vs untreated).

Mitragynine Impairs Place Learning and Reversal Learning in the IntelliCage

In the 5-day learning phase, two-way repeated measures ANOVA yielded highly significant treatment-by-day place learning in the IntelliCage system (**Figure 4A**; effect of treatment: $F_{5,\ 54} = 5.571$, $p = 0.023$; effect of day: $F_{4,\ 216} = 8.854$, $p = 0.005$; treatment × day interaction: $F_{20,\ 216} = 2.659$, $p = 0.014$). Untreated, Tween-20 vehicle, and low-dose mitragynine groups showed significant greater visit preference at the correct corner over days ($p < 0.0001$), signifying their improved place learning. However, chronic morphine, THC, and high-dose mitragynine groups showed significant deficits in the acquisition of place learning from Day 17–21 when compared with the control group (morphine: $p = 0.011$, THC: $p = 0.002$, mitragynine: $p = 0.02$). Analyses also discovered that the place learning deficiency in high-dose mitragynine mice did not differ significantly from the morphine- or THC-sensitized mice ($p = 0.810$ vs morphine; $p = 0.243$ vs THC).

Similarly, repeated measures ANOVA conducted on reversal learning data confirmed significant differences in treatment-by-day relearning (**Figure 4B**; effect of treatment: $F_{5,\ 54} = 17.00$, $p < 0.001$; effect of day: $F_{5,\ 270} = 24.88$, $p < 0.001$; treatment × day interaction: $F_{25,\ 270} = 6.981$, $p < 0.001$). Overall, the percentage of preference for the new correct corner on Day 22 (the first day of reversal learning) was generally lower than that recorded during Day 17 (the first day of learning phase). Significant increase in acquiring the newly placed correct water-reinforced corner was observed in untreated, Tween-20 vehicle, and low-dose mitragynine groups, implying their significant relearning abilities ($p < 0.05$). Consistent with place learning data, all drug-sensitized groups failed to acquire the reversed correct corner (morphine: $p = 0.004$, THC: $p = 0.002$, high-dose mitragynine: $p = 0.02$ vs control). There were no significant differences in reversal learning deficiency observed between high-dose mitragynine-, morphine-, and THC-sensitized groups (high-dose mitragynine: $p = 0.854$ vs morphine; $p = 0.898$ vs THC).

NIDA-41020 Reverses Mitragynine-Induced Place Learning and Reversal Learning Deficits

A two-way repeated measures ANOVA showed the significant effect of NIDA-41020 on drug-induced place learning (**Figure 4A**; effect of treatment: $F_{7,\ 72} = 8.246$, $p = 0.0006$; effect of day: $F_{4,\ 288} = 70.73$, $p < 0.0001$; treatment × day interaction: $F_{28,\ 288} = 5.624$, $p < 0.0001$) and reversal learning (**Figure 4B**; effect of treatment: $F_{7,\ 72} = 15.07$, $p < 0.0001$; effect of day: $F_{5,\ 360} = 94.25$, $p < 0.0001$; treatment × day interaction: $F_{35,\ 360} = 5.821$, $p < 0.0001$). Drugs paired with NIDA-41020 groups showed gradual increased preference for water-reinforced corner, revealing that NIDA-41020 significantly reversed morphine-, THC-, and high-dose mitragynine-induced impairment of place learning (**Figure 4A**; morphine: $p = 0.0475$ vs morphine + NIDA-41020; THC: $p = 0.0079$ vs THC + NIDA-41020; high-dose mitragynine: $p = 0.0466$ vs high-dose mitragynine + NIDA-41020) and reversal learning (**Figure 4B**; morphine: $p = 0.0152$ vs morphine + NIDA-41020; THC: $p = 0.0429$ vs THC + NIDA-41020; high-dose mitragynine: $p = 0.0445$ vs high-dose mitragynine + NIDA-41020).

Immunostaining of Positive and Negative Controls for CB_1 Receptor Antibody

The positive immunostaining reaction for mouse cerebellum was presented as brown deposits as seen in the immunoreactive fibers of the molecular layer (positive control; mean OD = 0.28; **Figure 5A**). Immunostaining of mouse CA1 hippocampal region as a negative control (primary antibodies were omitted) demonstrated the absence of immunostaining in the neurons and the surrounding fibers (negative control; mean OD = 0.00; **Figure 5B**).

Mitragynine Increases CB_1 Receptor Immunoreactivity in the Hippocampal CA1 Region

In the vehicle-treated mice, weak CB_1 receptor immunoreactivity was observed in CA1 pyramidal neurons surrounded by dense plexus of immunoreactive fibers (**Figure 6**). There was a statistically significant difference between the groups as determined by one-way ANOVA (**Figures 6B–L**; $F_{9,\ 140} = 208.7$, $p < 0.0001$). Tukey *post-hoc* test revealed that chronic treatment with morphine (**Figures 6D,L**; $p < 0.0001$) and high-dose mitragynine (**Figures 6H,L**; $p < 0.0001$), but not low-dose mitragynine (**Figures 6J,L**; $p = 0.1622$), significantly increased CB_1 receptor immunoreactivity in the CA1 field in comparison to the control groups. No significant different was detected in the CB_1 receptor immunoreactivity between morphine-sensitized and high-dose mitragynine-sensitized groups ($p = 0.8497$). By contrast, CB_1 receptor immunoreactivity in chronic THC was significantly decreased in the CA1 field (**Figures 6F,L**; $p = 0.0001$ vs control). There was no statistically significant difference in the morphine + NIDA-41020, THC + NIDA-41020, and mitragynine + NIDA-41020 groups compared with the control group (**Figures**

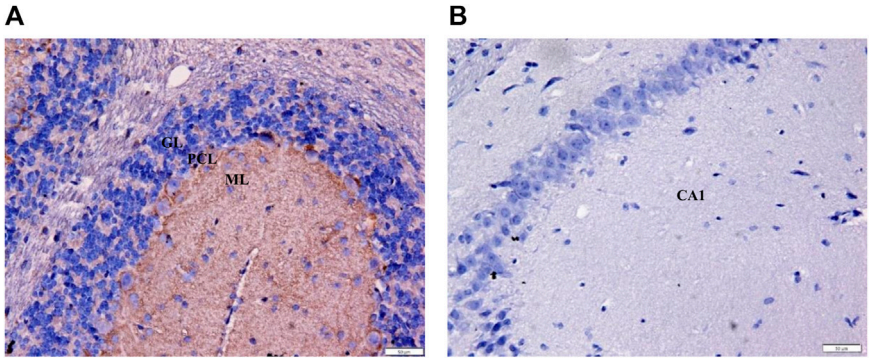


FIGURE 5 | Immunohistochemistry of positive and negative controls at ×400 magnification to validate staining specificity. **(A)** Immunoreactive fibers within the mouse cerebellar molecular layer served as the positive control for CB_1 receptor. The brown deposits confirm the presence of CB_1 receptors (OD = 0.28). **(B)** No stain was detected in the neurons and surrounding fibers of the CA1 hippocampal region (negative control; OD = 0.00). GL = granular layer; PCL = Purkinje cell layer; ML = molecular layer; Bars = 50 μm.

6E,G,I,L; morphine + NIDA-41020: $p = 0.7197$; THC + NIDA-41020 group: $p = 0.8868$; high-dose mitragynine + NIDA-41020 group: $p = 0.0364$ vs untreated). This suggests a reversal of morphine, THC, and mitragynine effects by CB_1 receptor antagonism.

Mitragynine Increases CB_1 Receptor Immunoreactivity in VTA

In the VTA, moderate CB_1 receptor immunoreactivity was observed in neuronal cell bodies and the surrounding fibers of vehicle-treated mice (**Figure 7**). A one-way ANOVA showed statistically significant difference between the groups (**Figures 7B–L**; $F_{9,\ 140} = 106.2$, $p < 0.0001$). Chronic morphine (**Figures 7D,L**; $p < 0.0001$) and high-dose mitragynine (**Figures 7H,L**; $p < 0.0001$) groups, but not low-dose mitragynine (**Figures 7J,L**; $p = 0.087$), showed significant increment of CB_1 receptor immunoreactivity in the VTA compared to the Tween 20 vehicle group. There was no significant difference between the morphine- and mitragynine-sensitized groups ($p = 0.0624$). By contrast, CB_1 receptor immunoreactivity in chronic THC was significantly decreased in the VTA (**Figures 7F,L**; $p < 0.0001$ vs vehicle). CB_1 receptor immunoreactivity in the VTA of morphine + NIDA-41020, THC + NIDA-41020, and mitragynine + NIDA-41020 groups did not show any significant difference compared with the Tween 20 group (**Figures 7E,G,I,L**; morphine + NIDA-41020: $p = 0.2282$; THC + NIDA-41020 group: $p = 0.9499$; high-dose mitragynine + NIDA-41020 group: $p = 0.1349$ vs vehicle), which suggests a reversal of the morphine, THC, and mitragynine effects.

Mitragynine-Induced Upregulation of CB_1 Receptor Levels in Brain Reward Area Reversed by CB_1 Receptor Antagonism

The CB_1 receptor protein levels in the brain reward mesolimbic area, as assessed by western blot, showed an overall significant difference between the groups (**Figures 8A,B**; $F_{9,\ 20} = 50.16$, $p < 0.0001$). The western blot analysis displayed that the protein levels of CB_1 receptor were upregulated in the morphine-sensitized ($p < 0.0001$) and high-dose mitragynine-sensitized groups ($p < 0.0001$), whereas downregulated in the THC-sensitized group ($p = 0.0022$), when compared with that in the vehicle group. There were no significant differences between the morphine- and mitragynine-sensitized groups ($p = 0.437$). No significant differences in CB_1 receptor protein levels of low-dose mitragynine were detected ($p = 0.712$). The administration of NIDA-41020 significantly reversed the drug-induced alterations of CB_1 receptor protein expressions as demonstrated in the respective drug + NIDA-41020 groups which do not differ significantly from the vehicle group (morphine + NIDA-41020 group: $p = 0.0734$; THC + NIDA-41020 group: $p = 0.3405$; high-dose mitragynine + NIDA-41020 group: $p = 0.6012$ vs vehicle).

The qPCR analysis was further performed to evaluate the effect of chronic drugs, with/without NIDA-41020 coadministration, on the CB_1 receptor at the mRNA level in the brain mesolimbic area (**Figure 8C**; $F_{9,\ 20} = 113.6$, $p < 0.0001$). Consistent with the western blot results, the qPCR analysis also showed that chronic morphine ($p < 0.0001$) and mitragynine ($p = 0.0006$) triggered the upregulation, while chronic THC triggered the downregulation ($p < 0.0001$), of the *Cnr1* gene level compared to vehicle. No significant differences were observed between the morphine- and mitragynine-sensitized groups ($p = 0.3225$). No significant difference was also observed in the low-dose mitragynine group compared with the control ($p = 0.251$). The drug-induced alteration of the *Cnr1* gene levels appeared to be absent in the respective drug + NIDA-41020 groups (morphine + NIDA-41020 group: $p = 0.1358$; THC + NIDA-41020 group: $p = 0.0568$; high-dose mitragynine + NIDA-41020 group: $p = 0.9995$ vs vehicle). These findings further affirm the likely CB_1 receptor antagonism of morphine, THC, and high-dose mitragynine at the protein and gene levels.

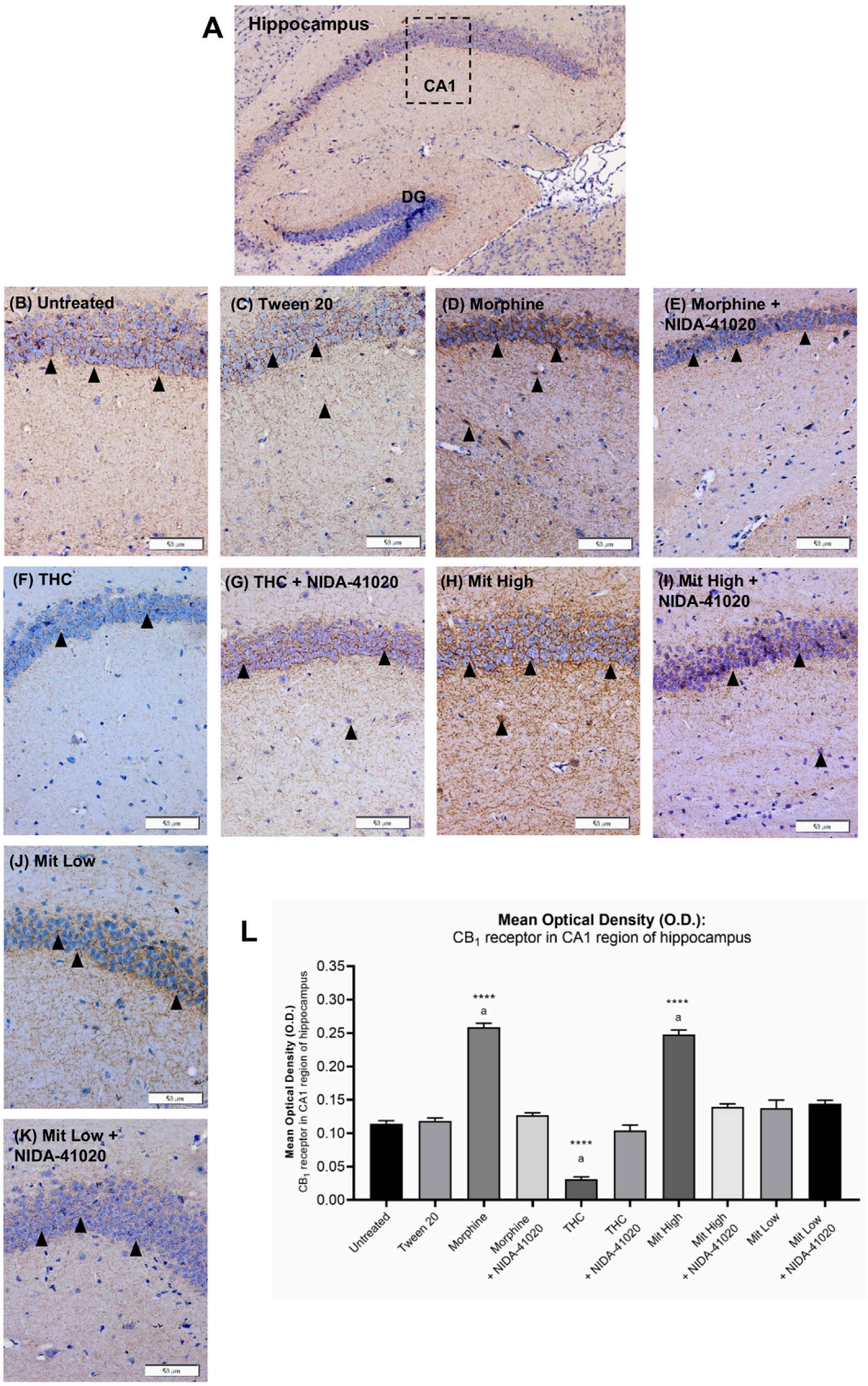


FIGURE 6 | Effects of drug intervention on CB_1 receptor staining in the **(A)** CA1 pyramidal region of the mice hippocampus (×100 magnification). The micrographs represent **(B)** untreated, **(C)** Tween 20, **(D)** morphine, **(E)** morphine + NIDA-41020, **(F)** THC, **(G)** THC + NIDA-41020, **(H)** high-dose mitragynine, **(I)** high-dose mitragynine + NIDA-41020, **(J)** low-dose mitragynine, and **(K)** low-dose mitragynine + NIDA-41020 groups at ×400 magnification. Arrowheads indicate CA1 pyramidal neurons surrounded by dense plexus of CB_1 receptor immunoreactive fibers. **(L)** Densitometric analysis are shown as the mean + SEM of 15 replicates per group. ****$p < 0.0001$ vs Tween 20; a: $p < 0.05$ vs drug + NIDA-41020 groups (one-way ANOVA followed by *post-hoc* Tukey test). Bars = 50 μm.

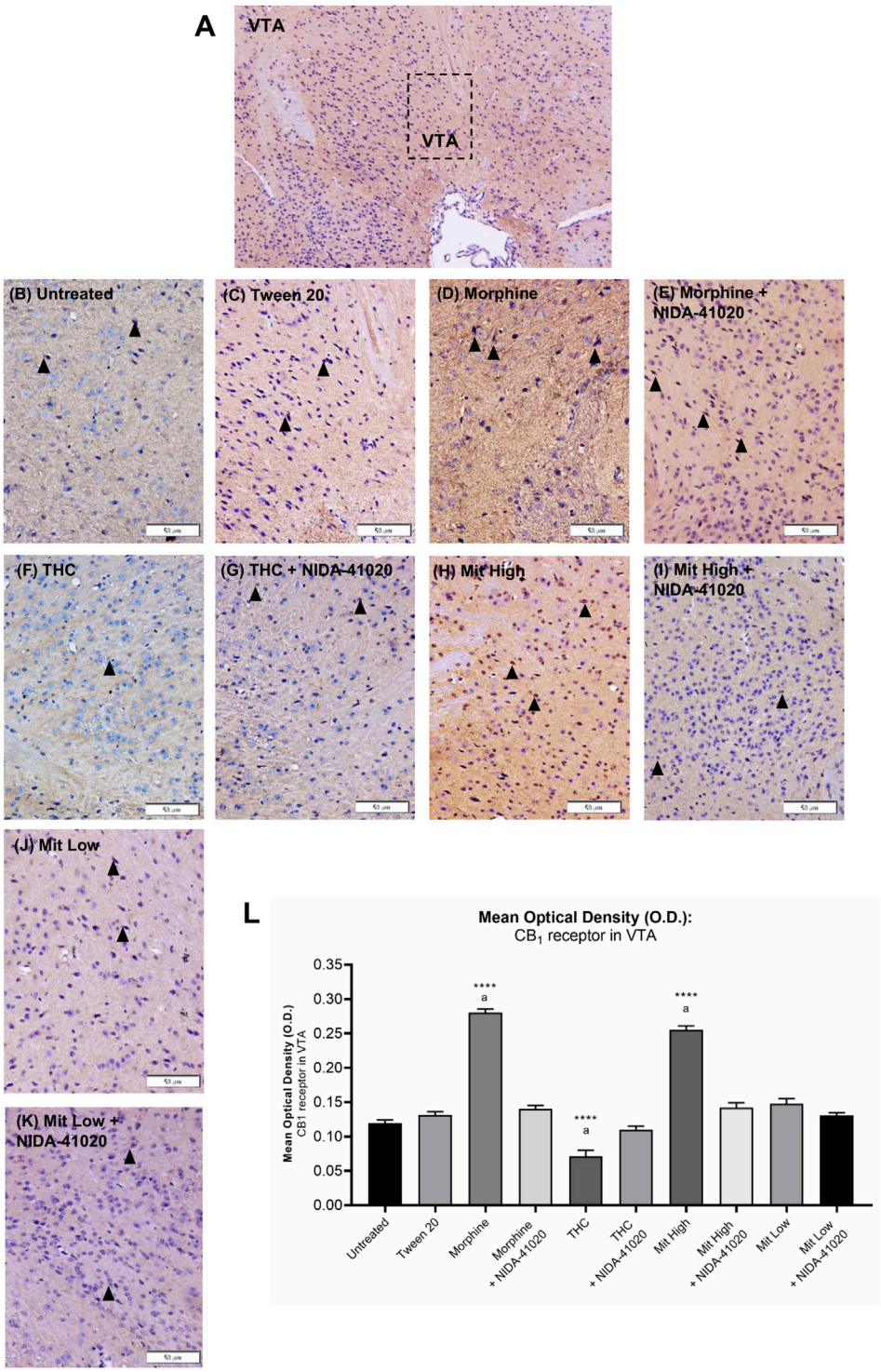


FIGURE 7 | Effects of drug intervention on CB_1 receptor staining in **(A)** mice VTA (×100 magnification). The micrographs represent **(B)** untreated, **(C)** Tween 20, **(D)** morphine, **(E)** morphine + NIDA-41020, **(F)** THC, **(G)** THC + NIDA-41020, **(H)** high-dose mitragynine, **(I)** high-dose mitragynine + NIDA-41020, **(J)** low-dose mitragynine, and **(K)** low-dose mitragynine + NIDA-41020 groups at ×400 magnification. Arrowheads indicate CB_1 immunoreactive neurons and the surrounding fibers. **(L)** Densitometric analysis are shown as mean + SEM of 15 replicates per group. ****$p < 0.0001$ vs Tween 20; a: $p < 0.05$ vs drug + NIDA-41020 groups (one-way ANOVA followed by *post-hoc* Tukey test). Bars = 50 μm.

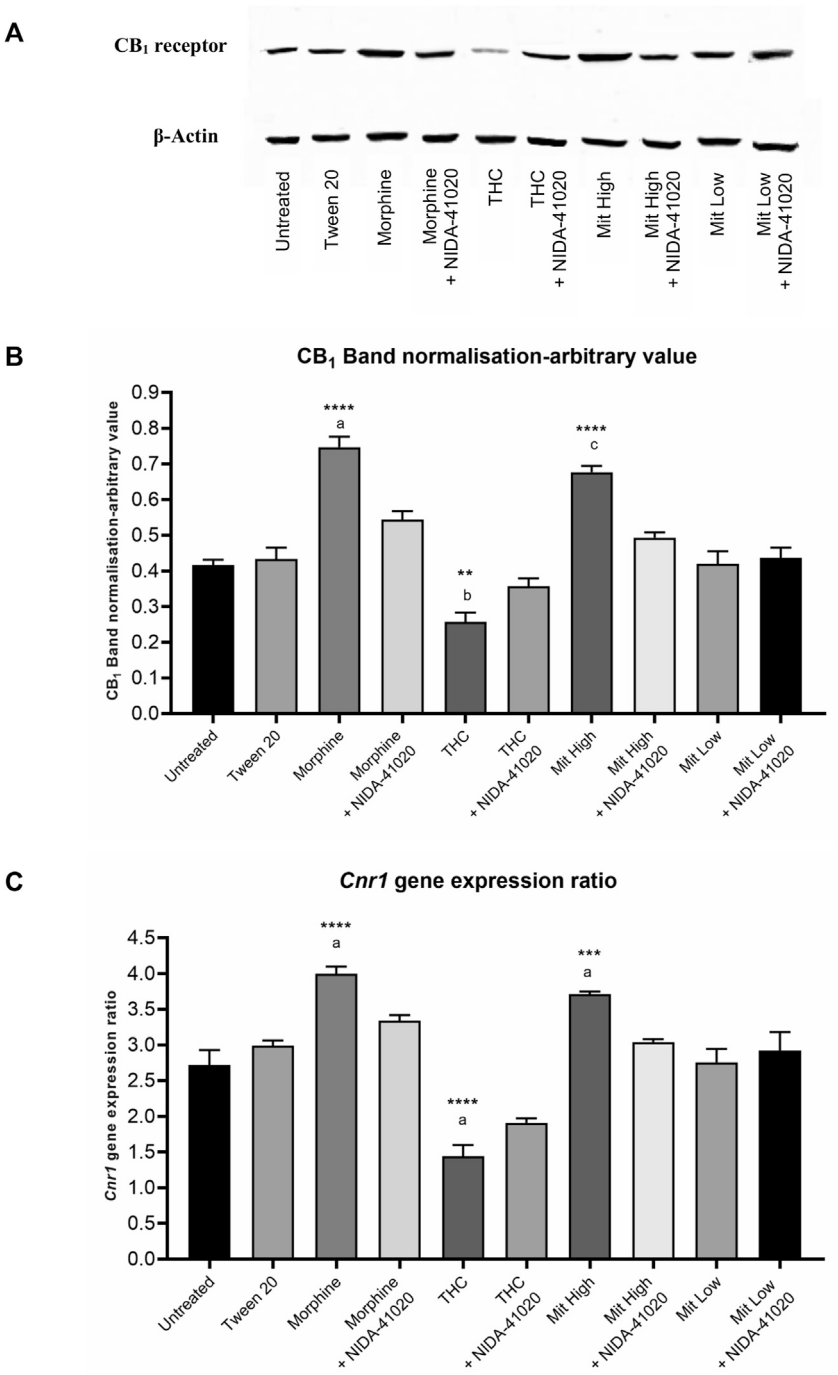


FIGURE 8 | CB_1 receptor **(A, B)** protein and **(C)** gene levels in mice brain mesolimbic area after 28 days of drug intervention. Data are shown as the mean + SEM of three biological replicates per group. The CB_1 receptor values have been normalized to β-actin controls. Data revealed that high-dose mitragynine and morphine groups significantly increased, whereas THC decreased; CB_1 receptor expression in the brain mesolimbic area vs controls and low-dose mitragynine group. NIDA-41020 reversed the drug-induced CB_1 receptor alteration. $^{***}p < 0.001$, $^{****}p < 0.0001$ vs Tween 20; a: $p < 0.05$ vs drug + NIDA-41020 groups (one-way ANOVA followed by *post-hoc* Tukey test).

DISCUSSION

The primary goal of the present study was to identify the involvement of cannabinoid CB_1 receptors in mediating the behavioral and cognitive effects of chronic kratom/mitragynine exposure. The IntelliCage system has been validated and widely used for addiction-related mouse models for various substances (Radwanska and Kaczmarek, 2012; Iman et al., 2017; Skupio et al.,

2017; Ajonijebu et al., 2018; 2019). In this study, the IntelliCage system was adapted for novelty-seeking trait (exploration of a novel environment) and basal horizontal locomotor activity levels in a familiar environment, in order to characterize the behavioral exploratory patterns (i.e., measured by the number of corner visits) in group-housed Swiss albino mice before drug intervention. During the initial adaptation period in the IntelliCage, all mice from all groups demonstrated similar pre-intervention novelty-seeking exploration and baseline locomotor activities shown by the number of visits to all corners. However, slight insignificant differences recorded between the groups may have originated because of individual differences or variations in the mice. Individual variations are often caused by the physical and social environment during development and adult life. This may be a key factor for the weak reproducibility of animal experiments (Koolhaas et al., 2010; Toth, 2015; Müller, 2018). Individual variations in drug vulnerability that may arise from poorly standardized housing, experimental protocols, and experimenter's handling, as well as social isolation, will jeopardize the reliability. To minimize these problems, an automated social group instrument such as the IntelliCage system was used in this study, as had been previously documented in addiction-related research (Radwanska and Kaczmarek, 2012; Marut et al., 2017).

This study revealed that chronic high-dose mitragynine (5–25 mg/kg) significantly enhanced exploratory activity in mice, as shown by the increased number of IntelliCage corner visits. Evidently, acute mitragynine administration was reported to induce a significant increase of arm explorations in Y-maze and elevated plus maze tests, as well as central zone explorations in the open-field test (Hazim et al., 2011; Hazim et al., 2014; Yusoff et al., 2016). Studies have suggested that in response to repeated administration of abused substances, locomotor sensitization occurs, resulting in the progressive amplification of behavioral and locomotor activity (Koob and Volkow, 2016; Uhl et al., 2019). Similarly, an acute low dose of mitragynine (1 mg/kg) induced profound hyperlocomotion and rearing activities in the open-field task (Yusoff et al., 2016). However, this finding contrasts with the findings of Apryani et al. (2010) that reported a significant reduction in locomotor activity in the open-field test after 28 days of mitragynine administrations (5, 10, and 15 mg/kg). The behavioral differences may reflect the psychostimulant effects with earlier exposures to mitragynine (i.e., within 7 days of administration) in this study. Subsequent behavioral and neural adaptations following chronic use may ultimately result in motor deficit. Despite the reported hypo- and hyperlocomotion induced by mitragynine at various treatment regimes and doses, the chronic low-dose mitragynine (1–4 mg/kg) in this study exhibited unaltered locomotor activity shown by a similar number of IntelliCage corner visits compared with the untreated and vehicle groups. This is in agreement with the previous findings of Moklas et al. (2008) that found unaltered locomotion activity when using 1 mg/kg mitragynine in a locomotor box, but is in contrast to the findings of Yusoff et al. (2016). Overall, this signifies the lack or no effect of chronic low-dose mitragynine on locomotor sensitization, unlike the effect induced by high-dose mitragynine, morphine, and THC.

Morphine-treated mice exhibit hyperlocomotion, which coincides with the induction of progressive behavioral sensitization in mice with intermittent morphine administration (Li et al., 2010; Kitanaka et al., 2018). By contrast, repeated exposure to THC produced a decrease in the number of visits to IntelliCage corners, signifying reduced locomotor activity in mice, which is consistent with several previous studies examining the locomotor effects of THC (Schramm-Sapyta et al., 2007; Dow-Edwards and Izenwasser, 2012; Bergman et al., 2016). Repetitive THC treatment in human and animal studies induced behavioral tolerance, which coincided with a rapid downregulation and desensitization of cannabinoid receptor–binding sites in several brain areas of the mesocorticolimbic circuitry and cerebellum (Justinova et al., 2009; Burston et al., 2010). This neural adaptation seems to be responsible for the development of cannabinoid tolerance, causing subsequently diminished locomotion. Furthermore, the anxiogenic effects of acute and chronic THC may contribute to hippocampal GABAergic dysfunction (Schramm-Sapyta et al., 2007), thereby exacerbating locomotor impairments.

In this study, the mice those were chronically treated with high-dose mitragynine (5–25 mg/kg), morphine, and THC showed significantly higher preferences for the sucrose-associated corner than did those untreated, vehicle treated, and treated with low-dose mitragynine (1–4 mg/kg). These findings suggest the escalation of sucrose hedonic value in drug-treated mice. Sweet preference has been speculated to be a predictor of substance abuse because of similar overlapping anatomical and functional mechanisms related with the rewarding effects of addictive substances and sucrose (Smith et al., 2011; Nieh et al., 2015). It is also feasible that the behavioral and neurological changes accompanying substance dependence result in enhanced impulsivity to securing immediate natural rewards (O'Brien et al., 2013; Harvey-Lewis et al., 2015). Contradictory to the increase of sucrose hedonic value by high-dose mitragynine sensitization, low-dose mitragynine did not induce any significant alteration in sucrose preference. This finding is in parallel with the findings of Yusoff et al. (2016) that supported the dose-dependent effects of mitragynine on its rewarding properties, where mitragynine at 1 and 5 mg/kg was found to suppress condition-placed preference (CPP) when compared with 10 and 30 mg/kg of mitragynine that induced CPP similar to 10 mg/kg morphine.

In addition, the present study also showed the consolidation of aversive memory to air-puff punishment as evident by the decreased persistency in sucrose seeking with a decrease in the frequency of licks at the sucrose-associated corner when paired with air-puff punishment. Liu et al. (2014) described a significant increase in mice heart rate in response to sudden air-puff stimuli, suggesting activation of a fearful emotional state. A fearful event leads to the alteration of the hippocampal CA1 region and anterior cingulate cortex, thus evoking aversive memory to that event (Xie et al., 2013). This may imply the possibility of enhanced resistance to air-puff punishment, as well as heightened persistency and motivation in securing salient reward in drug-dependent mice. Neuroplasticity in the mesolimbic dopaminergic system, especially in the amygdala that governs emotion and fear conditioning, may result in making sensitized mice exhibit an enhanced ability to resist punishment. Additionally, the extinction of long-lasting fear and aversive memory may occur with repeated exposure to addictive drugs. This is consistent with the literature on the impaired memory processes evoked by acute and chronic treatment with mitragynine (Yusoff et al., 2016; Iman et al., 2017), morphine (Lu et al., 2010), and THC (Justinova et al., 2009;

Calabrese and Rubio-Casillas, 2018). Studies have also demonstrated that rodents were willing to endure punishment in pursuit of abused drugs (Vanderschuren et al., 2017), which appears to mimic the pathological loss of control over substance abuse (or compulsive behaviors) seen in human addicts. Altogether, this compulsivity points to the underlying motivational shift from substance "liking" to "wanting" phenomenon even when the substance is no longer pleasurable. Nonetheless, mice from the high-dose mitragynine group showed significantly higher persistency in obtaining sucrose than those in the low-dose mitragynine group, which may indicate the dose-dependent effect on sucrose persistency that denotes dose-dependent compulsivity induced by mitragynine.

The place learning and reversal learning protocols were used to evaluate the effects of mitragynine sensitization on the learning abilities, as compared with the effects of morphine and THC. Learning abilities were determined by the percentage of visits (with successive nose pokes) performed at the water-reinforced corner. Untreated, Tween 20, and low-dose mitragynine groups showed place learning and reversal learning. The finding from the low-dose mitragynine group is in accordance with the findings of a study by Hassan et al. (2019) in which low-dose mitragynine (1 mg/kg)–treated mice showed preserved learning abilities in the Morris water maze task. In this study, we found that high-dose mitragynine-, morphine-, and THC-sensitized mice failed to learn the location of the water-reinforced corner. All mice from the three drug-sensitized groups showed no place learning and failed to show efficient reversal learning. Cognitive and learning deficits had been demonstrated in several studies after acute and chronic mitragynine treatment (Apryani et al., 2010; Hazim et al., 2011; Yusoff et al., 2016; Iman et al., 2017; Hassan et al., 2019). Recent human cross-sectional studies report progressive dependency, tolerance, craving, and withdrawal symptoms during abstinence from kratom consumption. These studies also suggest an association of poor cognitive performance with chronic kratom use in humans (Vicknasingam et al., 2010; Saingam et al., 2013; Cinosi et al., 2015; Singh et al., 2019). The long-lasting cognitive changes may eventually disrupt inhibitory control and impair decision-making, thereby contributing to a loss of control over drug intake, which reflects the persistent pursuits of drugs seen in human addicts. Additionally, cognitive deficits in the realm of learning and memory that persist even after prolonged abstinence may also impede the success of rehabilitation programs and thus provoke subsequent relapse despite achieving remission.

Reward-related place learning is a hippocampal-dependent task, paralleled by concomitant changes in VTA function and functional connectivity (Gomperts et al., 2015; Gruber et al., 2016; Pytka et al., 2020). Therefore, neuroplasticity in the hippocampus and VTA following chronic exposure to addictive substances may account for place and reversal learning impairment. This is consistent with reports of place learning deficits accompanied by reduced CA1 hippocampal synaptic transmission and LTP following high-dose mitragynine in rats (Hassan et al., 2019). CA1 hippocampal dysfunctions were also observed in rodents treated with a methanolic kratom extract (Harizal et al., 2012), morphine (Yang et al., 2013), and THC (Laaris et al., 2010). However, the causality remains unclear.

The CB_1 receptor antagonist NIDA-41020 was administered to mitragynine-sensitized mice to investigate the involvement of the endocannabinoid system in mitragynine-induced cognitive impairments. The selection of NIDA-41020 as the CB_1 receptor antagonist reaffirmed CB_1 receptor modulation that are known to occur with CB_1 receptor antagonism/reversal effect on morphine and THC (Pickel et al., 2004; Jin et al., 2014; Schindler et al., 2016). Behavioral alterations in both morphine and THC groups in the presence of NIDA-41020 when compared with the mitragynine groups became the surrogates for the likely CB_1 receptor involvement. Hence, NIDA-41020–alone group was not included in the present study. Interestingly, upon administration of NIDA-41020, mice treated with high-dose mitragynine showed a progressive ability in learning the location of the water-reinforced corner. This finding demonstrates that NIDA-41020 reversed the learning impairment in mitragynine-sensitized mice, thus providing the first clue to the interaction of mitragynine with the endocannabinoid system. In fact, this occurs during abstinence (i.e., 14 days without injection of drugs), and the reversal effect is comparable to the reversal effect produced by NIDA-41020 in the morphine- and THC-treated groups, suggesting that chronic high-dose mitragynine may exert its effect on CB_1 receptor signaling in probably the same way as would morphine and THC. It has been shown that long-term learning and memory impairment and the underlying neuronal alterations persist even after abstinence, particularly in the case of chronic administration of morphine, THC, and high-dose mitragynine (>5 mg/kg in mice) (Jin et al., 2014; Schindler et al., 2016; Iman et al., 2017; Hassan et al., 2019; Valentinova et al., 2019). Although this was adopted in the present study design, a similar interpretation cannot be made on the low-dose mitragynine (1–4 mg/kg) and limited by the absence of the NIDA-41020-alone group. Nevertheless, this gradual improvement in place learning ability with repetitive use of NIDA-41020 may suggest the prospective use of CB_1 receptor antagonists presumably by mitigating the effect of substance-induced learning memory deficits in chronic kratom users, and as a potential treatment for substance use disorder. The findings of this study also lend support to previous studies demonstrating that NIDA-41020 administration blocked THC, ethanol, and nicotine self-administration and reinstated substance-seeking behaviors in rodents (de Bruin et al., 2011; Maldonado et al., 2013; Schindler et al., 2016).

Previous molecular and histology studies showed that in the mesocorticolimbic reward pathway, a significantly high density of CB_1 receptor protein was localized on both GABAergic and glutamatergic axon terminals of the hippocampus, whereas a modest CB_1 receptor localization was reported throughout the neocortex, VTA, amygdala, periaqueductal gray nucleus, nucleus accumbens, and medial hypothalamus (Tsou et al., 1998; Katona, 2009). In the present study, the quantitative analysis of CB_1 expression focused on the hippocampal CA1 region and VTA. Tsou et al. (1998) showed that CB_1 receptors were expressed at the lightly stained cell bodies of CA1 pyramidal neurons, surrounded by a dense plexus of immunoreactive fibers. In the VTA, lightly stained CB_1 immunoreactive neurons and the surrounding fibers were located on the floor of the midbrain, adjacent to the substantia nigra (Tsou et al., 1998). The present study revealed an

upregulation of CB_1 immunoreactive fibers within the CA1 pyramidal region of the hippocampus, as well as CB_1 immunoreactive neurons and fibers in the VTA of mitragynine-sensitized mice. Consistently, the western blot and qPCR protocols also demonstrated a significant upregulation of CB_1 receptor protein and mRNA levels in these mice. CB_1 receptor protein upregulation may be a biochemical marker related to the development of tolerance and dependence after chronic mitragynine treatment. These upregulations suggest that adaptations within the endocannabinoid system may account for the observed learning impairments. The mitragynine-induced CB_1 receptor upregulations appeared to be attenuated by NIDA-41020 as seen in the immunohistochemical, protein, and mRNA studies. These findings suggest a potential mechanism for the beneficial effects of CB_1 receptor antagonism at the behavioral level. Therefore, the present results could also indicate a plausible chronic mitragynine–CB_1 receptor interaction in inducing the transition from "liking" to "wanting" response, which eventually culminates in the development of mitragynine/kratom addiction (Nanthini et al., 2015). Similarly, morphine-treated mice were also found to show an upregulation of CB_1 receptors in the hippocampal CA1 region and VTA at the immunohistochemical as well as protein and mRNA levels. Correspondingly, a study by Jin et al. (2014) demonstrated the upregulation of brain CB_1 receptor protein and mRNA levels occurring in morphine-dependent mice. In this study, we found that morphine effects were also reversed by CB_1 receptor antagonist treatment which confirms previous findings by Yamaguchi et al. (2001), extending the potential of CB_1 receptor antagonism as a treatment strategy to mitigate cognitive deficits in opiate addicts.

By contrast, THC-sensitized mice produced a significant downregulation of CB_1 receptor at the protein and mRNA levels in the CA1 hippocampal region and VTA. These findings are consistent with previous reports demonstrating the significant decline of CB_1 binding sites in several brain areas, including the mesolimbic system following chronic administration of cannabinoids in animals (Justinova et al., 2009) and humans (Hirvonen et al., 2012). Persistent abuse of marijuana or THC seems to be responsible for the development of profound cannabinoid tolerance which correlates with desensitization and downregulation of CB_1 receptors. Conversely, mice treated with chronic low-dose mitragynine exhibited unaltered regulation of CB_1 immunoreactivity within the hippocampal CA1 region and VTA, along with the unaltered CB_1 receptor protein and gene expressions, such as in the control group. These findings support the view of little or no endocannabinoid adaptations after chronic low-dose mitragynine.

CONCLUSION

The data of the present study demonstrate that high-dose mitragynine can induce spatial/place learning deficits in mice that resemble those of morphine and THC. This was paralleled by an induction of morphine-like CB_1 receptor alterations in mitragynine-sensitized mice, reaffirming the likelihood of common hijacking of the related brain pathways. This substantiates the kratom/mitragynine risk of abuse, dependence, and addiction after prolonged and unregulated use. The CB_1 receptor antagonist, NIDA-41020, reversed the behavioral and neural changes associated with prolonged mitragynine exposure. Furthermore, future research on binding dynamics, molecular docking studies of the CB_1 and CB_1-medicated signaling, and NIDA-41020–alone group in the experimental design may further substantiate CB_1 receptor involvement in kratom/mitragynine addiction. In conclusion, findings from the present study are the first to suggest a plausible role of CB_1 receptor in mediating the dose-dependent cognitive impairments after chronic high-dose mitragynine. This also highlights the potential of CB_1 receptor antagonism in ameliorating the cognitive deficits associated with long-term kratom/mitragynine consumption in humans.

DATA AVAILABILITY STATEMENT

The data sets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

ETHICS STATEMENT

The animal study was reviewed and approved by the USM Institutional Animal Care and Use Committee (USM IACUC), Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia.

AUTHOR CONTRIBUTIONS

Conceptualization: II, NA, and MM; methodology: II, NA, UT, MM, and NY; software: II and NA; investigation: II, NA, and UT; data collection: II, NA, and UT; writing—original draft preparation: II and NA; writing—review and editing: NY, AN, JK, MM, ZH, CM, and MM; supervision: NY and MM. All authors made substantial contribution to revising the manuscript critically for important intellectual content and approved the final manuscript.

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The Adverse Cardiovascular Effects and Cardiotoxicity of Kratom (*Mitragyna speciosa* Korth.): A Comprehensive Review

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Background: Kratom or *Mitragyna speciosa* (Korth.) has received overwhelming attention recently due to its alleged pain-relieving effects. Despite its potential therapeutic value, kratom use has been linked to many occurrences of multiorgan toxicity and cardiotoxicity. Accordingly, the current narrative review aimed to provide a detailed account of kratom's adverse cardiovascular effects and cardiotoxicity risk, based on *in vitro* studies, poison center reports, coroner and autopsy reports, clinical case reports, and clinical studies.

Methods: An electronic search was conducted to identify all research articles published in English from 1950 to 2021 using the major research databases, such as Google Scholar, Web of Science, PubMed, Scopus, Mendeley, EMBASE, Cochrane Library, and Medline. We then analyzed the literature's discussion of adverse cardiovascular effects, toxicity, and mortality related to kratom use.

Results: Our findings revealed that, although *in vitro* studies have found kratom preparations' most abundant alkaloid—*mitragynine*—to cause a prolonged QTc interval and an increased risk of torsades de pointes, a clinical study examining humans' regular consumption of kratom did not report such a risk. However, this latter study did show that regular kratom use could induce an increased QTc interval in a dose-dependent manner. A few case reports also highlighted that kratom consumption is associated with ventricular arrhythmia and cardiopulmonary arrest, but this association could have ensued when kratom was co-administered with another substance. Similarly, analyses of national poison data showed that kratom's most common adverse acute cardiovascular effects include tachycardia and hypertension. Meanwhile, coroner and autopsy reports indicated that kratom's cardiovascular sequelae encompass coronary atherosclerosis, myocardial infarction, hypertensive cardiovascular disease, left ventricular hypertrophy, cardiac arrhythmia, cardiomegaly, cardiomyopathy, focal band necrosis in the myocardium, and myocarditis. Given the available data, we deduced that all cardiac eventualities reported in the literature could have been compounded by polysubstance use and unresolved underlying medical illnesses.

Conclusion: Although kratom use has been associated with death and cardiotoxicity, especially at higher doses and when associated with other psychoactive drugs, the dearth

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of data and methodological limitations reported in existing studies do not allow a definitive conclusion, and further studies are still necessary to address this issue.

Keywords: cardiovascular adverse effects, cardiotoxicity, kratom related mortality, kratom use, QTc interval, literature review

INTRODUCTION

Mitragyna speciosa (Korth.) or kratom is an indigenous medicinal plant in the Rubiaceae family that can be widely found in its natural habitat of Southeast Asia, particularly in Thailand, Malaysia, and Indonesia. Its leaves are dark green in color and oval in shape, and they have been traditionally consumed by rural inhabitants of Southern Thailand and Northern Peninsular Malaysia for centuries. This traditional use has relied on kratom to symptomatically relieve muscle pain, cough, fever, and diabetes mellitus. Moreover, the plant has also been traditionally used in these areas as an aphrodisiac. For the past decade, kratom has become popular in the West (the United States and Europe), where it is mainly used for its broad antidepressant, anxiolytic, and analgesic properties as a safe substitute for prescription drugs and for illicit opioid or heroin use. Kratom has also been used in the West for its dose-dependent stimulant and sedative-like psychoactive effects. Unlike in Southeast Asia, where fresh kratom leaves are used to produce kratom decoctions (kratom tea or juice), kratom in the West is largely ingested as a dried leaf powder (Hassan et al., 2013; Singh et al., 2016; Leong Bin Abdullah et al., 2020; Domnic et al., 2021).

A wide variety of kratom products are currently sold online in the form of resin, dried leaves, or raw leaf extracts. However, these products' psychoactive content is unknown. Following reports about the addictive potential and various possible toxicities associated with kratom use, several countries have categorized kratom as a controlled substance. In Malaysia, mitragynine (the most abundant psychoactive alkaloid of kratom extracts) has been included in the Dangerous Poison Act 1953 since 2003. Although the planting of kratom trees is not considered an offense in Malaysia, the trafficking and possession of kratom leaves are illegal, and people convicted of these criminal acts could be penalized with prison sentences of up to 4 years, a maximum fine of 10,000 Malaysian Ringgit, or both of these punishments (Vicknasingam et al., 2010). In Thailand, kratom had previously been placed under Schedule 5 of the Thai Narcotic Act. Recently, however, kratom was removed from this schedule after an amendment to the act was passed. However, the cultivation of kratom products remains restricted under the country's new law (Vicknasingam et al., 2010; Bangkok Post, 2021). In Indonesia, the cultivation of kratom is permitted for commercial purposes, and kratom is exported to other countries in Asia, Europe, and America. However, under a new regulation of the Indonesian National Narcotics Agency (BNN) that will take effect in 2022, kratom will be an illegal substance.

In the international context, kratom is classified as a controlled substance in countries such as Myanmar, Australia, Sweden, Denmark, Poland, Latvia, Lithuania, and Romania. In the United Kingdom, the export, import, and sale of kratom are prohibited under the Psychoactive Substances Act. Although kratom is not a controlled substance in the United States, it has been scrutinized by the US Drug Enforcement Administration (Hassan et al., 2013; Eastlack et al., 2020). However, in 2018, the US Food and Drug Administration (FDA) issued a warning against the therapeutic use of kratom, claiming that the substance is an opioid with harmful effects that could cause abuse, dependence, and even death (Gershman et al., 2019). Due to kratom's potential to induce toxicity, it has been placed on the controlled substance lists of several US states—such as Alabama, Arkansas, Indiana, Tennessee, Wisconsin, Rhode Island, and Vermont (Eastlack et al., 2020).

Although more than 40 chemical compounds have been isolated from kratom leaves, only four alkaloids are known to be pharmacologically active: mitragynine, 7-hydroxymitragynine (7-HMG), corynantheidine, and speciociliatine (Chear et al., 2021). Among these compounds, mitragynine and its metabolite 7-HMG have been researched the most. Mitragynine is the most abundant alkaloid, contributing to 66% of kratom's total alkaloid content. Meanwhile, kratom preparations' 7-HMG content is much lower (only 0.02% of their total alkaloid content) (Takayama, 2004; Kruegel and Grundmann, 2018). Mitragynine and 7-HMG mainly bind to opioid receptors. Notably, mitragynine, and 7-HMG's affinities for the opioid receptor subtypes differ. Mitragynine has been reported to have a higher affinity for the μ and δ receptors while 7-HMG has exhibited a higher affinity for the μ and κ receptors. Unlike morphine, which is a μ and δ receptor agonist, mitragynine, and 7-HMG may be partial μ receptor agonists and δ receptor antagonists (Kruegel et al., 2016). Another notable difference is that mitragynine and 7-HMG are G-protein-coupled and not involved in the activation of β-arrestin signaling, unlike morphine. Therefore, kratom has been reported to induce less opioid-like adverse effects or toxicity than morphine, which has been shown to cause respiratory depression, constipation, and sedation (Raehal et al., 2011; Wisler et al., 2014).

Despite an expectation that kratom could induce less adverse or toxic effects than opioids, the toxicity related to kratom use has been reported cumulatively, and it involves many organ systems: 1) kratom-induced liver injury, such as hepatitis, raised liver enzymes, hepatomegaly, acute liver failure, intrahepatic cholestasis, and severe liver injury with jaundice (Dorman et al., 2015; Griffiths et al., 2018; Waters et al., 2018; Fernandes et al., 2019; Osborne et al., 2019; Ahmad et al., 2021); 2) endocrinal defects, such as hypothyroidism (Sheleg and Collins, 2011); 3) neurological defects, such as seizures, coma, and memory impairment (Nelsen et al., 2010; Tatum et al., 2018; Singh et al., 2019); 4) respiratory defects, such as pulmonary edema and congestion (McIntyre et al., 2015); 5) renal injury,

TABLE 1 | Summary of reviewed literature.

Author (year)	Study design, sampling, and sample size	Sample size calculation (Yes/No)	Objectives	Outcome measures	Findings	Limitations
Lu et al. (2014)	*In vitro* study with hiPSC-CMs	—	To investigate the cardiotoxicity of mitragynine and its analogs by studying their effects on hERG and APD	(1) IKr (2) ICa,L (3) APD	(1) Mitragynine, paynantheine, speciogynine, and speciociliatine suppressed IKr in hiPSC-CMs in a dose-dependent manner (2) Mitragynine significantly prolonged APD, which induced prolonged QTc and with the potential of causing torsades de pointes (3) Mitragynine did not cause synthesis or trafficking defects of hERG	(1) hiPSC-CMs contain different subtypes of cardiomyocytes (2) hiPSC-CMs are immature and embryonic-like compared to adult cardiomyocytes
Tay et al. (2019)	*In vitro* study with hERG1a/1b-transfected HEK293 cells	—	To determine the mechanisms of mitragynine-induced inhibition on hERG1a/1b current	The effects of mitragynine on: (1) hERG1a/1b expression (2) hERG1-cytosolic chaperones' interaction	(1) Mitragynine inhibited the cardiac IKr current in a concentration-dependent manner (2) Mitragynine had no inhibitory or induction effects on the mRNA expression of hERG1a and hERG1b (3) Mitragynine reduced fully glycosylated (fg) hERG1a but upregulated both core-glycosylated (cg) expression and hERG1a-Hsp90 complexes (4) In conclusion, mitragynine may impair hERG1a trafficking by preventing proper hERG1a channel protein folding through the plasma membrane of transfected HEK293 cells	(1) Used transfected HEK293 cells instead of cardiomyocytes
Aggarwal et al. (2018)	Case report	—	—	—	A 26-year-old man: (a) History: presented with cardiorespiratory arrest after ingesting an unknown quantity of kratom 24 h previously; no prior medical illness or regularly prescribed medication (b) Clinical findings: cardiorespiratory arrest with ventricular arrhythmia (c) Investigations (i) Urine toxicology: the presence of codeine (of which the patient had taken a standard dose just prior to admission) (ii) Other findings: imminent cerebral herniation in CT brain scan (d) Outcome: the patient died 12 h after initial ROSC	(1) The patient consumed a standard dose of codeine (2) Serum mitragynine and 7-HMG were not measured

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TABLE 1 | (*Continued*) Summary of reviewed literature.

Author (year)	Study design, sampling, and sample size	Sample size calculation (Yes/No)	Objectives	Outcome measures	Findings	Limitations
Abdullah et al. (2019)	Case report	—	—	—	A 35-year-old man: (a) History: presented with cardiorespiratory arrest and a history of taking kratom in powdered form as a tea numerous times daily; history of polysubstance abuse; used kratom as self-prescribed medication for opioid dependence (b) Clinical findings: cardiovascular, gastrointestinal, and respiratory examinations were otherwise unremarkable; a neurological examination revealed only evidence of cardiorespiratory arrest (c) Investigations (i) Arterial blood gas: respiratory acidosis, liver function test: liver impairment (ii) Cardiac enzyme analysis: high creatinine kinase (4,000 U/L) and troponin I (0.37 μ/L) (iii) ECG findings were normal and an echocardiogram only indicated a recent cardiac arrest (iv) Other investigations were unremarkable and a urine drug screen upon admission was negative for any drugs (d) Outcome: patient survived and recovered from opioid withdrawal symptoms 8 days after admission	(1) The kratom powder that the patient consumed could have been adulterated (2) Serum mitragynine and 7-HMG were not assessed
ELJack et al. (2020)	Case report	—	—	—	A 24-year-old man: (a) History: presented with cardiorespiratory arrest with a history of continually using illicit substances, particularly kratom, but had abstained from opioid use for approximately 1 year; history of polysubstance abuse but no history of medical illness prior to the incident (b) Clinical findings: physical examination revealed unremarkable findings (c) Investigations (i) Cardiovascular investigation: ventricular fibrillation (polymorphic ventricular tachycardia) and incomplete right bundle branch block in ECG (ii) Transthoracic echocardiography: normal (iii) Other investigation: indicative of tissue and organ hypoperfusion due to cardiac arrest	(1) Serum mitragynine and 7-HMG were not assessed (2) Likely co-exposure of kratom and other substances

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TABLE 1 | (*Continued*) Summary of reviewed literature.

Author (year)	Study design, sampling, and sample size	Sample size calculation (Yes/No)	Objectives	Outcome measures	Findings	Limitations
					(iv) Serum and urine toxicology screening: no evidence of any illicit drug use or medication overdose (d) Outcome: Patient fully recovered and was extubated 2 days after his hospital presentation	
Sheikh et al. (2021)	Case report	—	—	—	A 44-year-old man: (a) History: presented with cardiorespiratory arrest and a history of consuming kratom daily as an energy supplement, co-administered with an energy drink; otherwise, no history of underlying medical illnesses (b) Clinical findings: unremarkable (c) Investigations (i) Cardiovascular investigation: multiple episodes of ventricular fibrillation and later prolonged QT interval and intraventricular conduction block in ECG (ii) Chest x-ray: pulmonary vascular congestion (iii) Emergency cardiac catheterization, ECG (no left ventricular abnormalities), cardiac MRI, and serum troponin were all normal (d) Outcome: Patient fully recovered	(1) No assessment of serum mitragynine and 7-HMG (2) Co-exposure of kratom and other substances
Anwar et al. (2016)	(1) Retrospective survey (2) Sample size: 660 reports of kratom exposure	—	Not mentioned	(1) Single exposure versus multiple exposures (2) Common substances co-administered with kratom (3) Symptoms and signs of kratom exposure (4) Factors associated with outcomes' severity	Cardiovascular finding: (1) Common adverse cardiovascular effects were tachycardia (25%) and hypertension (11.7%) Other findings: (1) Isolated kratom exposure was reported in 64.8% of cases (2) Common co-administered substances included ethanol, other botanicals, benzodiazepines, narcotics, and acetaminophen (3) Multiple exposures (kratom co-administration with other substances) increased the risk of a severe outcome compared to a single exposure	(1) Unverified reports (2) Unknown health backgrounds in cases (3) Serum mitragynine and 7-HMG levels not available
Post et al. (2019)	(1) Retrospective survey	—	To analyze reports of kratom exposure to the US NPDS from 2011 to 2017	(1) Single exposure vs. multiple exposures by age group	Cardiovascular finding: (1) Adverse cardiovascular effects: tachycardia (21.4%), hypertension (10.1%), conduction defects (2.8%), chest pain (including non-	(1) Unverified reports

(Continued on following page)

TABLE 1 | (*Continued*) Summary of reviewed literature.

Author (year)	Study design, sampling, and sample size	Sample size calculation (Yes/No)	Objectives	Outcome measures	Findings	Limitations
					cardiac pain; 2.6%), hypotension (1.8%), bradycardia (1.2%), and cardiac arrest (0.4%)	
	(2) Sample size: 1,807 reports of kratom exposure			(2) Trend of kratom exposure from 2011 to 2017 (3) Clinical features and medical outcomes associated with kratom exposure	Other findings: (1) 65% of cases reported involved only kratom exposure (2) 11 kratom-related deaths were reported with only two cases associated with isolated kratom exposure	(2) Unknown health backgrounds in cases (3) Serum mitragynine and 7-HMG levels not available
Davidson et al. (2021)	(1) Retrospective survey (2) Sample size: 928 reports of kratom exposure	—	To analyze reports of kratom exposure with abuse potential to the US NPDS and Thai RPC from 2011 to 2017	(1) Characteristics of kratom exposure (2) Trend of kratom exposure from 2011 to 2017 (3) Single exposure vs. multiple exposures (4) Prevalence of co-ingested substances (5) Common clinical effects of kratom exposure (6) Factors associated with death and ICU admission	Cardiovascular findings: (1) Adverse cardiovascular effects and outcomes: tachycardia (30.4%) and hypertension (12.4%) Other findings: (1) Thailand registered a higher prevalence of co-exposure of kratom with other substances than the United States (2) The United States reported more co-ingestion with other sedatives than Thailand (3) Five out of six reported deaths were associated with the co-ingestion of kratom and other substances	(1) Unverified reports (2) Unknown health backgrounds in cases (3) Serum mitragynine and 7-HMG levels not available (4) Kratom dosing and formulation not available
Corkery et al. (2019)	(1) Retrospective survey (2) Sample size: 156 kratom-related mortality cases	—	To examine the nature of death reportedly associated with kratom exposure across the United Kingdom, United States, Europe, and Thailand until 2019	(1) The main characteristics of deaths associated with kratom use (2) Serum mitragynine and 7-HMG levels among patients who had died (3) Frequency of kratom exposure only and co-exposure (4) Main causes of death and autopsy reports associated with kratom exposure only and co-exposure	Cardiovascular finding: (1) Frequency of cardiovascular findings in deaths solely attributed to kratom: n = 9, 5.8% (2) Frequency of cardiovascular findings in deaths attributed to kratom combined with other substances: n = 18, 11.5% (3) Frequency of cardiovascular findings in deaths in which kratom's role was unclear: n = 5, 3.2% Other findings: (1) Exposure to kratom alone constitutes 23% of death cases while polysubstance use was reported in 87% of death cases (2) Serum mitragynine levels in mortality cases were as follows (a) Death solely attributed to kratom (mean = 0.398 mg/L, range 0.0035–0.890 mg/L; n = 3) (b) Death attributed to kratom combined with other substances (mean = 0.8903 mg/L, range 0.00089–16.000 mg/L; n = 62)	(1) Questionable quality of some data sources

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TABLE 1 | (*Continued*) Summary of reviewed literature.

Author (year)	Study design, sampling, and sample size	Sample size calculation (Yes/No)	Objectives	Outcome measures	Findings	Limitations
Leong Abdullah et al. (2021)	(1) Analytical, cross-sectional study (2) Snowball sampling (3) Sample size: regular kratom users (n = 100) vs. non-drug-using control participants (n = 100)	Yes	To investigate the prevalence of ECG abnormalities generally and QTc intervals particularly among regular kratom users versus non-kratom-using control participants	(1) Kratom use characteristics (2) Resting ECG	(1) Kratom users (8%) had significantly higher odds of sinus tachycardia than control participants (1%); no significant difference was found in other ECG abnormalities (2) An age during one's first experience of kratom consumption of >18 years old, a consumption duration of > 6 years, and daily kratom juice consumption quantity of one to four glasses significantly increased one's odds of a borderline QTc interval (QTc = 431–450 ms) but not of a prolonged QTc interval (QTc >450 ms)	(1) Cross-sectional design (2) No female participants (3) Participants were recruited from a single state in Peninsular Malaysia (4) Serum mitragynine analysis was not performed (5) Used Bazett's formula to calculate QTc intervals

Note: hiPSC-CMs = human-induced pluripotent stem cell-derived cardiomyocytes, hERG = human ether-a-go-go-related gene, APD = action potential duration, IKr = rapid delayed rectifier potassium current, ICa,L = L-type calcium current, hERG1a/1b = the human ether-a-go-go-related gene 1a/1b current, HEK293 cells = hERG1a/1b-transfected human embryonic kidney 293 cells, Hsp90 = heat shock protein 90, , 7-HMG = 7-hydroxymitragynine, ECG = electrocardiogram, NPDS = National Poison Data System, RPC = Ramathibodi Poison Center, ROSC = return of spontaneous circulation, MRI = magnetic resonance imaging, and CT = computerized tomography.

such as acute renal failure (Sangani et al., 2021); 6) muscular injury, such as rhabdomyolysis and compartment syndrome (Sangani et al., 2021); and neonatal abstinence syndrome among infants born to mothers who used kratom during pregnancy (Eldridge et al., 2018; Mitra and Virani, 2018). Evidence of possible cardiotoxicity due to kratom exposure was first documented in an *in vitro* study of human-induced pluripotent stem-cell-derived cardiomyocytes (hiPSC-CMs); this study reported that mitragynine and its analogs increased the risk of prolonged QTc interval and torsades de pointes (Lu et al., 2014). To the best of our knowledge, to date, comprehensive studies detailing the adverse cardiovascular effects and cardiotoxicity of kratom use have been lacking. Therefore, we conducted a comprehensive literature review incorporating *in vitro* studies, poison center reports, coroner and autopsy reports, clinical case reports, and clinical studies to provide a detailed view of this subject.

MATERIALS AND METHODS

An electronic search was conducted on literature published from 1950 to 2021. This search was conducted independently by this review's two authors (MFILA and DS) using the major research databases, such as Google Scholar, Web of Science, PubMed, Scopus, Mendeley, EMBASE, Cochrane Library, and Medline. The search terms and keywords used included "kratom," "*Mitragyna speciosa*," "*Mitragyna speciosa* Korth," "*M.speciosa* adverse effects," "kratom risks and benefits," "*M.speciosa* toxicity," "kratom cardiotoxicity," "*in vitro* study of kratom cardiotoxicity," "animal study of kratom cardiotoxicity," and "kratom-related death." An initial search yielded a total of 170 articles From these initially identified articles, our selection was refined according to our search criteria, which determined that literature was eligible for review if it was: 1) published in an English-language peer-reviewed journal, including in-press articles, 2) a research article, case report, or case series, and 3) related to the adverse cardiovascular effects and cardiotoxicity of kratom use. Literature was excluded from this review if it was: 1) published in non-English-language journals (because the current authors could not access an expert who could interpret non-English-language studies' content and findings), 2) a systematic review, narrative review, unpublished article, or thesis, 3) described *Mitragyna tubulosa*, *Mitragyna parvifolia*, *Mitragyna rotundifolia*, *Mitragyna hirsuta*, *Mitragyna savanica*, *Mitragyna inermis*, *Mitragyna africanus*, *Mitragyna Rubro stipulata*, or *Mitragyna ciliata*, or 4) addressed aspects of kratom-related toxicities other than cardiotoxicity. Therefore, after thorough analysis, only 11 identified articles were ultimately selected for inclusion in this review. A summary of these selected articles is presented in **Table 1**. The selected studies in **Table 1** are presented according to the hierarchy of evidence proposed by Sayre et al. (2017) from the lowest evidence level to the highest evidence level.

RESULTS

Kratom's Adverse Cardiovascular Effects

A few studies have extracted data from the National Poison Data System (NPDS) in the United States and reported several adverse cardiovascular effects associated with kratom use. Indeed, most of the reported cases involved multiple exposures to various substances, including kratom, and only a minority of cases reported exposure to kratom only. Anwar et al. (2016) reported a total of 660 calls to the National Poison Data System (NPDS) in the United States from 2010 to 2015, showing an upward trend in kratom exposure from 26 calls in

2010 to 263 calls in 2015. Isolated kratom exposure was documented for 64.8% of these calls, and healthcare provider reports were documented for 75.2% of the calls. The most common cardiovascular symptoms and signs that these callers complained about were hypertension (11.7%) and tachycardia (25.0%) (Anwar et al., 2016).

Next, Post et al. (2019) examined 1,807 cases of kratom exposure in the United States that had been reported to the NPDS from 2011 to 2017. Again, this study indicated that kratom-related exposure cases were rising in the United States. Although 65.0% of these exposure cases were due to a single exposure to kratom, multiple-substance exposure was associated with more severe medical outcomes. The most common adverse cardiovascular effects and toxidrome reported in this study were tachycardia (21.4%), hypertension (10.1%), conduction defects (2.8%), chest pain (including non-cardiac pain; 2.6%), hypotension (1.8%), bradycardia (1.2%), and cardiac arrest (0.4%). However, this study was notably limited by examining unverified reports of kratom-related adverse effects and toxicity since these cases were self-reported and not confirmed by a poison control center (Post et al., 2019).

Davidson et al. (2021) retrospectively analyzed 938 cases of kratom exposure that had been reported to the NPDS in the United States (760 cases) or the Ramathibodi Poison Center (RPC) in Thailand (168 cases) from 2010 to 2017. This study found that co-exposure to kratom and other substances was more common in Thailand than in the United States (64.8 vs. 37.4%). Notably, this study revealed that tachycardia (30.4%) and hypertension (12.4%) were the most common adverse cardiovascular effects associated with kratom use (Davidson et al., 2021).

Kratom's Effects on Heart Rhythm and Cardiac Arrest Reports

Two *in vitro* studies, one cross-sectional study of human subjects, and a few separate case reports examined kratom's effects on heart rhythm and cardiac arrest. The first study to identify evidence of kratom-related cardiotoxicity was an *in vitro* study which examined the effects of exposure of hERG-overexpressing human embryonic kidney (HEK) cells and hiPSC-CMs to mitragynine and its analogs (paynanthiene, speciogynine, and speciociliatine). The human ether-a-go-go-related gene (hERG) is a subunit of the potassium ion channel that regulates the rapid outward, delayed rectifier potassium current (I_{Kr}) in the cardiomyocytes. Since cardiomyocytes from the human heart are not available due to safety concerns and technical shortcomings, the HEK cell presents a reliable alternative cell model to assess cardiotoxicity in *in vitro* studies. Meanwhile, hiPSC-CMs are generated from human-induced pluripotent stem cells via cardiomyogenic differentiation. Thus, hiPSC-CMs exhibit ionic current characteristics that resemble adult human cardiomyocytes. This *in vitro* study found that mitragynine at a concentration of 10 mM had suppressed the I_{Kr} in hERG-HEK cells. Meanwhile, mitragynine at $IC_{50,}$ ranging from 0.91 to 2.47 mM, had also dose-dependently inhibited the I_{Kr} by 67–84% in hiPSC-CMs. Additionally, mitragynine had induced a marked hyperpolarization shift in the $V_{1/2}$ of steady-state inactivation, in turn prolonging the action potential duration (APD) at 50 and 90% repolarization (439.0 ± 11.6 vs. 585.2 ± 45.5 ms and 536.0 ± 22.6 vs. 705.9 ± 46.1 ms, respectively). This finding indicated mitragynine's potential to induce a prolonged QTc interval and increase the risk of torsades de pointes. However, mitragynine did not exhibit any tendency to suppress the voltage-gated calcium current ($I_{Ca,L}$). Moreover, this study did not indicate that mitragynine could induce defects in hERG channel protein synthesis or the trafficking of ions, nor induce apoptosis of the hiPSC-CMs (Lu et al., 2014).

Next, a second *in vitro* study of kratom-related cardiotoxicity evaluated the mechanism of mitragynine-induced inhibition of the human ether-a-go-go-related gene 1a/1b (hERG1a/1b) current in stable hERG1a/1b-transfected human embryonic kidney (HEK) 293 cells. This study confirmed the previous findings by Lu et al. (2014) that mitragynine at an IC_{50} value of 332.70 nM had inhibited the hERG1a/1b current in a dose-dependent manner. Indeed, the IC_{50} value of mitragynine that had induced an inhibitory effect was lower than in the study by Lu et al. (2014). Additionally, this study also reported that mitragynine had decreased the fully glycosylated (fg) hERG1a protein expression at a lower concentration—but upregulated both core-glycosylated (cg) hERG1a protein expression and hERG1a-Hsp90 complexes at a higher concentration—after the hERG1a/1b-transfected HEK 293 cells had been exposed to mitragynine for 24 h. This finding highlighted the possibility that mitragynine could induce defects in channel trafficking of the hERG channel. The authors hypothesized that the upregulation of the hERG1a-Hsp90 complexes may be due to a mitragynine-induced hERG1a channel misfolding that activates the unfolded protein response (UPR) and endoplasmic-reticulum-associated protein degradation (ERAD) system (Tay et al., 2019). However, this possibility has yet to be investigated.

So far, only one study has evaluated electrocardiogram (ECG) findings related to regular kratom users (human subjects) without a history of polysubstance use or significant health problems (Leong Abdullah et al., 2021). This cross-sectional study compared ECG findings between regular kratom users who consumed kratom daily and a control group. The mitragynine concentration in the kratom juice consumed by the studied kratom users was also quantified and reported as a daily mitragynine intake of 434.28 mg. Several ECG abnormalities were documented among this study's kratom users, such as sinus tachycardia (8% of all participants), left axis deviation (7%), prolonged QTc intervals (5%), a first-degree atrioventricular block (4%), left ventricular hypertrophy (4%), T inversion (4%), an incomplete right bundle branch block (3%), right axis deviation (2%), and sinus bradycardia (1%). The only ECG abnormality observed to be significantly prevalent among kratom users versus the control group was sinus tachycardia (OR = 8.61, 95% CI = 1.06–70.17, $p = 0.035$). Similarly, kratom users were also found to be more likely to experience borderline QTc intervals compared to the control group; however kratom users' odds of prolonged QTc intervals did not increase versus the control group. Therefore, this study concluded that regular kratom consumption (at an average daily quantity of four glasses or with a mitragynine intake of 434.28 mg) can apparently increase QTc intervals but does not induce

prolonged QTc intervals (Leong Abdullah et al., 2021). However, this study was limited in that it lacked serum mitragynine analysis. Therefore, further studies are needed to confirm these findings.

Despite a lack of human studies, a few case reports have pertained to kratom cardiotoxicity. Case 1 presented a 26-year-old man with no history of medical illness, who took no regular prescribed medication and who had visited an emergency department during cardiorespiratory arrest (primarily pulseless electrical activity). He had ingested an unknown quantity of kratom about 24 h prior to this incident. Upon examination, he was noted to have a brief period of ventricular arrhythmia. A computed tomography (CT) scan of the patient's brain revealed imminent cerebral herniation, but a urine toxicology report indicated traces of codeine without the presence of other substances (a finding that was confirmed by the patient's history revealing a standard dose of codeine prior to the incident). The patient died 12 h after an initial return of his spontaneous circulation, and his cause of death was suspected to be kratom-related cardiotoxicity. However, this report's authors did not assess the patient's serum mitragynine or 7-HMG levels. The quantity of kratom the patient had ingested prior to his death remained unknown (Aggarwal et al., 2018).

Case 2 presented a 35-year-old man with a significant past history of substance abuse. The patient had come under the care of emergency medical services (EMS) after suffering a cardiorespiratory arrest in his home. EMS and police personnel observed a large amount of kratom powder residue on the patient. Moreover, the patient had a history of alcohol, opioid, benzodiazepine, methamphetamine, and cannabis abuse. However, he had undergone rehabilitation treatment and, since then, abstained from all illicit drug use and alcohol. A systemic examination of the patient revealed no remarkable findings except for an examination of the central nervous system indicating marked reduced consciousness with a Glasgow coma scale of 3/15, as well as pinpoint, non-reactive pupils. A urine drug screen performed during the patient's admission was negative for illicit drugs. Laboratory tests indicated hyperkalemia (potassium of 5.9 mmol/L), raised liver enzymes (aspartate transaminase of 282 IU/L and alanine transaminase of 273 IU/L), acidic blood with a significant anion gap, raised serum creatinine (3.0 mg/dl from a baseline level of 0.6 mg/dl), and high serum creatine kinase (4,000 U/L) and troponin I (0.37 μ/L). The patient's other blood investigations were unremarkable. An echocardiography examination revealed cardiac arrest features while no other pathology was found. After treatment, the patient revealed a history of self-prescribed kratom consumption to treat his opioid dependence. He had consumed kratom multiple times daily to reduce his opioid withdrawal symptoms. In this case as well, however, the authors did not assess the patient's serum mitragynine or 7-HMG levels. Moreover, the amount of kratom that the patient had ingested daily was not well quantified (Abdullah et al., 2019).

Case 3 described a 24-year-old man with a history of polysubstance abuse of an amphetamine-type stimulant, opioids, and benzodiazepine who had visited the hospital during a cardiorespiratory arrest. His history revealed no other risk of sudden cardiac death. This patient was unresponsive to multiple intravenous doses of naloxone. He experienced two episodes of polymorphic ventricular tachycardia for which defibrillation was performed. The first episode occurred while he was traveling to the hospital, and the second episode occurred during his initial admission to the emergency unit. A systemic examination of the patient's cardiovascular system revealed no remarkable findings. The patient was placed on advanced cardiac life support, and his spontaneous circulation returned; however, his wide-complex tachycardia persisted. A urine drug screen was negative for opioids, cocaine, amphetamines, benzodiazepines, and tricyclic antidepressants. An investigation of the patient's blood indicated hypokalemia (potassium of 2.9 mmol/L), while his other blood tests revealed circulatory arrest features. ECG findings reported an incomplete right bundle branch block while the patient's echocardiogram was normal. After the patient recovered over 2 days, he described a history of continued polysubstance use, including kratom use. The amount of kratom he had consumed was not described, however, and the patient's serum mitragynine and 7-HMG levels were not assessed (ElJack et al., 2020).

Case 4 described a 44-year-old man with a history of hypertension and hyperlipidemia on pharmacotherapy. He was physically active, performing routine daily exercise, and had obtained unremarkable results from an annual cardiac examination. This patient visited an emergency department due to multiple episodes of ventricular fibrillation, which required defibrillation. A family history revealed that the patient consumed a mixture of energy supplements containing kratom and caffeine (172–688 mg) daily. Laboratory blood investigations did not demonstrate any remarkable findings, but urine toxicology screening indicated the presence of ethanol. ECG findings indicated a prolonged QTc interval and an intraventricular conduction block, while a chest x-ray showed pulmonary vascular congestion. A further investigation with a CT scan of the brain, emergency cardiac catheterization, and cardiac magnetic resonance imaging (MRI) reported no abnormal findings (Sheikh et al., 2021).

Kratom's Association With Ischemic Heart Diseases and Other Cardiovascular Toxicities

Corkery et al. (2019) conducted a retrospective study that critically examined coroner and medical examiner reports, including autopsy reports of mortality cases associated with kratom use in the United Kingdom and beyond (including the United States, Germany, Canada, Ireland, Norway, Sweden, and Thailand) from 2008 to 2019. The authors successfully identified 156 deaths associated with kratom use. Only 16.7% of these mortalities were solely due to kratom exposure alone. The mean serum mitragynine level reported among the patients whose deaths were solely attributed to kratom use was 0.398 mg/L (range = 0.0035–0.890 mg/L; three cases). Meanwhile, the mean serum mitragynine level reported among the patients whose deaths had been associated with polysubstance use was 0.890 mg/L (range = 0.00089–16.000 mg/L; 62 cases). The mean

serum level of 7-hydroxymitragynine among the patients whose deaths had involved polysubstance use was 0.662 mg/L (range = 0.0009–2.8 mg/L; five cases). Among the cardiovascular-system autopsy findings in cases linked to kratom exposure alone were coronary atherosclerosis (two cases), heart attack (one case), hypertensive cardiovascular disease (two cases), and left ventricular hypertrophy (three cases), totaling 5.1% of all studied mortality cases. Meanwhile, the autopsy findings linked to the co-administration of kratom with other substances included cardiac arrhythmia (one case), cardiomegaly (five cases), cardiomyopathy (one case), coronary atherosclerosis (five cases), focal band necrosis in the myocardium (one case), hypertensive cardiovascular disease (one case), left ventricular hypertrophy (three cases), and myocarditis (one case). However, this study's main limitation was that it had collected data from a wide range of sources, and some of these sources' quality was questionable (as data was extracted from case reports, coroner's and autopsy reports, and data from special national mortality registry related to substance use), rather than data from more reliable studies, such as case control or cohort studies, or randomized controlled clinical trials. Therefore, the hierarchy of evidence that these data had contributed was not sufficiently reliable (Corkery et al., 2019).

DISCUSSION

Our literature review aimed to provide a comprehensive and timely description of kratom use's adverse cardiovascular effects and cardiotoxicity risk. Based on our findings, we summarize a few salient features of the adverse cardiovascular effects and cardiotoxicity related to kratom use.

First, the most common acute adverse cardiovascular effects of kratom consumption were tachycardia and hypertension. Second, in the context of kratom's effects on cardiac rhythm, a few *in vitro* studies reported that mitragynine—the most abundant psychoactive alkaloid in the kratom leaf—could induce prolonged QTc intervals and precipitate the risk of torsades de pointes in a dose-dependent manner. A few case reports also speculatively suggested that kratom consumption may have induced ventricular arrhythmia, particularly ventricular tachycardia and fibrillation, resulting in cardiopulmonary arrest. However, the findings of a recent study demonstrated that regular kratom consumption (the ingestion of a brewed kratom decoction) appeared to increase QTc intervals but did not induce a prolonged QTc interval or torsades de pointes (Leong Abdullah et al., 2021). Similarly, data from the national poison data system and autopsy reports of mortality cases indicated that conduction defects and cardiac arrhythmia were, indeed, rare.

Third, autopsy and coroner reports of deaths related to kratom use recorded a few cardiac pathologies related to myocardial ischemia, such as coronary atherosclerosis, focal band necrosis in the myocardium, and hypertensive cardiovascular disease. However, a study of ECG findings by Leong Abdullah et al. (2021) proved that myocardial ischemia (T-wave inversion) did not occur differently among kratom users versus the control group.

Fourth, concerning the risk of heart failure related to kratom use, autopsy and coroner reports of fatalities noted a few related cardiac pathologies, including left ventricular hypertrophy, cardiomegaly, and cardiomyopathy. Again, however, no significant differences were observed in the occurrence of left ventricular hypertrophy between kratom users and a control group (Leong Abdullah et al., 2021). Moreover, case reports did not indicate any features of heart failure related to kratom use.

Fifth, the risk of cardiotoxicity may increase with the co-administration of kratom alongside other substances. The mechanism underlying this finding may result from mitragynine's role as a hepatic cytochrome P450 2D6 (CYP2D6) inhibitor that suppresses the metabolism of co-administered substances and increases their cardiotoxicity risk (Kong et al., 2011; Hanapi et al., 2013). Polymorphism of the CYP2D6 enzyme isoform categorized kratom users into a few sub-populations, such as ultra-rapid, extensive, intermediate, and poor metabolizers. Interestingly, co-administered substances that are also competitive CYP2D6 inhibitors of mitragynine could functionally convert kratom users who are extensive metabolizers to the poor metabolizers category *via* phenocopying (Bernard et al., 2006).

Finally, no animal studies have been conducted to investigate kratom's effects on cardiovascular function. Animal studies are vital for assessment of toxicity related to a particular drug or compound. Animal studies allow the estimation of the lethal dose (LD_{50}) related to cardiotoxicity of kratom or its pharmacoactive alkaloids, such as mitragynine or 7-HMG.

However, importantly, these findings should be interpreted with caution due to several limitations in these studies. First, human studies that have investigated the effects of kratom consumption on cardiac functioning and cardiotoxicity have been lacking—except for a cross-sectional study of ECG findings that was limited by its small sample size and lack of serum mitragynine concentration assessments among kratom users (Leong Abdullah et al., 2021). Furthermore, the findings of *in vitro* studies on cardiotoxicity should not be exclusively extrapolated to represent cardiotoxicity risk in humans. Second, despite a few case reports suggesting cardiotoxicity related to kratom use, the patients described in these case reports had either co-administered kratom with other substances (Aggarwal et al., 2018; Sheikh et al., 2021) or had a long, established history of polysubstance use that may have led them to co-administer kratom with other illicit substances (Abdullah et al., 2019; Eljack et al., 2020). Unfortunately, these case reports did not assess patients' serum mitragynine levels. Third, although a few studies investigating national poisoning data, coroner reports, and autopsy reports suspected cardiotoxicity linked to multiple kratom-induced outcomes, a significant number of these cases had involved polysubstance use. Moreover, whether the described pathologies were caused by kratom use *per se* or had been partially compounded by underlying medical disorders is unclear. Another vital concern among kratom researchers pertains to the validity of published data since cases have been self-reported, without verification by a poison center, and these data's hierarchy of evidence was not sufficiently reliable because most of these data had been obtained from case

reports and descriptive studies (Corkery et al., 2019; Post et al., 2019; Davidson et al., 2021).

Despite these limitations, the data we examined in this literature review have allowed us to offer a few recommendations for future research. Despite the lack of related studies using a rigorous methodology, our findings suggest that chronic, regular kratom consumption may affect the cardiac rhythm and be associated with a risk of myocardial ischemia. Given the gap in the related research and kratom's still unknown safety profile, more rigorous human studies with sufficiently large samples of respondents are urgently needed. Moreover, these studies should examine serum cardiac markers, echocardiograms, Holter monitoring, serum mitragynine levels, and serum 7-hydroxymitragynine levels in order to fully understand the potential cardiotoxicity risk of kratom use. Animal studies should, perhaps, also be conducted to determine the mechanisms underlying kratom use's effects on cardiovascular function. Additionally, since *in vitro* studies have suggested that the upregulation of the hERG1a-Hsp90 complexes may be due to a mitragynine-induced hERG1a channel misfolding (Tay et al., 2019), a human study investigating whether kratom consumption activates the UPR and ERAD system would be interesting, potentially indicating kratom-induced endoplasmic reticulum stress. Finally, future case reports can be more informative than previous reports by including an assessment of serum mitragynine levels and, in the case of polysubstance use, the serum levels of other co-administered substances.

Thus, we cannot offer a definitive conclusion about kratom's cardiotoxicity due to the lack of data and methodological limitations reported in existing studies. Nonetheless, our review offers two notable contributions to the literature. First, kratom's most common adverse cardiovascular effects include tachycardia and hypertension. And second, kratom use may affect the cardiac rhythm in a dose-dependent manner. Therefore, a kratom overdose or the concurrent use of kratom with other illicit substances or medications that affect the cardiac rhythm (e.g., antiarrhythmics, antipsychotics, calcium channel blockers, beta-blockers, and antidepressants) may lead to cardiac arrhythmia. Moreover, the psychoactive alkaloids in kratom's chemical profile remain poorly understood. Therefore, the question of whether kratom use can cause a cardiotoxicity risk merits further investigation.

AUTHOR CONTRIBUTIONS

ML and DS conceptualized and design the review. ML and DS involved in literature search. ML wrote the first draft of the manuscript. All authors involved in the revision of the manuscript and approved the submitted version.

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Evaluation of Kratom Opioid Derivatives as Potential Treatment Option for Alcohol Use Disorder

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Background and Purpose: *Mitragyna speciosa* extract and kratom alkaloids decrease alcohol consumption in mice at least in part through actions at the δ-opioid receptor (δOR). However, the most potent opioidergic kratom alkaloid, 7-hydroxymitragynine, exhibits rewarding properties and hyperlocomotion presumably due to preferred affinity for the mu opioid receptor (μOR). We hypothesized that opioidergic kratom alkaloids like paynantheine and speciogynine with reduced μOR potency could provide a starting point for developing opioids with an improved therapeutic window to treat alcohol use disorder.

Experimental Approach: We characterized paynantheine, speciociliatine, and four novel kratom-derived analogs for their ability to bind and activate δOR, μOR, and κOR. Select opioids were assessed in behavioral assays in male C57BL/6N WT and δOR knockout mice.

Key Results: Paynantheine (10 mg·kg^{-1}, i.p.) produced aversion in a limited conditioned place preference (CPP) paradigm but did not produce CPP with additional conditioning sessions. Paynantheine did not produce robust antinociception but did block morphine-induced antinociception and hyperlocomotion. Yet, at 10 and 30 mg·kg^{-1} doses (i.p.), paynantheine did not counteract morphine CPP. 7-hydroxypaynantheine and 7-hydroxyspeciogynine displayed potency at δOR but limited μOR potency relative to 7-hydroxymitragynine *in vitro*, and dose-dependently decreased voluntary alcohol consumption in WT but not δOR in KO mice. 7-hydroxyspeciogynine has a maximally tolerated dose of at least 10 mg·kg^{-1} (s.c.) at which it did not produce significant CPP neither alter general locomotion nor induce noticeable seizures.

Abbreviations: CPA: conditioned place aversion; CPP: conditioned place preference; DAMGO: (2S)-2-[[2-[[(2R)-2-[[(2S)-2-Amino-3-(4-hydroxyphenyl)propanoyl]amino]propanoyl] amino]acetyl]-methylamino]-N-(2-hydroxyethyl)-3-phenyl-propanamide; MTD: maximum tolerated dose; δOR: delta opioid receptor; κOR: kappa opioid receptor; μOR: mu opioid receptor; U50,488: 2-(3,4-dichlorophenyl)-N-methyl-N-[(1R,2R)-2-pyrrolidin-1-ylcyclohexyl]acetamide.

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Conclusion and Implications: Derivatizing kratom alkaloids with the goal of enhancing δOR potency and reducing off-target effects could provide a pathway to develop novel lead compounds to treat alcohol use disorder with an improved therapeutic window.

Keywords: kratom, alcohol use disorder, nociception, seizures, reward, delta opioid receptor, biased signaling

INTRODUCTION

Mitragyna speciosa, commonly known as kratom, is growing increasingly popular in the United States, with nearly 1% of the population aged 12 and older using kratom in 2019 (Palamar, 2021). While kratom is most commonly used to self-manage pain or reduce dependence to opioids and opiates (Coe et al., 2019), a recent online survey revealed that 18% of kratom users indicate reducing or quitting alcohol consumption as a reason they use kratom (Coe et al., 2019). This indication is in line with reports of individuals claiming that kratom was useful for reducing their alcohol intake (Havemann-Reinecke, 2011; Singh et al., 2014; Suhaimi et al., 2021). We have previously demonstrated that systemic injections of the kratom extract and kratom alkaloids (7-hydroxymitragynine, paynantheine, speciogynine, and mitragynine) decrease voluntary alcohol drinking in mouse models of moderate and binge alcohol consumption, with the kratom alkaloid 7-hydroxymitragynine being the most efficacious (Gutridge et al., 2020). Kratom alkaloids differ from opium-derived opioids and clinically used synthetic opioids in that upon binding to opioid receptors they activate the $G\alpha_{i/o}$ protein, without promoting β-arrestin recruitment to the receptor (Kruegel et al., 2016; Váradi et al., 2016; Faouzi et al., 2020; Chakraborty and Majumdar, 2021). Several preclinical studies in mice strongly suggest that β-arrestin recruitment at the delta opioid receptor (δOR) is a liability for enhanced alcohol use and should be avoided (Chiang et al., 2016; Robins et al., 2018; Gutridge et al., 2020). We have previously demonstrated that 7-hydroxymitragynine and other kratom alkaloids poorly recruit β-arrestin-2 at mu opioid receptors (μORs) and δORs and possess a degree of G-protein bias at this receptor (Gutridge et al., 2020). Moreover, our studies in δOR knockout mice revealed that 7-hydroxymitragynine's modulation of alcohol consumption was due to its activity at the δOR (Gutridge et al., 2020).

However, a possible concern is that 7-hydroxymitragynine and other kratom alkaloids generally have comparable, if not higher, affinity and potency at the μOR (Takayama et al., 2002; Matsumoto et al., 2004).While this μOR potency may be responsible for the alkaloids' ability to promote antinociception in mice (Matsumoto et al., 2004; Obeng et al., 2020; Wilson et al., 2020, 2021) and in humans (Vicknasingam et al., 2020), it appears that because of their μOR potency, kratom alkaloids, especially 7-hydroxymitragynine, are shown or predicted to share some of the same negative side effects associated with traditional opioids such as abuse liability. Accordingly, in rodent preclinical studies, 7-hydroxymitragynine has been shown to have rewarding qualities in models of conditioned place preference and self-administration, which indicates that it may have abuse liability (Yue et al., 2018; Hemby et al., 2019; Gutridge et al., 2020). Likewise, withdrawal symptoms following kratom exposure have also been recorded in rodents (Matsumoto et al., 2005; Wilson et al., 2021). Similarly, regular kratom use in humans leads to dependence problems in over 50% of users (Singh et al., 2014), and kratom withdrawal symptoms equally have been widely reported in humans (Singh et al., 2014; Saref et al., 2019; Stanciu et al., 2019; Anand and Hosanagar, 2021). Likely attributed to its potency at the μOR, another side effect of 7-hydroxymitragynine in mice is hyperlocomotion (Becker et al., 2000; Gutridge et al., 2020); this effect mirrors one of kratom's traditional uses as a stimulant (Suwanlert, 1975; Ahmad and Aziz, 2012). Still, relative to traditional opioids such as morphine, the negative side effect profile of kratom and kratom opioids is slightly lessened in regards to reward, respiratory depression, and withdrawal symptoms (Hemby et al., 2019; Wilson et al., 2020, 2021). This reduction in side effect profile was first attributed to G-protein–biased activity of the kratom alkaloids at the μOR (Kruegel et al., 2016; Váradi et al., 2016), but new research suggests that partial agonism at the μOR likely drives these effects (Gillis et al., 2020; Bhowmik et al., 2021; Uprety et al., 2021). Despite the reduced μOR-mediated side effects relative to traditional opioids, kratom use is not without risk, and this is reflected in controversial efforts to place 7-hydroxymitragynine and mitragynine under Schedule I regulation by the Drug Enforcement Agency (DEA, 2016; Griffin and Webb, 2018).

An additional side effect of kratom use is seizure activity (Coonan and Tatum, 2021). In rats, abnormal EEG activity has been reported following chronic exposure to mitragynine, the most abundant alkaloid in kratom (Suhaimi et al., 2021). In humans, several individual case reports have highlighted seizure side effects induced by kratom use or withdrawal (Boyer et al., 2008; Nelsen et al., 2010; Tatum et al., 2018; Burke et al., 2019; Afzal et al., 2020; Valenti et al., 2021), and a retrospective analysis of kratom exposure reports in the National Poison Data System reveals that 6.1% of reports detail seizure side effects (Eggleston et al., 2019). Currently, the mechanism underlying these reported seizure effects of kratom have not been defined.

We hypothesized that compared to 7-hydroxymitragynine, derivatizing kratom analogs with reduced μOR potency relative to δOR potency would reduce restrictive side effects such as abuse liability and hyperlocomotion, leading to an increased therapeutic window. Prior efforts have been made to utilize unique kratom alkaloid scaffolds to develop improved therapeutic options (Kruegel et al., 2016; Chakraborty et al., 2021a; Wilson et al., 2021). Similarly, here we investigate four novel kratom-derived analogs as well as two naturally occurring kratom alkaloids for their ability to decrease alcohol consumption, while monitoring lead compounds for their ability to produce seizure activity, induce reward properties, and affect general locomotion.

TAIL FLICK THERMAL NOCICEPTION ASSAY

Materials

Kratom "Red Indonesian Micro Powder" was purchased from Moon Kratom (Austin, TX, United States). Corynoxine and corynoxine B were purchased from BOC Sciences (NY, United States). Leu-enkephalin, forskolin, and morphine sulfate pentahydrate were purchased from Sigma-Aldrich (St. Louis, MO, United States). (2S)-2-[[2-[[(2R)-2-[[(2S)-2-Amino-3-(4-hydroxyphenyl)propanoyl] amino] propanoyl] amino]acetyl]-methylamino]-N-(2-hydroxyethyl)-3-phenylpropanamide (DAMGO), 2-(3,4-dichlorophenyl)-N-methyl-N-[(1R,2R)-2-pyrrolidin-1-ylcyclohexyl]acetamide (U50,488), and naloxone hydrochloride were purchased from Tocris Bioscience (Bio-Techne Corporation, Minneapolis, MN, United States). [3H] DAMGO (53.7 Ci/mmol, lot#2376538; 51.7 Ci/mmol, lot#2815607), [3H]U69,593 (60 Ci/mmol, lot#2367921 and lot#2644168; 49.2 Ci/mmol, lot#2791786), and [3H]DPDPE (49.2 CI/mmol, lot#2573313 and lot#2726659; 48.6 Ci/mmol, lot#2826289) were purchased from PerkinElmer (Waltham, MA, United States). For *in vivo* experiments, morphine and naloxone were prepared in a saline vehicle. Kratom-derived analogs were dissolved in a 1:1:8 ethanol:cremophor:saline vehicle for all behavioral experiments. For the two-bottle choice experiment in δOR KO mice, paynantheine was prepared in the same 1:1:8 ethanol: cremophor:saline vehicle. For all other experiments paynantheine and speciociliatine were dissolved in a slightly acidic saline solution that was adjusted to a pH of 6–7 before administration.

Chemistry

General

All chemicals were purchased from Sigma-Aldrich Chemicals and used without further purification. Reactions were carried out in flame-dried reaction flasks under Argon. Reaction mixtures were purified by silica flash chromatography on E. Merck 230–400 mesh silica gel 60 using a Teledyne ISCO CombiFlash Rf instrument with UV detection at 280 and 254 nm. RediSep Rf silica gel normal phase columns were used. The yields reported are isolated yields. NMR spectra were recorded on a Varian 400/500 MHz NMR spectrometer. NMR spectra were processed with MestReNova software. The chemical shifts were reported as δ ppm relative to TMS using residual solvent peak as the reference unless otherwise noted ($CDCl_3$ ^{1}H: 7.26, ^{13}C: 77.3). Peak multiplicity is reported as follows: s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet. Coupling constants (J) are expressed in Hz. High resolution mass spectra were obtained on a Bruker Daltonics 10 Tesla Apex Qe Fourier-Transform Ion Cyclotron Resonance–Mass Spectrometer by electrospray ionization (ESI). Accurate masses are reported for the molecular ion $[M + Na]^+$.

Isolation of Mitragynine From *Mitragyna speciosa* (Kratom)

Mitragynine was extracted from the powdered leaves by following our previously reported methods (Gutridge et al., 2020). Kratom powder (500 g) was heated to reflux in MeOH 700 ml for 40 min. The suspension was filtered and the methanolic extraction process was repeated (3 × 500 ml). The solvent of the combined methanolic extract was removed under reduced pressure and the content was dried using high vacuum. The dry residue was resuspended in 20% acetic acid solution (1 L) and washed with petroleum ether (4 × 500 ml). The aqueous layer was then cooled on ice bath and basified (pH ~9) with aqueous NaOH solution (3.5 M. ~1 L) slowly. Alkaloids were extracted in DCM (4 × 400 ml) from the aqueous layer. The combined DCM layer was washed with brine 300 ml, dried over anhydrous Na_2SO_4, and filtered. The solvent was removed under reduced pressure, and the residue was dried under high vacuum to obtain the kratom extract (9.8 g). Then this crude kratom extract was subjected to silica gel column chromatography, using 0–15% MeOH in dichloromethane to isolate mitragynine (4.7 g), paynantheine (568 mg), speciogynine (343 mg), and speciociliatine (754 mg), along with some minor alkaloids.

7-Hydroxypaynantheine (7OH Pay/7)

Paynantheine (100 mg, 0.25 mmol) was dissolved in acetonitrile (7 ml), and then water (2 ml) was added. The resulting suspension was cooled to 0°C, and PIFA (108 mg, 1.1 equiv) dissolved in acetonitrile (1.1 ml) was added slowly over the course of several minutes. The reaction mixture was stirred at 0°C for 45 min. Then saturated aqueous $NaHCO_3$ solution was added, and the mixture extracted with EtOAc (3 × 15 ml). The organic phase was washed with brine (20 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The residue was purified on a silica column using 10–75% EtOAc in hexanes as eluent. The fractions containing the product were evaporated to yield 42 mg (40%) of **9** as a light magenta amorphous powder. ^{1}H δ (400 MHz, ppm): 7.31 (1H, s, 17); 7.29 (1H, t, 3J = 7.7 Hz, 11); 7.19 (1H, t, 3J = 7.7 Hz, 12); 6.74 (1H, d, ^3J = 7.7 Hz, 10); 5.57 (1H, ddd, ^3J = 18.0, 10.3, 7.2 Hz, 19); 4.99 (1H, dd, ^3J = 18.0, ^2J = 1.5 Hz, 18 *trans*); 4.94 (1H, dd, ^3J = 10.3, ^2J = 1.5 Hz, 18 *cis*); 3.86 (3H, s, 9-OMe); 3.79 (3H, s, 17-OMe); 3.68 (3H, s, 16-COOMe); 3.46 (1H, s, 7-OH); 3.23 (1H, m, 3); 3.03 (1H, m, 21/1); 3.01 (1H, m, 20); 2.85 (1H, m, 5/2); 2.73 (1H, m, 5/1); 2.72 (1H, m, 15); 2.66 (1H, m, 6/1); 2.39 (1H, m, 14/1); 2.30 (1H, m, 21/2); 2.05 (1H, m, 14/2); 1.70 (1H, m, 6/2). ^{13}C δ (100 MHz, ppm): 183.5 (2); 168.8 (16-CO); 159.8 (17); 155.9 (9); 154.9 (13); 139.3 (19); 131.0 (11); 126.4 (8); 115.4 (18); 114.3 (12); 111.4 (16); 109.1 (10); 81.0 (7); 61.6 (21); 61.5 (17-OMe); 60.2 (3); 55.5 (9-OMe); 51.2 (16-COOMe); 49.8 (5); 42.8 (20); 38.2 (15); 35.9 (6); 30.4 (14). Relative configuration was determined based on the NOE cross peaks between the following 1H nuclei: 3 – 5/2; 3 – 14/2; 3 – 21/2; 3 – 5/2; 15 – 19; 19 – 21/2 (/1 always indicates the hydrogen pointing towards the reader from the paper; /2 indicate the hydrogen pointing behind the plain of the paper). HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd for $C_{23}H_{28}N_2NaO_5$ 435.189043; found. 435.189116.

Paynantheine Pseudoindoxyl (Pay PI/8)

7-Hydroxypaynantheine (**9**, 40 mg, 0.1 mmol) was dissolved in dry toluene (1.5 ml), and $Zn(OTf)_2$ (70 mg, 2 equiv) was added. The reaction mixture was stirred in a sealed tube for 30 min at 115°C. To the cooled mixture were added 2 ml sat. aqueous

$NaHCO_3$ solution and water (5 ml) and the organics were extracted with EtOAc (10 ml). The organic layer was rinsed with brine (10 ml) and dried over anhydrous Na_2SO_4. After evaporation of the solvent under reduced pressure, the residue was purified by flash column chromatography on silica (gradient: 40–75% EtOAc in hexanes) to yield 15 mg (38%) of product as a light yellow gum. ^{1}H δ (400 MHz, ppm): 7.32 (1H, t, ^3J = 8.2 Hz, 11); 7.18 (1H, s, 16); 6.37 (1H, d, ^3J = 8.2 Hz, 12); 6.13 (1H, d, ^{3}J = 8.2 Hz, 10); 5.49 (1H, ddd, ^3J = 18.2, 10.3, 7.4 Hz, 19); 5.25 (1H, br s, 1); 4.95 (1H, d, ^3J = 18.2, 18 *trans*); 4.9 (1H, d, ^3J = 10.3, 18 *cis*); 3.89 (3H, s, 9-OCH3); 3.73 (3H, s, 17-OCH3); 3.62 (3H, s, 16-$COOCH_3$); 3.23 (1H, m, 5/1); 3.11 (1H, m, 21/1); 2.87 (1H, m, 20); 2.49 (1H, m, 15); 2.39 (1H, m, 5/2); 2.39 (1H, m, 6/2); 2.34 (1H, m, 3); 1.98 (1H, m, 21/2); 1.94 (1H, m, 6/1); 1.79 (1H, br q ^3J = 11.3 Hz, 14/1); 1.26 (1H, br d, ^3J = 11.3 Hz, 14/2). ^{13}C δ (100 MHz, ppm): 199.8 (7); 168.2 (16-C=O); 162.1 (13); 159.7 (17); 158.7 (9); 139.5 (19); 139 (11); 115.6 (18); 111.9 (16); 109.5 (8); 104 (12); 99.2 (10); 74.7 (2); 72.4 (3); 61.5 (17-O-CH_3); 58.8 (21); 55.8 (9-OCH_3); 53.2 (5); 51.1 (COO-CH_3); 42.3 (20); 36.9 (15); 35.3 (6); 28.3 (14). Relative configuration was determined based on the NOE cross peaks between the following ^{1}H nuclei: 1 – 6/1; 3 – 14/2; 1 – 14/1; 14/1 – 20; 15 – 19; 19 – 21/2. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd for $C_{23}H_{28}N_2NaO_5$ 435.189043; found. 435.189219.

7-Hydroxyspeciogynine (7OH Spg/9)

Speciogynine (200 mg, 0.5 mmol) was dissolved in acetonitrile (15 ml), and then water (5 ml) was added. The resulting suspension was cooled to 0°C, and PIFA (216 mg, 1.1 equiv) dissolved in acetonitrile (2.2 ml) was added slowly over the course of several minutes. The reaction mixture was stirred at 0°C for 1 h. Then saturated aqueous $NaHCO_3$ solution was added, and the mixture extracted with EtOAc (3 × 40 ml). The organic phase was washed with brine (30 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The residue was redissolved in DCM and was purified using silica column chromatography 10–75% EtOAc in hexanes. The fractions containing the product were evaporated to yield 107 mg (57%) of **9** as a light brown amorphous powder. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.36–7.29 (m, 1H), 7.26 (dd, J = 8.8, 7.2 Hz, 1H), 7.17 (d, J = 7.7 Hz, 1H), 6.71 (d, J = 8.3 Hz, 1H), 3.84 (s, 3H), 3.75 (s, 3H), 3.66 (s, 3H), 3.21 – 3.08 (m, 2H), 2.82 (t, J = 12.3 Hz, 1H), 2.77–2.69 (m, 1H), 2.64 (d, J = 14.4 Hz, 1H), 2.54 (t, J = 11.2 Hz, 1H), 2.30 (d, J = 11.9 Hz, 1H), 2.17 (t, J = 10.5 Hz, 1H), 2.06 (t, J = 11.2 Hz, 2H), 1.80 (s, 1H), 1.69 (td, J = 13.5, 4.5 Hz, 1H), 1.40 (s, 1H), 1.02 (d, J = 17.1 Hz, 1H), 0.82 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-d) δ 183.9, 169.61, 160.10, 156.07, 155.15, 131.15, 126.52, 114.42, 111.44, 109.18, 81.16, 61.98, 61.49, 61.52, 55.66, 51.64, 50.21, 39.54, 38.87, 36.13, 24.49, 11.56, and 11.29. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd for $C_{23}H_{30}N_2NaO_5$ 437.204693; found. 437.204951.

Speciogynine Pseudoindoxyl (Spg PI/10)

7-hydroxyspeciogynine (**9**, 200 mg, 0.48 mmol) was dissolved in dry toluene (6 ml), and $Zn(OTf)_2$ (350 mg, two equivalent) was added. The reaction was stirred in a sealed tube for 2 h at 100°C. To the cooled mixture were added 10 ml sat. aqueous $NaHCO_3$ solution and water (20 ml), and extracted with EtOAc (30 ml). The organic layer was rinsed with brine (20 ml) and dried over anhydrous Na_2SO_4. After evaporation of the solvent under reduced pressure, the residue was redissolved in DCM and purified by flash column chromatography (gradient: 40–75% EtOAc in hexanes) to yield 78 mg (39%) of 10 as a light yellow amorphous powder. ^{1}H NMR (500 MHz, Chloroform-d) 7.31 (1H, t, ^3J = 8.2 Hz, 11), 7.23 (1H, s, 17), 6.36 (1H, d, ^3J = 8.2 Hz, 12), 6.12 (1H, d, ^3J = 8.2 Hz, 10), 5.34 (1H, br s, 1), 3.89 (3H, s, 9-OMe), 3.72 (3H, s, 17-OMe), 3.62 (3H, s, 16-COOMe), 3.25 – 3.23 (1H, m, 21/1), 3.22 – 3.21 (1H, m, 5/1), 2.37 – 2.35 (2H, m, 5/2; 6/2), 2.33 – 2.31 (1H, m, 15), 2.29 – 2.28 (1H, m, 3), 2.08 – 2.04 (1H, m, 20), 1.94 – 1.90 (1H, m, 6/1), 1.81 – 1.77 (1H, m, 14/1), 1.75 – 1.73 (1H, m, 21/2), 1.34 –1.30 (1H, br m, 19/1), 1.18–1.15 (1H, m, 14/2), 0.95–0.92 (1H, br m, 19/2), and 0.79 (3H, br, 18). ^{13}C NMR (100 MHz, Chloroform-d) 200.18 (7), 168.02 (16-CO), 162.25 (13), 160.27 (17), 158.83, (9), 139.17 (11), 112.22 (16), 109.5 (8), 104.26 (12), 99.17 (10), 74.94 (2), 72.94 (3), 61.51 (17-OMe), 58.42 (21), 55.99 (9-OMe), 53.57 (5), 51.07 (16-COOMe), 38.15 (20), 37.50 (15), 35.48 (6), 28.95 (4), 24.46 (9), and 11.35 (18). Relative configuration was determined based on the NOE cross peaks between the following ^{1}H nuclei: 1 – 6/1; 1 – 14/1; 15 – 19; 19 – 21/2 (/1 always indicates the hydrogen pointing towards the reader from the paper; /2 indicate the hydrogen pointing behind the plain of the paper). HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd for $C_{23}H_{30}N_2NaO_5$ 437.204693; found. 437.204760.

Cellular Assays and Associated Statistical Analysis

Membrane Isolation and Competitive Radioligand Binding Assay

Membrane isolation and subsequent binding assays were completed as described previously using membranes stably expressing the μOR, δOR, or κOR were isolated from CHO (μOR, δOR) or U2OS cells (κOR) (DiscoverX), and using OR specific radiolabels [^{3}H]DAMGO, [^{3}H]DPDPE, and [^{3}H]U69,593 (Cassell et al., 2019; Creed et al., 2021).

GloSensor cAMP Inhibition Assay

cAMP inhibition assays were performed in HEK cells and transiently transfected with pGloSensor22F, and either expressing FLAG-mouse δOR, HA-mouse μOR, or FLAG-mouse κOR, as previously described (Chiang et al., 2016).

PathHunter β-Arrestin-2 Recruitment Assay

β-Arrestin recruitment assays were performed in PathHunter cells stably expressing the μOR, δOR, or κOR and β-arrestin-2, as previously described (Chiang et al., 2016).

Statistical Analysis

Data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2018). The data analysis was completed using GraphPad 9 (GraphPad Prism software, La Jolla, CA, United States) and is presented as mean ± SEM. For findings from cellular assays, composite figures are shown consisting of an averaged curve from

TABLE 1 | Pharmacological characterization of kratom derivatives at the μ, δ, and κ opioid receptors.

Compounds	Binding		cAMP			β-arrestin-2	
μOR	**pK_i**	**K_i (μM)**	**pIC_{50}**	**IC_{50} (μM)**	**α**	**pEC_{50}**	**α**
DAMGO	9.6 ± 0.1 (1)	0.00024	8.0 ± 0.1 (6)	0.0099	100	6.6 ± 0.1 (6)	100
SPECIO	7.1 ± 0.1 (3)	0.086	6.4 ± 0.2 (5)	0.43	38 ± 3	ND (4)	ND
SPG PI	7.1 ± 0.1 (3)	0.077	6.6 ± 0.2 (5)	0.23	58 ± 4	ND (4)	ND
7OH SPG	7.7 ± 0.1 (3)	0.021	6.2 ± 0.2 (6)	0.61	66 ± 6	ND (4)	ND
7OH PAYN	5.2 ± 0.1 (3)	6.15	4.7 ± 0.5 (5)	21.8	80 ± 40	ND (3)	ND
PAYN PI	6.2 ± 0.1 (3)	0.68	5.3 ± 0.2 (4)	4.82	60 ± 6	ND (3)	ND
δOR	pK_i	K_i (μM)	pIC_{50}	IC_{50} (μM)	α	pEC_{50}	α
Leu-Enk	9.2 ± 0.1 (3)	0.00070	8.4 ± 0.1 (9)	0.0042	100	7.4 ± 0.1 (7)	100
SPECIO	5.4 ± 0.1 (3)	4.34	ND (3)	ND	ND	ND (5)	ND
SPG PI	6.0 ± 0.1 (3)	0.94	5.1 ± 0.3 (4)	8.53	80 ± 20	ND (4)	ND
7OH SPG	6.3 ± 0.1 (3)	0.46	5.6 ± 0.1 (6)	2.27	76 ± 6	ND (4)	ND
7OH PAYN	4.9 ± 0.2 (4)	12.7	5.2 ± 0.3 (5)	5.74	70 ± 20	ND (3)	ND
PAYN PI	6.0 ± 0.1 (3)	0.92	ND (5)	ND	ND	ND (3)	ND
κOR	pK_i	K_i (μM)	pIC_{50}	IC_{50} (μM)	α	pEC_{50}	α
U50,488	10.0 ± 0.2 (2)	0.000099	8.5 ± 0.1 (5)	0.0034	100	7.1 ± 0.1 (6)	100
SPECIO	6.2 ± 0.1 (4)	0.59	5.6 ± 0.2 (4)	2.50	60 ± 7	ND (5)	ND
SPG PI	6.1 ± 0.1 (3)	0.75	4.7 ± 0.5 (4)	20.6	80 ± 30	ND (3)	ND
7OH SPG	5.8 ± 0.2 (3)	1.63	5.1 ± 0.3 (3)	7.71	80 ± 20	ND (5)	ND
7OH PAYN	5.1 ± 0.1 (3)	7.46	ND (3)	ND	ND	ND (3)	ND
PAYN PI	5.9 ± 0.1 (4)	1.31	ND (3)	ND	ND	ND (3)	ND

Affinity (pKi, drug concentration at which 50% of receptors is occupied). cAMP inhibition potencies (pIC_{50}, drug concentration at 50% maximal efficacy) and efficacies (α, % inhibition at maximal efficacy normalized to DAMGO [μOR], Leu-enkephalin [δOR], or U50,488 [κOR]) of OR agonists to inhibit cAMP production are indicated ± SEM. β-arrestin-2 recruitment potencies (pEC_{50}) and efficacies (α, normalized to DAMGO, Leu-enkephalin or U50,488) of OR agonists to recruit β-arrestin 2 are indicated ± SEM. The number of repetitions for each drug is indicated in parentheses. ND, not detectable. Data for 7-hydroxymitragynine, speciogynine, and paynantheine in the GloSensor cAMP assay and PathHunter β-arrestin-2 recruitment assay was generated in a previous publication (Gutridge et al., 2020) and is shown in ***Supplemental Table S1*** *for easy comparison to the kratom derivatives.*

a minimum of three independent assays that were normalized to a positive control; best fit values in **Table 1** were generated by GraphPad Prism from composite figures.

Animals

General

The animal protocols (#1305000864 and #1605001408) describing the care and use of experimental animals was approved by the Purdue University Institutional Animal Care and Use Committee (https://www.purdue.edu/research/regulatory-affairs/animal-research/staff.php). Animal studies were carried out in accordance with the ARRIVE guidelines (Kilkenny et al., 2010) and recommendations made by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Wild-type C57Bl/6N mice (107 male, 10 female; 6–7 weeks old) were purchased from Envigo (Indianapolis, IN, United States) and were acclimated to the facility and to handling and injections for 1 week prior to any experimental procedures. δOR KO mice (27 male, 8–12 weeks old) with a C57Bl/6N background (re-derived in early 2021) were bred in-house and were similarly conditioned to handling and injections prior to experimentation. All mice were housed on a reverse 12-h light (21:30–9:30)/12-h dark cycle under controlled temperature (21–23°C) with *ad libitum* food access. The only exception to this is mice used in the rotarod assay; these mice were housed in 12-h light (6:00–18:00)/12-h dark cycle. All experiments were conducted between 10:30 and 15:00, and all mice were habituated to the test room at least 30 min prior to experimentation. Rotarod, nociception, and seizure experiments were conducted in well-lit rooms, whereas conditioned place preference, two-bottle choice, and locomotor experiments were conducted in the dark.

Experimental Groups

For the locomotor assays with 7-hydroxymitragynine, a group of 10 male mice was used. For the paynantheine agonist nociception assays, 10 male mice were treated on different days with 10 and 30 $mg{\cdot}kg^{-1}$ (i.p.) paynantheine. For the paynantheine antagonist nociception assays, a separate group of 10 mice were exposed to 6 $mg{\cdot}kg^{-1}$ morphine (s.c.) by itself, and then again after treatment with 10 and 30 $mg{\cdot}kg^{-1}$ paynantheine (i.p.). For agonist and antagonist antinociception assays with 7-hydroxyspeciogynine, a total of 11 wild-type male mice were used; all received 7-hydroxyspeciogynine for the agonist mode, and then for antagonist mode, n = 6 received morphine plus 7-hydroxyspeciogynine and n = 5 received vehicle plus 7-hydroxyspeciogynine. For specifics on drug administration timing in the nociception assays, see the Methods section titled **Tail Flick Thermal Nociception Assay**. For the two-bottle choice alcohol consumption experiments with WT male and female mice, separate groups of wild-type mice were used to test increasing doses of each analog (n = 8 males for 7-hydroxypaynantheine, n = 12 males and n = 10 females for 7-hydroxyspeciogynine). For the two-bottle choice experiments with δOR KO mice, a group of

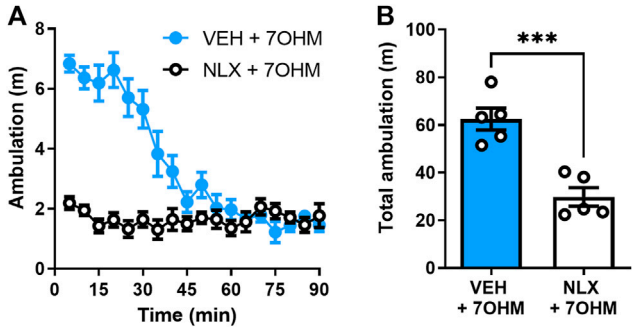

FIGURE 1 | Blocking μOR attenuates 7-hydroxymitragynine (7OHM) induced hyperlocomotion. **(A)** 90-min ambulation time course of wild-type, C57Bl/6 male mice (n = 5 per group) treated with 7-hydroxymitragynine (3 mg·kg^{-1}, i.p.) after pretreatment with the vehicle (s.c.) or naloxone (1 mg·kg^{-1}, s.c., NLX) injection (10 min prior to 7-hydroxymitragynine injection). **(B)** Total ambulation (area under the curve) for the same data set. ***p < 0.001 (for details, see **Supplementary Table S2**).

mice (n = 9) was repeatedly tested once per week with different drug treatments (consistent baseline ethanol consumption across the drug treatments is shown in **Supplementary Figure S5**). A second separate group of 10 male δOR KO mice was used to examine speciociliatine in the two-bottle choice paradigm. Following a 3-week period of alcohol withdrawal, five of the δOR KO mice from the first two-bottle choice group were used to examine seizure activity of paynantheine (30 mg·kg^{-1}, i.p.). Similarly, five wild-type mice from the naloxone-block locomotor experiment were reused to assess seizure activity of 30 mg·kg^{-1} paynantheine (i.p.) following a week of drug washout. In the rotarod assay, n = 8 wild-type male and n = 8 δOR KO male mice were used to assess motor incoordination effects following treatment with speciociliatine. Note that one δOR KO mouse died after experiencing severe level 5–6 seizures following i.p. administration of 30 mg/kg speciociliatine in the rotarod assay, leading to an overall n = 7 instead of n = 8 for this genotype. For the CPP paradigms, independent groups of wild-type male mice were used to examine paynantheine by itself (n = 16 total), paynantheine with morphine (n = 14 total), and 7-hydroxyspeciogynine (n = 8).

Behavioral Assays and Associated Statistical Analysis

Locomotor Evaluation

To assess drug-induced effects on ambulation for 7-hydroxymitragynine, locomotor activity was assessed in a 2-day protocol as previously described (Gutridge et al., 2020). To assess drug-induced effects on ambulation for paynantheine and 7-hydroxyspeciogynine, locomotor information was extracted from the data generated in the CPP experiments. Distance traveled during each drug and vehicle conditioning session were pulled from the 30- or 40-min conditioning session (extended or brief CPP paradigm, respectively), and all sessions per treatment were averaged for analysis. A summary of all statistical analyses for the locomotor data can be found in **Supplemental Table S2**. In brief, for 7-hydroxymitragynine locomotor data in **Figure 1**, an unpaired, two-tailed t test was used. For paynantheine locomotor data in **Figure 2G**, statistical significance of drug treatment vs. vehicle was obtained by a one-way ANOVA with Dunnett's multiple comparisons to VEH + VEH. For paynantheine + morphine locomotor data in **Figure 2G**, statistical significance of paynantheine + morphine vs. morphine alone was obtained *via* a one-way ANOVA with Dunnett's multiple comparisons to morphine (MOR). For 7-hydroxyspeciogynine locomotor data in **Figure 3B**, a two-tailed, paired t test was used; one mouse was removed from this analysis after being identified as an outlier with Grubb's test.

Brief and Extended Conditioned Place Preference Paradigms

Mice were conditioned to drugs and vehicle as described previously in two-chamber conditioned place preference (CPP) boxes in a counterbalanced, unbiased approach for either two drug conditioning sessions over 2 days (brief) or four drug conditioning sessions over 8 days (extended) (Váradi et al., 2015; Gutridge et al., 2020). For brief and extended conditioned place preference experiments, separate groups of mice were used for each drug dose. A summary of all statistical analyses for the CPP data can be found in **Supplemental Table S4**. In brief, all CPP data were analyzed with two-tailed, paired t tests comparing time spent on the drug-paired side pre- and post-conditioning.

Seizure Assay

To assess drug-induced seizurogenic activity, mice were placed in a clear plastic cylinder (25 cm diameter, 35 cm height) immediately following drug injection and their activity was recorded in a well-lit, quiet room using iSpy camera software (iSpyConnect.com). A recording time of 90 min was chosen for the tested compounds based on previous observations of seizures time lengths in experiments with 30 mg·kg^{-1} paynantheine. If animals were not presenting with seizure activity after 30 min, the recording time was shortened accordingly. Seizure severity was scored based on the modified Racine scale (half-scores allowed) in bins of 3–5 min. Onset to first seizure symptom, onset to highest Racine score, and highest Racine score were also assessed. A summary of all statistical analyses for the seizure data can be found in **Supplemental Table S3**. In brief, seizure-like behavior between wild type and δOR KO mice was compared with a two-tailed, unpaired t test with Welch's correction on area under the curve data generated from graphing the highest Racine score per time bin over 90 min for each mouse.

Tail Flick Thermal Nociception Assay

Antinociception *via* the tail flick assay was measured as previously described (van Rijn et al., 2012). Mice were first habituated to the handling restraint used during the experimentation. On subsequent test days, a radiant heat tail flick instrument (Columbus Instruments, Columbus, OH, United States) was used to collect duplicate measurements by testing two different regions on the mouse's tail. The beam intensity was adjusted between each group of mice to elicit

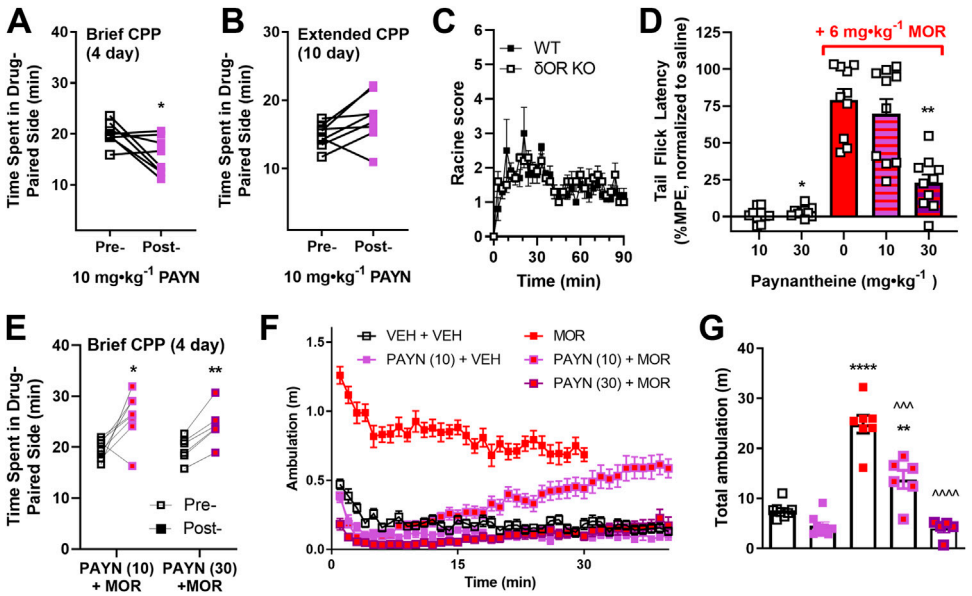

FIGURE 2 | Antagonistic action of paynantheine *in vivo*. The agonistic and antagonistic actions of kratom alkaloid paynantheine were further investigated in C57Bl/6 mice. Paynantheine (10 mg·kg^{-1}, i.p. PAYN) was evaluated in a **(A)** 4-day and **(B)** 10-day model of conditioned place preference (CPP, two vs. four drug conditioning sessions, respectively, n = 8 each). **(C)** Seizure activity induced by paynantheine (30 mg·kg^{-1}, i.p.) was evaluated in male δOR KO and WT mice (n = 5 per group). **(D)** Paynantheine (10 and 30 mg·kg^{-1}, i.p.) was tested for agonist and antagonistic properties in male mice (n = 10 per dose) *via* the tail flick thermal nociception assay. For the antagonist assays, morphine (6 mg·kg^{-1}, s.c., MOR) was administered 10 min following a dose of paynantheine (10 or 30 mg·kg^{-1}, i.p.). Nociception data are expressed as maximum possible effect (%MPE) normalized to a saline baseline (treatment–saline baseline). **(E)** Paynantheine (10 and 30 mg·kg^{-1}, i.p.) was evaluated for agonist and antagonist activity in an acute model of conditioned place preference by administering 10 min prior to morphine (6 mg·kg^{-1}) or the vehicle (n = 8 for 10 mg·kg^{-1} doses, n = 6 for 30 mg·kg^{-1} dose). Locomotor data were extracted from the conditioning sessions of the CPP experiments in **(A**,**E)** and is shown as **(F)** ambulation over time and **(G)** total ambulation (total area under curve). For comparison in **(F**,**G)**, locomotor data for morphine (6 mg·kg^{-1} morphine) was extracted from a previous CPP experiment with 30-min conditioning sessions. The vehicle locomotor data were extracted from the non–drug-paired side conditioning session for the 10 mg·kg^{-1} paynantheine + vehicle group. For locomotor data in **(G)**, statistical significance of drug treatment vs. vehicle (VEH + VEH) is shown with stars; statistical significance between paynantheine + morphine treatments and morphine-only treatment (MOR) is shown with carets. *p < 0.05, **p < 0.01, ^^^p < 0.001, *** or ^^^^p < 0.0001 (for details, see **Supplemental Tables S2–S5**).

reproducible responses between 2 and 3 s (beam intensity of 7–9). At a minimum, mice were given 2 days between experiments to recover from thermal stimuli. For each test day, a baseline tail flick response was collected for each mouse and was used to calculate the testing cutoff time (cutoff time = three times the baseline response time). To test antinociception by drug agonism, a vehicle injection was administered next (i.p. or s.c.), and tail flick responses were collected after 30 min. The drug was then administered (i.p. or s.c.), and tail flick responses were collected after 30 min. To test drug antagonism of morphine antinociception, a response to vehicle injections were similarly collected prior to drug administration with a first vehicle injection (i.p. or s.c.) at 0 min, followed by a second vehicle injection (s.c.) 10 min before collecting tail flick responses at 30 min (20 min after the second vehicle injection). The test compound was then administered (i.p. or s.c.), followed by 6 mg·kg^{-1} morphine (s.c.) 10 min later. Tail flick responses were collected 20 min after morphine administration. Data are represented as percent maximal possible effect (%MPE) and is calculated as %MPE = (treatment response time – baseline response time)/(cutoff time – baseline response time) * 100. Data are normalized to vehicle treatment: drug treatment %MPE – saline treatment %MPE. A summary of all statistical analyses for the antinociceptive data can be found in **Supplemental Table S5**. In brief, for agonist antinociception assays, significance was calculated *via* a two-tailed, paired t test to compare vehicle and drug treatment. For antagonist antinociception assays with three treatment groups in the same group of mice (**Figure 2D**), data were analyzed *via* repeated measures (RM) one-way ANOVA with Dunnett's multiple comparisons to the morphine-only treatment group. For antagonist antinociception assays with two treatment groups in two different groups of mice (**Figure 3D**), an unpaired t-test with Welch's correction was used to assess significance between the morphine-only group and the morphine plus "antagonist" group.

Two-Bottle Choice Alcohol Paradigm

Mice were subject to drinking in the dark (DID), limited access (4 h per day), two-bottle choice (10% ethanol vs. water) paradigm in which they were trained to consume alcohol voluntarily as previously described (Rhodes et al., 2005; van Rijn and Whistler, 2009). Mice reached stable alcohol consumption within 3 weeks of training, and after the third week, drug injections were administered prior to the daily drinking session on Friday. Drug's effect on alcohol consumption was measured as the change in Friday's alcohol intake minus the average alcohol intake from the preceding Tuesday–Thursday of that week

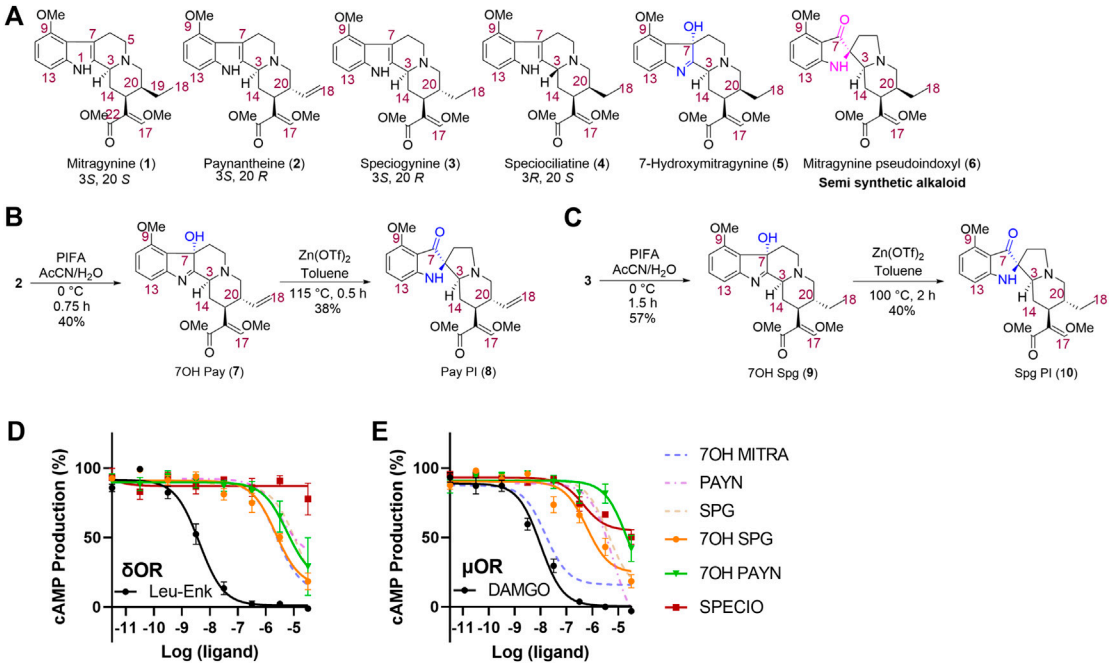

FIGURE 3 | Synthesis and characterization of kratom alkaloid analogs. Structures of naturally occurring kratom alkaloids paynantheine and speciogynine were used as scaffolds for analog synthesis. Analogs with pseudo-indoxyl (PI) rearrangements or hydroxyl group additions were made for both compounds, and a naturally occurring minor kratom alkaloid and speciogynine isomer, speciociliatine, was also synthesized for testing. **(A)** Chemical structures of selected indole-based kratom alkaloids; **(B)** synthesis of 7-hydroxypaynantheine (7) and paynantheine pseudoindoxyl (8); and **(C)** synthesis of 7-hydroxyspeciogynine (9) and speciogynine pseudoindoxyl (10). 7-hydroxyspeciogynine (7OH SPG, 9), 7-hydroxypaynantheine (7OH PAYN, 7), and speciociliatine (SPECIO, 4) are compared to kratom alkaloids (dashed lines; 7-hydroxymitragynine (7OH MITRA), paynantheine (PAYN), and speciogynine (SPG) for inhibition of forskolin-induced cAMP in a GloSensor assay in transfected HEK-293 cells at δOR **(D)** and μOR **(E)**. For additional *in vitro* characterization, see **Supplemental Figure S4**.

($g{\cdot}kg^{-1}$). A summary of all statistical analyses for the drinking data can be found in **Supplemental Tables S6–S9**. In brief, results from two-bottle choice alcohol consumption paradigms were assessed for statistical significance using RM two-way ANOVA for main effects of drug dose, treatment day, and drug dose × treatment day; Sidak's multiple comparisons (MC) between alcohol consumption baseline (Tuesday–Thursday average) vs. treatment day consumption (Friday) were then used as the post hoc test for each drug dose tested. The same RM two-way ANOVA and Sidak's MC *post hoc* analyses were used for water consumption and ethanol preference data. For the change in alcohol consumption, change in water consumption, and change in ethanol preference data for 7-hydroxyspeciogynine where male and female data were analyzed together, a mixed-effects model was used (due to missing values) with the Geisser–Greenhouse correction for main effects, followed by Dunnett's MC between alcohol consumption baseline vs. treatment day consumption. Sex differences between baseline data were evaluated using RM two-way ANOVA for main effects of sex, treatment baseline, and sex × treatment baseline; Sidak's multiple comparisons (MC) between male and female mice were then used as the post hoc test for each treatment week tested.

Accelerating Rotarod Test

Mice were trained to walk on a rotarod apparatus (IITC, United States) with 1.25" diameter drums 2 days prior to drug testing. The rotarod started at 3 rpm and was increased to 30 rpm over 300 s. A trial for a mouse ended when it fell and tripped the sensor, when it rode the rotarod for two consecutive revolutions, or after 300 s (the maximum trial time)(White et al., 2015). Mice received at least 3 min of rest between trials. On test day, baseline performance was assessed as the average latency to fall in three trials per mouse. Mice were then injected with 30 $mg{\cdot}kg^{-1}$ speciociliatine (i.p.) and immediately tested for performance on the apparatus (this first data point represented as latency to fall at 5 min), and then tested again at 15, 30, 60, and 120 min post-injection. Each mouse's performance was normalized to its own baseline and reported as a percentage. A summary of all statistical analyses for the rotarod data can be found in **Supplemental Table S2**. In brief, data for each tested timepoint were calculated as a percentage of the baseline, and thus statistical significance was calculated in a two-tailed, one sample *t* test vs. a hypothetical mean of 100 (baseline was 100%). Rotarod results between WT and δOR KO genotypes were compared with a mixed-effects model with fixed effects for timepoint, genotype, and timepoint × genotype.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently

archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

RESULTS

Hyperlocomotion Induced by the Kratom Alkaloid 7-Hydroxymitragynine Is Naloxone-Reversible

The kratom alkaloid 7-hydroxymitragynine was the most potent amongst kratom alkaloids in decreasing alcohol intake (Gutridge et al., 2020); however, it produces significant adverse effects in such as conditioned place preference and hyperlocomotion. This hyperlocomotion induced by 7-hydroxymitragynine was blocked by a low, 1 $mg{\cdot}kg^{-1}$ dose of naloxone (unpaired, two-tailed *t* test, t = 5.441, df = 8, $p = 0.0006$) (**Figure 1**).

Paynantheine Functionally Antagonizes Morphine Effects *in vivo*

Paynantheine is a naturally occurring G-protein–biased kratom alkaloid with micromolar potency and affinity at the μOR and δOR that dose-dependently decreases alcohol intake in male mice at 10 and 30 $mg{\cdot}kg^{-1}$, but unlike 7-hydroxymitragynine does not produce hyperlocomotion at its effective dose (Gutridge et al., 2020). In contrast to 7-hydroxymitragynine, paynantheine produces modest conditioned place aversion (CPA) in a brief CPP paradigm (paired, two-tailed *t* test, t = 2.606, df = 7, $p = 0.0351$) (**Figure 2A**). However, when using an extended CPP paradigm paynantheine did not produce CPP nor CPA (paired, two-tailed *t* test, t = 2.227, df = 7, $p = 0.0612$) (**Figure 2B**). Additionally, we observed Racine level 1–2 convulsive behaviors in wild type and δOR KO mice injected with a 30 $mg{\cdot}kg^{-1}$ dose (**Figure 2C**) with no difference between groups (Welch's *t* test, t = 0.9205, df = 6.738, $p = 0.3891$). In the GloSensor assay of cAMP inhibition, paynantheine displayed partial to full agonism at the ORs (Gutridge et al., 2020) (**Supplemental Table S1**); however, paynantheine has also been reported as weak antagonist in a BRET-based G-protein assay at human ORs (Kruegel et al., 2016). To obtain a better understanding of paynantheine's pharmacology *in vivo*, we assessed if paynantheine was antinociceptive in thermal nociception paradigms. Though the 30 $mg{\cdot}kg^{-1}$ dose of paynantheine produced a statistically significant difference in %MPE vs. vehicle (paired, two-tailed *t* test, t = 2.925, df = 9, $p = 0.0169$), neither the 10 nor 30 $mg{\cdot}kg^{-1}$ dose displayed meaningful antinociceptive effects (**Figure 2D**, first two columns). Instead, paynantheine dose-dependently blocked antinociception produced by 6 $mg{\cdot}kg^{-1}$ morphine (RM one-way ANOVA, overall effect: F (1.943,17.49) = 12.38, $p = 0.0005$, with Dunnett's MC to 6 $mg{\cdot}kg^{-1}$ morphine: $p = 0.6330$ for 10 $mg{\cdot}kg^{-1}$ dose, $p = 0.0019$ for 30 $mg{\cdot}kg^{-1}$ dose) (**Figure 2D**, last three columns). Because paynantheine blocked morphine action in a nociception assay and by itself did not produce CPP, we next sought to determine if it could block morphine CPP. However, neither pretreatment with 10 nor 30 $mg{\cdot}kg^{-1}$ paynantheine abolished 6 $mg{\cdot}kg^{-1}$ morphine CPP (paired, two-tailed t tests, t = 3.214, df = 7, $p = 0.0148$ for the 10 $mg{\cdot}kg^{-1}$ dose, t = 6.609, df = 5, $p = 0.0012$ for the 30 $mg{\cdot}kg^{-1}$ dose) (**Figure 2E**). However, when assessing locomotor data from the CPP experiments in **Figures 2A,E**, we did observe that paynantheine dose-dependently attenuated hyperlocomotion induced by 6 $mg{\cdot}kg^{-1}$ morphine (one-way ANOVA, overall effect: F (2,15) = 39.25, $p < 0.0001$, with Dunnett's MC to 6 $mg{\cdot}kg^{-1}$ morphine: $p = 0.0004$ for 10 $mg{\cdot}kg^{-1}$ dose, $p < 0.0001$ for 30 $mg{\cdot}kg^{-1}$ dose) (**Figures 2F,G**).

Kratom Analogs Are OR Partial Agonists With Minimal β-Arrestin-2 Recruitment

In order to produce better lead candidates to treat alcohol use disorder that lack adverse locomotor and rewarding effects, we next aimed to discover kratom alkaloids or alkaloid derivatives with increased δOR affinity and potency but with limited μOR potency. To this end, we extracted paynantheine (2), speciogynine (3), and speciociliatine (4) from dry kratom powder using a modified protocol reported by Varadi et al., 2016. Paynantheine (2) was converted to 7-hydroxypaynantheine (7), (**Figure 3B**) using PIFA in acetonitrile and water. This 7-hydroxypaynantheine was next transformed to paynantheine pseudoindoxyl (8) using $Zn(OTf)_2$ in refluxing toluene. We adopted the same strategy to synthesize 7-hydroxyspeciogynine (9) and speciogynine pseudoindoxyl (10) as shown in **Figure 3C**.

Affinity wise, we noted that the paynantheine analogs, especially the 7-hydroxyl analog, showed weak μOR affinity, whereas 7-hydroxyspeciogynine displayed the strongest μOR affinity (**Table 1** and **Supplementary Figure S3A**). At the δOR, 7-hydroxyspeciogynine displayed improved binding relative to speciogynine, which was on par with affinities for the two pseudoindoxyl analogs. 7-hydroxypaynantheine was a magnitude weaker in binding the δOR than 7-hydroxyspeciogynine; this same trend was apparent at the κOR (**Table 1** and **Supplementary Figures S4A–C**).

In terms of cAMP inhibition, we noted clear signs of partial agonism for the analogs at the μOR, with paynantheine pseudoindoxyl, 7-hydroxypaynantheine, and 7-hydroxyspeciogynine displaying the lowest potency at the μOR (**Figures 3D,E**; **Table 1**, **Supplementary Figure S3A**). 7-hydroxyspeciogynine was the strongest activator at the δOR (**Figure 3D**), whereas speciociliatine exhibited the strongest κOR potency out of the tested alkaloids (**Table 1** and **Suppementary Figure S3F**). Notably, while speciociliatine displayed binding at the δOR, it showed minimal activity at this receptor in regards to cAMP inhibition, suggestive of it acting as antagonist at the δOR (**Table 1** and **Supplemental Figures S3B,E**). At the κOR, we did not detect cAMP inhibition for 7-hydroxypaynantheine at the tested dose range (**Table 1** and **Supplementary Figure S3F**). We did not detect any β-arrestin-2 recruitment for speciociliatine and the pseudoindoxyl and 7-hydroxyl analogs within the tested dose range (**Table 1** and **Supplementary Figures 3G–I**), which is line with the reported G-biased nature of the

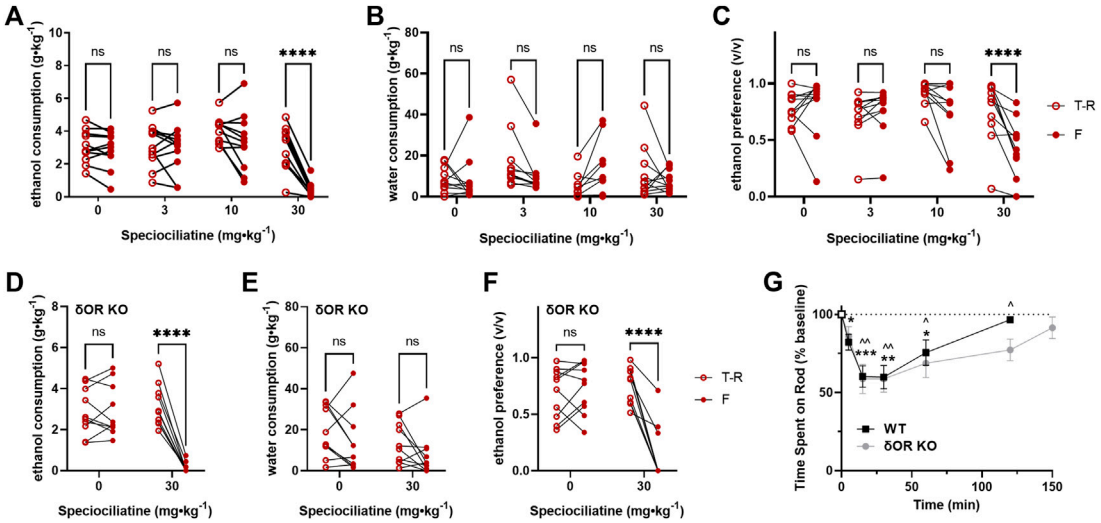

FIGURE 4 | Speciociliatine decreases voluntary ethanol consumption and impairs motor coordination in wild-type and δOR knockout mice. 10% ethanol consumption, water consumption and ethanol preference in male C57BL/6 **(A–C**, respectively) (n = 11) and δOR KO **(D–F**, respectively) mice (n = 10) in a voluntary twobottle choice, limited access, drinking-in-the-dark paradigm, following treatment with speciociliatine (3, 10, and 30 mg·kg^{-1}, i.p.) **(G)** 150-minute duration rotarod assessment of motor incoordination inWTmice (n = 8) and δOR KO mice (n = 7), immediately followed by a 30 mg·kg^{-1} dose of speciociliatine (i.p.); significance for WT mice and δOR KO mice is denoted with stars and carets, respectively. Open circles are the average intake/preference on the preceding 3 days (baseline), and closed circles are the intake on Fridays following drug exposure. * or$^{\wedge}p$ < 0.05, ** or$^{\wedge\wedge}p$ < 0.01, ***p < 0.001, ****p < 0.0001 (for details, see **Supplemental Tables S6–S8**).

kratom alkaloids (Kruegel et al., 2016; Váradi et al., 2016; Gutridge et al., 2020).

Speciociliatine Modulation of Alcohol Intake Is Compounded by Drug-Induced Locomotor Incoordination

Based on our hypothesis that G-protein–biased δOR agonism drives decreased alcohol intake following kratom alkaloid injection, we did not expect speciociliatine to decrease alcohol intake as it behaves *in vitro* as a partial agonist for μOR and κOR but antagonist at δOR (**Table 1**). However, speciociliatine significantly decreased ethanol consumption but only at the 30 mg·kg^{-1} dose (RM two-way ANOVA, dose: F (3, 30) = 36.48, p < 0.0001, time: F (1, 10) = 50.17, p < 0.0001, dose × time: F (3, 30) = 13.30, p < 0.0001, with Sidak's MC (T-R vs F), p < 0.0001 for the 30 mg·kg^{-1} dose) (**Figure 4A**) and with surprisingly strong efficacy (an average decrease of 2.5 ± 0.3 g·kg^{-1} ethanol or a 90 ± 3% reduction, **Supplementary Figure S5A**). However, the 30 mg·kg^{-1} dose demonstrated a similar alcohol modulating effect in δOR KO mice (RM two-way ANOVA, dose: F (1, 9) = 25.36, p = 0.0007, time: F (1, 9) = 61.69, p < 0.0001, dose × time: F (1, 9) = 83.26, p < 0.0001, with Sidak's MC (T-R vs F), p < 0.0001 for the 30 mg·kg^{-1} dose) (**Figure 4D**). Treatment with speciociliatine did not change water consumption at any of the tested doses in wild type or δOR KO mice (**Figures 4B,E**, respectively). Taking together the lack of compensatory increase in water consumption and the decrease in ethanol consumption at the 30 mg·kg^{-1} dose, the ethanol preference was thus significantly decreased at this dose in wild-type mice (**Figure 4C**) (RM two-way ANOVA, dose: F (3, 30) = 24.20, p < 0.0001, time: F (1, 10) = 17.10, p = 0.002, dose × time: F (3, 30) = 7.521, p = 0.0007, with Sidak's MC (T-R vs F), p < 0.0001 for the 30 mg·kg^{-1} dose) and δOR KO mice (**Figure 4F**) (RM two-way ANOVA, dose: F (1, 9) = 32.58, p = 0.0003, time: F (1, 9) = 23.26, p = 0.0009, dose × time: F (1, 9) = 64.72, p < 0.0001, with Sidak's MC (T-R vs F), p < 0.0001 for the 30 mg·kg^{-1} dose). The 30 mg·kg^{-1} dose also significantly reduced the ability of treated wild-type mice to perform in the rotarod assessment (**Figure 4G**). This motor effect had a rapid onset, where time spent on the device significantly decreased at 5 min (one sample t test, t = 3.478, df = 7, p = 0.0103), with the peak effect occurring between 15 and 30 min (t = 5.809, df = 7, p = 0.0007; t = 5.344, df = 7, p = 0.0011, respectively), and the mice fully recovering at 120 min (t = 1.953, df = 7, p = 0.0918). The same effect was observed in δOR KO mice (mixed effects model with matching for genotype × timepoint, F (1.941,11.26) = 1.930, p = 0.1906).

Kratom Analogs Decrease Ethanol Consumption in a δOR-Dependent Mechanism

Given the weak μOR potency of 7-hydroxyspeciogynine and 7-hydroxypaynantheine but the clear 0.5–1 log-fold difference in potency at the δOR between the two analogs (**Figures 3D,E**), we next assessed the *in vivo* potency of these two alkaloids in modulating volitional alcohol consumption in mice. In wild-type male mice, 7-hydroxyspeciogynine more potently

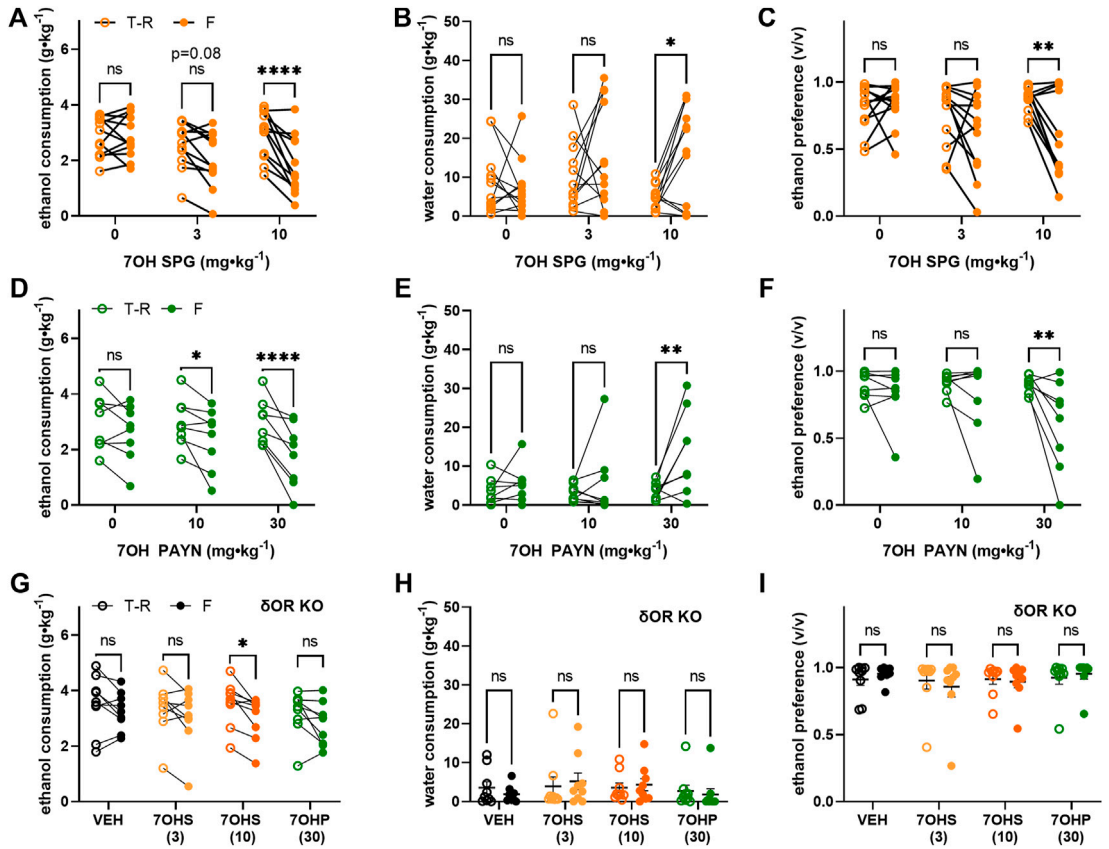

FIGURE 5 | Kratom analogs decrease voluntary ethanol consumption in mechanisms partially dependent on δOR. 10% ethanol consumption (left column), water consumption (middle column), and ethanol preference (right column) in male C57Bl/6 wild-type mice following treatment with **(A–C)** 7-hydroxyspeciogynine (3 and 10 mg·kg^{-1}, s.c., n = 12, 7OH SPG; 7OHS), **(D–F)** 7-hydroxypaynantheine (10 and/or 30 mg·kg^{-1}, s.c., n = 8, 7OH PAYN; 7OHP), and in **(G–I)** male δOR KO mice (n = 9), following treatment with effective doses of both analogs in a voluntary two-bottle choice, limited access, drinking-in-the-dark paradigm. Open circles are the average intake/preference on the preceding 3 days (baseline), and closed circles are the intake on Fridays following drug exposure. *p < 0.05, **p < 0.01, ****p < 0.0001 (for details, see **Supplemental Tables S6–S8**).

reduced alcohol intake in a dose-dependent manner at 3 and 10 mg·kg^{-1} (**Figure 5A**, RM two-way ANOVA, dose: F (2, 22) = 6.973, p = 0.0045, time: F (1, 7) = 13.79, p = 0.0006, dose × time: F (2, 22) = 8.675, p = 0.0017, with Sidak's MC (T-R vs F), p = 0.0802 for the 3 mg·kg^{-1} dose, p < 0.0001 for the 10 mg·kg^{-1} dose). This decrease in ethanol consumption at the 10 mg·kg^{-1} dose was accompanied by a concomitant increase in water consumption during the time course of the voluntary alcohol consumption paradigm (**Figure 5B**, RM two-way ANOVA, dose: F (2, 22) = 8.706, p = 0.0016, time: F (1, 11) = 4.161, p = 0.0661, dose × time: F (2, 22) = 3.489, p = 0.0483, with Sidak's MC (T-R vs F), p = 0.0112) as well as a corresponding decrease in ethanol preference (**Figure 5C**, RM two-way ANOVA, dose: F (2, 22) = 9.997, p = 0.0008, time: F (1, 11) = 8.284, p = 0.0150, dose × time: F (2, 22) = 4.140, p = 0.0298, with Sidak's MC (T-R vs F), p = 0.0036). We found that 7-hydroxypaynantheine was able to significantly reduce alcohol intake at a 10 and 30 mg·kg^{-1} dose (**Figure 5D**, RM two-way ANOVA, dose: F (2, 14) = 4.200, p = 0.0373, time: F (1, 7) = 13.79, p = 0.0075, dose × time: F (2, 14) = 5.515, p = 0.0171, with Sidak's MC (T-R vs F), p = 0.0219 for the 10 mg·kg^{-1} dose, p < 0.0001 for the 30 mg·kg^{-1} dose). Similarly, the decrease in ethanol consumption at the 30 mg·kg^{-1} dose of 7-hydroxypaynantheine was accompanied by a concomitant increase in water consumption during the time course of the voluntary alcohol consumption paradigm (**Figure 5E**, RM two-way ANOVA, dose: F (2, 14) = 4.129, p = 0.0389, time: F (1, 7) = 4.920, p = 0.0621, dose x time: F (2, 14) = 4.149, p = 0.0385, with Sidak's MC (T-R vs F), p = 0.0015) and a corresponding decrease in ethanol preference (**Figure 5F**, RM two-way ANOVA, dose: F (2, 14) = 3.845, p = 0.0467, time: F (1, 7) = 5.193, p = 0.0567, dose × time: F (2, 14) = 3.980, p = 0.0428, with Sidak's MC (T-R vs F), p = 0.0036). In δOR KO mice subject to the same voluntary alcohol consumption paradigm, 10 mg·kg^{-1} 7-hydroxyspeciogynine significantly decreased ethanol consumption (RM two-way ANOVA, dose: F (4, 32) = 6.407, p = 0.0007, time: F (1, 8) = 16.46, p = 0.0036, dose × time: F (4, 32) = 1.851, p = 0.1435, with Sidak's MC (T-R vs F), p = 0.0269) but not the 3 mg·kg^{-1}dose of 7-hydroxyspeciogynine or the 30 mg·kg^{-1}dose of 7-hydroxypaynantheine (**Figure 5G**). Water consumption (**Figure 5H**) and ethanol preference (**Figure 5I**) were not

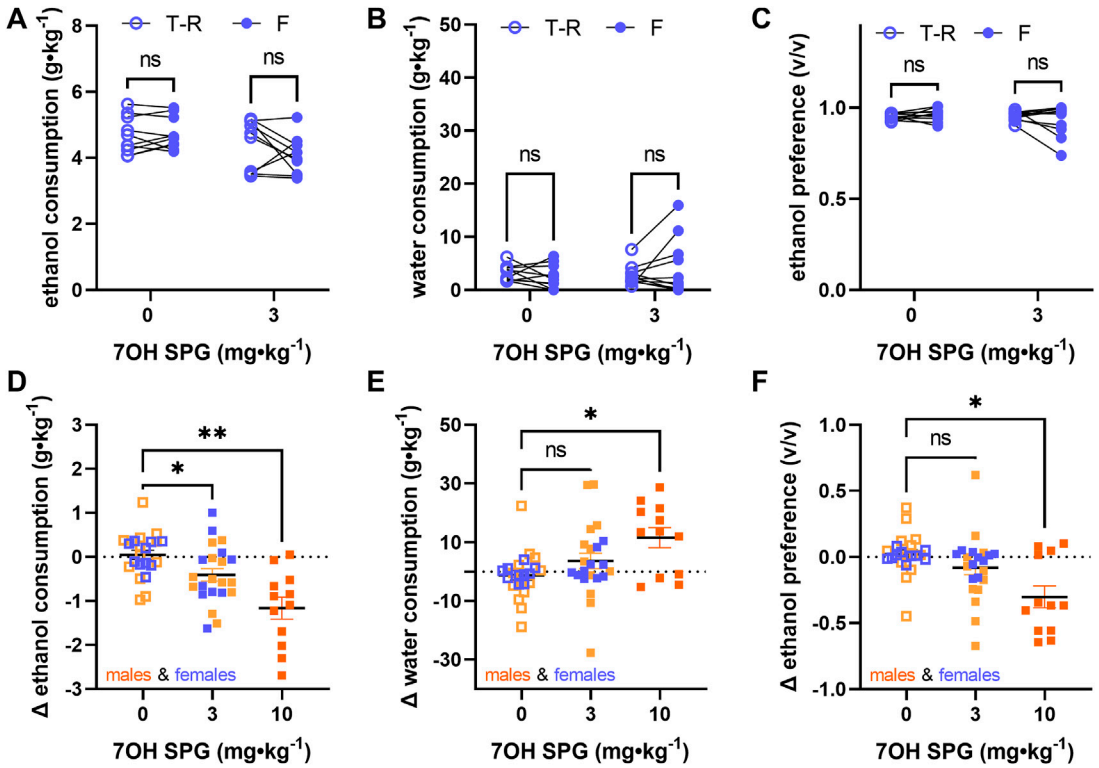

FIGURE 6 | Alcohol-modulating effects of 3 mg·kg^{-1} 7-hydroxyspeciogynine are not sex specific. In WT female mice (n = 10), effects of 3 mg·kg^{-1} 7-hydroxyspeciogynine (s.c., 7OG SPG) on 10% ethanol consumption **(A)**, water consumption **(B)**, and ethanol preference **(C)** were evaluated in a voluntary two-bottle choice, limited access, drinking-in-the-dark paradigm. Male and female responses to 7-hydroxyspeciogynine (3 and 10 mg·kg^{-1}, s.c.) in the two-bottle choice paradigm were pooled and are shown as **(D)** change (Δ) in 10% ethanol consumption, **(E)** change (Δ) in water consumption, and **(F)** change (Δ) in ethanol preference. In panels **(A–C)**, open circles are the average intake/preference on the preceding 3 days (baseline), and closed circles are the intake on Fridays following drug exposure. In panel **(D–F)**, female and male mice are depicted with blue and orange symbols, respectively. *p < 0.05, **p < 0.01 (for details, see **Supplemental Tables S6–S9**).

significantly changed in the δOR KO mice following treatment with the kratom analogs.

In female mice exposed to the voluntary alcohol consumption paradigm, 7-hydroxyspeciogynine did not significantly modulate ethanol consumption, water consumption, or ethanol preference at the 3 mg·kg^{-1} dose (**Figures 6A–C**; see **Supplemental Tables S6–S8** for statistical analyses). As previously reported (Rhodes et al., 2005), female mice exhibit a significantly higher baseline of alcohol consumption compared to males (**Supplemental Table S9**, RM two-way ANOVA, sex: F (1, 20) = 39.05, p < 0.0001, time: F (1, 20) = 6.295, p = 0.0208, dose × time: F (1, 20) = 0.1027, p = 0.7520, with Sidak's MC (male vs female), p < 0.0001 for the vehicle treatment baseline, p < 0.0001 for the 3 mg·kg^{-1} 7-hydroxyspeciogynine treatment baseline). However, no sex difference was apparent in the Δ ethanol intake (**Supplemental Table S9**, RM two-way ANOVA, sex: F (1, 20) = 0.1974, p = 0.6616, dose: F (1, 20) = 7.758, p = 0.0114, sex × dose: F (1, 20) = 0.2487, p = 0.6234, with Sidak's MC (male vs female), p = 0.9993 for the Δ ethanol consumption following vehicle treatment, p = 0.7635 for the Δ ethanol consumption following 3 mg·kg^{-1} 7-hydroxyspeciogynine treatment). Combining the Δ ethanol intake for males and females, we found that there was a significant ethanol modulation effect at the 3 mg·kg^{-1} dose when collectively analyzing male and female responses (**Figure 6D**, Mixed effects model (REML) with Geisser–Greenhouse correction, main effect of treatment: F (1.539, 40.80) = 13.36, p = 0.0001, with Dunnett's MC (treatment vs vehicle), p = 0.0165 for the 3 mg·kg^{-1} dose, p = 0.0064 for the 10 mg·kg^{-1} dose). After finding similar sex differences in water consumption and ethanol preference but not in the Δ of these parameters (see, **Supplemental Table S9** for details), pooled male and female responses were similarly analyzed for Δ in response of water consumption and ethanol preference. In the pooled data, a concomitant increase in water consumption was evident at a 10 mg·kg^{-1} dose (**Figure 6E**, Mixed effects model (REML) with Geisser–Greenhouse correction, main effect of treatment: F (1.733, 27.74) = 5.978, p = 0.0091, with Dunnett's MC (treatment vs vehicle), p = 0.1804 for the 3 mg·kg^{-1} dose, p = 0.0342 for the 10 mg·kg^{-1} dose). Accordingly, in the pooled data, a significant decrease in ethanol preference was noted at the 10 mg·kg^{-1} dose (**Figure 6F**, Mixed effects model (REML) with Geisser–Greenhouse correction, main effect of treatment: F (1.645, 43.58) = 7.889, p = 0.0022, with Dunnett's MC (treatment vs vehicle), p = 0.1644 for the 3 mg·kg^{-1} dose, p = 0.0255 for the 10 mg·kg^{-1} dose).

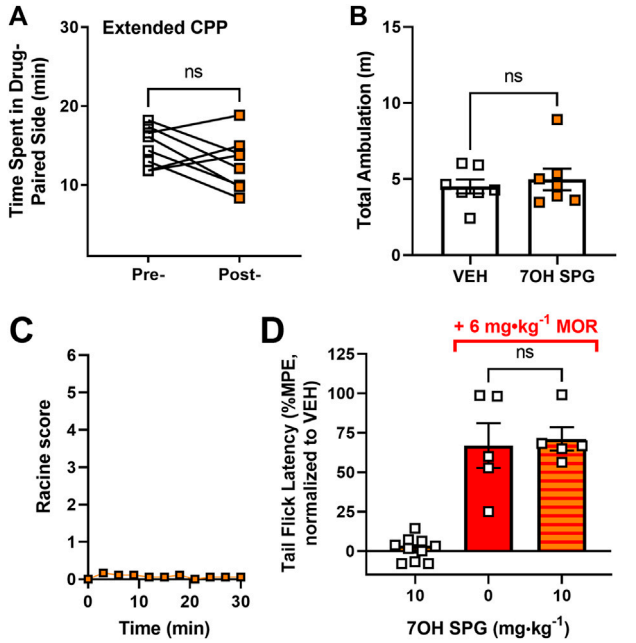

FIGURE 7 | Side effect profile of 10 mg·kg^{-1} 7-hydroxyspeciogynine. **(A)** In a 10-day conditioned place preference (CPP) paradigm, the rewarding effects of 7-hydroxyspeciogynine (s.c.) were evaluated in male, WT mice (n = 8). **(B)** Locomotor data were extracted from the CPP experiment in **(A)** and averaged across all vehicle/ drug treatment days (n = 7). **(C)** The highest Racine score collected every 3 min for 30 min following administration of 7-hydroxyspeciogynine was evaluated for 30 min after drug administration (n = 9). **(D)** 7-hydroxyspeciogynine was tested for agonist and analgesic properties in male mice *via* the tail flick thermal nociception assay (n = 10). In the same paradigm, antagonistic effects were evaluated after administering 7-hydroxypeciogynine, followed by morphine (6 mg·kg^{-1}, s.c.) 10 min later (n = 6), and were compared to vehicle plus morphine administration (n = 5) (for statistical details, see **Supplemental Tables S2–S5**).

7-Hydroxyspeciogynine Has Limited Side Effects Due to Its Decreased μOR-Dependent Pharmacology

From the cellular and behavioral experiments, 7-hydroxyspeciogynine emerged as the most promising kratom-derived analog for reducing alcohol use, with relatively equal *in vivo* potency as 7-hydroxymitragynine at the δOR but lower μOR potency. Next, we assessed whether 7-hydroxyspeciogynine exhibited a better side effect profile than 7-hydroxymitragynine due to its limited potency at the μOR. Additionally, to determine if 10 mg·kg^{-1} 7-hydroxyspeciogynine was the maximum tolerated dose (MTD), we assessed the side effect profile for the 10 mg·kg^{-1} dose. We found that mice treated with 10 mg·kg^{-1} 7-hydroxyspeciogynine did not develop conditioned place preference in our "extended" conditioned place preference protocol, which involves four conditioning sessions each for drug and vehicle (paired, two-tailed t test, t = 1.592, df = 7, p = 0.1554) (**Figure 7A**). The same 10 mg·kg^{-1} dose of 7-hydroxyspeciogynine did not significantly alter ambulation (paired, two-tailed t test, t = 0.7552, df = 6, p = 0.4787) (**Figure 7B**) or induce seizures (**Figure 7C**). Akin to 10 mg·kg^{-1} paynantheine, 10 mg·kg^{-1} 7-hydroxyspeciogynine did not produce antinociception (paired, two-tailed t test, t = 0.6193, df = 9, p = 0.5511) or block morphine analgesia (unpaired t test with Welch's correction, t = 0.2660, df = 5.994, p = 0.7991) (**Figure 7D**).

DISCUSSION

Over the past decade, kratom has been reported as a source for naturally occurring, G-protein–biased opioidergic alkaloids, and has been investigated for its effects on pain management (Matsumoto et al., 2004; Kruegel et al., 2019; Chakraborty et al., 2021b; Chakraborty and Majumdar, 2021), opioid withdrawal (Wilson et al., 2020, 2021), and alcohol abuse (Gutridge et al., 2020) as well as its decreased reward profile relative to traditional opioids (Hemby et al., 2019; Wilson et al., 2021). Here, we further probed the effects of kratom alkaloids and synthetic kratom alkaloid derivatives to obtain a better understanding of its *in vivo* pharmacology and in search of novel treatment options for alcohol use disorder. We report 7-hydroxyspeciogynine as an effective lead compound to reduce alcohol with an MTD of at least 10 mg·kg^{-1}.

We previously demonstrated that 7-hydroxymitragynine as well as paynantheine could decrease alcohol consumption (Gutridge et al., 2020). However, we were unable to obtain a MTD for 7-hydroxymitragyinine as it caused both hyperlocomotion and CPP at a 3 mg·kg^{-1} dose, which was the minimal effective dose to reduce alcohol intake (Gutridge et al., 2020). It has been well-established that μOR agonism can cause CPP, and that these rewarding effects can be blocked by μOR antagonists (Negus et al., 1993; Piepponen et al., 1997) as well as μOR KO (Matthes et al., 1996). Here, we show that 7-hydroxymitragynine–induced hyperlocomotion also appears to be μOR-mediated as it is completely blocked by a dose of naloxone considered to be μOR-selective (Takemori and Portoghese, 1984; Pastor et al., 2005). Since the alcohol-reducing effect of 7-hydroxymitragynine was dependent on δORs (Gutridge et al., 2020), μOR potency may be a liability when exploring kratom alkaloids as treatment option for AUD. Paynantheine has much lower μOR potency, while retaining δOR potency and decreases alcohol intake in mice at a 10 mg·kg^{-1} dose without causing hyperlocomotion (Gutridge et al., 2020). In line with the lower μOR potency, we find that 10 mg·kg^{-1} paynantheine does not produce place preference in an extended CPP paradigm. In a brief CPP paradigm, however, the same dose of paynantheine induces conditioned place aversion (CPA). Kratom use can lead to seizures (Coonan and Tatum, 2021) and we noticed that at 30 mg·kg^{-1}, paynantheine induced minor seizure activity. It is possible that mice administered a dose of 10 mg·kg^{-1} paynantheine did not feel well despite not showing overt tonic-clonic seizure activity that could contribute to the observed CPA at this dose. δOR agonism can cause seizures (Hong et al., 1998; Broom et al., 2002; Jutkiewicz et al., 2006); however, it is reported mostly for δOR agonists that are strong recruiters of β-arrestin, such as SNC80

and BW373U86 (O'Neill et al., 1997; Hong et al., 1998; Jutkiewicz et al., 2005). As such, we were not surprised that the G-protein–biased paynantheine-induced seizures were still present in δOR KO mice, indicating the seizures may be caused by an off-target interaction. Paynantheine can decrease alcohol consumption in wild-type mice (Gutridge et al., 2020); however, it also decreases alcohol consumption in δOR KO mice (**Supplementary Figure S5**; RM two-way ANOVA, dose: $F(4, 32) = 6.407$, $p = 0.0007$, time: $F(1, 8) = 16.46$, $p = 0.0036$, dose × time: $F(4, 32) = 1.851$, $p = 0.1435$, with Sidak's MC (T-R vs F), $p < 0.0001$). This analysis provides further evidence that many of paynantheine's *in vivo* effects are not mediated by δOR.

While antinociception has been reported for 7-hydroxymitragynine, the weaker μOR affinity alkaloid mitragynine reportedly lacks antinociceptive ability, and has been suggested to act as a μOR antagonist (Obeng et al., 2021); although in the cAMP assay, we previously identified mitragynine as a partial agonist (Gutridge et al., 2020), which is in line with a couple of other reports (Kruegel et al., 2016; Váradi et al., 2016). Paynantheine has weaker potency for the μOR than mitragynine in the cAMP assay but is more efficacious (Gutridge et al., 2020), which begged the question whether paynantheine possessed antinociceptive activity. However, both the 10 and 30 $mg{\cdot}kg^{-1}$ doses of paynantheine failed to produce meaningful antinociception in the tail flick paradigm. In contrast, paynantheine blocks morphine analgesia at a 30 $mg{\cdot}kg^{-1}$ dose but not at 10 $mg{\cdot}kg^{-1}$, yet neither dose blocks morphine CPP. Additionally, paynantheine both at 10 and 30 $mg{\cdot}kg^{-1}$ doses can block morphine hyper-ambulation. Paynatheine, at a 10 $mg{\cdot}kg^{-1}$ dose, only blocks morphine hyper-ambulation within the first 15–20 min of the 40-min conditioning session. Detailed pharmacokinetic data for paynantheine have yet to be reported, but a recent study has shown that following oral administration in rats, a 1.1 $mg{\cdot}kg^{-1}$ dose of paynantheine had a T_{max} of 10 min in plasma and was undetectable after an hour (Kamble et al., 2021). We suspect that in our hands paynantheine is similarly being rapidly metabolized and/or cleared from the brain and plasma, such that it may not block morphine's CPP long enough to inhibit it significantly. This may also explain why the 10 $mg{\cdot}kg^{-1}$ dose does not block morphine analgesia, which was tested at 20–30 min after administration. Furthermore, a day-by-day analysis of the locomotor activity revealed that the 30 $mg{\cdot}kg^{-1}$ dose of paynantheine does not fully block morphine hyper ambulation within the last 5 min of the day 2 conditioning session (**Supplementary Figures S1C,D**). Because even one exposure to morphine is known to cause place preference in mice (Bardo and Neisewander, 1986), it is possible that mice administered with 30 $mg{\cdot}kg^{-1}$ paynantheine experienced enough rewarding effects from morphine on day 2 to express CPP. However, since we did not measure CPP for 30 $mg{\cdot}kg^{-1}$ paynantheine, we cannot rule out that paynantheine is responsible or positively contributed to the observed CPP. Taking together previous findings and the data collected here, we conclude that paynantheine is a weak partial agonist at the μOR and δOR, with functional antagonistic activity at the μOR in the presence of a more potent agonist *in vivo*. Overall, our conditioned place preference findings indicate that paynantheine has a low risk of reward, but its use may be limited by its low potency *in vivo*, and seizure effects that are not δOR-mediated.

We next decided to utilize the G-protein–biased nature of the kratom alkaloid scaffold to discover opioids that have increased δOR potency but that exhibits relatively low μOR potency. 7-hydroxymitragynine and mitragynine pseudoindoxyl, two previously characterized analogs of mitragynine, had higher δOR as well as μOR affinity and activity in cell lines compared to the indole-based template of mitragynine, and showed unique binding poses in computational models (Váradi et al., 2016; Zhou et al., 2021). To extend the structure–activity relationship (SAR) to the paynantheine and related speciogynine templates, we synthesized the hydroxylated and spiropseudoindoxyl variants of these natural products. We identified 7-hydroxyspeciogynine and 7-hydroxypaynantheine as having reduced μOR potency but similar δOR potency relative to 7-hydroxymitragynine. In contrast to the mitragynine-derived spiropseudoindoxyls, no advantage with respect to potency at the ORs was seen with the pseudoindoxyls derived from paynantheine or speciogynine. Both the novel 7-hydroxyl analogs dose-dependently decreased alcohol consumption, with 7-hydroxyspeciogynine displaying efficacious activity at a dose of 3 $mg{\cdot}kg^{-1}$ and 7-hydroxypaynantheine at a 30 $mg{\cdot}kg^{-1}$ dose. We confirmed that the alcohol-modulating effects of these analogs are at least partially acting through a δOR-mediated mechanism as we did not observe statistically significant reductions alcohol consumption in δOR KO mice for the two analogs at their effective doses. Because 7-hydroxyspeciogynine decreases ethanol consumption in δOR KO at a 10 $mg{\cdot}kg^{-1}$dose but not 3 $mg{\cdot}kg^{-1}$, this suggests that 7-hydroxyspeciogynine's ethanol modulation is no longer solely mediated by δOR at higher doses.

Additionally, the *in vivo* potency of these compounds correlates well with their *in vitro* pharmacology at the δOR where 7-hydroxyspeciogynine is about 0.5–1 log-fold more potent than 7-hydroxypaynantheine (**Table 1**). While 7-hydroxyspeciogynine displays more potent activity at the μOR relative to 7-hydroxypaynantheine in the GloSensor assay (pIC_{50}s of 6.2 ± 0.3 and 4.7 ± 0.5, respectively), the activity at this receptor is still less potent than 7-hydroxymitragynine ($pIC_{50} = 7.8 \pm 0.1$). The G-protein–biased μOR activity of 7-hydroxyspeciogynine likely does not contribute to decreased alcohol use because of the lack of effect in δOR KO mice at the 3 $mg{\cdot}kg^{-1}$ dose and because we have previously shown that selective activation of μOR G-protein signaling using Oliceridine/TRV130 did not decrease alcohol consumption (Gutridge et al., 2020).

Kratom-based natural products, including paynantheine and speciociliatine examined here, have been predicted and shown to have activity at adrenergic 2A, 2B, and 2C receptors and serotonin 2A receptors (Boyer et al., 2008; Ellis et al., 2020; Foss et al., 2020; Obeng et al., 2020; León et al., 2021). Since we did not screen the kratom analogs for activity at these or other receptors, it is probable that non-δOR activity contributes to the observed alcohol intake modulation, especially at higher doses. Though there is support for targeting adrenergic and serotonin receptors for treatment of alcohol abuse (Haass-Koffler et al., 2018; DiVito and Leger, 2020; Berquist and

Fantegrossi, 2021; Sessa et al., 2021), our data about δOR KO animals shown here and in Gutridge et al., 2020 builds on our hypothesis of an ancillary, if not primary, role of δOR in decreasing alcohol consumption for kratom opioids and derivatives.

Relative to the GTPyS assay, the GloSensor assay of cAMP inhibition uses recombinant overexpressed cell systems and is amplified relative to measuring G-protein activity directly. As such, it is plausible that the partial agonism we detect for the kratom analogs *in vitro* does not resemble how they act *in vivo*. For example, at the δOR, mitragynine has partial agonism in the cAMP assay but acts as an antagonist in the GTPγS assay (Váradi et al., 2016; Gutridge et al., 2020). Therefore, it may be suggested that the kratom analogs are acting as functional δOR antagonists *in vivo*, competing with the fully efficacious activation of δORs by the endogenous Leu-enkephalin. However, our speciociliatine data counters this argument. At the δOR, speciociliatine binds with a pKi of 5.4 ± 0.1 which is in between the binding affinities of 7-hydroxyspeciogynine and 7-hydroxypaynantheine (6.3 ± 0.1 and 4.9 ± 0.2, respectively), yet speciociliatine acts as a δOR antagonist in the cAMP assay. When tested in mice, speciociliatine did cause a significant and sharp decrease in alcohol consumption at a relatively high 30 mg·kg^{-1} dose (**Supplementary Figure S4A–C**, an average decrease of 2.5 ± 0.3 g·kg^{-1} ethanol or a 90 ± 3% reduction, compared to a decrease of 1.2 ± 0.2 g·kg^{-1} ethanol (40 ± 7%) for 10 mg·kg^{-1} 7-hydroxyspeciogynine, and 1.1 ± 0.3 g·kg^{-1} ethanol (40 ± 11%) for 30 mg·kg^{-1} 7-hydroxypaynantheine), which indicates an off-target effect. In support of this explanation, a 30 mg·kg^{-1} dose of speciociliatine similarly decreases ethanol consumption in δOR KO mice and significantly impairs motor incoordination in wild-type and δOR KO mice, which likely contributes to the effects we see in the alcohol consumption paradigm. We did not test the kratom analogs or alkaloids in conjunction with δOR antagonists because the role of δOR antagonists in these behaviors is not well defined. For example, we have previously found that δOR-selective antagonist naltrindole does not decrease alcohol intake at a 10 mg·kg^{-1} dose in this alcohol model, whereas another δOR-selective antagonist, naltriben, dose-dependently decreases alcohol consumption at 6 and 10 mg·kg^{-1} doses (van Rijn and Whistler, 2009). Although in rats, both naltrindole and naltriben decrease alcohol intake (Krishnan-Sarin et al., 1995a; 1995b). These discrepant responses may be explained by mediation of distinct δOR subtypes by these specific antagonists (Dietis et al., 2011; van Rijn et al., 2013). Therefore, evaluating alcohol consumption responses in δOR KO mice provide a more straightforward and unambiguous approach for broadly determining δOR-mediated responses for the purposes of the experiments completed here.

At the μOR, it has recently been demonstrated that a reduction in G-protein efficacy is responsible for lessened adverse side effect profiles, rather than a lack of β-arrestin recruitment (Gillis et al., 2020). In the GloSensor cAMP assay, 7-hydroxyspeciogynine and 7-hydroxypaynantheine act as partial agonists at δOR and *in vivo* they reduce alcohol use. This begs the question whether partial agonism rather than full agonism is driving the δOR mediated effects on alcohol intake. The δOR agonist TAN-67 efficaciously reduces alcohol use in the two-bottle choice paradigm, and is a full agonist in the cAMP assay (Chiang et al., 2016) and the [^{35}S] GTPγS assay (Quock et al., 1997). However, a more recent [^{35}S] GTPγS study has suggested TAN-67 may be a partial agonist (Stanczyk et al., 2019), and thus the answer for now is not clear as to whether partial agonism and/or weak β-arrestin recruitment drives reduced alcohol use by δOR agonists.

Given that agonist-bound structures of both the μOR and δOR are available (Huang et al., 2015; Claff et al., 2019), it may be possible to identify strategies by which to enhance 7-hydroxyspeciogynine affinity selectively at δOR and not μOR. Additionally, *in vivo* characterization of 7-hydroxyspeciogynine for pharmacokinetic parameters including half-life and metabolism (e.g. role of CYP3A4 and CYP2D6) will be insightful. Further behavioral analysis, including modulation of respiratory depression and anxiety-like behavior (van Rijn et al., 2010; Ko et al., 2021) would establish 7-hydroxyspeciogynine's potential as clinical lead compound. Similarly, assessing off-target effects in a panel screen could identify other targets, including serotonin receptors (León et al., 2021) that contribute to 7-hydroxyspeciogynine's modulation of alcohol intake.

In summary, our current and past pharmacological characterization of kratom analogs suggest that alkaloids with sub-micromolar δOR potency, micromolar potency at the μOR, and G-protein bias provide the strongest opportunity to reduce alcohol use in mice with limited side effects. We discovered 7-hydroxyspeciogynine as a novel kratom-derived analog that decreases alcohol intake by activating δORs *in vitro* and *in vivo* but with limited μOR *in vivo* agonist activity, leading to a broadened therapeutic window as evident from a lack of rewarding, locomotive, and seizurogenic effects and a MTD of at least 10 mg·kg^{-1}. Our findings support the utility of targeting the δOR to reduce volitional alcohol consumption, and further demonstrate the effectiveness of using the kratom alkaloids as lead scaffolds for developing G-protein–biased δOR agonists for treatment of AUD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the Purdue Animal Care and Use Committee, Purdue University.

AUTHOR CONTRIBUTIONS

AG generated hypotheses, performed research, analyzed data, and prepared and edited the manuscript. ER, AF, AB, QR, HC, JY, and RC performed research and analyzed data. SC and BV synthesized ligands and edited the manuscript. MS helped

with analysis of NMR data. SM designed synthetic kratom-derived analogs, generated hypotheses, provided funding, provided supervision, analyzed data, and edited the manuscript. RvR generated hypotheses, provided funding, provided supervision, analyzed data, and prepared and edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.764885/full#supplementary-material

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frontiers in Pharmacology

REVIEW
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Kratom Alkaloids: Interactions With Enzymes, Receptors, and Cellular Barriers

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Parallel to the growing use of kratom, there is a wealth of evidence from self-report, preclinical, and early clinical studies on therapeutic benefits of its alkaloids in particular for treating pain, managing substance use disorder, and coping with emotional or mental health conditions. On the other hand, there are also reports on potential health risks concerning kratom use. These two aspects are often discussed in reviews on kratom. Here, we aim to highlight specific areas that are of importance to give insights into the mechanistic of kratom alkaloids pharmacological actions. This includes their interactions with drug-metabolizing enzymes and predictions of clinical drug-drug interactions, receptor-binding properties, interactions with cellular barriers in regards to barrier permeability, involvement of membrane transporters, and alteration of barrier function when exposed to the alkaloids.

Keywords: receptor-binding, mitragynine, *Mitragyna speciosa*, metabolism, kratom, alkaloids, drug-drug interactions, barrier permeability

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1 INTRODUCTION

Kratom (*Mitragyna speciosa* Korth.) use in the traditional settings in Southeast Asian countries particularly Malaysia and Thailand to treat minor ailments and to increase work endurance among manual laborers is not new. Reports on the use of kratom as a substitute for opium in Malaya have been published as early as in the 1930s (Burkill and Haniff, 1930; Burkill, 1935). Now, kratom use has spread to the West particularly in the United States of which kratom products are widely marketed online (Williams and Nikitin, 2020). Reasons for kratom use in the States include to self-treat acute and chronic pain, to reduce or abstain from using non-prescription opioids and/or heroin, and to a lesser extent as a substitute for the drugs, and to cope with emotional or mental health conditions such as anxiety, depression and post-traumatic stress disorder (Grundmann, 2017; Smith and Lawson 2017; Smith et al., 2021). The increasing use of kratom which is no longer limited to Southeast Asian countries has sparked many interests within the scientific community to investigate the therapeutic potential of the plant and possible health risks. A breadth of evidence is available on pharmacological actions of kratom preparations and alkaloids, primarily central actions of mitragynine and 7-hydroxymitragynine. Apart from the two most studied alkaloids, there is a growing number of other alkaloids being reported and to date, approximately 45 alkaloids were identified in kratom (Ramanathan et al., 2021). Chemical structures of kratom alkaloids which are discussed in the later sections of this review are shown in **Figure 1**. Findings from preclinical studies, for example, antinociceptive activity to some extent corroborated with data from self-report studies of which among the reasons for kratom use is to manage pain, further supported by the recent randomized controlled study in humans (Vicknasingam et al., 2020). This also seems to be the case for use of kratom to alleviate opioid withdrawal (Hassan et al., 2020) and to relieve anxiety (Hazim

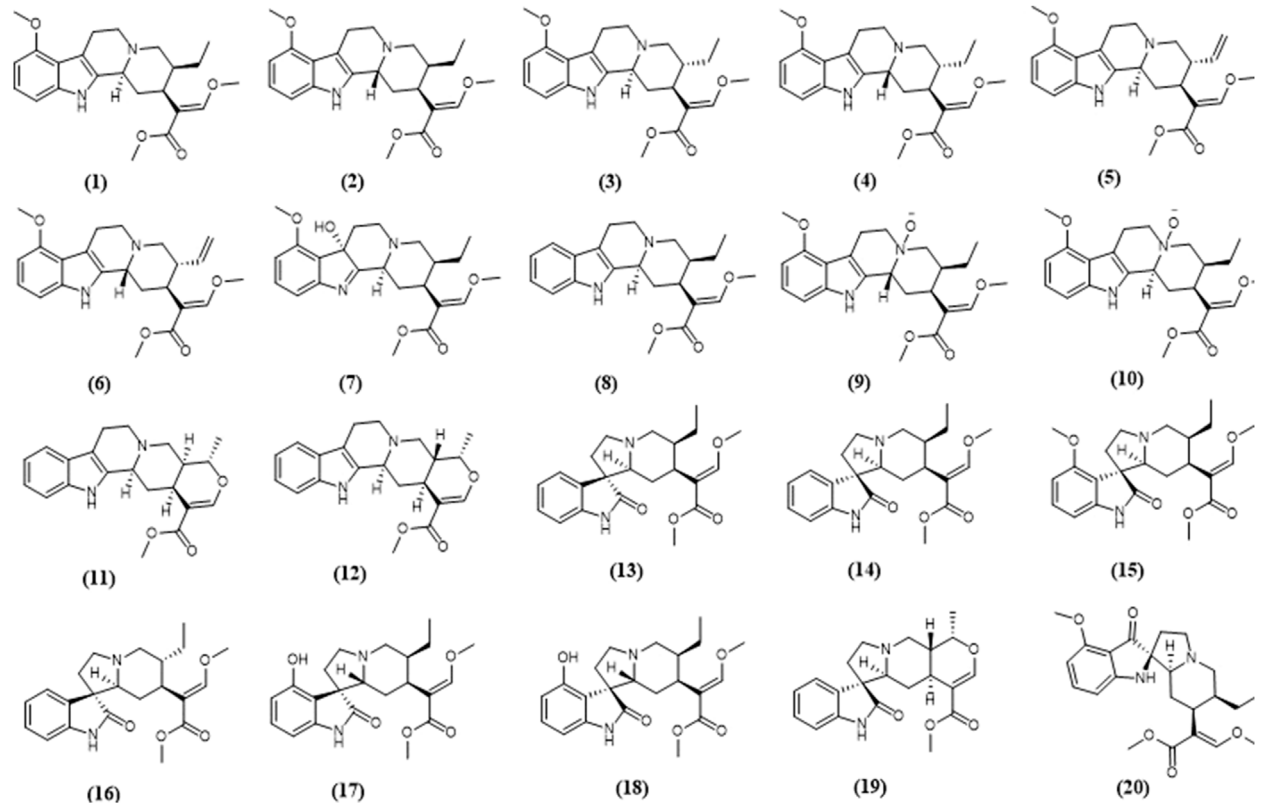

FIGURE 1 | Chemical structures of selected kratom alkaloids—(**1**) mitragynine, (**2**) speciociliatine, (**3**) speciogynine, (**4**) mitraciliatine, (**5**) paynantheine, (**6**) isopaynantheine, (**7**) 7-hydroxymitragynine, (**8**) corynantheidine, (**9**) speciociliatine n-oxide, (**10**) mitragynine n-oxide, (**11**) tetrahydroalstonine, (**12**) ajmalicine, (**13**) corynoxine, (**14**) corynoxine B, (**15**) mitragynine oxindole B, (**16**) isorynchophylline, (**17**) isospeciofoline, (**18**) speciofoline, (**19**) mitraphylline, (**20**) mitragynine pseudoindoxyl.

et al., 2014). Further investigations at the cellular and molecular level aid to gain an understanding of the mechanistic of kratom alkaloids actions.

Here, we highlight physiological interactions of kratom alkaloids focusing on interactions with enzymes, receptors, and cellular barriers. These are emerging areas of research concerning kratom alkaloids of which are of significant importance in determining potential development as therapeutics.

2 INTERACTIONS WITH ENZYMES

2.1 Metabolism of Kratom Alkaloids

Metabolism facilitates the elimination of drugs from animals and humans through the conversion of the drugs to more water-soluble metabolites. There are two phases of drug metabolism i.e., phase I and phase II. Phase I metabolism includes hydrolysis, oxidation, and reduction reactions which are mainly catalyzed by various drug-metabolizing enzymes (DMEs). Phase II consists of a conjugation reaction involving glucuronidation and sulfation (Shapiro and Shear, 2001).

To date, limited data are available concerning the metabolic pathways of kratom alkaloids and the involvement of various DMEs in the clearance of the alkaloids. Data from analyses of samples collected from rats and humans revealed that kratom alkaloids including mitragynine, speciogynine, paynantheine, speciociliatine, mitraciliatine, and isopaynantheine were extensively metabolized to multiple phase I and phase II metabolites. Phase I metabolism of the alkaloids involved hydrolysis of the methyl ester of the acrylic acid group at C-16, *O*-demethylation of the methoxy group at C-9 and C-17 positions, followed by oxidation to carboxylic acid or reduction to alcohol (Philipp et al., 2009; Philipp et al., 2010a; Philipp et al., 2010b; Philipp et al., 2011a; Philipp et al., 2011b). Following the phase I metabolism, some metabolites underwent phase II metabolism to produce glucuronide and sulfate conjugates (Phillip et al., 2009). The phase I and phase II metabolites of the alkaloids are tabulated in **Table 1**. In parallel to the list of metabolites, the proposed metabolic pathways for the alkaloids are illustrated in **Figures 2**–**7**.

Kamble et al. (2019) characterized the metabolic profile of mitragynine against various cytochrome P450 (CYP)-containing systems including human liver microsomes

TABLE 1 | Phase I and II metabolites of kratom alkaloids in rat and human urine samples.

Alkaloid	Phase I metabolites		Phase II metabolites	
	Rat urine	Human urine	Rat urine	Human urine
MG[a]	1) 9-*O*-demethyl MG 2) 16-carboxy MG 3) 9-*O*-demethyl-16-carboxy MG 4) 17-*O*-demethyl-16,17-dihydro MG 5) 9,17-*O*-bisdemethyl-16,17-dihydro MG 6) 17-carboxy-16,17-dihydro MG 7) 9-*O*-demethyl-17-carboxy-16,17-dihydro MG	1) 9-*O*-demethyl MG 2) 16-carboxy MG 3) 17-*O*-demethyl-16,17-dihydro MG 4) 17-carboxy-16,17-dihydro MG	Glucuronides of: 1) 9-*O*-demethyl MG 2) 16-carboxy MG 3) 9-*O*-demethyl-16-carboxy MG 4) 9,17-*O*-bisdemethyl-16,17-dihydro MG Sulfate of: 1) 9-*O*-demethyl-16-carboxy MG	Glucuronides of: 1) 9-*O*-demethyl MG 2) 16-carboxy MG 3) 17-*O*-demethyl-16,17-dihydro MG Sulfates of: 1) 9-*O*-demethyl MG 2) 9-*O*-demethyl-16-carboxy MG 3) 9,17-*O*-bisdemethyl-16,17-dihydro MG
PAY[b]	1) 9-*O*-demethyl PAY 2) 16-carboxy PAY 3) 9-*O*-demethyl-16-carboxy PAY 4) 17-*O*-demethyl-16,17-dihydro PAY 5) 9,17-*O*-bisdemethyl-16,17-dihydro PAY 6) 17-carboxy-16,17-dihydro PAY 7) 9-*O*-demethyl-17-carboxy-16,17-dihydro PAY 8) 17-*O*-demethyl PAY 9) 9,17-*O*-bisdemethyl PAY	1) 9-*O*-demethyl PAY 2) 16-carboxy PAY 3) 17-carboxy-16,17-dihydro PAY	Glucuronides of: 1) 9-*O*-demethyl PAY 2) 16-carboxy PAY 3) 9-*O*-demethyl-16-carboxy PAY 4) 17-*O*-demethyl-16,17-dihydro PAY 5) 9,17-*O*-bisdemethyl-16,17-dihydro PAY 6) 17-*O*-demethyl PAY 7) 9,17-*O*-bisdemethyl PAY Sulfate of: 1) 9,17-*O*-bisdemethyl-16,17-dihydro PAY	Glucuronides of: 1) 9-*O*-demethyl PAY 2) 16-carboxy PAY Sulfate of: 1) 9-*O*-demethyl PAY
SG[c]	1) 9-*O*-demethyl SG 2) 16-carboxy SG 3) 9-*O*-demethyl-16-carboxy SG 4) 17-*O*-demethyl-16,17-dihydro SG 5) 9,17-*O*-bisdemethyl-16,17-dihydro SG 6) 17-carboxy-16,17-dihydro SG 7) 9-*O*-demethyl-17-carboxy-16,17-dihydro SG 8) 17-*O*-demethyl SG 9) 9,17-*O*-bisdemethyl SG	1) 9-*O*-demethyl SG 2) 16-carboxy SG 3) 17-carboxy-16,17-dihydro SG	Glucuronides of: 1) 9-*O*-demethyl SG 2) 16-carboxy SG 3) 9-*O*-demethyl-16-carboxy SG 4) 17-*O*-demethyl-16,17-dihydro SG 5) 9,17-*O*-bisdemethyl-16,17-dihydro SG 6) 17-*O*-demethyl SG 7) 9,17-*O*-bisdemethyl SG Sulfate of: 1) 9,17-*O*-bisdemethyl-16,17-dihydro SG	Glucuronides of: 1) 9-*O*-demethyl SG 2) 16-carboxy SG Sulfate of 1) 9-*O*-demethyl SG
SC[d]	1) 9-*O*-demethyl SC 2) 16-carboxy SC 3) 9-*O*-demethyl-16-carboxy SC 4) 17-*O*-demethyl-16,17-dihydro SC 5) 9,17-*O*-bisdemethyl-16,17-dihydro SC 6) 17-carboxy-16,17-dihydro SC 7) 9-*O*-demethyl-17-carboxy-16,17-dihydro SC 8) 17-*O*-demethyl SC 9) 9,17-*O*-bisdemethyl SC	1) 9-*O*-demethyl SC 2) 16-carboxy SC 3) 9-*O*-demethyl-16-carboxy SC	Glucuronides of: 1) 9-*O*-demethyl SC 2) 16-carboxy SC 3) 9-*O*-demethyl-16-carboxy SC 4) 17-*O*-demethyl-16,17-dihydro SC 5) 9,17-*O*-bisdemethyl-16,17-dihydro SC 6) 9,17-*O*-bisdemethyl SC	Glucuronides of: 1) 9-*O*-demethyl SC 2) 16-carboxy SC 3) 17-*O*-demethyl-16,17-dihydro SC
MC[e]	1) 9-*O*-demethyl MC 2) 16-carboxy MC 3) 9-*O*-demethyl-16-carboxy MC 4) 17-*O*-demethyl-16,17-dihydro MC 5) 9,17-*O*-bisdemethyl-16,17-dihydro MC 6) 17-carboxy-16,17- dihydro MC 7) 9-*O*-demethyl-17-carboxy-16,17-dihydro MC 8) 17-*O*-demethyl MC 9) 9,17-*O*-bisdemethyl MC	1) 9-*O*-demethyl MC	Glucuronides of 1) 9-*O*-demethyl MC 2) 16-carboxy MC 3) 9-*O*-demethyl-16-carboxy MC 4) 17-*O*-demethyl-16,17-dihydro MC 5) 9,17-*O*-bisdemethyl-16,17-dihydro MC 6) 17-*O*-demethyl MC 7) 9,17-*O*-bisdemethyl MC	Glucuronide of: 1) 9-*O*-demethyl MC

(Continued on following page)

TABLE 1 | (*Continued*) Phase I and II metabolites of kratom alkaloids in rat and human urine samples.

Alkaloid	Phase I metabolites		Phase II metabolites	
	Rat urine	Human urine	Rat urine	Human urine
ISO-PAY[e]	1) 9-*O*-demethyl ISO-PAY 2) 16-carboxy ISO-PAY 3) 9-*O*-demethyl-16-carboxy ISO-PAY 4) 17-*O*-demethyl-16,17-dihydro ISO-PAY 5) 9,17-*O*-bisdemethyl-16,17-dihydro ISO-PAY 6) 17-carboxy-16,17-dihydro ISO-PAY 7) 9-*O*-demethyl-17-carboxy-16,17-dihydro ISO-PAY 8) 17-*O*-demethyl ISO-PAY 9) 9,17-*O*-bisdemethyl ISO-PAY	1) 9-*O*-demethyl ISO-PAY 2) 17-carboxy-16,17-dihydro ISO-PAY	Glucuronides of: 1) 9-*O*-demethyl ISO-PAY 2) 16-carboxy ISO-PAY 3) 17-*O*-demethyl-16,17-dihydro ISO-PAY 4) 17-*O*-demethyl ISO-PAY	

MG, mitragynine; PAY, paynantheine; SG, speciogynine; SC, speciociliatine; MC, mitraciliatine; ISO-PAY, isopaynantheine.
[a]*Phillip et al.(2009);*
[b]*Phillip et al. (2010a);*
[c]*Phillip et al. (2010b);*
[d]*Phillip et al. (2011a);*
[e]*Phillip et al. (2011b).*

(HLM), human liver S9 (HLS9), and recombinant CYP enzymes. In the HLM system, four oxidative species including 7-hydroxymitragynine and one *O*-demethylated metabolite i.e., 9-*O*-demethylmitragynine were detected as the prevalent metabolites of mitragynine, in accord with Basiliere and Kerrigan (2020a). None of the mitragynine phase I metabolites was discovered to be conjugated with glutathione. The metabolite profiling of mitragynine was comparable in HLM and HLS9, where both systems demonstrated a minor metabolic pathway. On the other hand, CYP3A4 was discovered as the major CYP isoform responsible for the metabolism of mitragynine with small or negligible contributions from CYP2C9, 2C19, and 2D6. The data on metabolic pathways of mitragynine via recombinant CYP enzymes were further evaluated against a series of multiple isoforms of 1A2, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, and 3A4 (Basiliere and Kerrigan, 2020b). Only 2C18, 2C19, 2D6, and 3A4 isoforms displayed metabolic activities among the tested enzymes. The results indicate that 9-*O*-demethylmitragynine was the most abundant metabolite produced by CYP2C19, 2D6, and 3A4, while 9-*O*-demethyl-16-carboxymitragynine was the least prevalent metabolite hydrolyzed by 2C19. 16-Carboxymitragynine was produced by CYP2C19 and 2D6 while 7-hydroxymitragynine was only produced by CYP3A4. Although data from Kamble et al. (2019) showed negligible metabolic activity expressed by CYP2C19 and 2D6, the data reported by Basiliere and Kerrigan (2020b) indicate that both isoforms were capable of metabolizing mitragynine to several important metabolites.

2.2 Potential Drug-Drug Interactions

This section deals with interactions of mitragynine and related alkaloids in modulating enzymes especially for enzymes that pose clinical importance. As the DMEs are the primary route of drug clearance in the human body (Di, 2014), modulation of the expression or function of DMEs through inhibition or induction by one or more chemicals that affect the metabolism of clinical drugs may lead to toxic effects or lack of clinical efficacy (Food and Drug Administration Center for Drug Evaluation and Research, 2020). The serious impact of DMEs modulation by other chemicals leads to the universal use of the term drug-drug interaction (DDI) to specifically refer to this type of interaction. DDI is often the primary obstacle in drug discovery and development and causes many clinically approved drugs to be withdrawn from the market (Wienkers and Heath, 2005). DDI can be recognized earlier in the preclinical phase by good experimental designs and by following guidelines provided by regulating agencies such as the U.S. Food and Drug Administration (FDA) (Food and Drug Administration Center for Drug Evaluation and Research, 2020) and European Medicines Agency (European Medicines Agency, 2012). Interactions of mitragynine with enzymes other than DMEs have also been reported. However, studies on these enzymes were very limited, with just acetylcholinesterase (Innok et al., 2021) and cyclooxygenase (Utar et al., 2011) being evaluated. Findings from these studies were less clinically relevant as the experiments were performed using enzymes from non-human sources i.e., electric eel for acetylcholinesterase, and rodent macrophage cell lines for cyclooxygenase, or the inhibition data showed mitragynine concentration that is hardly attained in human plasma, for example, IC_{50} of 264 μM for acetylcholinesterase.

It is obvious from the preceding section that mitragynine and other related alkaloids are substrates for multiple CYP isoforms and hence may interfere with metabolisms of clinical drugs. Preclinical research on mitragynine and related alkaloids on DDI is limited but has been gaining attention within the last 10 years (Hanapi et al., 2013; Lim et al., 2013; Kamble et al., 2020; Todd et al., 2020; Tanna et al., 2021). Here, the focus is on the effect of mitragynine and related alkaloids on DMEs from *in vitro* preclinical research, and their utility to predict clinical DDI. Only studies on human DMEs that make clinical prediction possible were included in this review (**Table 2**).

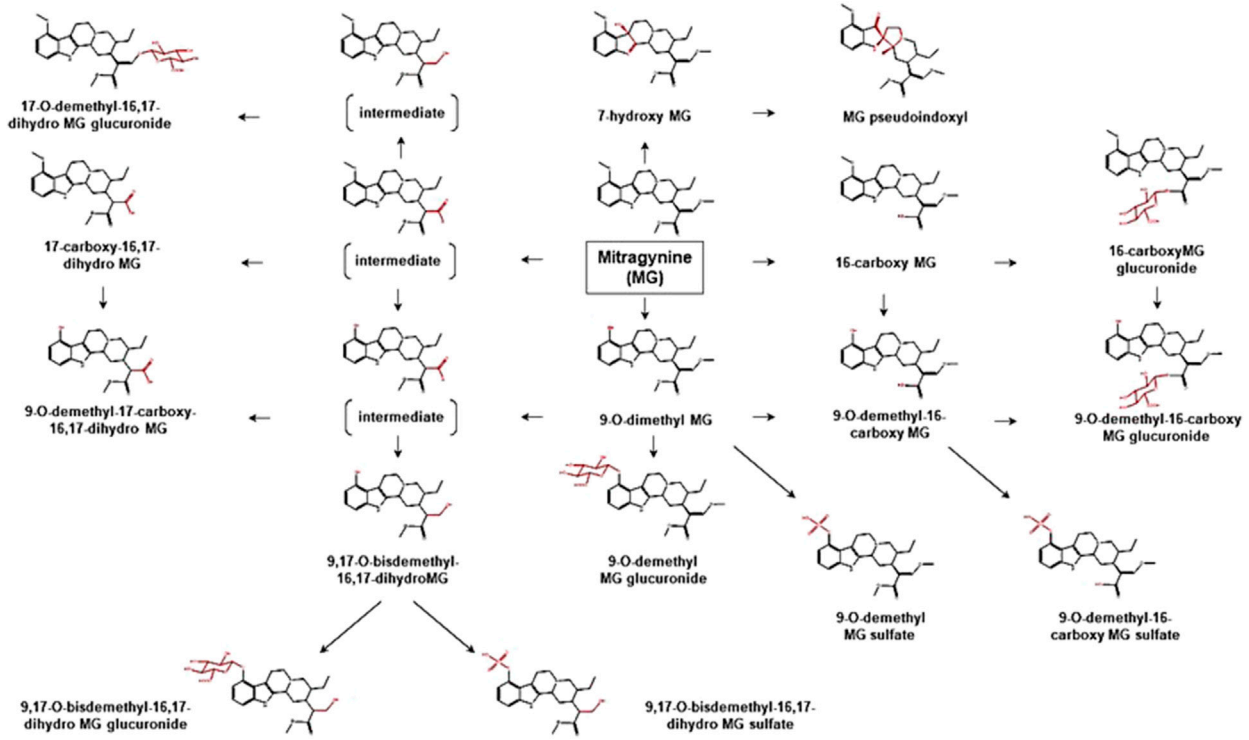

FIGURE 2 | Proposed metabolic pathways of MG based on data obtained in rodents and humans. Structures highlighted in red denote structural transformation from parent molecule MG. Figure was modified from Philipp et al. (2009) and Kamble et al. (2020).

The CYPs 1A2, 2C9, 2C19, 2D6, 2E1, and 3A4 isoforms are the major phase I DMEs responsible for the metabolism of over 90% of commercially marketed drugs (Lynch and Price, 2007; Bibi, 2008). The first study of mitragynine interactions with CYPs by Hanapi et al. (2013) set precedence to the subsequent studies that revealed more detailed information on the mechanisms and strength of DDI. The inhibitory constant parameter such as IC_{50} and K_i gathered from these studies were useful to support the prediction of potential clinical DDI through a static mechanistic model or physiologically based pharmacokinetic modeling (Obach et al., 2006; Templeton et al., 2016; Tod et al., 2016). For example, using the static mechanistic model, a ratio of the area under the plasma versus concentration-time curve (AUCR) for known CYP isoform substrate in the presence to absence of inhibitor could be estimated. An AUCR > 1.25 may suggest potential clinical DDI (Food and Drug Administration Center for Drug Evaluation and Research, 2020). The cut-off point of K_i < 12 μM to group mitragynine and other alkaloids as a potential clinical inhibitor of DMEs in this review was based on the guidelines provided by Tanna et al. (2021). Mitragynine K_i value from DMEs inhibition study <12 μM denote potential clinical relevance of CYP inhibition, determined relative to the highest mitragynine concentration quantified from autopsy blood samples of kratom-related death (Gershman et al., 2019; Tanna et al., 2021). For enzyme induction, a recent FDA guideline was used to classify CYP induction as potentially clinically significant (Food and Drug Administration Center for Drug Evaluation and Research, 2020). According to the guideline, a drug is interpreted as an inducer if the fold change of CYP mRNA expression relative to the vehicle control is ≥2-fold at the expected hepatic concentrations of the drug and/or if the increase is >20% of the response of the positive control *in vitro* cell-based assay.

Mitragynine and other alkaloids inhibited the *O*-deethylation reaction of CYP1A2 substrate phenacetin with a K_i value greater than the concentration that could be obtained in human plasma (Kamble et al., 2020). However, a study with human liver cancer cell line HepG2 showed the imminent potential of CYP1A2 induction by mitragynine (Lim et al., 2013). The mRNA expression for CYP1A2 when exposed to 10 μM mitragynine exceeded 2-fold relative to negative control and the increase was approximately 28% of the response of the CYP1A2 known inducer omeprazole in the cell-based assay (Lim et al., 2013). For the CYP2C subfamily, mitragynine and related alkaloids did not appreciably inhibit CYP2C8 and CYP2C9 (Kamble et al., 2020). However, mitragynine and speciociliatine inhibited CYP2C19 catalyzed S-mephenytoin hydroxylation with IC_{50} values of 10.6 and 8 μM respectively (Kamble et al., 2020). Although the study by Kamble et al. (2020) did not measure K_i for mitragynine and speciociliatine, estimation through Cheng-Prusoff equation (Yung-Chi and Prusoff, 1973; Haupt et al., 2015) for

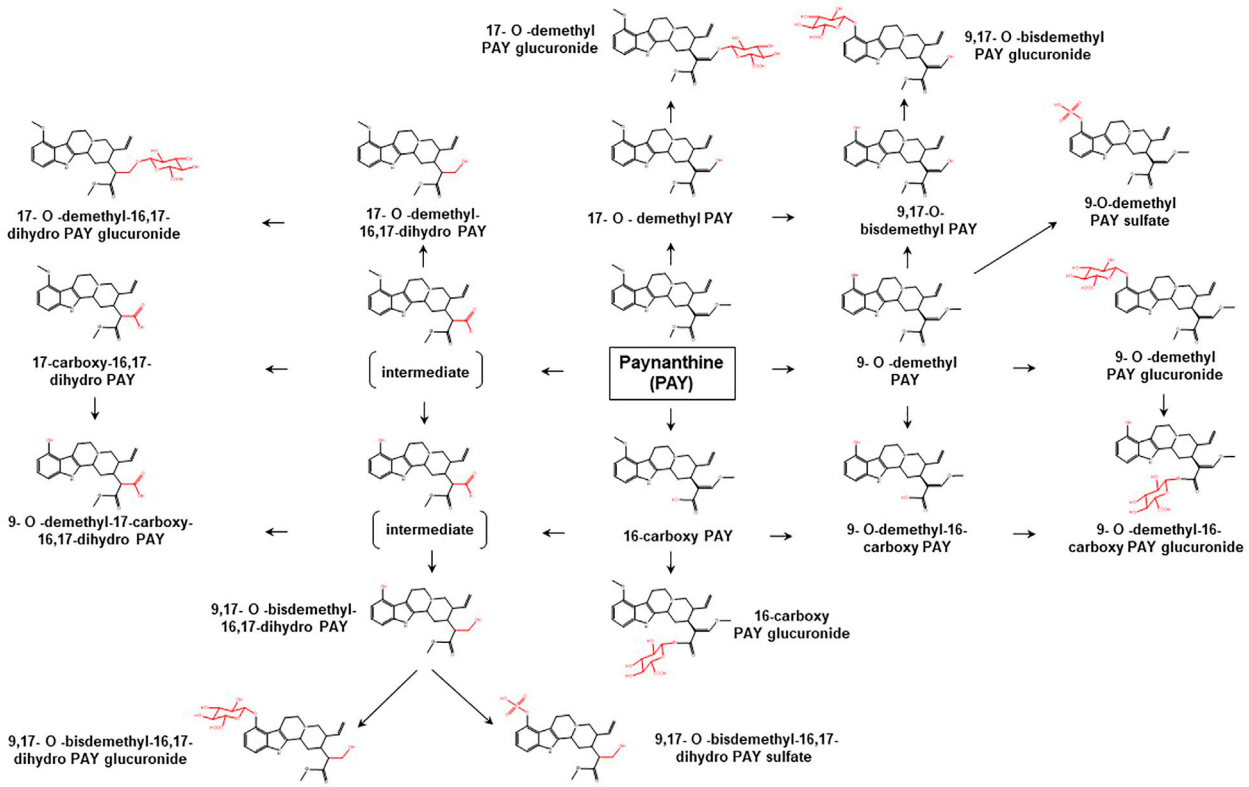

FIGURE 3 | Proposed metabolic pathways of PAY based on data obtained in rodents and humans. Structures highlighted in red denote structural transformation from parent molecule PAY. Figure was modified from Philipp et al. (2010a).

competitive inhibitor (K_i = IC_{50}/2) may suggest potential clinical DDI between mitragynine (K_i ~ 5 μM) and speciociliatine (K_i ~ 4 μM) for drugs mainly metabolized by CYP2C19. Speciociliatine was the third [0.29% (w/w)] major alkaloid after mitragynine [3.8% (w/w)] in the Malaysian strain of kratom juice preparation and may significantly reach the K_i concentration in chronic kratom users assuming the intestinal absorption similar to mitragynine (Singh et al., 2020a).

Mitragynine has been repeatedly shown in different *in vitro* studies to potently inhibit CYP2D6 with K_i values ranging from 1.1 to 13 μM (Hanapi et al., 2013; Kamble et al., 2020; Todd et al., 2020; Tanna et al., 2021). Using the static mechanistic model, Tanna et al. (2021) revealed that kratom preparation sold in the U.S. market could cause significant DDI with drugs primarily metabolized by CYP2D6 if more than 9 g kratom extract containing 83 mg mitragynine (Todd et al., 2020) was taken with AUCR > 1.25. Based on this finding, the reported daily intake average i.e., 2.7 glasses of traditionally prepared kratom juice among chronic kratom users in Malaysia (Singh et al., 2020a) is sufficient to cause significant DDI with dextromethorphan (AUCR ~ 1.4). Mitragynine did not appear to have a significant effect on the mRNA expression of CYP2D6. Although there was a substantial protein induction based on a qualitative technique for protein expression, the fold-induction did not qualify mitragynine as a clinically relevant CYP2D6 inducer (Lim et al., 2013).

Mitragynine was initially thought not to effectively inhibit the CYP3A4 isoform in a bioluminescent experiment with an IC_{50} of 41.32 μM (Hanapi et al., 2013). Subsequent studies with HLM support the previous data but with a much lower IC_{50} of < 20 μM when FDA recommended CYP3A4 probe substrate midazolam was used (Kamble et al., 2020; Tanna et al., 2021). Mitragynine also appears to inhibit CYP3A4 catalyzed midazolam hydroxylation in human intestinal microsomes (HIM) with IC_{50} = 21.9 μM (Tanna et al., 2021). Interestingly, mitragynine IC_{50} for CYP3A4 reduced substantially to 2.6 μM (HLM) and 3.2 μM (HIM) in a time-dependent inhibition experimental design (Tanna et al., 2021). The time-dependent inhibition observed from the study highlighted a mechanism-based inhibition that was irreversible and more potent for CYP3A4. In this type of inhibition, a product of mitragynine metabolism is covalently bound to the CYP3A4 active site instead of being released, which rendered the enzyme unavailable for other reactions (Deodhar et al., 2020). This observation was frequently missed in classical IC_{50} assays as the study design limit sufficient formation of active metabolites to form and deactivate the CYP. The impact of this finding is huge as roughly 40% of clinical drugs are substrates for CYP3A4 metabolism (Schaffenburg et al., 2021). The static mechanistic model demonstrated that as little as 2 g kratom powder containing 21 mg mitragynine (Todd et al.,

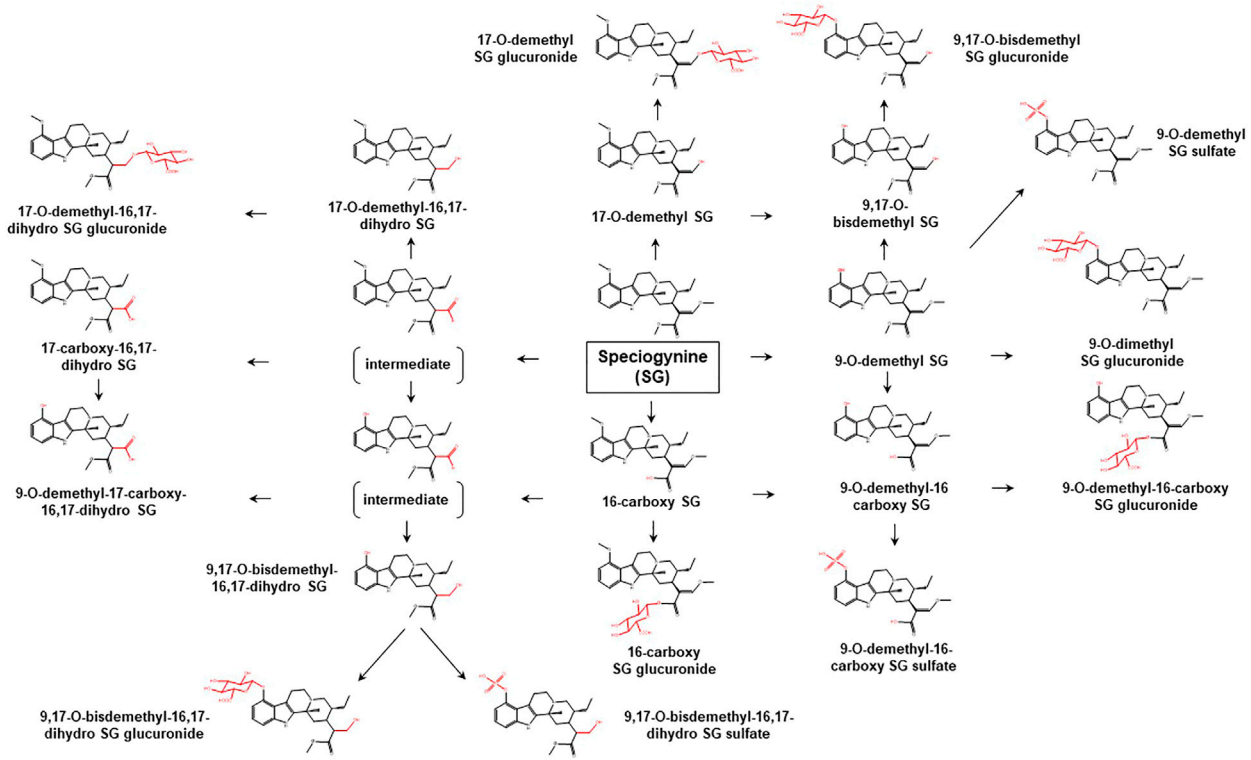

FIGURE 4 | Proposed metabolic pathways of SG based on data obtained in rodents and humans. Structures highlighted in red denote structural transformation from parent molecule SG. Figure was modified from Philipp et al. (2010b).

2020) will precipitate DDI with CYP3A4 substrate midazolam (AUCR = 5.7) (Tanna et al., 2021). Similarly, about half-glass (~150 ml) of traditionally prepared Malaysian kratom juice daily (Singh et al., 2020a) would be estimated to progressively increase the plasma level of midazolam by ~ 6 fold. The influence of mitragynine on midazolam clearance would be substantially greater among chronic kratom users in Malaysia with AUCR > 12. On the other hand, the effects of mitragynine on the CYP3A4 mRNA, protein, and enzymatic activity in HepG2 cells were all below the criteria to suggest a significant *in vitro* induction effect (Lim et al., 2013).

3 INTERACTIONS OF KRATOM ALKALOIDS WITH CENTRAL NERVOUS SYSTEM RECEPTORS

The effects of kratom alkaloids on central nervous system (CNS) receptors have been extensively studied *in vitro* and *in vivo* assays. *In vitro* radioligand binding studies revealed that kratom alkaloids interact with opioid μ, δ, κ subtypes, and non-opioid receptors including alpha-1A, alpha-2A, 5-HT1A, 5-HT2A, D1, and D2 (Takayama et al., 2002; Boyer et al., 2008; Kruegel et al., 2016; Ellis et al., 2020; Obeng et al., 2020; Chear et al., 2021; Obeng et al., 2021). *In vivo* studies demonstrated that kratom alkaloids exert central analgesic, anti-anxiety, anti-drug addiction, and antipsychotic effects primarily through activation of central opioidergic, adrenergic, serotoninergic, and dopaminergic neurotransmission systems (Matsumoto et al., 1996a; Matsumoto et al., 1996b; Matsumoto et al., 1997; Takayama et al., 2002; Hazim et al., 2014; Vijeepallam et al., 2016; Foss et al., 2020; Obeng et al., 2020; Chear et al., 2021; Obeng et al., 2021). To better understand the CNS pharmacological targets of kratom alkaloids, this section is structured as follows: opioid receptors and non-opioid receptors (adrenergic, serotonin, and dopamine receptors).

3.1 Opioid Receptors

Kratom extracts (alcoholic, water, and alkaloid-enriched extracts) and the main alkaloid i.e., mitragynine demonstrated significant central analgesic activity in rodents and humans, and were fully antagonized by the non-selective opioid antagonists such as naloxone or naltrexone in most cases (Matsumoto et al., 1996a; Shaik Mossadeq et al., 2009; Sabetghadam et al., 2010; Carpenter et al., 2016; Vicknasingam et al., 2020). These suggest that the central analgesic effects of kratom and mitragynine are primarily mediated by opioid receptors (Ramanathan et al., 2021). Takayama et al. (2002) were the first to report on the interaction of mitragynine and its related indole alkaloids, 7-hydroxymitragynine, corynantheidine, and speciociliatine with μ-opioid receptors derived from guinea pig (Takayama et al., 2002). The opioid agonistic activity of

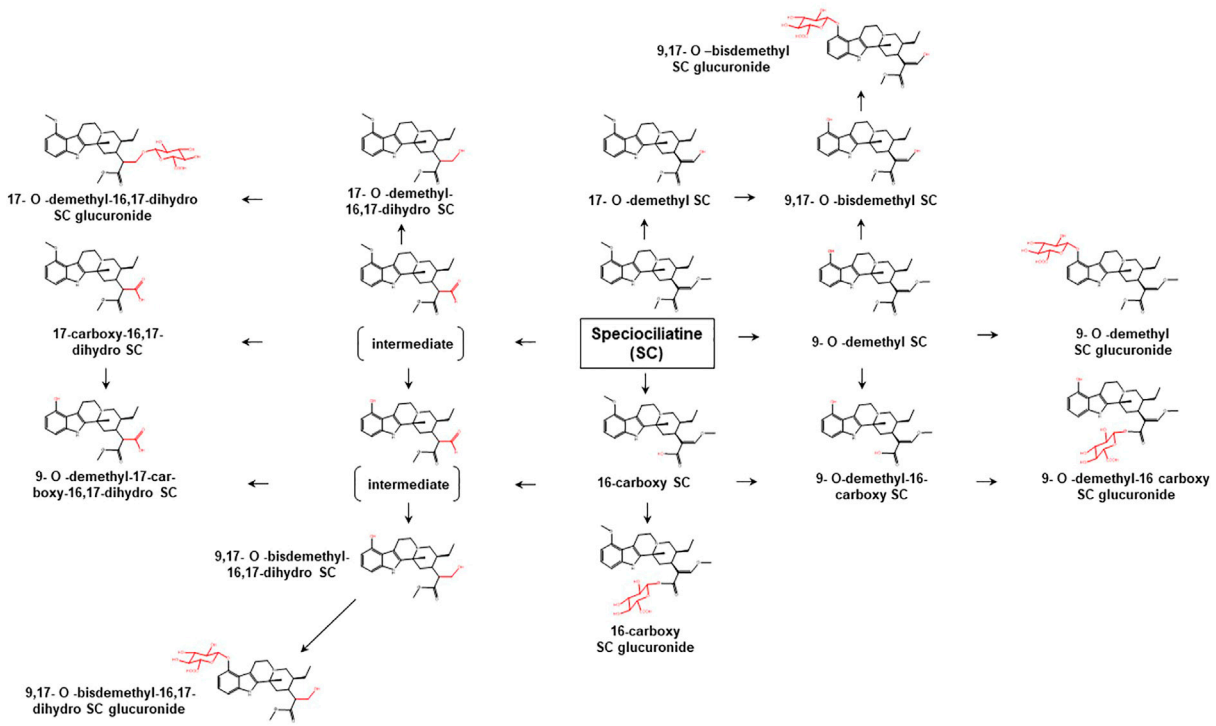

FIGURE 5 | Proposed metabolic pathways of SC based on data obtained in rodents and humans. Structures highlighted in red denote structural transformation from parent molecule SC. Figure was modified from Philipp et al. (2011a).

the alkaloids was evaluated *ex vivo* by measuring electrically-induced twitch contraction in guinea pig ileum. Receptor binding affinities of the alkaloids at guinea pig μ, κ, and δ opioid receptors were determined by radioligand displacement assay against [^{3}H]DAMGO, [^{3}H]DPDPE, and [^{3}H]U69593 respectively. In guinea pig ileum, mitragynine inhibited the electrically-induced twitch contraction with a pD2 value of 6.59, which was reversed by naloxone (300 nM). The pD2 or also known as pEC_{50} is the negative logarithm to base 10 of the EC_{50} of an agonist which indicates the potency but not the efficacy of the agonist. This suggests that mitragynine acted as an opioid agonist, but one that is weaker than morphine (pD2 = 7.17). Both the oxidized mitragynine i.e., 7-hydroxymitragynine and mitragynine pseudoindoxyl showed greater opioid agonistic activity than their precursor with pD2 values of 8.20 and 8.71 respectively. The two alkaloids were also more potent than morphine with relative potencies of 1,071 and 3,467%. Relative potency is defined as a percentage of the pD2 value of the tested compound against the reference drug, in this case, morphine. Mitragynine, 7-hydroxymitragynine, and mitragynine pseudoindoxyl showed selective binding affinities to the μ-opioid receptor in the radioligand binding assay against [^{3}H]DAMGO, indicating that the alkaloids were μ-opioid receptor agonists. Speciociliatine on the other hand was found to weakly inhibit the twitch contraction with a relative potency of 2% (pD2 = 5.40). Corynantheidine (9-demethylated mitragynine) did not show opioid agonistic activity in the guinea pig ileum model. However, corynantheidine was later discovered to inhibit morphine-induced twitch contraction in guinea pig ileum with selective binding affinity to the μ-opioid receptor. This finding suggests that corynantheidine is a functional and selective μ-opioid receptor antagonist. Based on the above findings, it could be postulated that 1) *S*-orientation at the C-3 position of mitragynine is important for opioid-agonistic activity; 2) oxidation at indole B-ring enhances the opioid-agonistic activity; 3) the loss of Nb lone pair at C-ring abolishes the opioid agonistic activity; 4) the loss of 9-methoxy group abolishes the opioid-agonistic activity.

For the past 5 years, the interactions of kratom alkaloids with human opioid receptors have been extensively studied using various *in vitro* and *in vivo* assays. Kruegel et al. investigated binding affinity and functionality of mitragynine, 7-hydroxymitragynine, speciociliatine, speciogynine and paynantheine at human μ (hMOR), δ (hDOR) and κ (hKOR) opioid receptors using radioligand displacement and bioluminescence resonance energy transfer (BRET) functional assays (Kruegel et al., 2016). In general, the indole-based kratom alkaloids showed greater binding affinities at hMOR and hKOR with K_i values in submicromolar and micromolar ranges compared to hDOR (K_i > 10 μM). Among the tested alkaloids, 7-hydroxymitragynine had the highest and selective affinity for hMOR with a K_i value of 47 nM, followed by mitragynine (K_i = 233 nM), paynantheine (K_i = 410 nM), speciociliatine (K_i = 560 nM), and speciogynine (K_i = 728 nM). In addition, 7-

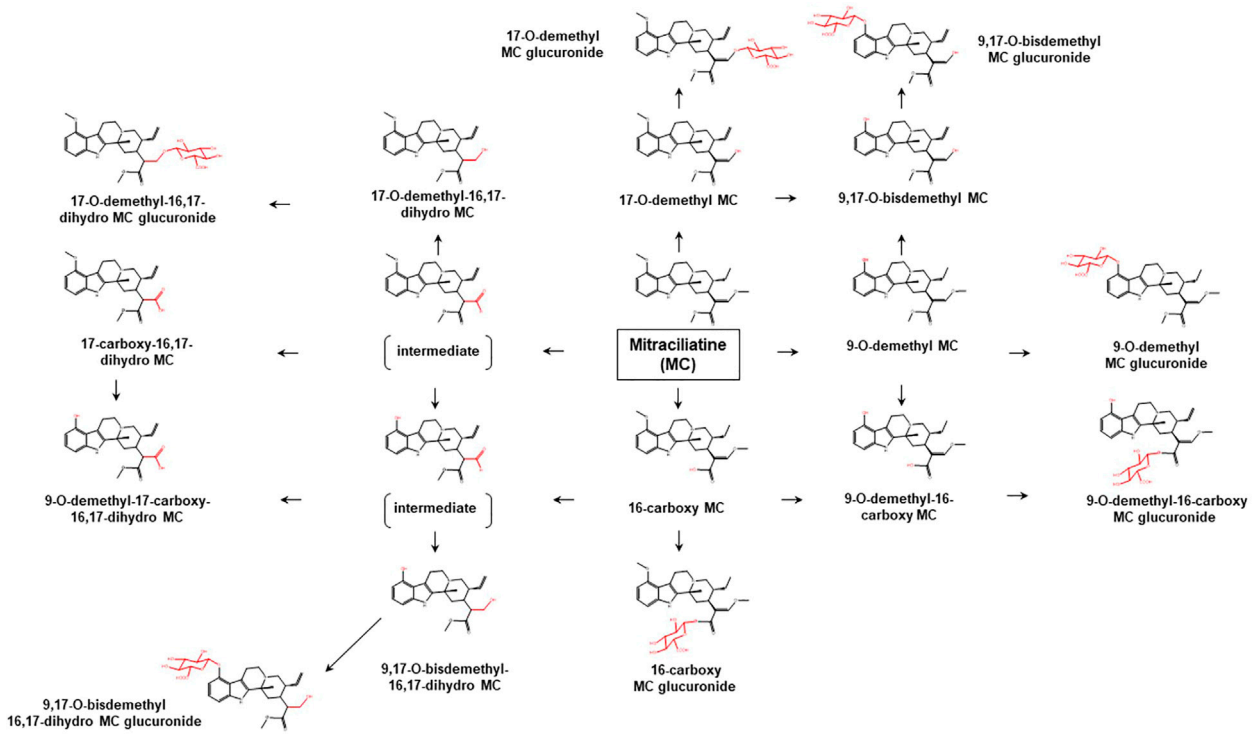

FIGURE 6 | Proposed metabolic pathways of MC based on data obtained in rodents and humans. Structures highlighted in red denote structural transformation from parent molecule MC. Figure was modified from Philipp et al. (2011b).

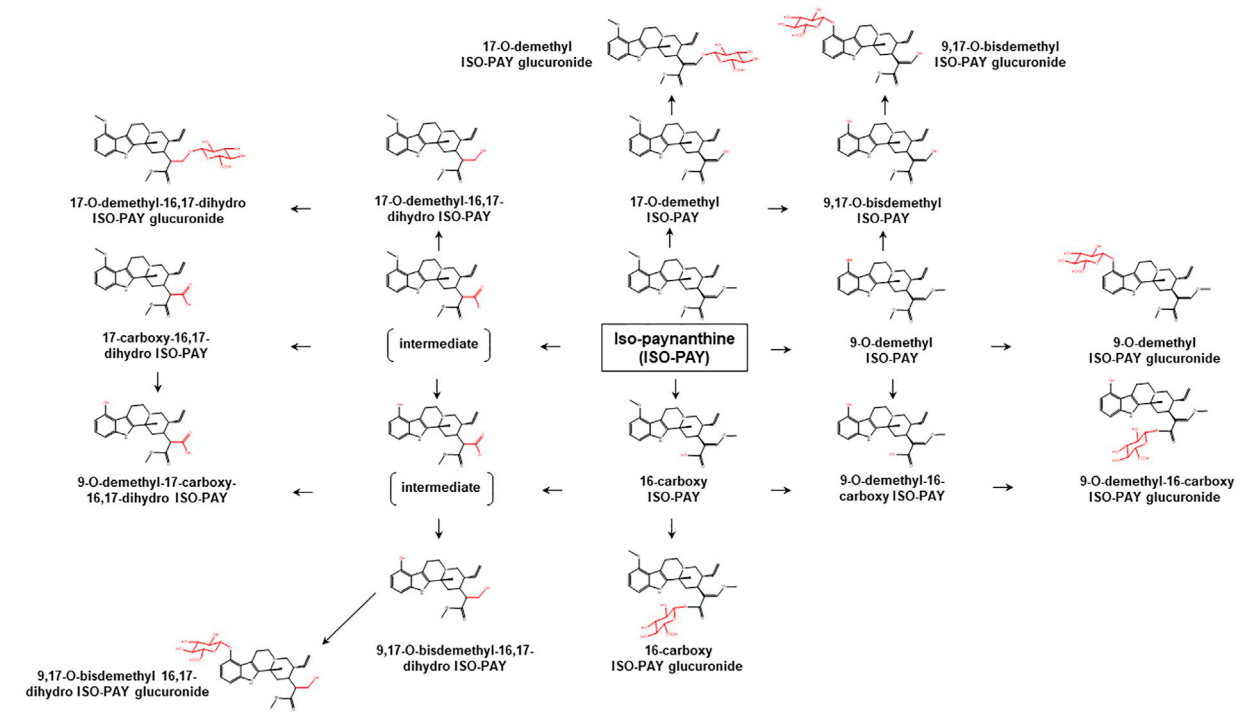

FIGURE 7 | Proposed metabolic pathways of ISO-PAY based on data obtained in rodents and humans. Structures highlighted in red denote structural transformation from parent molecule ISO-PAY. Figure was modified from Philipp et al. (2011b).

TABLE 2 | Extent of mitragynine and related alkaloids inhibition on major human drug metabolizing enzymes (DMEs).

References	Enzyme system	CYPs isoform	Alkaloids	Key findings	Prediction to clinical DDI is possible?	Following FDA/EMA guideline?
Tanna et al. (2021)	Human liver microsomes; human intestinal microsomes	2C19, 2D6, 3A	Mitragynine	Mitragynine is a competitive inhibitor for CYP2D6 (K_i = 1.17 μM) Mitragynine is a mechanism-based inhibitor for CYP3A4 (HLM: K_I = 4.1 μM, K_{inact} = 0.068 min^{-1}; HIM: K_I = 4.2 μM, K_{inact} = 0.079 min^{-1})	Yes	Yes
Todd et al. (2020)	Human liver microsomes	2C9, 2D6, 3A	Mitragynine, 7-hydroxymitragynine, and speciofoline	Mitragynine at 100 μM inhibit >80% for CYP2C9, CYP2D6, and CYP3A 7-Hydroxymitragynine at 100 μM inhibit >80% for CYP2D6 Speciofoline at 100 μM inhibit >80% for CYP2C9 and CYP3A	No	No
Kamble et al. (2020)	Human liver microsomes	1A2, 2C8, 2C9, 2C19, 2D6, 3A4/5	Mitragynine, speciogynine, speciociliatine, corynantheidine, 7-hydroxymitragynine, and paynantheine	Mitragynine and corynantheidiene is a competitive inhibitor for CYP2D6 activity with K_i values of 1.1 and 2.8 μM respectively	Yes	Yes
Hanapi et al. (2013)	Baculovirus hCYP450 Expression system (baculosomes); human liver cancer cell line (HepG2)	1A2, 2D6, 3A4	Mitragynine	Mitragynine is a non-competitive inhibitor for CYP2C9 (K_i = 61.48 μM) and CYP2D6 (K_i = 12.86 μM) Mitragynine is a competitive inhibitor for CYP3A4 (K_i = 379.18 μM)	Yes	No
Lim et al. (2013)	Baculovirus hCYP450 Expression system (baculosomes)	2C9, 2D6, 3A4	Mitragynine	Mitragynine inhibit CYP3A4 with IC_{50} value 3.98 μM (testosterone) and 17.3 μM (midazolam) Mitragynine induce CYP1A2 mRNA and protein expression as well as enzyme activity	Yes	No

HLM, human liver microsomes; HIM, human intestinal microsomes; K_i reversible inhibition constant; K_I, time-dependent inhibition constant; K_{inact} maximum rate of inactivation.

hydroxymitragynine was also bound to hKOR and hDOR with K_i values of 188 and 219 nM respectively. In the BRET assay, mitragynine and 7-hydroxymitragynine showed potent agonistic activity at hMOR with EC_{50} values of 339 and 34.5 nM respectively. The two alkaloids acted as partial agonists at hMOR with maximal efficacy, E_{max} of 34 and 47% respectively, when compared to the full agonist DAMGO in antagonist experiments. In contrast, at hKOR, mitragynine and 7-hydroxymitragynine acted as competitive antagonists with IC_{50} values of 8.5 and 7.9 μM, and pA2 values of 1.4 and 0.49 μM respectively, when compared to the reference agonist U-50488. The pA2 reflects the affinity of an antagonist to a receptor. The value of pA2 is a negative logarithm of the molar concentration of the competitive antagonist, implying that the agonist concentration must be doubled to compensate for the antagonist's action. Paynantheine, speciogynine, and speciociliatine showed weak competitive antagonist activities at both hMOR and hKOR with EC_{50} or IC_{50} values in micromolar ranges. Interestingly, all tested kratom alkaloids were also reported as competitive antagonists at mouse MOR, indicating the possibility of intra-species variation between the *in vitro* functional assays. Later in 2020, Obeng et al. also reported the opioid-like activity of selected indole-based kratom alkaloids i.e., 7-hydroxymitragynine, mitragynine, speciociliatine, and corynantheidine using radioligand displacement and homogenous time-resolved fluorescence (HTRF) assays (Obeng et al., 2020). In the study, 7-hydroxymitragynine was predominantly bound to hMOR (Ki = 7.16 nM), followed by hKOR (K_i = 74.1 nM) and hDOR (K_i = 236 nM). The binding strength of kratom alkaloids at hMOR was reported as follows: 7-hydroxymitragynine (K_i = 7.16 nM) > speciociliatine (K_i = 54.5 nM) > corynantheidine (K_i = 118 nM) > mitragynine (K_i = 161 nM). Similarly, 7-hydroxymitragynine also exhibited the highest binding affinity to hKOR with a K_i value of 74.1 nM, followed by speciociliatine (K_i = 116 nM), mitragynine (K_i = 198 nM), and corynantheidine (K_i = 1910 nM). In the HTRF assay, 7-hydroxymitragynine acted as a full agonist at hMOR (EC_{50} = 7.6 nM), and competitive antagonist at both hKOR and hDOR. Both mitragynine and speciociliatine were partial agonists at hMOR with EC_{50} values of 307.5 and 39.2 nM respectively. The *in vivo* opioid agonistic activities of 7-hydroxymitragynine, speciociliatine, and mitragynine were then evaluated using the hot-plate test in rats. Speciociliatine produced antinociceptive response with an ED_{50} value of 6.25 mg/kg, which was

comparable to morphine (ED_{50} = 5.10 mg/kg) but weaker than 7-hydroxymitragynine (ED_{50} = 1.91 mg/kg). Similar to the *in vitro* hMOR binding and functional studies, mitragynine also exhibited the weakest antinociceptive effect (E_{max} 17.3%) among the tested alkaloids at the highest dose tested (10 mg/kg, i.v.). The antinociceptive action of speciociliatine, 7-hydroxymitragynine, mitragynine, and morphine were fully antagonized by naltrexone (0.1 mg/kg, i.v.). In the study, speciociliatine demonstrated opioid agonistic activity at hMOR, which is in contrast with findings reported by Kruegel et al. (2016) where the compound showed weak antagonistic activity at hMOR *in vitro*. The finding also contrasted with Takayama et al. (2002), who found that speciociliatine had negligible opioid agonistic activity in the guinea pig ileum model. The discrepancies could be due to the different types of assays used to assess the functional effect of speciociliatine. Nonetheless, based on both *in vitro* and *in vivo* functional assays, it is possible to hypothesize that the *R* orientation at the C-3 position of speciociliatine enhances its interaction with hMOR, resulting in improved antinociceptive activity compared to mitragynine.

Although indole-based kratom alkaloids have received a lot of attention, little is known about the binding affinity and functional activity of minor oxindole alkaloids. A recent study by Chear et al. (2021) showed that the oxindole alkaloids i.e., corynoxine, corynoxine B, mitragynine oxindole B, and isospeciofoline were predominantly bound to hMOR (K_i < 2 μM) compared to hKOR and hDOR (K_i > 10 μM). At hMOR, corynoxine exhibited the highest binding affinity with a K_i value of 16.4 nM, which is approximately 5 times greater than its C-7 stereoisomer, corynoxine B (K_i = 109.8 nM). On the other hand, mitragynine oxindole B and isospeciofoline were moderately bound to hMOR indicating the substitution at the C-9 position of corynoxine/corynoxine B reduces the affinity to hMOR (K_i > 1,000 nM). The *in vivo* functional effect of corynoxine was then evaluated using the hot-plate test in rats. The results showed that corynoxine dose-dependently increased antinociception with an ED_{50} value of 6.72 mg/kg which is more potent than morphine (ED_{50} = 12.1 mg/kg). The antinociception of both corynoxine and morphine was also reversed by naltrexone (0.1 mg/kg, i.v.), suggesting that the compounds act as μ-opioid receptor agonists. Interestingly, corynantheidine, an indole precursor of corynoxine/corynoxine B, was discovered to be a functional μ-opioid receptor antagonist (Takayama et al., 2002). The oxidative rearrangement of the indole B-ring caused the shift in μ-opioid antagonistic to agonistic activity. Overall, the above findings suggest that indole and oxindole-based kratom alkaloids could be useful leads for developing new analgesics with fewer side effects that are not derived from morphinan analgesics.

3.2 Adrenergic Receptors

In addition to opioid receptors, the adrenergic neurotransmitter system is another major pharmacological target of kratom in treating pain and opioid withdrawal symptoms. Mitragynine was the first kratom alkaloid proven to exert antinociceptive action in rodents via activation of the central adrenergic system. In the hot-plate test, pretreatment with idazoxan (10 μg) was able to reverse the antinociceptive action of mitragynine (10 μg, i.c.v.) in mice (Matsumoto et al., 1996b). Yohimbine (alpha-2 adrenoreceptor antagonist) and prazosin (alpha-1 adrenoreceptor antagonist) also totally and partially suppressed mitragynine antinociceptive activity in a chemotherapy-induced neuropathic pain rat model respectively (Foss et al., 2020). However, information on the specific binding of mitragynine or other kratom alkaloids to various subtypes of alpha-1 and alpha-2 adrenergic receptors is still lacking. As a result, the potential radioligand binding affinities of mitragynine and other indole-based kratom alkaloids i.e. speciogynine, 7-hydroxymitragynine, speciociliatine, corynantheidine, ajmalicine, and tetrahydroalstonine at alpha-1A, 1B, and 1D, and alpha-2A, 2B, and 2C adrenergic receptors were investigated using a high throughput screening approach (Ellis et al., 2020; Obeng et al., 2020). Mitragynine was found to have moderate and non-selective binding affinities at alpha-1A, 1B, and 1D, and alpha-2A, 2B, and 2C, with K_i values in the low micromolar range (1.3–9.29 μM). Corynantheidine exhibited high and selective binding affinity at alpha-1D but not alpha-2 adrenergic receptors, with a K_i value of 41.7 nM, which is comparable to prazosin, a selective alpha-1D blocker (K_i = 0.17 nM) (Obeng et al., 2020). Interestingly, the binding affinity of both mitragynine diastereoisomers i.e. speciociliatine and speciogynine varied at the alpha-2 subtypes. Speciogynine displayed non-selective binding affinities for alpha-2A, 2B, and 2C adrenergic receptors, with K_i values ranging from 0.36 to 2.6 μM, similar to its diastereoisomer at the C-20 (mitragynine). Speciociliatine on the other hand was discovered to be less active (K_i > 10 μM), implying that the *S*-orientation at the C-3 of mitragynine (speciogynine) is required for binding to alpha-2A, 2B, and 2C adrenergic receptors.

Unlike mitragynine, 7-hydroxymitragynine had little to no binding affinity to both alpha-1 and alpha-2 adrenoreceptors indicating that oxidation at the C-7 abolishes the interaction with these receptors. Both pentacyclic kratom alkaloids i.e. ajmalicine and tetrahydroalstonine showed higher binding affinities on alpha-2A, 2B, and 2C receptors, with K_i values in the submicromolar range (K_i = 18–65 nM) than tetracyclic kratom alkaloids (K_i values in the micromolar range). This shows that, like yohimbine (a potent but non-selective alpha-2 adrenergic antagonist with K_i values <5 nM), the ring-D of ajmalicine and tetrahydroalstonine is a critical characteristic for displaying binding to alpha-2A, 2B, and 2C adrenergic receptors (Obeng et al., 2020). The major kratom alkaloids such as mitragynine and speciogynine showed significant binding affinities at alpha-2A, 2B, and 2C adrenergic receptors, which could contribute to kratom overall antinociceptive effect. However, additional research is needed to determine whether the alkaloids work as agonists or antagonists on human adrenergic receptors.

3.3 Serotonin Receptors

Serotonin (5-HT) receptors are a class of G-protein-coupled receptors (GPCRs) and ligand-gated ion channels that regulate physiological functions including mood, cognition, sleep, sociability, blood pressure, body temperature, and sexual behavior, through their natural ligand serotonin (Hoyer et al., 1994; Beliveau et al., 2017). 5-HT receptors are known to have at

least 14 subtypes from seven distinct families, 5-HT1–5-HT7 (Nichols and Nichols, 2008). Kratom has long been used as a mood enhancer, mild stimulant, or aphrodisiac in traditional settings in Malaysia and Thailand (Singh et al., 2019; Singh et al., 2020b). However, research into its potential interaction with the human serotonin neurotransmission system is still in its early stages.

Matsumoto et al. (1997) reported that mitragynine has a suppressive effect on the central serotonin neurotransmission system. In rodents, pretreatment with mitragynine (i.p.) or ritaserin (i.p.) significantly suppressed the 5-HT2A agonist (5-methoxy-N,N-dimethyltryptamine)-induced head twitch response. The results showed that mitragynine, like ritaserin, acts as a competitive antagonist, blocking the stimulation of the 5-HT2A receptor. Further, mitragynine and its diastereoisomer speciogynine measured K_i at 5-HT2A receptor were 7.3 and 2.9 μM respectively in a radioligand binding assay against [^{3}H] clozapine (Ellis et al., 2020). In the same study, Ellis et al. also evaluated the 5-HT2A binding affinity of other kratom alkaloids including 7-hydroxymitragynine, corynoxine B, isorhynchophylline, tetrahydroalstonine, and ajmalicine. However, the alkaloids were found to weakly inhibit binding of the radioligand [^{3}H]clozapine with K_i values >10 μM except for tetrahydroalstonine (K_i = 2.6 μM).

Along with the 5-HT2A receptor, indole-based kratom alkaloids such as mitragynine, speciogynine, speciociliatine, and paynantheine have been shown to interact with the 5-HT1A receptor (Obeng et al., 2021). Using *in vitro* displacement of [^{3}H]8-OH-DPAT, paynantheine was found to have the highest binding affinity at the human 5-HT1A receptor, with a K_i value of 32 nM, followed by speciogynine (39 nM), mitragynine (>1,000 nM), and speciociliatine (>1,000 nM). The *in vivo* binding functionality of the alkaloids at the 5-HT1A receptor was further evaluated by induction of lower lip retraction (LLR) in rats (i.p.) in reference to ipsapirone, a selective 5-HT1A partial agonist. Among the tested alkaloids, speciogynine induced the strongest LLR effect with an ED_{50} value of 23 mol/kg, followed by paynantheine (26 mol/kg) and mitragynine (62 mol/kg). However, the effects were weaker than ipsapirone (ED_{50} = 1.1 mol/kg). The LLR effects of the alkaloids and ipsapirone were reversed by the 5-HT1A receptor antagonist WAY100635 (0.019 μmol/kg, i.p.), suggesting that the alkaloids potentially act as 5-HT1A agonists or partial agonists, in a similar way to ipsapirone. Based on the *in vitro* and *in vivo* findings, it can be assumed that the *R* orientation at C-20 of speciogynine and paynantheine is critical for 5-HT1A agonistic activity. The binding affinity of the alkaloids for the 5-HT1A receptor is dramatically reduced when their orientation is switched from *R* to *S* (mitragynine/speciociliatine). Taking all of this into account, it is hypothesized that the traditional use of kratom as a mood enhancer is due in part to the interaction of its indole-based alkaloids with the 5-HT1A and 5-HT2A receptors.

3.4 Dopamine Receptors

The level of dopamine neurotransmitter in the brain is primarily regulated by a group of GPCRs known as dopamine receptors (Beaulieu and Gainetdinov, 2011). There are a total of 5 dopamine receptor subtypes i.e., D1, D2, D3, D4, and D5 regulating emotion, locomotion, memory and learning, sleep, decision making, and the reward system in the human brain (Mishra et al., 2018). Several studies have suggested that dopaminergic receptors are involved in the antipsychotic, antidepressant, anxiolytic, and anti-addiction activities of kratom or its main alkaloid, mitragynine.

Boyer et al. (2008) were the first to report the binding potential of mitragynine to dopamine receptors, specifically the D2 subtype. In the study, mitragynine was found to moderately inhibit the radioligand binding to the D2 receptor, with a percentage inhibition of 54.22%. The *in vitro* finding was supported by several *in vivo* studies utilizing approaches such as elevated plus-maze, apomorphine-induced climbing behavior, haloperidol-induced catalepsy, and ketamine-induced social withdrawal in rodents. Hazim et al. (2014) investigated the potential role of the dopaminergic system in the anxiolytic-like activity of mitragynine in the elevated plus-maze test. The findings showed that a single oral administration of mitragynine (40 mg/kg) increased the percentage of open arm entries and the time spent on open arms, in a similar way to apomorphine, a non-selective dopamine agonist. The effects were significant but not fully antagonized by sulpiride and SCH 23390. Sulpiride is a non-selective D2-like antagonist while SCH 23390 is a selective D1 antagonist (Holanda et al., 2019). These observations suggest that mitragynine is a moderate dopamine agonist, and its anxiolytic-like activity was partly mediated by D1 and D2-like receptors. However, the findings are in contradiction to the findings reported by Vijeepallam et al. (2016) where they discovered that kratom leaf extract exhibited an antipsychotic-like effect in mice through the blockage of the central D2 receptor. Vijeepallam et al. (2016) found that pretreatment with kratom leaf extract (75 and 100 mg/kg, p.o.) significantly reversed apomorphine-induced cage climbing behavior, ketamine-induced hyperactivity, and social withdrawal deficit in mice. Moreover, co-treatment with the leaf extract significantly enhanced the haloperidol-induced catalepsy in mice. Haloperidol is an antipsychotic that acts as a dopamine D2 receptor antagonist. The antidopaminergic action of the leaf extract (1–100 μg/ml) was further assessed in an *ex-vivo* study using isolated rat vas deferens preparation, and the results showed that the extract inhibited the contractility evoked by dopamine in a dose-dependent manner. However, their pEC_{50} values (pEC_{50} 1.01–1.40 μg/ml) were not significantly altered at different treatment doses (1–20 μg/ml) similar to what observed in the treatment with haloperidol (1.6–12.8 μg/ml) (pEC50 1.31–1.53 μg/ml). These results affirm kratom leaf extract acts as a dopamine D2 blocker/antagonist, similar to haloperidol. However, Vijeepallam's findings are in contradiction with what was reported by Hazim et al. (2014) of which mitragynine acts as a dopamine D1 or D2 agonist, and this could be caused by several factors: 1) mitragynine as a pure compound has a narrow receptor binding profile compared to kratom extract; 2) kratom leaf extract contains multicomponent which might interact with a broad range of CNS receptors leading to the differences in the observed effect;

TABLE 3 | Radioligand binding and functional profiles of selected kratom alkaloids.

References	Membrane source	Receptor	Radioligand	Alkaloids	Key findings	Binding affinity	Functional
Takayama et al. (2002)	Guinea pig (rodent)	μ-opioid	[^{3}H]DAMGO	Mitragynine, speciociliatine, 7-hydroxymitragynine, mitragynine pseudoindoxyl, corynantheidine, mitragynine n-oxide	Mitragynine, 7-hydroxymitragynine, and mitragynine pseudoindoxyl act as agonists at μ-opioid receptor 7-hydroxymitragynine and mitragynine pseudoindoxyl are more potent than morphine Corynantheidine is a selective and functional μ-opioid antagonist	Yes	Yes (*In vivo*)
Kruegel et al. (2016)	Transfected cells (human and rodent)	μ-opioid κ-opioid δ-opioid	[^{3}H]DAMGO [^{3}H]U69593 [^{3}H]DADLE	Mitragynine, 7-hydroxymitragynine, speciociliatine, paynantheine, speciogynine	7-hydroxymitragynine and mitragynine are partial agonists at human μ-opioid receptor and competitive antagonists at human κ-receptor Paynantheine, speciogynine and speciociliatine are competitive antagonists at both human κ- and μ-opioid receptor subtypes Except for 7-hydroxymitragynine and mitragynine, other kratom alkaloids show no notable agonistic or antagonistic effects at rodent opioid receptors. Mitragynine acts as a competitive antagonist at rodent μ-opioid receptor, 7-hydroxymitragynine remains as partial agonist	Yes	Yes (*In vitro*)
Obeng et al. (2020)	Transfected cells (human)	μ-opioid κ-opioid δ-opioid	[^{3}H]DAMGO [^{3}H]U69593 [^{3}H]DADLE	Mitragynine, Speciociliatine, corynantheidine, 7-hydroxymitragynine	7-hydroxymitragynine is a full agonist at μ-opioid receptor and a competitive antagonist at κ- and δ-opioid receptors Mitragynine and speciociliatine are partial agonists at μ-opioid receptor. Speciociliatine (K_i 54.6 nM; EC_{50} 39.2 nM) is a stronger partial agonist than mitragynine (K_i 161 nM; EC_{50} 307.5 nM) Corynantheidine binds selectively to μ-opioid receptor (K_i 118 nM)	Yes	Yes (*In vivo*) (*In vitro*)
Ellis et al. (2020)	Transfected cells (human)	μ-opioid κ-opioid δ-opioid	[^{3}H]DAMGO [^{3}H]U69593 [^{3}H]DADLE	Mitragynine, speciogynine, ajmalicine, tetrahydroalstonine, corynoxine B, isorhynchophylline	Mitragynine and speciogynine bind to μ- and κ- opioid receptors at low micromolar range (K_i 0.74–3.6 μM) 7-hydroxymitragynine shows non-selective and greatest binding affinity to all opioid subtypes (K_i < 1 μM) Ajmalicine shows weak or no binding affinity to all opioid receptor subtypes ($K_i \geq$ 10 μM). Corynoxine B and isorhynchophylline bind selectively to μ-opioid receptor with K_i 1.6 and 0.54 μM, respectively	Yes	No

(Continued on following page)

TABLE 3 | (*Continued*) Radioligand binding and functional profiles of selected kratom alkaloids.

References	Membrane source	Receptor	Radioligand	Alkaloids	Key findings	Binding affinity	Functional
Chear et al. (2021)	Transfected cells (human)	μ-opioid κ-opioid δ-opioid	[^{3}H]DAMGO [^{3}H]U69593 [^{3}H]DADLE	Corynoxine, corynoxine B, isospeciofoline, mitragynine oxindole B, Speciociliatine n-oxide	Corynoxine and corynoxine B exhibit strong and selective binding affinity to μ-opioid receptor with K_i 16.4 and 109.8 nM, respectively Corynoxine acts as a μ-opioid receptor agonist in hot-plate test (10 mg/kg) and the effect is reversed by naltrexone	Yes	Yes (*In vivo*)
Obeng et al. (2020)	Transfected cells (human)	Alpha-1A Alpha-1B Alpha-1D Alpha-2A Alpha-2B Alpha-2C	[^{3}H]prazosin [^{3}H]prazosin [^{3}H]prazosin [^{3}H]RX821002 [^{3}H]RX821002 [^{3}H]RX821002	Mitragynine, speciociliatine, corynantheidine, 7-hydroxymitragynine	Mitragynine binds to alpha-1 and alpha-2 subtypes (K_i at low micromolar range). Mitragynine is a partial agonist at alpha-1A,D, but acts as a competitive antagonist at alpha-1B,2C Corynantheidine binds to alpha-1D receptor (K_i 41.7 nM)	Yes	Yes (*In vitro*)
Ellis et al. (2020)	Transfected cells (human)	Alpha-2A Alpha-2B Alpha-2C	[^{3}H] rauwolscine	Mitragynine, speciogynine, 7-hydroxymitragynine, ajmalicine, corynoxine B, isorhynchophylline	Mitragynine and speciogynine show non-selective binding affinity to all subtypes at low micromolar range (K_i 0.36–4.9 μM) Oxygenated or oxindole alkaloids K_i > 10 μM for adrenergic receptors (not active) Ajmalicine exhibits non-selective binding affinity to all alpha-2 subtypes (K_i 18–65 nM)	Yes	No
Ellis et al. (2020)	Transfected cells (human)	5-HT1A 5-HT2A	[^{3}H]8-OH-DPAT [^{3}H]clozapine	Mitragynine, speciogynine, ajmalicine, tetrahydroalstonine, corynoxine B, isorhynchophylline	Mitragynine and speciogynine K_i 0.54–7.3 μM Ajmalicine and tetrahydroalstonine 5-HT1A K_i < 0.5 μM. Oxygenated indole and oxindole alkaloids	Yes	No
Obeng et al. (2021)	Transfected cells (human)	5-HT1A	[^{3}H]8-OH-DPAT	Mitragynine, paynantheine, speciogynine, speciociliatine	Binding affinity: paynantheine (32 nM) > speciogynine (39 nM) > mitragynine (>1,000 nM) and speciociliatine (>1,000 nM) Speciogynine, paynantheine and mitragynine are 5-HT1A agonists	Yes	Yes (*In vivo*)
Boyer et al. (2008)	Not specified	μ-opioid; κ-opioid; δ-opioid; Alpha-2; D2; 5-HT2C; 5-HT7; A2A	Not specified	Mitragynine	Mitragynine binds to μ and κ-opioid receptors (~90% inhibition) but not δ-opioid receptor	No (% inhibition of radioligand binding at single dose screening)	No

3) dose-dependent presynaptic (functional antagonistic) and postsynaptic (agonistic) action of kratom and mitragynine at dopamine receptors. Therefore, more research is needed to determine the specific binding profile of mitragynine and other kratom alkaloids at dopamine receptors using *in vitro* radioligand binding assays.

Summary of interactions of kratom alkaloids with CNS receptors is tabulated in **Table 3**.

4 INTERACTIONS OF KRATOM ALKALOIDS WITH CELLULAR BARRIERS

Cellular barriers formed by epithelium that lined tissue cavities and endothelium that lined blood vessels delineate tissue compartments and play a pivotal role in maintaining homeostasis, and protecting the tissue microenvironment. The barriers function as a gatekeeper, regulating the passage of substances across the tissue compartments through restrictive tight junctions between adjacent cells; and concerted action of transporters that transport essential nutrients required by the tissues, and keeping out xenobiotics and other harmful substances (Abbott et al., 2010; Vancamelbeke and Vermeire, 2018). In drug discovery and development, it is acknowledged that the barriers imposed a significant hurdle due to the restrictive nature of the barriers which would limit successful delivery of therapeutic molecules to the site of action. It is also known that the functions of the barriers are altered in pathophysiology (Chelakkot et al., 2018; Sweeney et al., 2019). Here, interactions of kratom alkaloids with cellular barriers are discussed within the scope of barrier permeability of the alkaloids, involvement with transporters expressed at the barriers, and effects of the alkaloids on the barrier function.

4.1 Barrier Permeability

The most widely used method to measure barrier permeability is by utilizing two-dimensional *in vitro* cell-based models. The models are established by culturing epithelial or endothelial cells on semi-permeable membrane of well-plate inserts to yield confluent cell monolayers. Determination of barrier properties of the cells particularly tight junction tightness and functional expression of polarized membrane transporters are carried out to evaluate the goodness of purpose of the models. Following the model validation, *in vitro* permeability assay of a compound of interest is conducted. Quantitative analysis of the compound present in assay buffer sampled from the apical and the basolateral compartments which are separated by the cell monolayer enables determination of apparent permeability coefficient, P_{app} of the compound. Comparison of the P_{app} with P_{app} of reference drug would give insights to the potential of barrier permeation of the compound.

For the intestinal barrier, the Caco-2 cell line developed from human colorectal adenocarcinoma epithelium is commonly used to establish a model for the barrier, to determine intestinal absorption (Volpe, 2020). The model was used to investigate intestinal permeability of kratom alkaloids mitragynine (Manda et al., 2014; Rusli et al., 2019), 7-hydroxymitragynine, and mitraphylline (Manda et al., 2014). Mitragynine was found to be the most permeable across the Caco-2 cells, followed by 7-hydroxymitragynine and mitraphylline with P_{app} of 24.2×10^{-6} cm/s, 16.1×10^{-6} cm/s and 6.3×10^{-6} cm/s respectively, when tested at 5 μM in the absorptive direction (apical to basolateral). P_{app} values in the absorptive direction for the three compounds were similar when tested at 10 μM (Manda et al., 2014). Rusli et al. (2019) reported comparable mitragynine P_{app} of 18.8×10^{-6} cm/s. The permeability of mitragynine across the intestinal barrier was also measured using *in situ* single-pass perfusion technique in rats (Jagabalan et al., 2019). *In situ* perfusion technique enables measurement of barrier permeability in an intact functional barrier with membrane transporter machinery in place (Jeong et al., 2004). The findings showed that mitragynine P_{eff} was 111×10^{-6} cm/s. Manda et al. (2014) and Jagabalan et al. (2019) both included high permeability reference drug i.e. propranolol in their studies. Mitragynine showed comparable permeability coefficients to the drug where P_{app} of 24.2×10^{-6} cm/s (5 μM) and 25.3×10^{-6} cm/s (10 μM) were measured using the *in vitro* Caco-2 model while propranolol showed P_{app} of 34.2×10^{-6} cm/s (Manda et al., 2014); P_{eff} of 111×10^{-6} cm/s was measured using the *in situ* technique while propranolol showed P_{eff} of 127×10^{-6} cm/s (Jagabalan et al., 2019).

Previous studies on the BBB permeability of kratom alkaloids utilized *in vitro* models from epithelial and endothelial cells (Manda et al., 2014; Yusof et al., 2019). Compared to endothelial cells, epithelial cell monolayer more readily shows restrictive tight junctions which is the hallmark of the BBB. However, use of primary brain endothelial cells or differentiated stem cells, and co-culture of endothelial cells with other cells of the neurovascular unit for example astrocytes could contribute to having endothelial cell monolayer with restrictive tight junctions and close phenotypic resemblance to the BBB *in vivo* (see Helms et al., 2016 for the different *in vitro* BBB models available). In the MDR-MDCK epithelial cell model, mitragynine and 7-hydroxymitragynine showed apical to basolateral, or blood to brain side P_{app} of 15.3×10^{-6} cm/s and 12.4×10^{-6} cm/s when tested at 5 μM; 16.2×10^{-6} cm/s and 13.2×10^{-6} cm/s when tested at 10 μM respectively (Manda et al., 2014). When the two alkaloids were assayed using primary porcine brain endothelial cells, mitragynine showed apical to basolateral P_{app} of 31.8×10^{-6} cm/s, while 7-hydroxymitragynine P_{app} was 15.3×10^{-6} cm/s (Yusof et al., 2019). Based on the two studies, mitragynine showed approximately 1.2–2.1 times higher BBB permeability than 7-hydroxymitragynine. This could potentially be contributed by differences in physicochemical properties of the alkaloids. Mitragynine being more lipophilic and 7-hydroxymitragynine being more polar might eased and hampered passive transcellular permeation respectively. Another possibility is the involvement of membrane transporters to transport the alkaloids. Meanwhile, mitraphylline apical to basolateral permeability was more restricted with P_{app} of 3.3 ×

TABLE 4 | Functional interactions of kratom alkaloids with efflux transporters.

Alkaloid	Concentration tested	Methods	Findings	Subjected to efflux	Efflux transporter inhibition	References
Mitragynine	5, 10 μM	*In vitro* bidirectional permeability assay using Caco-2 and MDR-MDCKII cells	No polarization of transport. Efflux ratio = 1.0 and 1.1	No	—	Manda et al. (2014)
	5 μM	*In vitro* permeability assay using Caco-2 cells with or without P-gp inhibitor verapamil (5 μM)	Permeability was unaltered in presence of verapamil	No	—	Meyer et al. (2015)
	10 μM	*In vitro* bidirectional permeability assay using Caco-2 cells	No polarization of transport. Efflux ratio = 0.9	No	—	Rusli et al. (2019)
	0.3 μM	*In vitro* permeability assay using primary porcine brain endothelial cells with or without P-gp inhibitor valspodar (PSC833; 1 μM)	Increased apical to basal permeability (blood to brain side) in presence of valspodar	Yes (P-gp)	—	Yusof et al. (2019)
		Brain extent study—combinatory approach of *in vivo* neuropharmacokinetic, *in vitro* drug tissue binding and brain slice assays	$K_{p,uu,brain}$ < 1 indicating net efflux	Yes		
	40 μg/ml	*In situ* single pass intestinal perfusion in small intestine of rats with or without P-gp inhibitor azithromycin (200 μg/ml)	Permeability was unaltered in presence of azithromycin	No	—	Jabagabalan et al. (2019)
	—	*In vitro* uptake assay of P-gp substrate calcein-AM in presence of mitragynine at different concentrations	Increased uptake of calcein-AM in MDR-MDCKII cells in presence of mitragynine dose-dependently (EC_{50} = 18.2 μM)	—	Yes (P-gp)	Manda et al. (2014)
	5 μM	*In vitro* permeability assay of P-gp substrate rhodamine 123 across Caco-2 cell monolayers with or without mitragynine in basolateral to apical (secretory) direction	Reduced basolateral to apical permeability of rhodamine 123 in presence of mitragynine	—	Yes (P-gp)	Meyer et al. (2015)
	10 μM	*In vitro* permeability assay of P-gp substrate digoxin across Caco-2 cell monolayers with or without mitragynine	Reduced basolateral to apical permeability of digoxin in presence of mitragynine	—	Yes (P-gp)	Rusli et al. (2019)
	0.3 μM	*In vitro* permeability assay of P-gp substrate digoxin across primary porcine brain endothelial cell monolayers with or without mitragynine in apical to basolateral (absorptive) direction	Increased apical to basolateral permeability of digoxin in presence of mitragynine	—	Yes (P-gp)	Yusof et al. (2019)
	5, 50, 500 μM	Human BCRP (hBCRP) ATPase activity	Mitragynine stimulated hBCRP ATPase at all concentrations tested, and inihibited hBCRP ATPase at 500 μM	Yes	Possibly weak inhibition due to IC_{50} value	Wagmann et al. (2018)
	5–2,500 μM	Determination of IC_{50}	IC_{50} = 359 μM			
7-hydroxy-mitragynine	5, 10 μM	*In vitro* bidirectional permeability assay using Caco-2 and MDR-MDCKII cells	No polarization of transport. Efflux ratio = 1.2	No	—	Manda et al. (2014)
	0.3 μM	*In vitro* bidirectional permeability assay using primary porcine brain endothelial cells	Higher basolateral to apical (brain to blood side) permeability. Efflux ratio = 1.39	Yes (P-gp)	—	Yusof et al. (2019)
	0.3 μM	*In vitro* permeability assay with or without P-gp inhibitor valspodar (PSC833; 1 μM)	Increased apical to basolateral permeability (blood to brain side) in presence of valspodar			
	—	Brain extent study—combinatory approach of *in vivo* neuropharmacokinetic, *in vitro* drug tissue binding and brain slice assays	$K_{p,uu,brain}$ < 1 indicating net efflux	Yes		

(Continued on following page)

TABLE 4 | (*Continued*) Functional interactions of kratom alkaloids with efflux transporters.

Alkaloid	Concentration tested	Methods	Findings	Subjected to efflux	Efflux transporter inhibition	References
	—	*In vitro* uptake assay of P-gp substrate calcein-AM in presence of 7-hydroxymitragynine at different concentrations	Increased uptake of calcein-AM in MDR-MDCKII cells in presence of 7-hydroxymitragynine dose-dependently (EC_{50} = 32.4 μM)	—	Yes (P-gp)	Manda et al. (2014)
	0.3 μM	*In vitro* permeability assay of P-gp substrate digoxin across primary porcine brain endothelial cell monolayers with or without 7-hydroxymitragynine in apical to basolateral (absorptive) direction	Increased apical to basolateral permeability of digoxin in presence of 7-hydroxymitragynine	—	Yes (P-gp)	Yusof et al. (2019)
Mitraphylline	5, 10 μM	*In vitro* bidirectional permeability assay using Caco-2 and MDR-MDCKII cells	Higher basolateral to apical (secretory) permeability with efflux ratio of 3.3–6.6	Yes		Manda et al. (2014)
	—	*In vitro* uptake assay of P-gp substrate calcein-AM in presence of mitraphylline at different concentrations	No effect on calcein-AM uptake		No	Manda et al. (2014)

10^{-6} cm/s when tested at 5 μM, and 3.4 × 10^{-6} cm/s when tested at 10 μM (Manda et al., 2014).

4.2 Interactions With Membrane Transporters

Physiological barriers not only act as a physical barrier which is contributed by the restrictive tight junctions, but also as metabolic and transport barriers to permeation of molecules (Abbott et al., 2006). The transport barrier is imposed by solute carrier (SLC) and ATP-binding cassette (ABC) transporters, which function to either facilitate or impede transcellular permeability across the barriers. In drug discovery and development, prediction or determination of compounds' potential substrates for the ABC transporters particularly the P-glycoprotein (P-gp) is often considered. Efflux by the P-gp which has broad substrate specificity could affect the pharmacokinetics of a compound such as limiting intestinal absorption, impeding CNS penetration, and thus influencing drug delivery and targeting (Lin and Yamazaki, 2003; Miller, 2015). To overcome this, modulation of the P-gp function to reduce efflux, or alteration of the P-gp expression are some of the approaches being explored to improve drug delivery (Miller, 2015).

Evidence on mitragynine and 7-hydroxymitragynine P-gp-mediated efflux are conflicting (**Table 4**). Lack of polarization in bidirectional transport measured *in vitro* indicated no potential efflux, and unaltered permeability in presence of P-gp inhibitors suggested that the alkaloids were not substrates of P-gp (Manda et al., 2014; Meyer et al., 2015; Jagabalan et al., 2019; Rusli et al., 2019). However, when the alkaloids were tested at submicromolar concentration, an increase in apical to basolateral permeability was observed in presence of the P-gp inhibitor, valspodar (PSC833), suggesting P-gp-mediated efflux (Yusof et al., 2019). The differences in concentrations used to test the alkaloids in the studies may explain the discrepancies of the findings, as higher concentrations can cause transporter saturation, and this, in turn, affects readouts of *in vitro* bidirectional permeability assay (Saaby and Brodin, 2017). On the other hand, P-gp-mediated efflux of mitraphylline was evident from Manda et al. study.

Previous studies are in agreement that mitragynine and 7-hydroxymitragynine inhibited P-gp-mediated efflux of known substrates of the transporter (**Table 4**). The alkaloids dose-dependently increased MDR-MDCK cell uptake of calcein-AM, with mitragynine and 7-hydroxymitragynine EC_{50} of 18.2 and 32.4 μM respectively, comparable to the P-gp inhibitor verapamil which shown EC_{50} of 22.3 μM (Manda et al., 2014). In the Caco-2 model, mitragynine was demonstrated to reduce the permeability of rhodamine 123 and digoxin in the basolateral to the apical direction (secretory direction) at 5 and 10 μM (Meyer et al., 2015; Rusli et al., 2019). We also found evidence for mitragynine and 7-hydroxymitragynine inhibition of P-gp-mediated efflux of digoxin at a concentration of 0.3 μM, comparable to inhibition by valspodar (Yusof et al., 2019). The inhibition of P-gp-mediated efflux by kratom alkaloids needs careful considerations as this could potentially cause interactions with drugs that are substrates of the P-gp. Co-presence of the alkaloids and the drugs may lead to an increase in the drugs absorption and tissue distribution, and decrease elimination. While this could be a strategy for the drugs to reach sites of action, the non-specific inhibition of the P-gp in non-targeted tissues could contribute to cytotoxicity.

Another important ABC transporter which expression includes at the gastrointestinal tract and at the BBB is the breast cancer resistance protein (BCRP). At the human BBB, the BCRP expression was found to be the most abundant among the ABC transporters, 1.34 fold higher than the P-gp expression; while the opposite was found for mice where the P-gp expression was 3.20 fold higher than the BCRP expression (Uchida et al., 2011). This need to be taken into consideration when extrapolating data from mice to human. The two transporters have been reported to work cooperatively in limiting the entry of chemotherapeutic drugs into the brain, and inhibition of

one transporter can be compensated by the other (Agarwal et al., 2011). Based on human BCRP ATPase activity where the formation of ADP was quantified as an indicator for either stimulation or inhibition of the transporter in presence of test compounds, mitragynine was reported as a potential substrate of the BCRP and could inhibit the transporter function with an IC_{50} value of 359 μM (Wagmann et al., 2018). Findings reported by Wagmann et al. (2018) and Yusof et al. (2019) provide evidence for mitragynine dual substrate of the P-gp and the BCRP.

Efflux of mitragynine and 7-hydroxymitragynine was also determined in the study of the alkaloids extent in the brain (Yusof et al., 2019). By using a combinatory approach of *in vivo* neuropharmacokinetic, *in vitro* drug tissue binding and brain slice assays to calculate total whole brain to plasma concentration ratio ($K_{p,brain}$), fraction of unbound alkaloids in plasma ($f_{u,plasma}$), and volume of distribution of unbound alkaloids in the brain ($V_{u,brain}$) respectively, the extent of unbound alkaloids in the brain ($K_{p,uu,brain}$) yielded values of approximately 0.1, which is below the value of unity (1), thus indicating efficient efflux of the alkaloids (Yusof et al., 2019).

Apart from interactions with the efflux transporters, mitragynine could also potentially be transported by influx transporters into the brain (Yusof et al., 2019). However, further investigations are needed to confirm this.

4.3 Alteration of Barrier Function

In vitro cell-based models of physiological barriers not only are great tools to investigate mechanisms of permeability but can also be used to determine the effects of exposure to compounds on the structure and function of the barriers. Exposure to mitragynine at 40 and 60 μM for 48 h reduced the viability of human aortic endothelial cells, which was linked to an increase in intracellular reactive oxygen species (ROS) production, leading to caspase-3 activation, DNA fragmentation, and apoptosis (Matsunaga et al., 2017). The LC_{50} determined was 43.1 μM. The effect of mitragynine on the tight junction function of the human aortic endothelial cells was also investigated. The cells grown on semi-permeable inserts were exposed to mitragynine at 5 μM either for a short, or long-term incubation of 5 days. The cells were also incubated with 10 and 20 μM mitragynine for 5 days. The transendothelial electrical resistance was then measured as an indicator for tight junction integrity. Tight junction leakage to FITC-dextran with a molecular weight of approximately 150 kDa was assessed. Findings from the study showed that the long-term exposure to mitragynine caused a decrease in tight junction tightness of the human aortic endothelial cells at all concentrations tested i.e. 5, 10, and 20 μM, which might contribute to leakage of the FITC-dextran at 20 μM (Matsunaga et al., 2017). The decrease in tightness of the tight junction was not observed in cells pre-treated with ROS inhibitor, while ROS generators made it worse. This indicates the involvement of ROS in the disruption of tight junction integrity of the human aortic endothelial cells upon exposure to mitragynine (Matsunaga et al., 2017).

Mitragynine was found to alter the expression of the P-gp. The Caco-2 cells incubated with mitragynine at 0.1, 1, and 10 μM for 72 h showed downregulation of mRNA and protein expression of the P-gp in a concentration-dependent manner (Rusli et al., 2019). The downregulation of expression correlates with reduced intensity of P-gp staining of the cells. The number of cells expressing P-gp was also reduced (Rusli et al., 2019).

Evidence of alteration of cellular barrier function by mitragynine in long-term exposure is concerning. In particular when the concentrations that affected the function falls within the range of mitragynine concentrations reported in human plasma, of which a range of 1.13–5.77 μM was reported in a recent study by Vicknasingam et al. (2020). Future studies should look into other potential alterations to the barrier structure and function as part of safety evaluations.

5 CONCLUSION

Here, we have gathered and discussed physiological interactions of kratom alkaloids within the scope of interactions with drug-metabolizing enzymes and potential for drug-drug interactions, interactions with central nervous system receptors to relate with pharmacological actions, and interactions with cellular barriers of which are not limited to mechanisms of barrier permeability, but also effects of exposure to kratom alkaloids on the barrier function. Although the interactions with enzymes and the receptors may not be necessarily new in regards to kratom research, these areas have gained renewed interest among researchers in recent years due to the wealth of evidence on pharmacological actions of the alkaloids in preclinical studies, the rise of kratom use for self-treatment purposes, and the controversies surrounding the consumption of kratom. Meanwhile, interactions of kratom alkaloids with cellular barriers are largely unexplored.

Highlights from the discussion include the potential for clinically relevant drug-drug interaction due to modulation either in expression or function of drug-metabolizing enzymes, particularly the cytochrome P450 enzymes by the alkaloids. Secondly, kratom alkaloids have been known as atypical opioids stem from the discoveries of their opioids and non-opioids mechanistic. This multimechanistic property of the alkaloids could provide interesting avenues for the development of multi-targeted therapeutics for better efficacy and reduced side effects. As traditional uses generally involve consumption of a brewed drink, the mechanistic of the alkaloids as single compounds and in combination need to be delved deeper. Thirdly, cellular barriers imposed formidable hurdles in the development of therapeutics due to their protective nature and dynamic regulation of the tissue microenvironment. Therefore, a good understanding of the alkaloids' molecular traffic between physiological interfaces will aid future delivery strategies. As kratom alkaloids have been demonstrated to interact with membrane transporters particularly the efflux transporters, this could also imply the potential for drug-drug interaction with the transporter substrates. Taken together, interactions of kratom alkaloids with drug-metabolizing

enzymes and cellular barriers not only affect their tissue distributions and the concentrations at target receptor sites to elicit functional responses, but also distributions, and functional responses of other drugs. As always, more work is needed to understand the physiological interactions of kratom alkaloids in the course of further development as potential therapeutics.

AUTHOR CONTRIBUTIONS

SY, JA, and NC conceptualized the content of the manuscript. SY, JA, NC, and AH wrote sections in the manuscript. All authors reviewed the manuscript and approved it for submission.

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Kratom Use in the US: Both a Regional Phenomenon and a White Middle-Class Phenomenon? Evidence From NSDUH 2019 and an Online Convenience Sample

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Kratom products available in the United States are becoming increasingly diverse both in terms of content and in terms of how they are marketed. Prior survey research indicates that kratom has been primarily used in the US to self-treat anxiety, depression, pain, fatigue, and substance use disorder (SUD) symptoms. Kratom is also well-known for its use as a short- or long-term full opioid agonist substitute. Therefore, use may be greater in regions particularly impacted by addiction to prescription opioids. Use may also be greater in demographic groups targeted by media outlets (such as specific podcasts) in which kratom is touted. Here, we aimed to determine whether lifetime and past-year kratom use were associated with region of residence and with being young, White, post-secondary educated, and employed. To strengthen confidence in our findings, we analyzed data from two sources: our own crowdsourced online convenience sample and the 2019 National Survey on Drug Use and Health (NSDUH). In our sample (N = 2,615), 11.1% reported lifetime and 6.7% reported past-year kratom use, and the odds of kratom use were higher among people who were White, younger, at least high school educated, employed, and above the poverty line, as well as those reporting nonmedical opioid use, past-year SUD, or lifetime SUD treatment; residence was not a significant predictor. In NSDUH data, suburban residence and other demographic factors, concordant with those from the crowdsourced sample, were associated with kratom use. Taken together, the findings support a general "White middle-class suburban" profile of the modal kratom user, but more research is needed to understand it. In the interim, focus should be on our finding that lifetime nonmedical opioid use was associated with an up to five times greater likelihood of past-year kratom use, suggesting that drug-use history may presently be the strongest predictor of kratom use.

Keywords: rural drug use, substance use disorder treatment, substance use disorder, opioids, mitragyna speciosa, kratom

INTRODUCTION

Kratom, the lay term referring to the Mitragyna speciosa Korth [Rubiaceae] tree native to Southeast Asia, has leaves that contain at least over 40 alkaloids with pharmacologic activity. Most notable among these with dose-dependent psychoactive effects are mitragynine (MG) and 7-hydroxymitragynine (7-HMG). Both alkaloids bind to and partially agonize the mu-opioid receptor, producing analgesic, stimulatory, and anxiolytic effects (Kruegel and Grundmann, 2018; Kruegel et al., 2019; Obeng et al., 2021; Todd, et al., 2020). While some of these effects can likely be attributed to mu-opioid receptor activity, others may occur through separate mechanisms (Hiranita et al., 2019).

Although use of the kratom leaf in Southeast Asia dates back at least to the early 1800s (Jansen and Prast, 1988), kratom use was not generally noted in the United States (US) until the early-mid 2000s (Boyer et al., 2007; Babu et al., 2008) and did not become widespread until approximately 2015 (Grundmann, 2017; Smith and Lawson, 2017). Currently, a variety of kratom products (loose leaf, powder, capsules, concentrate) can be legally purchased from online retailers, smoke shops, convenience stores, and specialty supplement shops in 46 US states (Griffin et al., 2016; Fowble and Musah, 2019). Exploratory surveys in the US seeking to better understand kratom use, motivations, and effects have found that many people report using kratom to "self-manage" chronic pain, fatigue, psychiatric, and symptoms of substance use disorders (SUDs), including opioid-withdrawal symptom relief and/or as a replacement for full opioid agonists (Bath et al., 2020; Boyer et al., 2008; Coe, et al., 2019; Garcia-Romeu, et al., 2020; Grundmann, 2017; Smith and Lawson, 2017; Swogger and Walsh, 2018).

Kratom's Relevance to Rural Regions

These latter two opioid-related motivations for use indicate that kratom use in the US may vary by region. Kratom's relevance to people's needs (and thus its prevalence of use) may be greater in rural communities that experienced higher per capita rates of opioid prescribing during the early 2000s and subsequently experienced changes in the licit and illicit prescription opioid market (Thomas et al., 2020). Findings consistently indicate high opioid-related risk for those living in rural settings: opioid prescribing is up to 33% higher in rural counties than elsewhere; rural-residing adolescents are more likely than those in urban-metro counties to initiate nonmedical use of opioids; rural justice-involvement carries a five-fold greater likelihood of nonmedical use of opioids; and overdose death rates for nonmedical use of opioids are 20–30% higher in rural counties (Havens et al., 2007; Paulozzi and Xi, 2008; Havens et al., 2011; Mack et al., 2017; Mosher et al., 2017; Ayres and Jalal, 2018; Luu et al., 2019). These outcomes are compounded by the practical and social difficulties of accessing treatment for opioid use disorder (OUD) in rural counties, including stigma surrounding medication for OUD (MOUD) (Bunting et al., 2018; Jones, 2018; Jacobson et al., 2020; Lister et al., 2020; Cole et al., 2021; Franz et al., 2021). Recent findings suggest that only half of physicians authorized to prescribe MOUD had the availability to accept new patients (Andrilla et al., 2018), and though MOUD access is increasing nationally and gains have been made to increase prescriber capacity in underserved areas (Barnett et al., 2019), more than half of small and rural counties lack a physician waivered by the Drug Enforcement Administration to prescribe MOUD (Andrilla and Patterson, 2021). Given the high prevalence of prescription opioid misuse, poor psychiatric (Snell-Rood and Carpenter-Song, 2018) and physical health (including high rates of chronic pain) (Meit et al., 2017), and the difficulty in obtaining MOUD in rural areas (Sexton et al., 2008; Prunuske et al., 2014; Woolf et al., 2019; Monnat, 2020), it is possible that kratom use might be more prevalent in rural counties than in urban-metro counties. Although heroin use is increasing in many rural communities that had elevated rates of opioid prescribing (Nolte et al., 2020; Schnell et al., 2020; Hedegaard and Spencer, 2021; Strickland et al., 2021), kratom might be more accessible or more attractive than heroin to people whose sole prior opioid use had involved prescribable pills. To date, kratom use has not been well characterized in terms of rural/suburban/urban differences. Only two large US survey studies have noted the geographic region of kratom users in their sample, both finding that a slightly greater proportion resided in the US South (Coe et al., 2019; Garcia-Romeu et al., 2020). However, in separate analyses, Nicewonder et al. (2019) found that kratom use was more widely distributed across the US, with higher rates in Florida, as well as Oregon, California, and Idaho, and still noteworthy use in the Northeast. These findings were from data collected in 2017; given the relatively recency of kratom's emergence in the US, an update would probably be informative.

But is Kratom Use More Than Self-Treatment and Opioid Replacement?

As kratom popularity in the US has grown substantially, there may be new subpopulations of kratom users that are distinct from those using kratom to address pain, psychiatric symptoms, and/or SUDs. In our own analyses of social-media posts, we found that some people are using kratom not to "self-treat" symptoms but rather to enhance mood and performance and to boost energy (Smith et al., 2021a; Smith et al., 2021b).

Using articles and books published in popular media outlets as a proxy, we can observe that kratom is now being advertised and sought out as a performance-boosting ("nootropic") or wellness supplement (Mun and Wong, 2020; Carcache de Blanco and Kinghorn, 2021; Ng et al., 2021). A recent content analysis of over 42,000 comments made on kratom-related YouTube videos found that 50% reported use of kratom for its energy-boosting effects and 25% for its purported nootropic effects (Prevete et al., 2021). Though these motivations do not seem to represent a majority of kratom-using people, interest in kratom as a nootropic could expand interest in kratom and increase purchasing and use for groups other than those seeking to self-manage health conditions. For such groups, kratom products would likely be conceptualized as a wellness or performance-enhancing supplement, not a medication to alleviate underlying health symptoms. Indicative of expanding interest and popularization, discussions about kratom and its effects have been featured on popular media outlets such as The

Joe Rogan Experience, one of the most downloaded podcasts in 2019 and 2020, with individual episodes garnishing up to 45 million views on YouTube alone (Rogan, 2018; Rogan, 2019; Jarvey, 2020). Precise figures on the demographics of podcast listenership are not readily available, but one informal survey estimates that The Joe Rogan Experience listenership is 24 years of age on average, 71% male, 50% post-secondary educated, and fairly high in income (76% reported earning over $50,000 USD annually). The male skew appears to be driven mostly by trends in overall podcast listenership, as equal proportions of podcast-listening men and women reported listening to The Joe Rogan Experience (Media Monitors, 2021). From these findings, we may expect to see greater likelihood of kratom initiation among people who constitute the demographic being more frequently exposed to promotion of kratom in specific types of content—people who are young, White, post-secondary educated, and employed.

Some evidence from national surveys does suggest greater kratom use prevalence among White, educated men, although the findings are mixed. A national-level convenience sample of over 8,000 kratom users was majority non-Hispanic White (89%) and male (57%), with at least some college-educated (82%), and earning annual incomes exceeding $35,000 USD (Grundmann, 2017). More recent nationally representative data from the Cross-sectional Survey of Non-Medical Use of Prescription Drugs (NMURx) Program 2018 – 2019 found that kratom use was not associated with income or race/ethnicity but was represented by a male majority (Schimmel et al., 2021). Meanwhile, data from the nationally representative National Survey on Drug Use and Health (NSDUH) in 2019 suggest decreased odds for past-year kratom consumption among people of Hispanic and Black race/ethnicity compared with White people, but found no robust associations with education level or annual family income (Palamar, 2021).

Aims

We sought to address each of the two demographic considerations just discussed: whether kratom use is associated with rurality (versus urbanicity), and, in parallel, whether there is also an emerging culture of kratom use (possibly for different reasons, though we did not address that here) among people who are young, White, post-secondary educated, and employed. We used two independent data sources: our own crowdsourced online convenience sample of people reporting past 6 month alcohol, opioid, and/or stimulant use, and the 2019 National Survey on Drug Use and Health (NSDUH). By examining data from two distinct surveys with divergent sampling and assessment methods we hoped to find some convergence in results. Still, we did not have a priori hypotheses as to whether we would find such convergence, or even whether indirect evidence of a kratom-user typology, characteristic of the one described above, would be found.

METHODS

This secondary data analyses examined responses from two different US-based surveys, neither of which sought to recruit based on kratom use. Each data source is described below.

Crowdsourced Online Convenience Sample

Using Amazon Mechanical Turk (mTurk), a crowdsourcing platform for data collection (Chandler and Shapiro, 2016; Miller et al., 2017; Mortensen and Hughes, 2018; Peer et al., 2014; Strickland and Stoops, 2018; 2019), we notified people with registered mTurk accounts between September 2020 and March 2021 that they could complete a screening questionnaire to determine their eligibility for a large online survey pertaining to drug use and social conditions. People were eligible for inclusion into that survey study convenience sample if they were >18 years, US residents, English language proficient, reported using: alcohol only (nicotine and caffeine use permitted), opioids (licit or illicit), and illicit stimulants during the 6 month period prior to screening (people reporting opioid and/stimulant use could report other drug use and remain eligible). Because the survey did not solicit personally identifiable information, the study was considered exempt by the National Institutes of Health Institutional Review Board (NIH IRB). For a more detailed description of the methods, see Smith et al., 2021c; Smith et al., 2021d.

Convenience Sample Survey Measures

Items assessed included basic demographic information (age, gender, race/ethnicity, highest education attained, employment status, annual income and zip code), lifetime and past-year substance use, DSM-5 SUD symptom checklist for all diagnostic items, and a single-item question asking respondents to indicate whether they had ever received SUD treatment. Lifetime nonmedical use of opioids was defined as any medically unsupervised use of prescription opioids, heroin, or fentanyl. For modeling purposes and to increase concordance with measures employed by NSDUH, age was coded as under versus over 35. Sex/gender was coded as male versus nonmale (an arbitrary, admittedly imperfect solution to the small cell size for respondents who identified as nonbinary).

To test for greater kratom use likelihood among people who could reasonably be described as "young, white, and at least middle class," we created an indicator variable for both men and women who were: under the age of 35 years, of White race/ethnicity, at least high school educated, employed, and making above US poverty line annual household incomes.

Rural and metropolitan classifications were assigned according to the 2013 rural-urban continuum (Beale) codes, a classification scheme primarily developed by the United States Department of Agriculture (USDA) that classifies counties as being "large metropolitan", "small metropolitan", or "non-metropolitan" and the degree to which each is influenced by population size, metropolitan area, urbanization, or adjacency to a metro area. For our online convenience sample, we converted participants' zip codes to county-level codes and assigned respondents to one of the three aforementioned categories, reflecting the county they reported residing in for the majority of the past year.

Nationally Representative Sample

Data from the 2019 NSDUH (questionnaire items on kratom use were included in the NSDUH for the first time in 2019) included

survey responses from a nationally representative US sample of persons aged 12 and older. Here, we included only responses from persons >18 years of age. The NSDUH employs a "probability proportional to size" sampling design to collect responses from noninstitutionalized civilians in all 50 states and the District of Columbia, but not from people who are housing insecure, incarcerated, institutionalized, or actively deployed in military service. These data are therefore considered representative for approximately 97% of the US population (Lofquist et al., 2012). Analysis of publicly available NSDUH data is also considered exempt from institutional review by the NIH IRB.

NSDUH Survey Items

We used all measures from the NSDUH that were concordant with measures from our online convenience sample: basic demographic information (age, sex/gender, race/ethnicity, highest education attained, employment status, annual income), an indicator variable for lifetime nonmedical use of opioids (either prescription opioids or heroin), ever having received SUD treatment, and an indicator variable for past-year SUD (by DSM-IV criteria: see next paragraph). NSDUH provides a recoded variable of rural-urban continuum codes (COUTYP4) in which people are classified as living in a large metro, small metro, or non-metro country in the same fashion as in our online convenience sample. We constructed a "young, white, and at least middle class" indicator variable in the same fashion as in our convenience sample.

To ensure maximal comparability of the analyses from the two data sources, we dichotomized demographic variables to match exactly, and we selected concordant indicators of substance use. The only included variable that differed between data sources was the indicator for moderate to severe past-year SUD. In our online convenience sample, past-year SUD was measured using a DSM-5 checklist for SUD for any substance by endorsing >3 DSM-5 SUD diagnostic criteria. Participants were prompted to complete the DSM-5 SUD checklist for one of two conditions: 1) for the substance (alcohol included) they believed they had the biggest problem with during the past year or 2) for those who did not believe they had any alcohol/drug problems, for the substance they had used most frequently. Those endorsing >3 diagnostic criteria were coded moderate-severe). Because NSDUH does not administer the DSM-5 SUD questionnaire, we selected a proxy variable (UDPYILL) that indicates past year DSM-IV dependence on or abuse of an illicit substance.

Analytic Plan

We generated descriptive results, displayed in **Table 1**, for the full online convenience sample and the subsets of participants reporting lifetime and past year kratom use. For the 2019 NSDUH data, we describe the sample by reporting nationally representative proportion estimates of lifetime and past year kratom use split by demographic factors and substance use factors associated with kratom use in **Table 2**.

Because one primary aim was to examine regionality, we fit multinomial logistic regression models predicting both lifetime and past-year kratom use from the metropolitan classification of participants' residence while controlling for demographic factors and substance use factors that have been previously associated with kratom use. Survey sampling weights and survey design-based variance estimation were employed on all NSDUH models to produce nationally representative estimates. All analyses were conducted using R version 4.1.1. The R analyses syntax and datasets generated and analyzed from the NSDUH 2019 dataset for this study can be found on Open Science Framework at doi: 10.17605/OSF.IO/M7DW4 [https://osf.io/m7dw4/]. Lifetime and past-year kratom use proportion estimates for the online convenience sample and NSDUH 2019 are plotted in **Figures 1** through **4**, respectively.

Additionally, because we were interested in detecting a signal for higher kratom use prevalence among white, middle-class men and women, we fit two multiple-logistic-regression models from each data set predicting lifetime and past year kratom use from a single combined factor indicating the aforementioned population while controlling for the same non-demographic terms entered into the previously employed models.

All logistic regression model results are displayed in **Table 3** through **Table 6**. Regression coefficients are **Table 4** reported as odds ratios and their 95% confidence intervals. We computed variance inflation factors for each predictor term, also displayed in the tables, to ensure that multicollinearity did not substantially influence predictor performance.

RESULTS

Crowdsourced Online Convenience Sample

Between September 2020 and March 2021 a total of 13,608 people completed screening questionnaires on mTurk, 3,414 (25.1%) meet study inclusion criteria and were invited to participate, 2,864 (21.0%) completed the survey, and 2,615 (19.2%) passed all data quality checks and constituted the final analyzable sample.

Table 1 shows descriptive data. The respondents were majority female (58.6%), White (74.7%), college educated (54.2%), employed (78.6%), and living in large metropolitan counties (53.5%). Lifetime kratom use was reported by 289 (11.1%) respondents. Past-year kratom use was reported by 174 (6.7%). The subset of participants who reported lifetime and past-year kratom use contained a higher proportion of male, high school-educated people, people making below US poverty line annual incomes, people living in non-metro (rural) counties, people having ever received SUD treatment, and people meeting criteria for a severe SUD.

Increased likelihood of lifetime kratom use was predicated by young (<35) age (OR = 1.64, 95% CI = 1.24, 2.16), male sex/gender (OR = 1.79, 95% CI = 1.37, 2.34), being high school educated (OR = 1.39, 95% CI = 1.04, 1.87), lifetime nonmedical use of opioids (OR = 5.13, 95% CI = 3.80, 6.94), at least moderate SUD (OR = 2.00, 95% CI = 1.49, 2.68), and having ever received SUD treatment (OR = 1.53, 95% CI = 1.09, 2.14).

Similarly, increased likelihood of past-year kratom use was indicated by male sex/gender (OR = 1.47, 95% CI = 1.06, 2.03), being high school educated (OR = 1.44, 95% CI = 1.00, 2.08),

TABLE 1 | Demographics for our online crowdsourced sample, by lifetime and past-year kratom use.

	Complete sample		Lifetime kratom use		Past-year kratom use	
	N	**M ± SD**	**N**	**M ± SD**	**N**	**M ± SD**
Age	2,615	36.65 ± 11.35	289	33.58 ± 8.67	174	33.58 ± 8.67
	N	%	*N*	%	*N*	%
Young Age (<35 Years)	1,335	51.05	179	61.94	103	59.20
Sex/gender						
Male	1,052	40.23	154	53.29	87	50.00
Female	1,531	58.55	126	43.60	81	46.55
Nonbinary	32	1.22	9	3.11	6	3.45
Race/Ethnicity						
White	1954	74.72	209	72.32	124	71.26
US Minority	661	25.28	80	27.68	50	28.74
Education						
HS Graduate	1,199	45.85	189	65.40	115	66.09
College Graduate	1,416	54.15	100	34.60	57	32.76
Employment						
Employed	2054	78.55	216	74.74	125	71.84
Unemployed	561	21.45	73	25.26	49	28.16
Annual Income						
Below Poverty Line	541	20.69	79	27.34	47	27.01
Above Poverty Line	2074	79.31	210	72.66	127	72.99
Rural-Urban Continuum						
Large Metro	1,398	53.46	137	47.40	81	46.55
Small Metro	797	30.48	93	32.18	55	31.61
Non-Metro	420	16.06	59	20.42	38	21.84
Ever SUD Treatment	284	10.86	82	28.37	46	26.44
Moderate - Severe SUD	949	36.29	193	66.78	117	67.24
Lifetime NMO	801	30.63	209	72.32	129	74.14
"White Middle-Class" indicator						
Male	289	11.05	48	16.61	25	14.37
Nonmale (Female or nonbinary)[a]	341	13.04	31	10.73	18	10.34

[a]*Our use of "male" as the reference category, with female and nonbinary collapsed into the other category, was our admittedly imperfect solution to the smallness of the cell size for respondents identifying as nonbinary in our survey. Despite misgivings about the categorization, we think it is preferable to excluding respondents who did not fall into one of the two large categories. The issue did not arise for the nationally representative NSDUH, data* ***(Tables 2, 5, 6)*** *because the NSDUH, survey did not included "nonbinary" as a response choice.*

lifetime nonmedical use of opioids (OR = 5.22, 95% CI = 3.56, 7.66), and at least moderate SUD (OR = 1.94, 95% CI = 1.34, 2.79).

Residence in a rural county was not significantly associated with either lifetime or past-year kratom use in our crowdsourced sample, although there was a trend towards an association of non-metro county residence with increased odds of lifetime (OR = 1.25, 95% CI = 0.97, 1.60) and past-year kratom use (OR = 1.30, 95% CI = 0.96, 1.75).

The "White, middle-class" indicator was associated with increased likelihood of lifetime kratom use among men (OR = 1.94, 95% CI = 1.33, 2.82) but not women or nonbinary respondents (OR = 0.89, 95% CI = 0.58, 1.36). Past-year kratom use was not associated with the "White, middle-class" indicator for either sex/gender category.

Nationally Representative Sample

Table 2 shows weighted prevalence estimates for lifetime and past-year kratom use nested within demographic and substance use factors. For complete model results, see **Table 5** and **Table 6**.

In these models, which duplicated as closely as possible the models we used for our online crowdsourced sample, increased likelihood of lifetime kratom use was associated with young age (OR = 2.25, 95% CI = 1.79, 2.83), male sex/gender (OR = 1.41, 95% CI = 1.14, 1.73), White race/ethnicity (OR = 2.41, 95% CI = 1.91, 3.03), being employed (OR = 1.22, 95% CI = 1.01, 1.48), lifetime nonmedical use of opioids (OR = 6.34, 95% CI = 5.19, 7.69), past-year drug dependence or abuse (OR = 3.17, 95% CI = 2.33, 4.33), and having ever received SUD treatment (OR = 2.07, 95% CI = 1.57, 2.71).

Findings for past-year kratom use differed slightly from those for lifetime use; past-year use was associated with young age (OR = 2.06, 95% CI = 1.59, 2.68), White race/ethnicity (OR = 2.21, 95% CI = 1.69, 2.88), being employed (OR = 1.52, 95% CI = 1.14, 2.02), lifetime nonmedical use of opioids (OR = 4.62, 95% CI = 3.36, 6.37), past-year drug dependence/abuse (OR = 2.99, 95% CI = 2.11, 4.24), and having ever received SUD treatment (OR = 2.02, 95% CI = 1.40, 2.90).

Compared to those residing in large metro counties, those in small metro counties had greater odds of both lifetime (OR = 1.32, 95% CI = 1.05, 1.66) and past-year (OR = 1.41, 95% CI = 1.03, 1.93) kratom use in NSDUH 2019 binomial models. Also, unlike in the convenience sample, we found greater odds of lifetime (OR = 3.10, 95% CI = 2.44, 3.93) and past-year kratom use (OR = 2.30, 95% CI = 1.59, 3.33) among men categorized as "White and at least middle class." For women categorized as "White and at least middle class," the association

TABLE 2 | Survey-weighted proportions of respondents with lifetime and past-year kratom use, National Survey on Drug Use and Health (NSDUH), 2019.

	Past-year kratom use		Lifetime kratom use	
	Proportion	**95% CI**	**Proportion**	**95% CI**
Sex/gender				
Female	0.006	[0.005, 0.007]	0.011	[0.010, 0.012]
Male	0.009	[0.008, 0.011]	0.019	[0.016, 0.021]
Race/Ethnicity				
Minority	0.004	[0.003, 0.004]	0.007	[0.006, 0.009]
White	0.009	[0.008, 0.011]	0.019	[0.018, 0.021]
Age				
Under 35	0.011	[0.009, 0.012]	0.022	[0.020, 0.012]
Over 35	0.005	[0.004, 0.006]	0.010	[0.009, 0.021]
Education				
Neither	0.005	[0.003, 0.008]	0.010	[0.007, 0.014]
High School Educated	0.007	[0.005, 0.008]	0.013	[0.011, 0.016]
College Educated	0.008	[0.007, 0.009]	0.017	[0.015, 0.019]
Employment				
Unemployed	0.005	[0.004, 0.006]	0.011	[0.010, 0.013]
Employed	0.009	[0.008, 0.010]	0.017	[0.016, 0.019]
Annual Income				
Below Poverty Line	0.007	[0.005, 0.009]	0.016	[0.013, 0.020]
Above Poverty Line	0.007	[0.006, 0.008]	0.014	[0.013, 0.016]
Rural-urban Continuum				
Rural Zip Code	0.008	[0.005, 0.013]	0.015	[0.011, 0.020]
Urban Zip Code	0.007	[0.006, 0.008]	0.015	[0.013, 0.016]
Lifetime Non-Med Opioid Use				
Yes	0.034	[0.028, 0.040]	0.078	[0.070, 0.086]
No	0.004	[0.004, 0.005]	0.008	[0.007, 0.009]
Past-year Drug Dependence/Abuse				
Yes	0.060	[0.046, 0.078]	0.134	[0.108, 0.165]
No	0.006	[0.005, 0.007]	0.012	[0.011, 0.013]
Lifetime SUD Treatment				
Yes	0.031	[0.024, 0.039]	0.068	[0.057, 0.081]
No	0.006	[0.005, 0.007]	0.011	[0.010, 0.012]
"White Middle-Class" indicator				
Male	0.020	[0.013, 0.029]	0.049	[0.040, 0.061]
Nonmale	0.014	[0.010, 0.019]	0.024	[0.017, 0.032]

was similar but weaker, for both lifetime (OR = 1.86, 95% CI = 1.32, 2.60) and past-year (OR = 2.05, 95% CI = 1.51, 2.79) kratom use.

DISCUSSION

We aimed to determine whether rurality was associated with lifetime or past-year kratom use while controlling for potentially confounding factors, and, at the same time, whether there was an emerging subpopulation of kratom users who we believe are increasingly being exposed to kratom-related media content, namely younger, White, educated, employed people. The use of two separate data sources helps increase confidence in our findings.

Rurality, Opioid-Related Harms, and Kratom Use

Though there is substantial survey evidence indicating that kratom is often used as a form of self-managed MOUD (Grundmann, 2017; Smith and Lawson, 2017; Coe et al., 2019; Garcia-Romeu et al., 2020)—for which the need might be greatest in rural communities—we did not find a significant association between past-year rural residence and kratom use. We did, however, find that residency in a small metro (suburban) county was associated with a 32% greater likelihood of lifetime kratom use and a 41% greater likelihood of past-year kratom use. Our ability to detect an association between rurality and kratom use may have been hindered by rural/urban classification in the NSDUH data and the relatively small proportion of rural-residing respondents in our online convenience sample. The NSDUH dataset provides only three levels of rural-urban classification: large metro, small metro, and non-metro. We can only conclude that non-metro residents do not display increased odds of kratom use compared with those living in metro counties, and cannot attest to varying degrees of rurality in comparison to the varying degrees of metropolitan size and their association (or lack thereof) with kratom use. Previous investigations suggest that opioid-related harms are relatively greater, and subsequent public health policy response is relatively slower, in the most remote US counties (Thomas et al., 2020; Andrilla and Patterson, 2021). Thus, it is important

TABLE 3 | Multiple logistic regression models using online crowdsourced data to examine the relationship between county residence and kratom use while controlling for demographic and substance use factors.

Lifetime kratom use - mTurk	OR	95% CI		Z	p	VIF
		Lower	Upper			
Intercept	0.02	0.00	0.03	-17.77	<0.001	
Young Age (<35)	**1.64**	1.24	2.16	3.52	<**0.001**	1.03
Sex/gender (Male – Nonmale)	**1.79**	1.37	2.34	4.24	<**0.001**	1.02
Race (White - US Minority)	0.81	0.60	1.09	-1.39	0.16	1.01
Education (Highschool - College)	**1.39**	1.04	1.87	2.20	**0.03**	1.16
Employed (Unemployed - Employed)	1.10	0.79	1.54	0.55	0.58	1.16
Below Poverty Line Annual Income	1.01	0.72	1.40	0.03	0.98	1.20
Rural - Urban Continuum						
Non-metro - Large Metro	1.23	0.95	1.59	1.57	0.12	1.05
Small Metro - Large Metro	1.04	0.82	1.32	0.32	0.75	1.05
Lifetime Non-Medical Opioid Use	**5.13**	3.80	6.94	10.62	<**0.001**	1.17
Moderate to Severe SUD	**2.00**	1.49	2.68	4.61	<**0.001**	1.17
Lifetime SUD Treatment	**1.53**	1.09	2.14	2.47	**0.01**	1.16
Past-Year Kratom Use - mTurk	**OR**	**95% CI**		**Z**	**p**	**VIF**
		Lower	**Upper**			
Intercept	0.02	0.00	0.03	-15.97	<0.001	
Young Age (<35)	1.37	0.98	1.90	1.84	0.07	1.03
Sex/gender (Male - Nonmale)	**1.47**	1.06	2.03	2.29	**0.02**	1.03
Race (White - US Minority)	0.77	0.54	1.10	-1.42	0.15	1.01
Education (Highschool - College)	**1.44**	1.00	2.08	1.97	**0.05**	1.17
Employed (Unemployed - Employed)	1.30	0.88	1.93	1.31	0.19	1.17
Below Poverty Line Annual Income	0.92	0.61	1.37	-0.42	0.67	1.20
Rural - Urban Continuum						
Non-metro - Large Metro	1.26	0.93	1.72	1.51	0.13	1.05
Small Metro - Large Metro	1.08	0.80	1.44	0.49	0.62	1.05
Lifetime Non-Medical Opioid Use	**5.22**	3.56	7.66	8.46	<**0.001**	1.17
Moderate to Severe SUD	**1.94**	1.34	2.79	3.54	<**0.001**	1.18
Lifetime SUD Treatment	1.20	0.80	1.79	0.87	0.39	1.16

$\chi^2(11) = 331.38$; Pseudo-R^2, 0.24; p = *<0.01; AIC, 1,510.53.* $\chi^2(11) = 192.74$; *Pseudo-R^2, 0.18;* p = *<0.01; AIC, 1,110.48. Statistically significant explanatory variables are denoted by bolded text.*

for future work in this realm to distinguish between the most rural counties and those closer to metropolitan areas. Here, we approached an approximation in measurement, but more granular work will be needed as kratom products continue to be uniquely branded, marketed, and sold in the US.

Kratom Use and the White Middle Class

In our online convenience sample, we observed significantly greater odds of lifetime kratom use among White men, and greater odds of past-year kratom use among men only. When examining combined factor(s) through which we operationalized "White and middle-class," we found that men in the White, middle-class group were nearly twice as likely to report lifetime kratom use as other men. Further, we observed much stronger and consistent associations between this indicator and both lifetime and past-year kratom use in NSDUH data. Lifetime kratom use was 3.10 times more likely to be reported by White, middle-class men and 1.86 times as likely to be reported by White, middle-class women. With respect to past-year kratom use, White, middle-class men and women were 2.30 times and 2.05 times as likely to report use, respectively. We suspect that these kratom users are not only of people with SUD histories, but also people who represent far more socially normative substance-use sub-groups who are using kratom for wellness purposes or enhancement (e.g., to boost cognitive and physical performance), as these motivations have been expressed by kratom-using people in prior investigations (Smith et al., 2021a; Smith et al., 2021b). Given the cross-sectional nature of these data and that these analyses are the first to use a "White, middle-class" indicator variable to represent a specific demographic of kratom users, we cannot claim that kratom use is increasing among this demographic. Rather, we can only assert that kratom use prevalence is significantly higher with this demographic intersection, seemingly among those with suburban residence, when compared with the rest of the US population (using NSDUH data), or when compared to other survey respondents with normative and illicit substance use (in our crowdsourced convenience sample). That kratom use has been associated with similar "middle class" attributes in kratom-specific online surveys in the US suggests that at least a sizeable proportion of people using kratom can be characterized in this way, even though we do not dismiss the heterogeneity that likely exists within this group. For instance, there are people who use kratom to address anxiety, chronic pain, fatigue, or SUD who are also among such a demographic group, but this does not suggest that

TABLE 4 | Multiple logistic regression models using online crowdsourced data to examine the relationship between a "white middle-class" indicator and kratom use while controlling for substance use factors.

Lifetime kratom use - mTurk	OR	95% CI		Z	p	VIF
		Lower	Upper			
Intercept	0.03	0.03	0.04	-24.09	<0.001	
White Middle-Class indicator						
Male	**1.94**	1.33	2.82	3.45	**<0.001**	1.03
Nonmale	0.89	0.58	1.36	-0.54	0.59	1.03
Rural - Urban Continuum						
Non-metro - Large Metro	1.25	0.97	1.60	1.72	0.09	1.02
Small Metro - Large Metro	1.05	0.83	1.33	0.40	0.69	1.02
Lifetime Non-Medical Opioid Use	**5.41**	4.02	7.28	11.16	**<0.001**	1.14
Moderate to Severe SUD	**2.22**	1.66	2.96	5.41	**<0.001**	1.14
Lifetime SUD Treatment	**1.56**	1.12	2.18	2.66	**0.01**	1.14
Past-year Kratom Use - mTurk	**OR**	**95% CI**		**Z**	**p**	**VIF**
		Lower	**Upper**			
Intercept	0.02	0.01	0.03	-21.77	<0.001	
White Middle-Class indicator						
Male	1.45	0.90	2.32	1.54	0.12	1.02
Nonmale	0.83	0.49	1.40	-0.71	0.48	1.02
Rural - Urban Continuum						
Non-metro - Large Metro	1.30	0.96	1.75	1.70	0.09	1.02
Small Metro - Large Metro	1.09	0.81	1.45	0.56	0.58	1.02
Lifetime Non-Medical Opioid Use	**5.56**	3.81	8.11	8.91	**<0.001**	1.15
Moderate to Severe SUD	**2.13**	1.49	3.05	4.12	**<0.001**	1.15
Lifetime SUD Treatment	1.24	0.83	1.84	1.06	0.29	1.13

$\chi^2(7) = 179.39$; Pseudo-R^2, 0.17; p = *<0.01;* AIC, *1,115.83.* *$\chi^2(7) = 304.80$; Pseudo-R^2, 0.22;* p = *<0.01;* AIC, *1,529.11. Statistically significant explanatory variables are denoted by bolded text.*

there are no other motivations within this demographic groups, or other demographic groups for whom kratom use will become more prevalent for these or other reasons. With respect to the former, the lack of greater understanding of heterogeneity of kratom-using people may be an artifact of the questions that have been typically asked in surveys.

Does it all Just Come Back to Opioids?

The kratom narrative in the US is increasingly framed as part of a broader opioid narrative. Here, while we see greater rates of lifetime and past-year kratom use among people we have conceptualized as a "White, suburban middle class," these analyses still provide strong support for the association between kratom use and nonmedical use of prescribed or illicit opioids. Both our convenience sample models and our nationally representative models indicate that people who have ever used opioids nonmedically display five times greater likelihood of having used kratom in the past year. We also saw a smaller but still sizeable set of associations between past-year kratom use and moderate to severe SUD, past-year drug dependence/abuse, and having ever received SUD treatment.

Again, the cross-sectional nature of our data and that of others who have found similar associations (Grundmann et al., 2021; Palamar, 2021; Schimmel et al., 2021) prevent us from speculating as to which preceded the other. Currently, kratom use has yet to predict incident SUD at later time points (except for the logical inevitability of its having to precede kratom use disorder, a diagnostic entity that is not yet formally recognized but has been documented by our group and others). Because kratom is often used by people to mitigate or reduce symptoms of OUD or other SUDs, including withdrawal, we know that at least some portion of people initiating kratom use are doing so only after initiating nonmedical use of opioids.

Importance of Sampling to Current and Future Kratom Research

As noted above, though we have found some evidence of greater kratom use among people that we operationalize as being "White and middle class" and who reside in small metro ("suburban") areas in the US, kratom-using people remain a heterogenous group in terms of motivation(s) for use (which may be shifting and which are likely dynamic) and substance use history/experience. The differences we observed in comparing results from our two data sources highlight the importance of improving how we study kratom use and the people who use it. This includes purposeful sampling, improved survey methods (and survey question wording), investing in longitudinal study designs, and adopting real-time ambulatory assessment where possible. All of these can help to produce a more complete picture than currently exists. Moreover, there is a need for ongoing assessment of the kratom market and changes in the US commercial kratom industry (which we believe will increase and become more diverse in terms of what consumer groups are targeted with unique kratom product branding). In our online convenience sample, which contained a much greater proportion of people using stimulant and/or opioid drugs

TABLE 5 | Survey-weighted multiple logistic regression models using nationally representative NSDUH 2019 data to examine the relationship between county residence and kratom use while controlling for demographic and substance use factors.

Lifetime kratom use - NSDUH	**OR**	**95% CI**		**t**	**p**	**VIF**
		Lower	**Upper**			
Intercept	0.00	0.00	0.00	-25.17	<0.001	
Young age (< 35 years)	**2.25**	1.79	2.83	6.93	<**0.001**	1.33
Sex/gender (Male - Female)	**1.41**	1.14	1.73	3.24	<**0.001**	1.44
Race/ethnicity (White - US Minority)	**2.41**	1.91	3.03	7.49	<**0.001**	2.07
Education						
Highschool Grad - Not Highschool Grad	1.20	0.77	1.86	0.80	0.43	1.65
College Grad - Not Highschool Grad	1.55	1.00	2.40	1.94	0.06	1.65
Employment (Employed - Unemployed)	**1.22**	1.01	1.48	2.08	**0.04**	1.24
Below Poverty Line Annual Income	1.19	0.93	1.52	1.41	0.17	1.57
Rural - Urban Continuum						
Non-metro - Large Metro	1.11	0.79	1.56	0.59	0.56	2.41
Small Metro - Large Metro	**1.32**	1.05	1.66	2.33	**0.03**	2.41
Lifetime Non-Medical Opioid Use	**6.32**	5.19	7.69	18.40	<**0.001**	1.50
Past Year Drug Dependence/Abuse	**3.17**	2.33	4.33	7.31	<**0.001**	1.46
Lifetime SUD Treatment	**2.07**	1.57	2.71	5.21	<**0.001**	1.96
Past-year Kratom Use - NSDUH	**OR**	**95% CI**		**t**	**p**	**VIF**
		Lower	**Upper**			
Intercept	0.00	0.00	0.00	-21.19	0.00	
Young age (< 35 years)	**2.06**	1.59	2.68	5.40	<**0.001**	1.92
Sex/gender (Male - Female)	1.29	0.95	1.76	1.64	0.11	1.80
Race/ethnicity (White - US Minority)	**2.21**	1.69	2.88	5.85	<**0.001**	1.28
Education						
Highschool Grad - Not Highschool Grad	1.15	0.66	2.02	0.50	0.62	3.36
College Grad - Not Highschool Grad	1.35	0.79	2.28	1.10	0.28	3.36
Employment (Employed - Unemployed)	**1.52**	1.14	2.02	2.88	**0.01**	2.06
Below Poverty Line Annual Income	1.01	0.69	1.49	0.06	0.95	2.04
Rural - Urban Continuum						
Non-metro - Large Metro	1.26	0.74	2.16	0.86	0.40	3.95
Small Metro - Large Metro	**1.41**	1.03	1.93	2.17	**0.04**	3.95
Lifetime Non-Medical Opioid Use	**4.62**	3.36	6.37	9.40	<**0.001**	2.01
Past Year Drug Dependence/Abuse	**2.99**	2.11	4.24	6.17	<**0.001**	1.57
Lifetime SUD Treatment	**2.02**	1.40	2.90	3.77	<**0.001**	2.16

Pseudo R^2 *= 0.12;* p = *<0.01; AIC = 3,922.74; Est. Dispersion Parameter = 0.99. Pseudo* R^2 *= 0.17;* p = *<0.01; AIC = 6,744.25; Est. Dispersion Parameter = 0.98. Statistically significant explanatory variables are denoted by bolded text.*

than in the US population, we saw that substance use factors (opioid use, SUD, treatment) were the most important variable for explaining the incidence of kratom use. It may be that for such groups, kratom will continue to be marketed in terms of its potential for harm reduction as a form self-managed MOUD. Likewise, when analyzing data representative of the greater US population, we observed greater associations with demographic factors (i.e., the "White middle class" factor) that, when examined in the convenience sample models, did not contribute significantly. It may be that for this group, kratom will come to be marketed as a wellness or energy-enhancing supplement that is specific to boosting performance (e.g., pre-workout, "nootropic"). These and other subgroups are likely to be identified as research continues. In the interim, continuing methods such as the ones we used here, wherein we analyzed data from two unique survey sources, have clear benefits: our convenience sample provided us with greater insight into the nuances that may exist among people who use illicit substances, whereas the NSDUH sample provided greater insight into substance use phenomena among the US population as a whole. Ultimately both converged to suggest that kratom use is, for now, a mostly middle-class and suburban phenomenon with possibly greater prevalence among men. However, given kratom's relative novelty in the US, this is subject to change, making continued assessment critical.

LIMITATIONS

Findings from the analyses here should be interpreted with several limitations in mind, including the cross-sectional nature of the data collection for both our crowdsourced convenience sample using mTurk and the NSDUH survey. While NSDUH 2019 data are considered representative for approximately 97% of US residents, the crowdsourced convenience sample contains greater proportions of White people and people earning more than $50,000 USD than the US population, which could hinder the results' generalizability to people from US minority communities. Each may limit generalizability to the larger kratom-using community in the

TABLE 6 | Survey-weighted multiple logistic regression models using nationally representative NSDUH 2019 to examine the relationship between a "white middle-class" indicator and kratom use while controlling for substance use factors.

Lifetime kratom use - NSDUH	OR	95% CI		t	p	VIF
		Lower	Upper			
Intercept	0.01	0.00	0.01	-55.52	<0.001	
White Middle-Class indicator						
Male	**3.10**	2.44	3.93	9.31	<**0.001**	1.11
Female	**1.86**	1.32	2.60	3.57	<**0.001**	1.37
Rural - Urban Continuum						
Non-metro - Large Metro	1.22	0.89	1.69	1.22	0.23	1.24
Small Metro - Large Metro	**1.41**	1.12	1.77	2.94	<**0.001**	1.24
Lifetime Non-Medical Opioid Use	**6.77**	5.53	8.29	18.56	<**0.001**	1.45
Past Year Drug Dependence/Abuse	**3.50**	2.56	4.79	7.83	<**0.001**	1.60
Lifetime SUD Treatment	**2.11**	1.61	2.75	5.48	<**0.001**	1.64
Past-year Kratom Use - NSDUH	**OR**	**95% CI**		**t**	**p**	**VIF**
		Lower	**Upper**			
Intercept	0.00	0.00	0.00	–49.11	<0.001	
White Middle-Class indicator						
Male	**2.30**	1.59	3.33	4.41	<**0.001**	1.05
Female	**2.05**	1.51	2.79	4.61	<**0.001**	1.10
Rural - Urban Continuum						
Non-metro - Large Metro	1.36	0.82	2.28	1.19	0.24	1.37
Small Metro - Large Metro	**1.49**	1.09	2.04	2.48	**0.02**	1.37
Lifetime Non-Medical Opioid Use	**5.03**	3.66	6.93	9.91	<**0.001**	1.61
Past Year Drug Dependence/Abuse	**3.27**	2.30	4.64	6.63	<**0.001**	1.37
Lifetime SUD Treatment	**2.01**	1.39	2.90	3.73	<**0.001**	1.54

Pseudo-R^2 = 0.16; p = <0.01; AIC = 6,945.21; Est. Dispersion Parameter = 0.95. Pseudo-R^2 = 0.16; p = <0.01; AIC = 6,945.21; Est. Dispersion Parameter = 0.95. Statistically significant explanatory variables are denoted by bolded text.

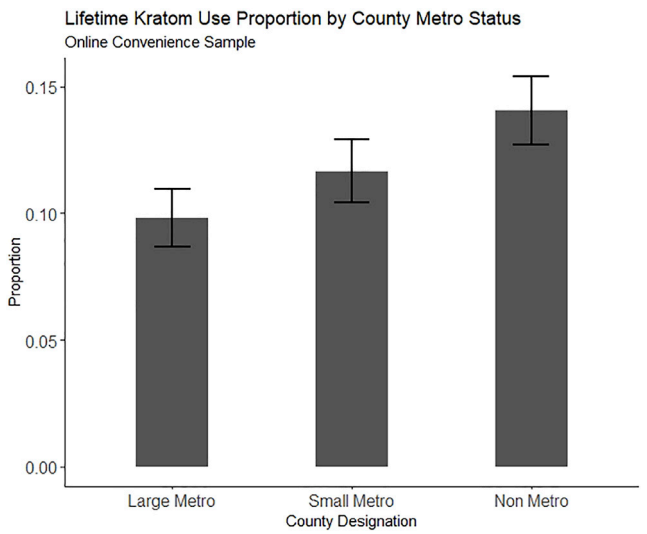

FIGURE 1 | Proportion of people reporting lifetime kratom use in an online convenience sample (N = 2,615), split by county metro status. Error bars represent the estimate 95% confidence interval.

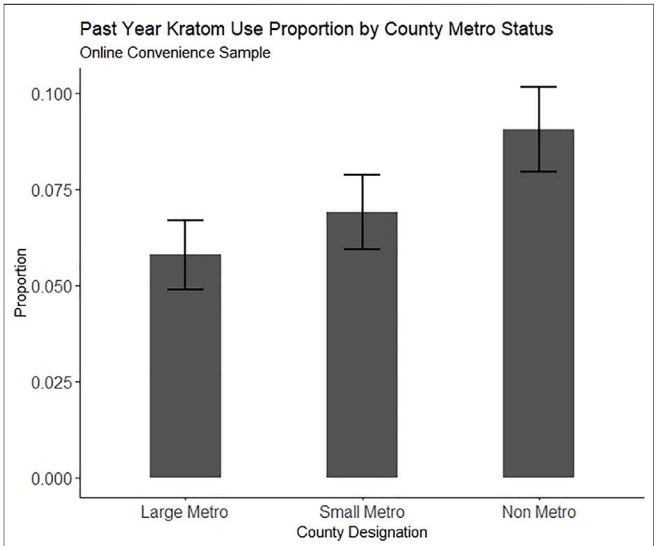

FIGURE 2 | Proportion of people reporting past-year kratom use in an online convenience sample (N = 2,615), split by county metro status. Error bars represent the estimate 95% confidence interval.

US: mTurk represents only one platform for crowdsourcing (and our sampling strategy included only people who reported past-6 months alcohol or past 6 months opioid and/or psychostimulant use), and the NSDUH may not have captured respondents who had used kratom, given the survey item wording (which does not reflect all kratom slang names nor all kratom product types), nor as noted earlier, people who were institutionalized, homeless, or in military service.

Our use of similar data collected from two separate sources is, in some respects, a limitation, in that survey item order and, most

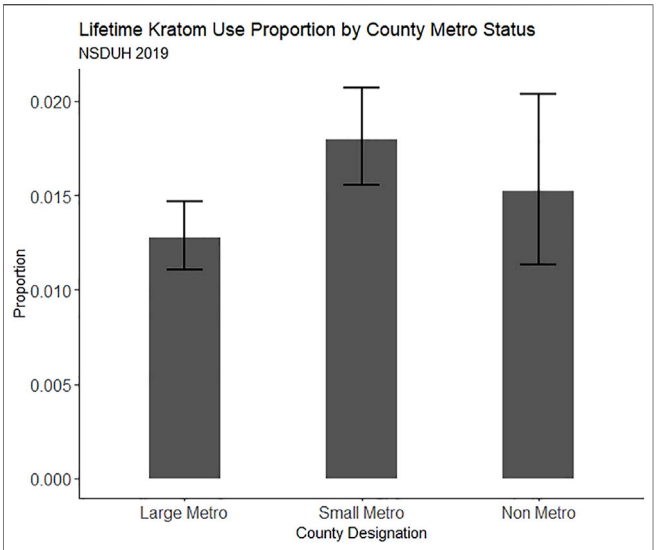

FIGURE 3 | Proportion of people reporting lifetime kratom use in NSDUH 2019, split by county metro status. Error bars represent the estimate 95% confidence interval.

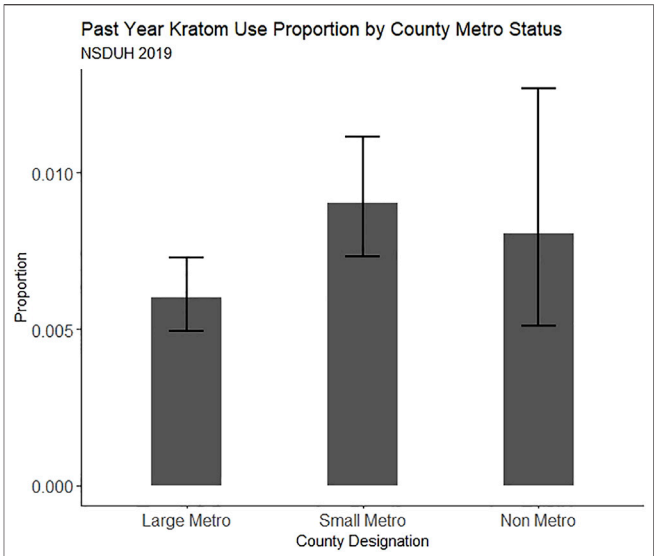

FIGURE 4 | Proportion of people reporting past-year kratom use in NSDUH 2019, split by county metro status. Error bars represent the estimate 95% confidence interval.

importantly, phrasing was different. This means that while we examined variables that were similar, they were not measured or collected using identical methods. We believe that the items selected and/or re-coded, including the composite variable created for the "White middle class" factor, were nonetheless meaningfully similar indicators (or were merely identical indicators for such things as age cut-offs). It may be that differences in methodology between the two surveys are more a strength than a limitation, helping to address what has been called a "generalizability crisis" in behavioral research (Yarkoni, 2020).

Perhaps the greatest limitation of the findings presented here is also among the greatest limitations to all self-report kratom survey research: we ultimately cannot be sure what respondents meant when they reported having used "kratom," as this term could have meant many different things practically and experientially: alkaloid content varying among specific types of kratom products (e.g., different leaves were used to make different batches of a product at different times by different distributors or vendors), variation between kratom products (e.g., extracts, loose leaf, pulverized plant matter), and different route of administration or dosing (e.g., slowly sipping tea versus consuming one kratom shot versus ingesting prepared capsules). A related limitation is that motivations for kratom use among respondents were not measured, leaving us only to speculate based on prior literature and changes in kratom product marketing.

FUTURE DIRECTIONS

All nationally representative surveys designed to assess substance use prevalence should now assess for kratom use. Unlike synthetic novel psychoactive substances, kratom remains largely legal in the US and has not resulted in widespread reports of misuse or toxicity, relative to novel synthetics or even traditional illicit drugs (e.g., heroin, cocaine). This means that there remains the potential for kratom to become adopted into US culture with less stigma than other emerging psychoactive substances, possibly facilitating the perception of kratom to be more like cannabis, alcohol, or caffeine—substances that may produce psychological or physical dependence, but which are perceived by some users to be mostly compatible with or even helpful to improving the quality of everyday life (e.g., work, recreation; Smith et al., 2021b; Smith et al., 2021d). Although we cannot be certain, there are no clear indicators that kratom use is decreasing in the US. Rather, it seems that kratom products are poised to be used by a more diverse group, in that kratom is now being framed not only a self-treatment for psychiatric or SUD symptoms, but as a means for enhancing mood, performance, and recreation. Given the unknown current and future prevalence of kratom use, and the limitations of any single survey method, we recommend that future survey data, wherever possible, be analyzed with data collected by similar, but still distinct, methods in order to increase the ability to detect similar (or dissimilar) findings of public health significance. While we cannot claim that any finding from one of the datasets used here validated another in the strictest sense, we do believe that when methods such as these are repeated often enough there will be clear signals that are apparent and which can be followed up on using more precise methods, which are desperately needed in this nascent body of research.

DATA AVAILABILITY STATEMENT

The R syntax and datasets generated and analyzed from the NSDUH 2019 dataset for this study can be found on Open Science Framework at DOI: 10.17605/OSF.IO/M7DW4 [https://osf.io/m7dw4/].

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the National Institute on Drug Abuse IRB. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KS and DE conceived of and designed the original online crowdsourced survey. JR implemented the survey protocol, collected all data, and conducted statistical analyses. JR took the lead in writing this manuscript with significant contributions from JS, KS, and DE. JS conceived the idea to include nationally representative data and analyses from NSDUH 2019, contributed to statistical inference, and provided critical feedback.

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REVIEW
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Kratom Abuse Potential 2021: An Updated Eight Factor Analysis

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Drugs are regulated in the United States (US) by the Controlled Substances Act (CSA) if assessment of their abuse potential, including public health risks, show such control is warranted. An evaluation via the 8 factors of the CSA provides the comprehensive assessment required for permanent listing of new chemical entities and previously uncontrolled substances. Such an assessment was published for two kratom alkaloids in 2018 that the Food and Drug Administration (FDA) have identified as candidates for CSA listing: mitragynine (MG) and 7-hydroxymitragynine (7-OH-MG) (Henningfield et al., 2018a). That assessment concluded the abuse potential of MG was within the range of many other uncontrolled substances, that there was not evidence of an imminent risk to public health, and that a Schedule I listing (the only option for substances that are not FDA approved for therapeutic use such as kratom) carried public health risks including drug overdoses by people using kratom to abstain from opioids. The purpose of this review is to provide an updated abuse potential assessment reviewing greater than 100 studies published since January 1, 2018. These include studies of abuse potential and physical dependence/withdrawal in animals; *in-vitro* receptor binding; assessments of potential efficacy treating pain and substance use disorders; pharmacokinetic/pharmacodynamic studies with safety-related findings; clinical studies of long-term users with various physiological endpoints; and surveys of patterns and reasons for use and associated effects including dependence and withdrawal. Findings from these studies suggest that public health is better served by assuring continued access to kratom products by consumers and researchers. Currently, Kratom alkaloids and derivatives are in development as safer and/or more effective medicines for treating pain, substances use disorders, and mood disorders. Placing kratom in the CSA via scheduling would criminalize consumers and possession, seriously impede research, and can be predicted to have serious adverse public health consequences, including potentially thousands of drug overdose deaths. Therefore, CSA listing is not recommended. Regulation to minimize risks of contaminated, adulterated, and inappropriately marketed products is recommended.

Keywords: dietary supplement, safety, abuse potential, epidemiology, substance use disorder treatment, opioid pharmacology, Controlled Substances Act

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1 INTRODUCTION

This is an update to the Henningfield et al. (2018) assessment of the abuse potential of kratom based on the eight factors of the United States Controlled Substances Act (US CSA) (Henningfield et al., 2018a) and summarizes new scientific findings from January 2018 through August 2021. The CSA eight factors evaluate pharmacological actions in the brain or central nervous system (CNS) that may lead to dependence, substance use disorders, or addictions (American Psychiatric Association, 2013; National Institute on Drug Abuse, 2019; World Health Organization, 1994; O'Brien et al., 2011). Abuse potential assessments determine whether substances and medicinal products should be controlled by the CSA (scheduled), and if so the restrictiveness or level of control. Substances are only placed in Schedule I (heroin, LSD, cannabis) when there is no FDA approved therapeutic use and sufficient abuse potential to merit control. Substances with approved therapeutic uses and sufficient abuse potential must be placed in Schedules II–V. By law, an eight-factor analysis (8-FA) provides the primary pharmacological and public health basis for drug scheduling (Food and Drug Administration, 2017a; Belouin and Henningfield, 2018; Johnson et al., 2018). This assessment focuses on kratom and its alkaloids, in particular mitragynine (MG), the primary alkaloid in kratom present in sufficient amounts to account for its effects.

Kratom and its alkaloids are not approved for any therapeutic use by the FDA, are not federally controlled in the US, nor in the International Drug Control Conventions; however some countries do control kratom and/or its two primary alkaloids, MG and 7-OH-MG (Prozialeck et al., 2019; International Narcotics C, 2020a; International Narcotics C, 2020b). Six states in the US (Alabama, Arkansas, Indiana, Tennessee, Vermont and Wisconsin) have banned kratom, while five have passed consumer protection legislation to ensure consumer access to kratom with a framework for regulatory oversight (Arizona, Georgia, Nevada, Oklahoma and Utah). Maryland rejected a proposed ban and passed a minimum age of purchase law (age 21), and at this writing, several states are considering their own kratom consumer protection laws to ensure consumer access but with regulatory oversight. In 2021, Thailand decriminalized kratom farming, possession and sales. In December, 2021, the World Health Organization Expert Committee on Drug Dependence concluded "there is insufficient evidence to recommend a critical review of kratom mitragynine and 7-hydroxymitragynine" [for potential scheduling] but should be kept under surveillance (Commission on Narcotic Drugs, 2021).

In August 2016 the US Drug Enforcement Agency (DEA) proposed scheduling kratom on a temporary "emergency" basis but withdrew the proposal due to thousands of comments from kratom consumers and bipartisan members of Congress, and out of concern that people who were managing their opioid use disorder with the aid of kratom would return to opioid use. The DEA requested that FDA perform a full 8-FA and develop its own independent recommendations related to scheduling (Ingraham, 2016a; Ingraham, 2016b). Subsequently, Dr. Henningfield and his colleagues at PinneyAssociates were commissioned by the American Kratom Association's legal regulatory counsel to develop an 8-FA (Pinney Associates (2016)) for submission to DEA by December 2, 2016. In November 2017, FDA Commissioner Scott Gottlieb announced that kratom carried "narcotic like" risks of addiction and death but did not make public its October 17th recommendation to DEA to permanently place MG and 7-OH-MG in Schedule I of the CSA (Food and Drug Administration, 2017b; Food and Drug Administration, 2017c).

DEA typically responds to formal 8-FA scheduling requests within 90 days, though there is no legal timeline; however, a formal scheduling rescission order was issued on August 18, 2018 from the Assistant Secretary of Health, US Department of Health and Human Services (DHHS) (Giroir, 2018). The order included conclusions based on a DHHS review consistent with those of the Henningfield et al. (2018) 8-FA (Henningfield et al., 2018a). The DHHS rescission letter stated "mitragynine does not satisfy the first of the three statutory requisites for Schedule I"; "There is still debate among reputable scientists over whether kratom by itself is associated with fatal overdoses"; and "there is a significant risk of immediate adverse public health consequences for potentially millions of users if kratom or its components are included in Schedule I." The letter also raised concerns about "the stifling effect of classification in Schedule l on critical research needed on the complex and potentially useful chemistry of components of kratom." This letter was not made public until January 2021.

In 2017, the National Institute on Drug Abuse (NIDA) substantially increased its active research program on kratom's alkaloids and derivatives as potentially safer and less abusable medicines for pain and addiction and other disorders. The purpose of this review is to provide an update of our 2018 article on the abuse potential of kratom. It includes more than 100 new studies related to kratom abuse potential, safety, patterns of use, and potential therapeutic and public health benefits.

2 METHODS

The intent was to include all new studies published in English relevant to kratom abuse potential, safety and mechanisms of action published in since January 1, 2018 with some essential earlier studies mentioned and referenced to our 2018 review.[1] This was by comprehensive online literature searches, and direct requests to leading kratom researchers worldwide. To be concise, factors 4, 5, and 6 are considered a single group of public health related factors.[2] (Henningfield et al., 2018a; Johnson et al., 2018). Factor 8 is unchanged as neither kratom nor its constituents are scheduled.

[1]The authors welcome communications from readers on abuse-potential and safety related kratom research published since 2018 that we might have missed.

[2]For formal FDA submissions Factors 4, 5, and 6 are considered separately (see Henningfield et al., 2018a and Johnson, Griffiths, Hendricks and Henningfield, 2018 as examples), however, for temporary (also known as "emergency") scheduling, determining if a substance poses an imminent health risk is based on the analysis of all three factors combined similarly to our approach in this review.

3 RESULTS

3.1 Factor 1: Actual or Relative Potential for Abuse

A summary of the references used, along with main findings and comments from the authors of this review are included in **Table 1**.

3.1.1 Summary of 2018 Findings

There were no animal intravenous drug self-administration (IV DSA), intracranial self-stimulation (ICSS) brain reward, or physical dependence/withdrawal studies of kratom's alkaloids; however, other data suggested relatively low abuse potential as compared to opioids and other drugs of abuse (Henningfield et al., 2018a). There was evidence of morphine opioid receptor (MOR) mediated effects, and preliminary drug discrimination and conditioned place preference (CPP) studies with rats suggested abuse related effects at high intolerable human dose equivalents.

Survey data from the US and field studies in Southeast Asia (SEA) showed most kratom use was for health-related benefits, and to facilitate occupational performance. Data indicated that problem abuse and addiction were not common and was generally more tolerable and readily self-manageable as compared to opioids. A frequent reason for use was as an opioid substitute for pain and self-management of opioid, alcohol, and other drug dependence.

3.1.2 Factor 1 Science Updates

3.1.2.1 Intravenous Drug Self-Administration Trials

Rates of MG self-administration were similar to those of saline, and MG pretreatment produced dose-related reductions in morphine self-administration rates (Hemby et al., 2019). The authors concluded "The present findings indicate that MG does not have abuse potential and reduces morphine intake, desired characteristics of candidate pharmacotherapies for opiate addiction and withdrawal … ". 7-OH-MG was self-administered at high doses and pretreatment increased morphine self-administration.

MG self-administration rates in rats did not exceed those obtained with saline and MG pretreatment decreased heroin self-administration, with little effect on methamphetamine self-administration (Yue et al., 2018). The authors noted "These results suggest limited abuse liability of mitragynine and the potential for mitragynine treatment to specifically reduce opioid abuse. With the current prevalence of opioid abuse and misuse, it appears currently that mitragynine is deserving of more extensive exploration for its development or that of an analog as a medical treatment for opioid abuse." These results are consistent with human reports that kratom is useful in the management of opioid craving and withdrawal and to support opioid abstinence (Grundmann et al., 2018; Coe et al., 2019; Prozialeck et al., 2019; Garcia-Romeu et al., 2020).

Intracranial Self-Stimulation

In the ICSS model, rats equipped with brain electrodes self-deliver rewarding electrical brain stimulation. Opioids, amphetamine-like stimulants, cocaine, and other classic drugs of abuse reduce the stimulation threshold and increase the strength of the rewarding effects of drugs on ICSS (Negus and Miller, 2014). Neither MG nor 7-OH-MG showed evidence of brain rewarding effects, whereas morphine robustly and dose-dependently decreased the stimulation threshold (Behnood-Rod et al., 2020). Thus, the ICSS results suggest lower brain rewarding effects of MG as compared to morphine.

Drug Discrimination Studies

The discriminative stimulus effects of MG were evaluated in studies designed to assess generalization to morphine as well as the delta-opioid receptor agonist SNC80 and kappa-opioid receptor agonist U69593, alpha adrenergic agonists lofexidine, clonidine and phenylephrine, alpha adrenergic antagonists yohimbine and atipamezole, and the cannabinoid agonist Δ-9-tetrahydrocannabinol (Reeve et al., 2020). The strongest generalization was to lofexidine and phenylephrine, both unscheduled drugs: phenylephrine is in some over-the-counter cold medicines; lofexidine is approved for several indications including the first nonopioid for alleviating opioid withdrawal.

In a comparison of MG and 7-OH-MG across studies that included *in vitro* receptor binding and an antinociception test, MG partially generalized to morphine, whereas 7-OH-MG fully generalized to morphine in rats (Obeng et al., 2021). Similarly, Hiranita et al. (2020) found only partial generalization of oral MG to i.p. morphine in rats (Hiranita et al., 2020).

3.1.2.4 Conditioned Place Preference

Various MG preparations produced mixed CPP effects with some suggesting abuse potential at high doses. A low priming injection of MG or morphine reinstated CPP after establishment with either drug, suggesting rewarding effects for both (Japarin et al., 2021). Baclofen pretreatment prevented the acquisition and expression of MG-induced CPP (Yusoff et al., 2018). CPP was achieved in mice with a high dose methanolic extract of kratom leaves (Vijeepallam et al., 2019). In a fourth study (see also Factor 2), lyophilized (freeze-dried) kratom tea (LKT), a potential treatment for pain and opioid dependence, did not induce CPP in mice (Wilson et al., 2020).

3.1.2.5 Physical Dependence and Withdrawal

Discontinuation of morphine administration produced response rate disruptions indicating significant signs of spontaneous withdrawal, whereas discontinuation of MG administration did not produce significant signs of spontaneous withdrawal. Naloxone administration did precipitate response rate disruptions indicating withdrawal in both MG and morphine treated rats, however, this withdrawal effect was weaker and shorter lived in MG treated rats as compared to morphine treated rats (Harun et al., 2020). MG treatment also reduced naloxone precipitated withdrawal in animals receiving chronic morphine, consistent with human reports. Hassan, Pike, See, Sreenlivasan et al. (2020) compared the efficacy of MG to methadone for treating morphine withdrawal in rats concluding that MG treatment attenuated withdrawal symptoms significantly, similar to methadone and buprenorphine, and potentially with less undesired effects (Hassan et al., 2020).

TABLE 1 | Summary of references.

Factor/Description	Citations	Main findings	Comments
Factor 1: Actual or relative potential for abuse			
Intravenous Self-Administration (IV SA)	(Prozialeck et al., 2019), (Grundmann et al., 2018; Yue et al., 2018; Coe et al., 2019; Hemby et al., 2019; Garcia-Romeu et al., 2020)	No evidence of reward	MG pretreatment reduced morphine self-administration
Intracranial Self-Stimulation (ICSS)	(Negus and Miller, 2014)-(Behnood-Rod et al., 2020)	No evidence of reward for MG or 7-OH-MG	
Drug Discrimination	(Hiranita et al., 2020; Reeve et al., 2020; Obeng et al., 2021)	MG showed partial generalization to multiple drugs, including morphine 7-OH-MG showed full generalization to morphine	Strongest generalization of MG was to unscheduled drugs: phenylephrine and lofexidine
Conditioned Place Preference (CPP)	(Yusoff et al., 2018; Vijeepallam et al., 2019; Wilson et al., 2020; Japarin et al., 2021)	Mixed evidence of CPP	
Physical Dependence/ Withdrawal	(Harun et al., 2020; Hassan et al., 2020; Johari et al., 2021; Hassan et al., 2021; Hassan et al., 20211778; Harun et al., 2021a)	Mixed evidence of weak withdrawal across studies relative to morphine	MG reduces morphine withdrawal and differs from morphine withdrawal on some measures
Survey Data	(Prozialeck et al., 2019), (Grundmann et al., 2018; Coe et al., 2019; Garcia-Romeu et al., 2020), (Singh et al., 2014; Galbis-Reig, 2016; Swogger and Walsh, 2018; Smith et al., 2019; Harun et al., 2021b)	Majority use is for health benefits, not recreational use or to get high. Use is almost exclusively oral, without the tendency of many recreational substance to smoke, inject, and/or nasally insufflate	Most people reporting "kratom addiction" found it weaker and more tolerable and acceptable than "drug" addiction and were more likely so use it to manage other addictions than to use addictively
Factor 2: Scientific evidence of pharmacological effect			
Potential Therapeutic Effects	(Behnood-Rod et al., 2020; Obeng et al., 2021), (Vicknasingam et al., 2020; Chakraborty et al., 2021a)	Kratom's antinociceptive effects appear to be mediated at least partly by 7-OH-MG metabolite formation	Animal study findings are consistent for use to manage opioid use disorder and withdrawal, pain and suggest exploration for other disorders
Mechanisms of Action	(Prozialeck et al., 2019), (Behnood-Rod et al., 2020), (Hassan et al., 2019; Hiranita et al., 2019; Kruegel et al., 2019; Gutridge et al., 2020; Todd et al., 2020; Suhaimi et al., 2021)	Kratom alkaloids, including 7-OH-MG may interact with opioid receptors, but do not recruit β-arrestin 2	These are consistent with little or no respiratory depression across a broad range of doses and conditions
Kratom Minor Alkaloids and Metabolites	(Kruegel et al., 2019; Chakraborty et al., 2021a; León et al., 2021; Sharma and McCurdy, 2021), (Newman and Cragg, 2016; Sharma et al., 2019; Domnic et al., 2021a; Domnic et al., 2021b; Chear et al., 2021)	Most minor kratom alkaloids and metabolites are in de minimis levels	Some minor alkaloids might influence kratom's pharmacological effects and merit evaluation for potential therapeutic uses at much higher doses than provided by kratom
Metabolism and Metabolite Profiling	(Kamble et al., 2019; Kamble et al., 2020a; Kamble et al., 2020b)	7-OH-MG appears to metabolize differently in humans than in other species (e.g., rats, dogs, monkeys)	Animal models for kratom alone may not be fully predictive of human effects
Factor 3: Current state of scientific knowledge			
MG and 7-OH-MG PK/PD	(Hiranita et al., 2020), (Avery et al., 2019; Jagabalan et al., 2019; Maxwell et al., 2020)	Greater exposure observed with natural kratom formulations than with oral MG	
Minor Alkaloids PK/PD	(King et al., 2020; Berthold et al., 2021; Kamble et al., 2021)	Approximately one third of minor alkaloids are characterized	
Clinical Studies	(Singh et al., 2018a; Singh et al., 2018b; Singh et al., 2019a; Singh et al., 2020a; Leong Bin Abdullah et al., 2020; Leong Abdullah et al., 2021)	Long term users of kratom have no significant differences in most physiological measures compared to nonusers	These should not be considered definitive safety data but provide a foundation for further studies
Factors 4, 5, and 6—History and Current Patterns of Abuse; The Scope, Significance and Duration of abuse; What, if any, Risk is there to the Public Health			
U.S. National and Federal Survey Data	(National Institute on Drug Abuse, 2019), (Coe et al., 2019)-(Garcia-Romeu et al., 2020), (U.S. Department of health and Human Services, 2020; Schimmel et al., 2021; Covvey et al., 2020; Grundmann, 2017; Drug Abuse Warning Network, 2020; Drug Enforcement Adm, 2020a; Substance Abuse and Menta,	NSDUH Lifetime Use: 1.4%; Past Year Use 0.7%. Little evidence of use on other federal surveys either because kratom was not specifically included or did not meet the threshold for reporting	Federal survey data provide no evidence that kratom poses an imminent threat to public health but merits continuing monitoring to better understand trends in use

(Continued on following page)

TABLE 1 | (*Continued*) Summary of references.

Factor/Description	Citations	Main findings	Comments
	2020; Drug Enforcement Adm, 2020b; Grundmann et al., 2021; Miech et al., 2021) (U.S. Department of Health and Human Services, 2020; Schimmel et al., 2021; Covvey et al., 2020), (Botanical Education Alliance, 2016)		
Kratom Use Prevalence		Estimates range from 1.8 million to over 16 million users in the US	It appears likely that there are at least 10 million kratom users in the US but more definitive studies are needed
Kratom Use Associated Mortality	(National Institute on Drug Abuse, 2019), (Giroir, 2018), (Food and Drug Admini, 2018; Gershman et al., 2019; Henningfield et al., 2019; Olsen et al., 2019)	Risk of kratom associated death appears to be at least a thousand times lower than for morphine-like opioids	Approximately 80% of kratom positive or "involved" deaths also detected other drugs of abuse or the decedent had a history of substance use disorders in one study contribution by other drugs not possible to rule out
Mortality Risks Projected as a Result of Banning Licit Kratom	(Henningfield et al., 2018a), (Ingraham, 2016b), (Giroir, 2018), (Grundmann et al., 2018; Coe et al., 2019; Garcia-Romeu et al., 2020), (Grundmann, 2017), (Henningfield et al., 2018b; Henningfield et al., 2018c; Henningfield et al., 2018d; Prozialeck et al., 2020)	Surveys suggest that it is likely that some kratom users would return to opioid use if kratom use and possession is banned	Fears of relapse to opioid use was a serious concern voiced by thousands of users in surveys and comments to DEA and FDA
Public Health and Individual Benefits of Kratom	(Henningfield et al., 2018a), (Prozialeck et al., 2019), (Coe et al., 2019)-(Garcia-Romeu et al., 2020), (Swogger and Walsh, 2018), (Grundmann, 2017), (Drug Enforcement Adm, 2016), (Raffa, 2014)-(Pain News Network, 2018)	Kratom is used by millions of people in the US to manage substance use disorders, pain, mood disorder, and for energy and improved mental focus and alertness	Reasons for use of kratom rather than FDA approved medications included better efficacy, presumed lower risks and because it is more accessible and tolerable, and/or preferred as a "natural product". Note: such data should not be used to support therapeutic claims in labeling or marketing
Kratom Use for Managing Opioid Use/Withdrawal and Other Health Reasons	(Coe et al., 2019), (Grundmann, 2017), (Singh et al., 2019b; Singh et al., 2020b; Singh et al., 2020c)	Surveys in US and SEA report kratom is used mostly for its health benefits, including opioid withdrawal	Although management of opioid use and withdrawal is prominent, nonclinical data suggest that use for other substance use disorder management and many other disorders merit further exploration
Comment on Therapeutic Use in Context of FDA Standards	(Katz, 2004; DiMasi et al., 2016; Food and Drug Admini, 2016; Dabrowska and Thaul, 2018; Wouters et al., 2020)	While research has yet to meet FDA's standard for therapeutic efficacy (NDA), there is substantial evidence of its use and efficacy in treating opioid withdrawal symptoms, and other disorders	
Factor 7—The psychic or physiological dependence liability			
Science Updates	(Hemby et al., 2019), (Coe et al., 2019)-(Garcia-Romeu et al. 2020), (Swogger and Walsh, 2018), (Harun et al., 2021b)-(Vicknasingam et al., 2020), (Grundmann, 2017), (Grundmann et al., 2021), (Swogger et al., 2015; Smith and Lawson, 2017; Singh et al., 2018c; Leong Bin Abdullah et al., 2021)	Some chronic, frequent kratom users report dependence/addition and/or withdrawal, but this is generally more readily self-managed compared to use disorders of other drugs of abuse	

Although MG withdrawal signs are weak in rats compared to those from morphine withdrawal, there does appear to be evidence of physical dependence; however, MG withdrawal unlike morphine was not associated with anxiogenic-like subjective symptoms. When using a pentylenetetrazol (PTZ) discrimination trial to evaluate anxiogenic signs in rats after MG or morphine withdrawal precipitated by naloxone, MG showed no substitution to the PTZ discriminative stimulus, while morphine produced a dose-related PTZ-like stimulus, further supporting MG as a novel pharmacotherapeutic intervention for managing opioid use disorder (Johari et al., 2021).

Other studies of opioid or MG withdrawal suggested that specific brain proteins might serve as more sensitive biomarkers for physiological dependence in rats as compared to behavioral signs (Hassan et al., 2021). Clonidine treatment may attenuate MG withdrawal signs in rats (Hassan et al., 2021). Another recent study employed an open-field test and an elevated-plus maze test to evaluate naloxone-precipitated withdrawal from MG as compared to morphine, and provided additional evidence confirming that MG can induce physical dependence and measurable signs of withdrawal in rats (Harun et al., 2021a). Overall, the research is consistent with human reports that

kratom withdrawal is generally more modest and more readily self-manageable than that produced by opioids (e.g., 22 and as discussed in Factor 7).

3.1.2.6 Real World Evidence of Abuse and Dependence

Factors 4–6 discuss the public health aspects of kratom use; however, many of the same studies address Factor 1 concerning evidence for abuse and are mentioned here.

As reported by Henningfield, et al. (2018), although surveys and anecdotal reports in the US and SEA confirm that some kratom consumers reported "addiction" those studies also indicated that use "to get high" is relatively low as compared to opioids and other recreational drugs of abuse, and that use by smoking, injecting, and/or insufflating was rare as compared to opioids, stimulants and other recreational drugs (Henningfield et al., 2018a). Recent studies confirm that kratom intake can lead to dependence and withdrawal in some kratom users, but these are substantially less likely to interfere with family, social and occupational life and commitments as compared to opioid dependence. Moreover, kratom is widely viewed as a healthier and less life-impairing substance to replace drugs such as opioids, alcohol, and stimulants (Singh et al., 2014; Galbis-Reig, 2016; Swogger and Walsh, 2018; Prozialeck et al., 2019).

A variety of reports confirm kratom use to self-manage opioid withdrawal and that abstinence from high chronic kratom use is typically associated with milder symptomatology than abstinence from classical opioids (Grundmann et al., 2018; Smith et al., 2019; Garcia-Romeu et al., 2020). The conclusion of Prozialeck et al. (2019) and Grundmann et al. (2018) (Grundmann et al., 2018; Prozialeck et al., 2019) were further strengthened by two published US surveys which found that the overwhelming majority of kratom consumers reported that their use was for various health benefits and not for recreational purposes (Coe et al., 2019; Garcia-Romeu et al., 2020; Harun et al., 2021b).

3.1.3 Factor 1 Updated Conclusion

Diverse scientific approaches were employed to profile MG's abuse potential, finding no evidence of rewarding effects in the IV self-administration and ICSS models, and weak evidence of potential reward in the CPP procedure. MG only partially generalizes to morphine and more fully generalizes to the nonscheduled alpha-adrenergic agonists, phenylephrine and lofexidine. The new data suggest relatively low abuse potential as compared to morphine-like opioids, stimulants, and other drugs of abuse that demonstrate robust rewarding effects across all such abuse potential models. Similarly, MG's potential to produce physical dependence and withdrawal appears relatively low, but not absent, as compared to opioids in animal models. These findings are generally consistent with human reports that MG has a relatively low abuse and withdrawal potential as compared to recreationally used opioids but can reduce opioid self-administration and withdrawal. Surveys indicate that reducing opioid self-administration and withdrawal are among the most common reasons for kratom use in the US (also discussed in Factors 4, 5, and 6). New studies discussed in Factors 2–7 contribute further to the understanding of kratom's abuse potential, including its public health risks and benefits, that are part of the 8-factor abuse potential assessment.

3.2 Factor 2—Scientific Evidence of its Pharmacological Effects

3.2.1 Summary of 2018 Findings

MG and 7-OH-MG have some MOR mediated effects, but 7-OH-MG occurs at low concentrations in kratom leaves and is absent in many kratom product derivatives suggesting that the effects reported by kratom consumers are due primarily to MG. Some kratom effects were shown to be naloxone reversible (e.g., "pain" tolerance); however, MG and 7-OH-MG mechanisms of action were diverse and mediated by non-opioid transmitters and pathways (Kruegel and Grundmann, 2018). Thus, characterization of MG as an opioid "analog" or "narcotic like opioid" is not consistent with the overall evidence, leading Henningfield et al. (2018) to conclude "More research is clearly needed to elucidate receptor binding profiles and the diverse and probably complex mechanisms of action of the kratom alkaloids singly, in combination, and as commonly occur in marketed products and brewed extracts" (Henningfield et al., 2018a).

3.2.2 Factor 2 Science Updates

3.2.2.1 Potential Therapeutic Effects

Although neither kratom nor any of its alkaloids are approved for therapeutic use for any disorder, surveys discussed in *Factors 4, 5, and 6—History and Current Patterns of Abuse; the Scope, Significance and Duration of Abuse; what, if Any, Risk is There to the Public Health* and elsewhere (Henningfield et al., 2018a; Grundmann et al., 2018; Swogger and Walsh, 2018; Coe et al., 2019; Prozialeck et al., 2019; Garcia-Romeu et al., 2020) show individuals in the US and around the world describe using kratom for its health benefits. Research characterizing kratom's effects, mechanisms of action, and therapeutic kratom alkaloid use rapidly advanced since 2018. In a placebo-controlled cold pressor task evaluating anti-nociceptive effects, pain tolerance was significantly increased following consumption of a kratom tea-type decoction similar to Malaysian preparations (Vicknasingam et al., 2020). These data provided "the first objectively measured evidence obtained in controlled research with human subjects that are preliminarily supporting or confirming previously published reports of kratom pain relieving properties based on self-reports collected in observational studies".

Consistent with Vicknasingam et al. (2020)'s clinical findings, oral LKT administration to mice produced dose-related antinociceptive effects at doses that did not alter locomotion or produce CPP; there were brief, non-life threatening decreases in respiration (Behnood-Rod et al., 2020). Repeated LKT administration produced no physical dependence, but significantly decreased naloxone-precipitated withdrawal in morphine dependent mice, confirming MOR agonist activity and therapeutic LKT effect for treating pain and opioid physical dependence.

After investigating *in vitro* receptor binding affinity and *in vivo* morphine discrimination, antinociception in the "heated plate" pain test, and naloxone challenge tests in rats, the authors concluded "At human m-opioid receptor (MOR) *in vitro*, mitragynine has low affinity and is an antagonist . . . ". Overall, 7-OH-MG had stronger MOR mediated effects including antinociception (Obeng et al., 2021). An extensive series of tests characterized several minor indole and oxindole alkaloids that the authors suggest are insufficient in abundance to account for the biological effects of kratom but may show promise for the development of potential medicines including potential new chemical entities (Chakraborty et al., 2021a).

Several of these studies showed MOR mediated antinociceptive effects with little evidence of respiratory depression suggesting the potential to contribute to new generations of nonopioid analgesics.

3.2.2.2 Mechanisms of Action

Although kratom produces some effects in common with opioids, and some of its alkaloid's actions are mediated by MOR receptors, its effects and mechanisms of action are diverse and include non-opioid mechanisms of action and non-opioid acting constituent alkaloids, as discussed in earlier reviews (Henningfield et al., 2018a; Kruegel and Grundmann, 2018; Prozialeck et al., 2019). In 2021, Leon et al. (2021) investigated several alkaloids, including mitragynine, paynantheine and speciogynine that produce serotonergic effects potentially mediated by their metabolites. As the authors discuss, such actions would be consistent with some of the mood enhancing effects attributed to kratom (Kruegel and Grundmann, 2018; Sharma and McCurdy, 2021).

Kratom contains approximately 1–2% MG by weight, as well as other alkaloids (including 7-OH-MG) that typically are present at such low levels in kratom leaf material that it is uncertain if they contribute to kratom effects (Prozialeck et al., 2019). 7-OH-MG is present in low concentrations in natural kratom products, but gradually emerges *in vivo* as a MG metabolite. Kruegel et al. (2019) studied its role as a mediator of MG effects (Kruegel et al., 2019) summarizing "7-hydroxymitragynine is formed from mitragynine in mice and . . . brain concentrations of this metabolite are sufficient to explain most or all of the opioid-receptor-mediated analgesic activity of mitragynine . . . it suggests a possible explanation for the seemingly improved safety profile of mitragynine compared to classical opioid agonists . . . We believe mitragynine and related compounds have great potential as future therapeutics, but metabolic processes must be carefully considered as the field continues to advance." Hiranita, Sharma, Oyola et al. (2020) reported although "the conversion rate of 7-hydroxymitragynine from p.o. mitragynine is low, 7-hydroxymitragynine is a more potent and efficacious μ-opioid receptor agonist than mitragynine, suggesting that conversion to this metabolite may contribute to the *in vivo* μ-opioid activity of mitragynine" (Behnood-Rod et al., 2020).

Kratom is commonly consumed to enhance occupational performance and as a coffee substitute for energy at low doses. In an animal model of spatial learning and memory, high doses impaired memory (Hassan et al., 2019). Suhaimi, Hassan, Mansor and Müller (2021) reported changes in brain electroencephalogram (EEG) activity after acute and chronic MG exposure in rats, with strong effects on some measures at high doses, supporting the importance of more research on brain function and potential cognitive effects (Suhaimi et al., 2021).

Gutridge et al. (2020) pharmacologically characterized interactions between kratom extracts, kratom alkaloids, and synthetic carfentanil-amide opioids with G-proteins and beta-arrestin at mu, delta and kappa opioid receptors *in vitro,* and assessed whether they had rewarding properties and the degree to which they reduced alcohol intake (Gutridge et al., 2020). The authors concluded that "kratom alkaloids do not recruit β-arrestin 2 at the μOP, δOP, and κOP and can significantly reduce both moderate and binge alcohol intake in male and female mice. This pharmacological profile and effect on alcohol intake in rodents may explain why some find kratom useful to self-medicate for alcohol use disorder." These findings were further supported by the findings by Todd et al. (2020) who concluded "mitragynine and 7-hydroxymitragynine demonstrate functional selectivity for G-protein signaling, with no measurable recruitment of β-arrestin. Overall, the study demonstrates the unique binding and functional profiles of the kratom alkaloids, suggesting potential utility for managing pain, but further studies are needed to follow up on these *in vitro* findings" (Todd et al., 2020).

Hiranita et al. (2019) compared the effects of MG to morphine in behavioral and antinociception assays in rat models finding "Opioid receptors do not appear to mediate the disruptive effects of mitragynine on learned behavior. Mitragynine had lesser antinociceptive effects than morphine, and these did not appear to be mediated by opioid receptors. The pharmacology of mitragynine includes a substantial non-opioid mechanism" (Hiranita et al., 2019).

3.2.2.3 Studies of Kratom Minor Alkaloids and Their Metabolites, and Analogs

Advances in analytical methods are accelerating our understanding of the effects of numerous kratom alkaloids including liquid chromatography–tandem mass spectrometry assays that quantify kratom alkaloids in kratom leaf extracts and commercial products (Sharma et al., 2019).

Most of these alkaloids are present at de minimis levels with respect to human experience, effects, and safety; however, it is possible that while the majority of natural plant-based kratom preparation effects are mediated by MG, one or more minor alkaloids may also play a minor role and account for differences in kratom strains (Kruegel et al., 2019; Chear et al., 2021).

An *in vitro* pharmacological characterization of five kratom based minor alkaloids found that their low abundance made it unlikely that these alkaloids play a major mediating role in the biological actions of kratom consumed by humans, but this research may contribute to furthering the understanding of kratom mechanisms of action and opioid receptor function (Chakraborty et al., 2021a).

Kratom alkaloids are of interest as templates for novel synthesized molecules (i.e., analogs) for new medicines. One third to one half of FDA-approved medicines are based on natural plant product substances from which novel chemical entities were developed (Newman and Cragg, 2016; Domnic et al., 2021a). Such efforts are actively in progress characterizing a variety of indole and oxindole alkaloids, determining their chemical structures, and binding affinities for opioid and other receptors (Chear et al., 2021). One approach to the synthesis of novel MG analogs produced several partial MOR agonists with low G-protein activation (Chakraborty et al., 2021b). These analogs demonstrated robust analgesic effects but low respiratory depressant, locomotor, and conditioned place preference suggesting lower adverse effects including abuse potential.

Combinations of kratom alkaloids may inhibit cell proliferation and migration of nasopharyngeal carcinoma cells suggesting alkaloid or new analogs as potential cancer treatments (Domnic et al., 2021b).

3.2.2.4 MG Metabolism and Metabolite Profiling

Thirteen MG metabolites were identified in human liver microsomes (HLM) and S9 fraction studies (Kamble et al., 2019), and potential MG and other kratom alkaloids drug interactions were investigated including with pharmaceutic products (Kamble et al., 2020a).

7-OH-MG can be converted to pseudoindoxyl-MG in human plasma to a greater extent than is produced in mice, rats, dogs and cynomolgus monkeys, possibly explaining potential human effects and benefits that may not be predicted in animal studies alone (Kamble et al., 2020b).

3.2.3 Factor 2 Updated Conclusion

Kratom's main effects are due to the consumption of MG, but other minor alkaloids and metabolites, including 7-OH-MG, may also contribute to effects reported by consumers. Since 2018, many scientific advances improved our understanding of how these alkaloids and metabolites interact. Some alkaloids that contribute little to the effects of kratom may ultimately contribute to safer and more effective new medicines for a variety of disorders, as well as for general health and well-being. Development and approval of such products may be a decade or more in the future, but this rapidly advancing science is explaining how kratom works, and why its pain relieving, and other benefits occur with relatively low levels of abuse, dependence, and harmful decreases in respiration compared to opioids.

3.3 Factor 3—The State of Current Scientific Knowledge Regarding the Drug

3.3.1 Summary of 2018 Findings

The 2018 8-FA highlighted kratom's pharmacodynamic effects. Preclinical anti-nociceptive studies suggested that MG and 7-OH-MG produced such effects mediated by MOR receptors. Most information about the effects of long-term use in humans on various physiological, and cognitive parameters was based on anecdotal reports, case histories, and preliminary field studies in SEA. A two-compartment model best described human oral MG pharmacokinetics (Trakulsrichai et al., 2015).

3.3.2 Factor 3 Science Updates

New kratom pharmacokinetics studies in rats, mice and dogs document plasma MG, 7-OH-MG, and other alkaloids and minor metabolites over 12 h or more, with accompanying safety assessments. Six new clinical studies following long-term kratom use provide safety data on health, and organ and brain function were also conducted.

3.3.2.1 Pharmacokinetics and Pharmacodynamics Findings Related to MG and 7-OH-MG Safety

After oral administration of traditional or other natural kratom formulations to rats, greater systemic exposure was observed than that of an equivalent oral MG dose alone; no adverse events were reported (Avery et al., 2019).

Administration of 5 mg/kg oral MG (equivalent to approximately 3 mg/kg in humans) and 0.1 mg/kg IV MG to beagle dogs was well tolerated and produced no adverse events or major abnormalities in clinical parameters (Maxwell et al., 2020).

The estimated MG clearance (CL/F) was 2.21 L/h, absorption rate (Ka) 0.82/h, and volume of distribution (Vd) 30.8 L after oral 20, 40, and 80 mg/kg MG doses to rats (Jagabalan et al., 2019). Oral 55 mg/kg MG produced 85 ng/ml Cmax for 7-OH-MG, 14 times lower than the MG Cmax. Anti-nociception after IV MG and 7-OH-MG suggested that 7-OH-MG was more potent and efficacious than MG, and metabolic formation of 7-OH-MG contributes to *in vivo* MOR mediated effects of oral MG (Hiranita et al., 2020).

3.3.2.2 Pharmacokinetic and Pharmacodynamic Findings With Kratom's Minor Alkaloids

MG, 7-OH-MG, corynantheidine, speciogynine, speciociliatine, paynantheine, corynoxine, corynoxine-B, mitraphylline, ajmalicine, and isospeciofoline were analyzed in rat plasma after a variety of oral kratom products, with only MG, 7-OH-MG, speciociliatine, and corynantheidine quantifiable at 8 h (Kamble et al., 2021).

Speciociliatine pharmacokinetics were characterized following IV and oral dosing to help understand the potential contribution of this alkaloid to *in vivo* kratom administration effects (Berthold et al., 2021). Speciociliatine had higher systemic exposure and lower clearance compared to the other kratom alkaloids mitragynine and corynantheidine. Similarly, the pharmacokinetics of corynanthidine, a minor kratom alkaloid and perhaps a MOR antagonist, were determined after 2.5 mg/kg IV and 20 mg/kg oral doses to rats, yielding a 50% oral bioavailability, a 4.1 h Tmax and extensive distribution including in brain corpus callosum and hippocampus regions (King et al., 2020).

3.3.2.3 Safety Assessments From Clinical Studies

Kratom's anti-nociceptive effects in the cold pressor test are described in Factor 2 and its potential for physiological dependence and withdrawal are discussed in Factor 7 (Vicknasingam et al., 2020). This section summarizes six new clinical studies that assessed health and safety endpoints.

Leong Bin Abdullah et al. (2020) studied the lipid profiles, liver function and blood chemistries in 100 chronic kratom users and 100 healthy nonusers in Malaysia finding that the "liver parameters of the study participants were within normal range. The serum total cholesterol and LDL of kratom users were significantly lower than those of healthy subjects who do not use kratom. There were no significant differences in the serum triglyceride and HDL levels. However, higher average daily frequency of kratom use and increasing age were associated with increased serum total cholesterol among kratom users."

Singh, Muller, Murugaiyah et al. (2018) studied various hematological and clinical-chemistry parameters of kratom users in Malaysia (Singh et al., 2018a). They interviewed and collected blood samples from 58 "regular kratom users" and 19 "healthy controls." Findings showed there were no significant differences in the hematological and clinical-chemistry parameters of traditional kratom users and healthy controls, except for HDL and LDL cholesterol values; these were found to be above the normal reference range for the users. Similarly, long-term kratom consumption (>5 years), and quantity of daily kratom use (≥3 ½ glasses; mitragynine content 76.3–114.8 mg) did not appear to alter the hematological and biochemical parameters of kratom users. These data suggest that even long-term and heavy kratom consumption did not significantly alter the hematological and clinical-chemistry parameters of kratom users in a traditional setting.

Singh, Narayanan, Grundmann et al. (2020), studied the long-term effects of kratom use in thirteen people in Malaysia who used kratom longer than 20 years in a cross-sectional pilot study (Singh et al., 2020a). They summarized their results as follows: "Respondents were required to undergo a blood-test and laboratory analysis was conducted to determine the mitragynine content in an acquired street sample of kratom. The regular, long-term consumption of brewed kratom decoction did not cause any significant alterations in haematological, kidney, liver, thyroid, inflammatory and gastrointestinal analytes in a cohort of kratom users who had no history of substance misuse. However, those who had a higher intake (>3 glasses per day) of kratom exhibited higher lipid values (except for HDL-cholesterol), and a moderate elevation of homocysteine level. Long-term (>20 years with a daily intake of ≥87.54 mg mitragynine) kratom consumption was not associated with altered biochemical levels, although prolonged and chronic, frequent use (>3 glasses daily) may result in cardiovascular risks." Note that this study was not designed to determine if kratom or other factors contributed to higher lipid values.

Singh, Chye, Suo et al. (2018) conducted a preliminary study of the impact of kratom use on brain function, as assessed by brain magnetic resonance imaging, among chronic kratom users in Malaysia. They reported "There were no significant differences ($p > 0.05$) in the intracranial volume (ICV), cortical volumes (frontal, parietal, temporal, occipital, or cingulate lobe), or subcortical volumes (striatum, hippocampus, or amygdala), as well as in the diffusion tensor imaging (DTI) metrics, fractional anisotropy (FA) and mean diffusivity (MD) between kratom users and the controls. This preliminary study showed long-term consumption of kratom decoction is not significantly associated with altered brain structures in regular kratom users in traditional settings" (Singh et al., 2018b).

Singh, Narayanan, Muller et al. (2019) studied potential long-term cognitive effects associated with kratom use in kratom uses in Malaysia with assessments performed using the Cambridge Neuropsychological Test Automated Battery (Singh et al., 2019a). Relative to control participants, higher consumption (>3 glasses daily or mitragynine doses between 72.5 and 74.9 mg) of kratom tea was selectively associated with impaired performance on the Paired Associates Learning task of the Cambridge Neuropsychological Test Automated Battery, reflecting deficits in visual episodic memory and new learning.

Leong Bin Abdullah, Tan, et al., evaluated the prevalence of ECG abnormalities and QTc intervals in kratom users without histories of illicit drug use. Sinus tachycardia was higher in kratom users. Daily kratom consumption was associated with borderline QTc intervals (Leong Abdullah et al., 2021). Another study by Leong Bin Abdullah and Singh found that people who consumed four or more glasses of kratom tea daily had higher MG concentrations than lower intake consumers and this higher intake was associated with prolonged QTc intervals (Leong Bin Abdullah and Singh, 2021a). The same authors published a comprehensive review of the cardiovascular and cardiotoxic effects of kratom and came to the conclusion that limitations in studies to date do not permit definitive conclusions about the cardiovascular risks (Leong Bin Abdullah and Singh, 2021b).

3.3.3 Factor 3 Updated Conclusion

Pharmacokinetics and safety data from multiple species, kratom preparations, alkaloids, and metabolites; advances in bioanalytical assays providing more accurate and reliable findings; and data from multiple studies with MG doses many times higher than those human kratom users take are now available. These studies add to those described in Factors 1 and 2 confirming little evidence of serious adverse or life-threatening effects over a broad range of doses, dosage forms, and in four species (mouse, rat, dog, and monkey).

Other major advances in kratom science come from six clinical studies of long term kratom use effects and safety, as well as the study of anti-nociceptive effects of kratom and physiological dependence described in Factors 2 and 7. These important advances in kratom science evaluated the effects of long-term kratom use on a variety of physiological parameters including kidney and liver function, hematological parameters, cognition, and on brain function by brain magnetic resonance imaging. Although relatively small studies, none suggest serious adverse consequences of use. It is important to note that these are not definitive safety studies and cannot be used to claim that kratom has no adverse effects on any of the studied

physiological domains and limitations of each study were noted in the publications. Nonetheless, the findings are encouraging and should facilitate the conduct of more comprehensive follow-up studies.

3.4 Factors 4, 5, and 6—History and Current Patterns of Abuse; the Scope, Significance and Duration of Abuse; what, if Any, Risk is There to the Public Health

3.4.1 Summary of 2018 Findings

Note that for this update, Factors 4, 5, and 6 are considered together because they all contribute to understanding nonmedical use, recreational use and abuse, and public health impact, relying on some of the same surveys across factors. The Henningfield et al., 2018 8-FA considered all major relevant federal surveys, as well as data from internet monitoring, and more than 20,200 comments to the DEA, and concluded that there was no evidence of an imminent public health threat associated with kratom (Henningfield et al., 2018a). To the contrary, the review concluded that there were foreseeable health risks including opioid overdose and deaths if lawful kratom was banned and possession criminalized. Moreover, although kratom is not approved as safe and effective for therapeutic use, it was evident that most kratom use in the US was for health and well-being by people who personally found kratom to be more effective, tolerable, accessible and/or preferred a natural product as compared to FDA approved medicines.

3.4.2 Factors 4, 5, and 6 Science Updates

3.4.2.1 U.S. National and Federal Survey Data

Table 2 summarizes the main findings from the major national and federal surveys and other data sources. Overall, there are more similarities than differences with respect to demographics reported in this table as well as in other demographics reported in the published survey results. Prevalence appears to be substantially underestimated by the NSDUH and RADARS surveys (U.S. Department of health and Human Services, 2020; Schimmel et al., 2021).

NSDUH, RADARS, and Covvey et al. did not report reasons for use; however, many kratom users reported past or present use of opioids and/or drug addiction treatment consistent with past findings that self-management of addiction and withdrawal is a common reason for kratom use (National Institute on Drug Abuse, 2019; Coe et al., 2019; Garcia-Romeu et al., 2020; U.S. Department of health and Human Services, 2020; Schimmel et al., 2021; Covvey et al., 2020; Grundmann, 2017). Survey data incidence reports for DAWN, MTFS, NFLIS, and TEDS are apparently below the threshold for reporting as confirmed in an inquiry to NFLIS (Drug Enforcement Administration, 2020a; Drug Abuse Warning Network, 2020; Substance Abuse and Mental, 2020).

These findings do not support the conclusion that kratom use represents an imminent health threat and in fact kratom is not listed in the most recent DEA National Drug Threat Assessment (Drug Enforcement Administration, 2020b). There is no evidence that kratom is "fueling" or otherwise contributing to the opioid epidemic, though the survey data suggest that it is an informal self-management approach supporting the efforts of many opioid users to reduce and discontinue opioid use (Grundmann, 2017; Coe et al., 2019; Garcia-Romeu et al., 2020; Grundmann et al., 2021).

3.4.2.2 Kratom Use Prevalence

As mentioned in **Table 2**, the NSDUH and RADARS surveys may greatly underestimate the US prevalence and incidence of kratom use, with estimates of past year kratom use of 1,790,00–2,040,000.[3] (U.S. Department of health and Human Services, 2020; Schimmel et al., 2021). In contrast, a credible estimate based on market data suggested prevalence of 3–5 million in 2014–2015 (Botanical Education Allia, 2016).

Experts and marketers agree that the kratom market substantially expanded since that time, with kratom export data from Indonesia to the US and major marketer consensus finding that the US consumer base was likely 10–16 million. This is consistent with a nationally projectable survey estimate from 2020 concluding past year kratom use as 4.1% or 10,500,000 kratom users (Covvey et al., 2020).

3.4.2.3 Kratom Use Associated Mortality

The two most widely cited estimates of kratom associated mortality are based on world-wide reports over nearly 10 years (Food and Drug Administration, 2018; Olsen et al., 2019). FDA's statement noted that all but one involved other substances, and that case was under further investigation.[4] Medical examiners or coroners reported kratom as the cause of death in 91 (59.9%) of 152 kratom positive decedents (out of 27,338 overdose deaths in 27 states), including seven for whom kratom was the only substance positive on postmortem toxicology, although other substances could not be ruled out (Olsen et al., 2019). In approximately 80% of kratom positive or "involved" deaths, decedents had a history of "substance misuse", with 65% of cases listing fentanyl as the cause of death, 32.9% heroin, followed by benzodiazepines, prescription opioids, and cocaine. An earlier study (Gershman et al., 2019) cautioned that comprehensive toxicology might identify other substances contributing or causing death. We are not aware that any of the 93,000 drug overdoses estimated for 2020 included deaths due to kratom. That does not mean that there were no deaths in which kratom was the primary cause or a contributing factor; however, the signal is clearly low.

An assessment of various survey data concluded that the risk of kratom associated death was at least a thousand times lower than for morphine-like opioids (Henningfield et al., 2019). This is consistent with NIDA's position (National Institute on Drug Abuse, 2019) and with the 2018 DHHS kratom scheduling rescission letter and the conclusions drawn by Assistant Secretary of Health Brett P. Giroir, MD, ADM who stated:

[3]Note in a summary of RADARS data presented a few months after the Schimmel et al., 2020 publication, it was reported that the national projected past year prevalence estimate was 3.35 million.

[4]FDA never reported the results of that investigation, however, the US DHHS review that led to the 2018 withdrawal of the 2017 MG and 7-OH-MG CSA scheduling recommendation determined that the incident in question was an automobile crash not attributable to kratom use.

TABLE 2 | Summary of data sources.

Survey/Data source	Main results and comment	Other comments
Drug Abuse Warning Network (Drug Abuse Warning Networ, 2020)	No reports in DAWN from 1970 to 2011 "New DAWN" began in 2019 and has not listed kratom	
Monitoring the Future Study (Miech et al., 2021)	Kratom use is not assessed	Note that 9% of NSDUH Reports were from age 12–17 year olds
National Forensic Laboratory Information Service (Drug Enforcement Adm, 2020a)	Since 2016 NFLIS did not include MG/kratom reports because the rates are below the threshold for inclusion	
National Survey on Drug Use and Health (U.S. Department of health and Human Services, 2020)	Paid responders on national panel (n = 67,625).[6] 2019 Prevalence Lifetime Use: 1.4%; Past Year Use: 0.7%	See Grundmann et al., 2021 and Henningfield et al., 2021 comment on apparent underestimation of kratom use prevalence (Grundmann et al., 2021; Henningfield et al., 2021)
Treatment Episodes Data Set (Substance Abuse and Menta, 2020)	No reports. This does not mean there were no reports but suggests subthreshold signal	Internet chatrooms and SUD treatment clinic advertising suggests some kratom users are seeking cessation assistance
Coe et al. (2019) (Coe et al., 2019)	Internet Survey of self-identified kratom users age ≥18 (n = 2,867) 48% use for self-management of pain 10% for self-management of opioid UD or withdrawal 22% use for mood management 2.4% use to get high	
Garcia-Romeu et al. (2020) (Garcia-Romeu et al., 2020)	Internet Survey of self-identified kratom users, age ≥18 (n = 2,798) 91% use for self-management of pain 41% for self-management of opioid UD or withdrawal 67% for management of anxiety 65% for depression <3% report kratom dependence	2% met DSM-5 criteria for past-year moderate or severe kratom-related SUD, but it was rated very low on scale of concern and adverse impact
Covvey et al. (2020) (Covvey et al., 2020)	Nationally representative Internet survey of persons aged 18–59 (n = 1842) 112 (6%) reported lifetime kratom use 72% were 25–44 years old, male, employed, and at higher educational levels 24–47% of respondents indicated self-reported diagnoses for any addiction, and 43% reported previously received treatment for addiction	Similar demographics as Grundmann 2017, Coe et al., 2019 and Garcia-Romeu et al., 2020 but may have underestimated % over 50 due to 59 year old upper age limit of survey. ((Coe et al., 2019), (Garcia-Romeu et al., 2020), (Grundmann, 2017)) Reasons for use were not asked, e.g., to self-manage pain, addiction, mood
Schimmel et al. (2021) (Schimmel et al., 2021)	RADARS® survey of paid survey responder on national panel age >18 (n = 59,714) 0.8% lifetime use 44% age >35 61% male 59% past year opioid use	Reasons for use were not asked, e.g., pain, addiction, mood. See Grundmann et al., 2021 and Henningfield et al., 2021 comment on apparent under estimation of kratom use prevalence (Grundmann et al., 2021; Henningfield et al., 2021)

"There is still debate among reputable scientists over whether kratom by itself is associated with fatal overdoses" (Giroir, 2018).

3.4.2.4 Mortality Risks Projected as a Result of Banning Licit Kratom

Surveys and more than 20,000 comments to the DEA suggest that many kratom users fear resumption of opioid use and the need to resort to illicit kratom markets (Drug Enforcement Adm, 2016; Grundmann, 2017; Coe et al., 2019; Garcia-Romeu et al., 2020). It is not possible to project how many people would relapse to opioids and potentially overdose (Henningfield et al., 2018a; Henningfield et al., 2018b; Henningfield et al., 2018c; Henningfield et al., 2018d; Grundmann et al., 2018; Prozialeck et al., 2020). This was a concern of the DEA in withdrawing its 2016 kratom scheduling proposal (Ingraham, 2016b) and in the US DHHS kratom scheduling recission letter (Giroir, 2018).

3.4.2.5 Public Health and Individual Benefits of Kratom

In 2018, a systematic review of kratom use and mental health by Swogger and Walsh concluded ". . .kratom use appears to have several important mental health benefits that warrant further study. Kratom dependence is a risk for some people, though the dependence syndrome appears to be mild in its psychosocial and physiological effects relative to that of opioids. More and better research, including well-controlled, prospective studies, is necessary to further elucidate kratom's potential for good and harm and the moderators of its effects" (Swogger and Walsh, 2018). The

therapeutic potential of kratom based on surveys, anecdotal reports, and nonclinical research supports the plausibility of such benefits as discussed by other reviewers (Prozialeck et al., 2019; Hemby et al., 2019; Yue et al., 2018; Grundmann et al., 2018; Kruegel and Grundmann, 2018; Sharma and McCurdy, 2021; Swogger et al., 2018; Prozialeck et al., 2021).

The most important public health benefits with respect to mortality are widely agreed upon by kratom experts and surveys, and that is its use to self-manage opioid and other drug addiction and withdrawal symptoms, and thereby reduce use and overdose from far deadlier substances. This type of use is not unique in the US but was long reported in SEA (Raffa, 2014; Henningfield et al., 2018a). This was also reported in the first major US Internet survey of kratom use (Grundmann, 2017), as well as in subsequent surveys (Coe et al., 2019; Garcia-Romeu et al., 2020; Pain News Network (2018)). This was also a conclusion of a systematic review of 13 studies addressing kratom use and mental health in the US, SEA, and other countries and regions of the world, and a review by an international consortium of kratom researchers (Swogger and Walsh, 2018; Prozialeck et al., 2019).

While the opioid epidemic represents a highly visible and deadly epidemic in its own right, it is important to recognize that many millions use kratom as their preferred approach to managing other life-threatening disorders including pain, depression, anxiety, post-traumatic stress, fibromyalgia and more (Drug Enforcement Adm, 2016; Grundmann, 2017; Coe et al., 2019; Garcia-Romeu et al., 2020).

3.4.2.6 Kratom Use for Managing Opioid Use/Withdrawal and Other Health Reasons

In the first half-year of the COVID-19 pandemic, there was uncertainty about kratom supply by vendors and consumers, however, overall US supply was not affected. The main reasons for kratom use are pain relief (48%), anxiety, "PTSD" or depression (22%), increase energy or focus (10%), and "help cut down on opioid use and/or relieve withdrawal" (10%) (Coe et al., 2019). Side effects were generally minor, e.g., stomach upset, rarely required medical attention and rates and severity of "bad reactions" were generally similar to those reported by Grundmann (Grundmann, 2017).

Field studies with face-to-face interviews in Malaysia provide complementary evidence to US Internet surveys regarding reasons for use and potential benefits (Singh et al., 2019b). Motives related to mood and other factors in 116 regular kratom users employed the Drinking Motives Questionnaire (DMQ) to measure motives for kratom use, reported "heavy" kratom use as drinking more than three glasses daily (estimating that 1 glass contains 48.24–50.4 mg of mitragynine), with use associated with significantly higher means scores on the Coping and Enhancement scales. A field face-to-face survey of 92 long-term male kratom users found that 72 participants (78%) reported using kratom to "enhance sexual performance" and all but one found did their sexual performance did improve. Interestingly, among participants who described kratom intake for other reasons, 35% reported enhanced sexual performance (Singh et al., 2020b).

Patterns and reasons for use and demographics were investigated in 142 current and 62 former opioid polydrug users in Malaysia (Singh et al., 2020c). The alkaloid content of a kratom street sample was primarily MG, followed by paynantheine, speciociliatine, speciogynine, and "low levels" of 7-OH-MG. There were no significant differences in demographic characteristics between current and former opioid polydrug users except with respect to marital status, with current kratom users having a higher odds ratio of being single. While both current and former opioid users reported using kratom to ameliorate opioid withdrawal, current users had significantly higher likelihood of using kratom for that purpose; however, former opioid users were more likely to use kratom for mood elevating effects.

3.4.2.7 Comment on Therapeutic Use in Context of FDA Standards

It is important to note that the benefits documented in published surveys do not constitute the basis for therapeutic claims and no kratom product or kratom alkaloid is approved for therapeutic use in the US. The FDA and other federal agencies state that there is no proven therapeutic use for kratom despite evidence that millions of people in the US and many more in SEA use kratom primarily for therapeutic, beneficial use. That evidence includes peer reviewed surveys and field studies in the US and SEA, clinical and preclinical studies showing that MG's mechanisms of action are consistent with such effects. Moreover, several animal models used to predict efficacy for treating opioid use disorder, opioid withdrawal and pain demonstrated efficacy.

None of this research meets FDA's standard for therapeutic efficacy that is determined by evaluation of a New Drug Application (NDA). The NDA must be supported by "substantial evidence of effectiveness," and is defined as "evidence consisting of adequate and well-controlled investigations" (Katz, 2004; Dabrowska and Thaul, 2018). The time and cost to develop and achieve FDA approval of a product as therapeutically effective and acceptably safe varies widely but is often approximated at 10 years and 1 billion dollars (DiMasi et al., 2016; Wouters et al., 2020). Only two botanical substances, Veregen® (sinecatechins) and Mytesi™ (crofelemer), were developed as drug products consistent with FDA's Botanical Drug Guidance and both are available only as prescription drugs that is typical of new drug approvals (Food and Drug Admini, 2016).

3.4.3 Factor 4, 5, and 6 Updated Conclusions

The most important finding from new US survey evidence is that the conclusion that kratom products and kratom's primary active alkaloid, MG, pose a "serious imminent threat to public health" is not supported. This extensive survey update agrees with the Henningfield et al. (2018) conclusion: "There has been no documented threat to public health that would appear to warrant emergency scheduling of the products and placement in Schedule I of the CSA carries risks of creating serious public health problems. . .. Although kratom appears to have pharmacological properties that support some level of scheduling, if it was an approved drug, placing it into Schedule I, thus banning it, risks creating public health problems that do not presently exist" (Henningfield et al., 2018a).

The evidence shows that millions of people in the US purchase and use kratom products for improving health and are preferred to FDA approved medicines because for them, kratom products are more effective, accessible, and tolerable. Furthermore, many prefer managing health problems with natural products.

For those using kratom products in place of opioids, which appears to be approximately 1/3 of all kratom users, it is foreseeable that removing kratom from the legal marketplace would put many at risk of returning to opioid use and risking opioid overdose death. This was clearly stated in comments to the DEA and public hearings as reported in the 2018 8-FA, and in surveys. Assistant Secretary Dr. Giroir noted "... there is a significant risk of immediate adverse public health consequences for potentially millions of users if kratom or its components are included in Schedule I, such as: ... Kratom users switching to highly lethal opioids, including potent and deadly prescription opioids, heroin, and/or fentanyl, risking thousands of deaths from overdoses and infectious diseases associated with IV drug use ... " (Giroir, 2018).

3.5 Factor 7—The Psychic or Physiological Dependence Liability

3.5.1 Summary of 2018 Findings

Recently, psychic dependence is referred to simply as "dependence" or "substance use disorder" and more commonly as "addiction" though definitions of addiction vary widely (American Psychiatric Asso, 1994; World Health Organization, 1994). Physiological dependence is often used interchangeably with the most common measure of physiological dependence, namely "withdrawal" which is also considered a clinical disorder (American Psychiatric Asso, 2013).

In the 2018 8-FA, Henningfield, Fant and Wang (2018) concluded "There have not been laboratory studies of physical or psychological dependence or abuse potential in humans caused by kratom." Nor had classic animal studies employing the drug self-administration and physical dependence/withdrawal model been conducted (see Factor 2 in this report)".

Nonetheless, the real-world evidence in the published literature supported the following conclusions: "...abrupt discontinuation [of kratom use] may be accompanied by withdrawal symptoms that are qualitatively similar but generally weaker than those observed following discontinuation of opioids. However, such reports make it difficult to disentangle the emergence of preexisting symptoms that had been mitigated by kratom use from those that occur as a physiological rebound accompanying the abrupt discontinuation of kratom use in kratom-dependent people. More studies of kratom's potential to produce physical dependence, tolerance, and withdrawal are needed to characterize the nature and severity, and determinants of abstinence-associated symptoms."

3.5.2 Factor 7 Science Updates

In addition to the animal laboratory studies predictive of abuse potential, dependence, and withdrawal summarized in Factor 1, there are several new studies, surveys, and expert reviews addressing the risk and factors associated with dependence and withdrawal. A major category of kratom use is related to the typically mild and tolerable dependence and withdrawal that occurs in some frequent kratom users and the resulting use of kratom as an approach to self-management. In this context, kratom provides a harm reduction alternative to opioids in particular, but also potentially for alcohol, methamphetamine, and other drugs.

Dependence and withdrawal were addressed in a systematic review of kratom use for mental health reasons that concluded "Kratom dependence is a risk for some people, though the dependence syndrome appears to be mild in its psychosocial and physiological effects relative to that of opioids ... kratom use does not appear to result in significant respiratory depression" (Swogger and Walsh, 2018). A major category of kratom use globally was to self-manage substance use disorders, consistent with the findings discussed in Factor 1 that demonstrated low abuse and physical dependence potential, and that MG administration reduced morphine and heroin self-administration, and withdrawal signs (Hemby et al., 2019; Harun et al., 2021b).

The Vicknasingam et al. (2021) study included in Factor 2 also assessed potential withdrawal signs using the Clinical Opiate Withdrawal Scale (COWS), comparing scores when participants were administered placebo or a kratom concoction (Vicknasingam et al., 2020). Although this study was not designed to be a definitive withdrawal assessment study, and did not include an opioid comparator, it was likely that people using kratom multiple times per day for many years would have experienced pronounced withdrawal symptoms. The authors concluded "None of the participants reported withdrawal symptoms either using spontaneous self-report or had significant withdrawal symptoms based on the COWS scores... All participants reported long histories of daily kratom consumption, with high frequency of daily consumption and substantial amounts consumed. It is not possible to quantify these reports into markers that could be used to approximate amounts of plant material or active ingredients consumed. However, despite the reported long duration and high levels of daily kratom consumption, during documented kratom discontinuation lasting from 10 to 20 h, no participant reported or displayed discomfort, symptoms, or signs of potential withdrawal symptoms."

100 long term kratom users and 100 non-users in Malaysia were interviewed to assess potential symptoms related to kratom dependence and withdrawal (Leong Bin Abdullah et al., 2021). Kratom use longer than 6 years and 3 or more times per day were more likely to be associated with dependence, reduced quality of life and/or withdrawal symptoms when kratom use is discontinued. However, the authors noted that the study did not allow causative conclusions as to whether quality of life reductions are a

result of increased kratom use or if such quality of life and other demographic factors contribute to more frequent kratom use.

An internet survey assessing reasons for use and effects of use in 2,798 present and past kratom users included questions about kratom dependence, withdrawal symptoms associated with discontinuation, and use to self-manage opioid dependence (Garcia-Romeu et al., 2020). Kratom-related withdrawal symptoms were reported by 9.5% of respondents with another 17.5% reporting possible kratom-related withdrawal. This supports results of previous studies (Swogger et al., 2015; Grundmann, 2017; Smith and Lawson, 2017; Coe et al., 2019) by suggesting that kratom has a relatively benign risk profile compared to typical opioids, with only a minority of respondents endorsing kratom-related adverse effects, withdrawal symptoms, or problematic use.

Coe et al. (2019) conducted an internet survey (2,867 current, 157 former kratom users) that included similar questions as Garcia-Romeu et al. and Grundmann (2017) (Grundmann, 2017; Garcia-Romeu et al., 2020), related to opioid use and effects. Kratom use was less likely to interfere with social, family, and occupational functioning compared to conventional opioids. Kratom was used by many to reduce or completely replace prescription and nonprescription opioid withdrawal and was generally considered "very effective" for managing opioid withdrawal. Relief of anxiety (including associated with post-traumatic stress disorder), depression, as well as to increase focus or energy were other major reasons for use. The foregoing conclusions are also consistent with those of Grundmann, Babin, Henningfield et al. (2021) who stated: "Some user reports suggest that regular kratom consumption carries risks of dependency and addiction, though with generally self-manageable withdrawal" (Grundmann et al., 2021).

Singh, Narayanan, Muller et al. (2018) employed widely used psychiatric instruments (Beck Depression Inventory and Beck Anxiety Inventory) to assess potential symptoms of anxiety and depression that may accompany abrupt discontinuation of kratom use in apparently frequent chronic kratom consumers in Malaysia (Singh et al., 2018c). Most respondents (70%) experienced symptoms of mild anxiety, while 81% reported symptoms of mild depression during kratom cessation. Those who consumed higher quantities of kratom tea daily (≥4 glasses) had "higher odds of reporting longer duration of kratom use history . . . , higher frequency of daily kratom use (≥4 times), . . . and were more likely to experience moderate symptoms of depression during kratom cessation" than those who consumed less. Cessation from regular and long-term kratom tea consumption was not associated with symptoms of high anxiety or depression.

3.5.3 Factor 7 Updated Conclusion

Kratom's potential to produce psychic dependence (aka "dependence" or "use disorder") and physiological dependence (aka, "withdrawal") advanced considerably due to surveys, and preclinical and clinical studies. Several surveys in the US, field studies in Malaysia, and a clinical trial of pain relief efficacy that included assessment of withdrawal support the conclusions of the 2018 8-FA (Henningfield et al., 2018a). Some kratom users report dependence/addiction and/or withdrawal with a greater likelihood with higher levels of chronic daily consumption. In general, it is more readily self-managed and less likely to interfere with occupational, social and family activities and responsibilities compared to dependencies to opioids, alcohol, stimulants and other drugs of abuse.

It is also important to note that there is wide individual variability, and some people do experience what they consider to be strong addiction and withdrawal to kratom. At present, it appears likely that many if not most individuals had prior histories of dependence to opioids and/or other drugs. Their conditions remain of concern nonetheless and is another area warranting further study. People for whom kratom use is considered a serious problem should have the same access to treatment as anyone with a substance use disorder. Many addiction treatment providers already advertise and offer kratom use disorder treatment assistance.

Use of opioids such as methadone and buprenorphine should be judicious in people seeking help to manage their kratom use disorder and/or withdrawal. If they formerly or are perhaps still using opioids, then the possibility of treatment with buprenorphine or methadone may be more helpful and appropriate if kratom is not satisfactory. However, for people without prior histories of recreational opioid use and dependence, treating with buprenorphine or methadone may introduce individuals to opioids and may not be the best option. This could be like treating unwanted caffeine dependence with amphetamine to replace the caffeine.

4 DISCUSSION AND CONCLUSION

In 2018, there was sufficient evidence to conclude that there was no imminent public health threat nor high degree of pharmacological abuse potential that would justify scheduling, taking into consideration the serious foreseeable adverse public health consequences of thousands of former opioid users returning to opioids and risking overdose, as well as the *de facto* criminalization of millions of US citizens. Approximately 8 months after the Henningfield et al. 8-FA was published, the US DHHS came to the same conclusion and rescinded the 2017 recommendation to place MG and 7-OH-MG in Schedule I of the CSA (Giroir, 2018). Since January 2018, there was remarkable research relevant to the abuse potential and safety of kratom from the perspective of the CSA eight factors.

Two intravenous drug self-administration studies showed that MG did not substitute for morphine (Hemby et al., 2019) or heroin (Yue et al., 2018), and that MG pretreatment reduced morphine and heroin self-administration. An intracranial brain self-stimulation (ICSS) study showed that whereas morphine produced robust decreases in the brain stimulation threshold, MG and 7-OH-MG did not (Behnood-Rod et al., 2020).

In the evaluation of physical dependence and withdrawal potential, four studies showed MG did not carry morphine-like physical dependence or withdrawal potential (Harun et al., 2020; Hassan et al., 2020; Wilson et al., 2020; Johari et al., 2021). Moreover, MG pretreatment of animals reduced spontaneous

morphine withdrawal (Hassan et al., 2020). In MG physically dependent animals, withdrawal signs were qualitatively different and much weaker than morphine, consistent with its mixed mechanisms of action (Johari et al., 2021). In a mouse physical dependence model (Wilson et al., 2020), naloxone precipitated withdrawal in morphine- but not MG LKT-maintained animals, while LKT pretreatment significantly reduced withdrawal in the morphine-maintained mice.

These findings are consistent with new US survey data showing relatively low self-reported kratom addiction rates, with most people describing MG use to manage pain, depression, anxiety, opioid and other drug use disorders and withdrawal, and to increase alertness, focus and work performance. In addition, kratom dependence and withdrawal are generally weaker and more readily self-managed relative to opioids.

Extensive *in vitro* and *in vivo* animal neuropharmacology studies of the mechanisms of action of MG, 7-OH-MG and other alkaloids illustrate that they are not appropriately designated as opioids, opioid analogs, or "atypical opioids," though some are partial agonists with low potential to recruit beta arrestin and produce respiratory depression. 7-OH-MG produces stronger MOR mediated opioid effects on abuse potential related measures and antinociception, but naturally occurs at levels so low as to not contribute meaningfully to kratom effects. This supports recommendations that regulations should prohibit kratom products with 7-OH-MG concentrations greater than occur safely in nature.[5]

Safety assessments in pharmacokinetic and pharmacodynamic studies confirm that kratom based extracts and individual alkaloids at far higher doses than consumed by humans do not appear to carry substantial mortality risk, with one analysis suggesting a mortality risk at least 1000 times less than illicit opioids (Henningfield et al., 2019). Results support the US DHHS conclusion that "experts disagree on whether kratom by itself causes overdose deaths" (Giroir, 2018; National Institute on Drug Abuse, 2019). This does not imply that kratom does not carry a mortality risk—most substances do under certain conditions and exposure levels, another important area for further research.

As to the question of whether or not kratom poses an imminent public health threat, no analysis of factors 4–6 of the 8 CSA factors, including the FDA analysis (Food and Drug Administration, 2017b), revealed kratom to pose an imminent public health risk. The US has the most comprehensive survey data to address the need for temporary or "emergency" placement of substances into CSA Schedule I. Yet none of the major surveillance systems identified such a public health threat. This includes the old and new Drug Abuse Warning Network, Monitoring the Future, National Survey on Drug Use and Health, RADARS®, or the Treatment Evaluation Data Set. DEA's National Forensic Laboratory Information System mentioned kratom reports from 2010–2016 but none thereafter because the signal remained low. Neither has kratom been included in any DEA Annual National Drug Threat Reports.

The primary public health consequences of kratom use are well documented by four surveys of more than 20,000 kratom consumers summarized in this review, by Henningfield et al., 2018 (Henningfield et al., 2018a), and more than 20,000 comments to DEA (Drug Enforcement Adm, 2016) suggesting that millions of US citizens use kratom for health and well-being and many to self-manage opioid and other drug withdrawal and use disorders as their preferred approach. Many kratom users believe kratom is more effective, tolerable and/or accessible than other pharmaceuticals (Grundmann et al., 2018; Swogger and Walsh, 2018; Prozialeck et al., 2019; Prozialeck et al., 2020).

There are problems with kratom product purity (e.g., Prozialeck et al., 2020) (Prozialeck et al., 2020) and adulteration (Prozialeck et al., 2019) in the consumer marketplace. A scheduling imposed kratom ban would likely worsen these problems because kratom marketing would not discontinue and consumer demand would not cease, rather marketing would switch from regulatable lawful to illicit kratom suppliers. More states and ideally the US federal government could address these issues by product performance standards and regulatory approaches guided by science and informed through a federal rule-making approach.

Remarkably, as discussed in several reports (Henningfield et al., 2019; Prozialeck et al., 2019; Henningfield et al., 2021), there has yet to emerge a generally accepted estimate of the number of current US kratom consumers, which current ranges from approximately 2 to more than 10 million (see factors 4–6 and Henningfield et al., 2021) (Henningfield et al., 2021). As noted by Henningfield et al., 2018 and bluntly stated in the US DHHS scheduling rescission letter (Giroir, 2018), surveys need to address such issues before any action to ban consumer kratom sales and possession is contemplated. As stated in the DHHS letter:

"Further analysis and public input regarding kratom and its chemical components are needed before any scheduling should be undertaken. It is important that we have additional information to justify scheduling, such as:

- A scientific assessment of how many Americans utilize *kratom,* and an understanding of the geographic and demographic distribution of these users (Factors 4, 5);
- A scientific assessment of the actual scale and degree of dependence and/or addiction of Americans utilizing *kratom* (Factors 1, 5, 7);
- A scientific determination based on data whether *kratom* actually serves as a gateway drug that promotes further use of more dangerous opioids (Factors 1, 4, 5).
- A valid prediction of how many kratom users will suffer adverse consequences if kratom is no longer available, including among people with intractable pain, psychological distress, risk for suicide; and/or people who might transition to proven deadly opioids such as prescription opioids, heroin, or fentanyl.

[5]Five states (AZ, GA, NV, OK, and UT) have taken this approach in their kratom consumer protection regulations and law but setting actual performance standards to address the variety of kratom based products would be seem best done by FDA which has extensive experience in such matters and could take a federal rule making approach that ensures input from diverse stakeholders representing science, public health, consumers, and the industry that prepares and manufactures kratom products.

- A scientifically valid assessment of causality in the current few deaths in which *kratom* was co-utilized with known lethal drugs such as fentanyl (Factors 1, 2, 3, 5 and 6)" (Giroir, 2018).

By law, scheduling considers diverse evidence including chemistry and pharmacology, level of abuse potential, physiological dependence determined in animal and human studies, as well as assessment of individual and public health risks and benefits. Taking all of these factors into account, this review provides stronger evidence than was available to Henningfield et al., or the US DHHS in 2018 (Henningfield et al., 2018a; Giroir, 2018) to recommend not only that CSA scheduling is not warranted but that CSA scheduling carries a substantial foreseeable risk of thousands of opioid overdose deaths as well as depriving millions of US citizens of one of their preferred health management assets. The fact that possession of kratom by millions of US citizens would be criminalized as a heroin-like drug felony offense is not a CSA consideration but should not be ignored.

In conclusion, we do not recommend scheduling kratom or any of its alkaloids in the CSA. We do recommend accelerated research to address the many questions raised in this review, including support of the potential development of new medicines with potential better safety and/or efficacy profiles for a variety of diseases. Finally, we recommend that the US federal government and other nations consider approaches to kratom regulation as are presently being pioneered in five US states.

AUTHOR CONTRIBUTIONS

JH was the primary scientist/investigator, and lead the identification of articles, writing, and analysis. DW supported writing, research, and analysis. MH provided toxicological analysis of articles and supported writing and analysis.

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REVIEW
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The Chemical and Pharmacological Properties of Mitragynine and Its Diastereomers: An Insight Review

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Mitragynine, is a naturally occurring indole alkaloid that can be isolated from the leaves of a psychoactive medicinal plant. *Mitragyna speciosa*, also known as kratom, is found to possess promising analgesic effects on mediating the opioid receptors such as μ (MOR), δ (DOR), and κ (KOR). This alkaloid has therapeutic potential for pain management as it has limited adverse effect compared to a classical opioid, morphine. Mitragynine is frequently regarded to behave like an opioid but possesses milder withdrawal symptoms. The use of this alkaloid as the source of an analgesic candidate has been proven through comprehensive preclinical and clinical studies. The present data have shown that mitragynine is able to bind to opioid receptors, particularly MOR, to exhibit the analgesic effect. Moreover, the chemical and pharmacological aspects of mitragynine and its diastereomers, speciogynine, speciociliatine, and mitraciliatine, are discussed. It is interesting to know how the difference in stereochemical configuration could lead to the difference in the bioactivity of the respective compounds. Hence, in this review, the updated pharmacological and toxicological properties of mitragynine and its diastereomers are discussed to render a comprehensive understanding of the pharmacological properties of mitragynine and its diastereomers based on their structure–activity relationship study.

Keywords: mitragynine, diastereomers, indole alkaloids, analgesic, opioid receptor

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INTRODUCTION

Mitragynine **(1)** is an interesting natural product in the class of alkaloids that can be primarily isolated from the leaves of a medicinal plant, known as *Mitragyna speciosa* Korth (Gogineni et al., 2015). *M. speciosa* (**Figure 1**) is an indigenous and popularly cultivated plant from the Rubiaceae (coffee) family that grows in Southeast Asia, especially Malaysia and Thailand (Brown et al., 2017). In Malaysia, it is called ketum or biak-biak, while in Thailand, the plant is commonly known as kratom (Papsun et al., 2019; Chakraborty and Majumdar, 2020; Goh et al., 2021). Locals from southern Thailand and northern Malaysian Peninsular traditionally consumed the aqueous decoction for its distinctive medicinal properties to treat a variety of ailments such as diarrhea, muscle pain, and hypertension (Ilmie et al., 2015; Meireles et al., 2019).

The mitragynine **(1)** content in the leaves of *M. speciosa* varies considerably and is affected by geographical and climate conditions (Boffa et al., 2018). For example, Takayama (2004) found that

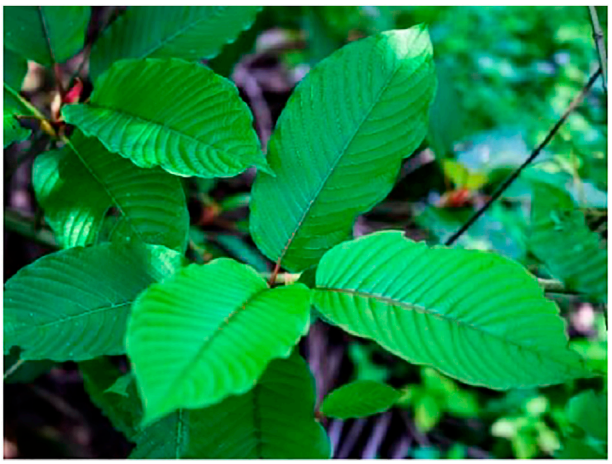


FIGURE 1 | *M. speciosa* leaves.

mitragynine **(1)** contained about 12% mitragynine content in *M. speciosa* cultivated in Malaysia; meanwhile, Thailand's *M. speciosa* possessed a much higher concentration of 66% mitragynine content. However, a recent finding by Goh et al. (2021) found that the mitragynine content in the Malaysian *M. speciosa* variant to be between 6.53% and 7.19%. The variation in mitragynine content could be attributed to several factors that may intrinsically affect the main constituent content, such as different chemotypes, climate, environmental pressures, and soil types (Chear et al., 2021).

Apart from mitragynine **(1)**, other interesting alkaloids are also present in considerable amounts in *M. speciosa*, especially in leaves. To date, about 54 alkaloids have been successfully isolated and identified from this species, which begin from the isolation of indole-alkaloid, mitragynine **(1),** by Ellen Field in the year 1921 (Flores-Bocanegra et al., 2020). Subsequently, other alkaloids from *M. speciosa* were discovered. Mitragynine's **(1)** diastereomers, speciogynine **(2)**, speciociliatine **(3),** and mitraciliatine **(4)** were some of the prominent indole alkaloids that were found in *M. speciosa*. The biosynthesis of mitragynine **(1)** and its diastereomers is a complex system that involves various enzymatic steps in forming the respective phytoconstituents. Jumali et al. (2011) and Chear et al. (2021) have proposed putatively possible biosynthetic pathways where these phytoalkaloids were synthesized *via* a typical indole alkaloid pathway, starting from the shikimic acid pathway together with the methyl-erythritol phosphate pathway (MEP). Corresponding to mitragynine **(1)**, the content of the analogues of mitragynine **(1)** also varies significantly based on regional varieties and the maturity of the plant (Pearson et al., 2018). For instance, the leaves of matured *M. speciosa* plant contain the main alkaloid mitragynine **(1)**, its diastereomers speciogynine **(2)**, and speciociliatine **(3)**, as well as paynantheine, while the leaves of the younger plants contain mitragynine **(1)**, speciogynine **(2)**, speciociliatine **(3)**, and some small amounts of the mitragynine's **(1)** diastereomer, mitraciliatine **(4)** (Philipp et al., 2010; Boffa et al., 2018).

Although this plant has been widely consumed due to its medicinal properties such as analgesic effect, mitragynine **(1)** have been found to be the major alkaloid in this traditional herb despite having opium-like effect at higher doses (Wilson et al., 2020). The local agriculture community commonly consumed it as a stimulant to increase endurance and counteract fatigue while working under the hot sun (Veltri and Grundmann, 2019; Ya et al., 2019). Several studies indicate that mitragynine **(1)** exhibits similar effects as cocaine (coca-like effects) and opium on the human body (Abdullah and Ismail, 2018; Wilson et al., 2020).

Moreover, mitragynine **(1)** and its diastereomers were reported to be widely metabolized by phase I and phase II metabolic enzymes into relevant metabolites based on animal and human studies (Kruegel et al., 2019; Chakraborty et al., 2021a). Metabolism of mitragynine **(1)** was first elucidated by Philipp et al. (2009). Phase I metabolism of mitragynine **(1)** and its diastereomers involved the hydrolysis of methyl ester of the propenoic acid at C-16 whereas *O*-demethylation of the methoxy group positioned at C-9 and C-17, respectively followed by oxidation or reduction reactions to form carboxylic acid or alcohol (Hanapi et al., 2021). In the human liver microsomes (HLM) system, 7-hydroxymitragynine and 9-*O*-demethylmitragynine were discovered as the most prevalent metabolites of mitragynine **(1)** (Basiliere and Kerrigan, 2020).

CHEMISTRY

Mitragynine **(1)** is a corynanthe-type monoterpene indole alkaloid. Mitragynine congeners especially its diastereomers were found to be present in the leaves of *M. speciosa* which are speciogynine **(2)**, speciociliatine **(3)**, and mitraciliatine **(4)** (Raffa, 2015). Since the diastereomeric phytoconstituents are congeners of mitragynine that have the tetracyclic indole alkaloid core structure, these compounds can be distinguished through the structural configuration at certain important

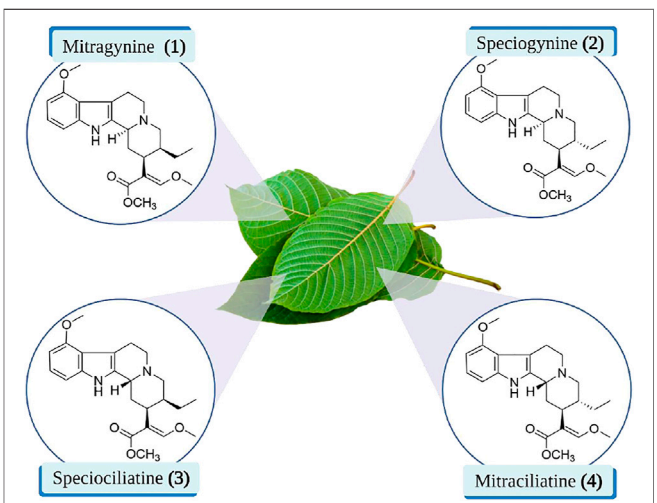


FIGURE 2 | Chemical structures of mitragynine **(1)** and its diastereomers.

TABLE 1 | The absolute configurations of mitragynine **(1)** and its diastereomers.

Compound	C-3	C-15	C-20
Mitragynine **(1)**	*S*	*S*	*S*
Speciogynine **(2)**	*S*	*S*	*R*
Speciociliatine **(3)**	*R*	*S*	*S*
Mitraciliatine **(4)**	*R*	*S*	*R*

positions (**Figure 2**) (Ellis et al., 2020). Based on the chemical structure of these compounds, the difference in the configurational positioning at C-3, C-15, and C-20 results in the occurrence of mitragynine **(1)**, speciogynine **(2)**, speciociliatine **(3)**, and mitraciliatine **(4)**, respectively. The summary of absolute configurations (*R* or *S*) at the positioning of C-3, C-15, and C-20 of the respective compounds **1**–**4** are shown in **Table 1**.

Despite the discovery of over 54 alkaloids from the leaves of *M. speciosa*, most research focused on the major constituent in the plant, which is mitragynine **(1)** (Flores-Bocanegra et al., 2020). Historically, mitragynine **(1)** was first isolated by Ellen Field in 1921, and later, the structure was completely characterized and elucidated by Beckett and Zacharias in 1965 (Gogineni et al., 2015). Furthermore, the diastereomers of mitragynine **(1),** speciogynine **(2)**, speciociliatine **(3)**, and mitraciliatine **(4)** were also reported to be isolated from the leaves of *M. speciosa*.

Based on the stereochemical configuration on the structure of mitragynine **(1)** and its diastereomers at position C-3, mitragynine **(1)** and speciogynine **(2)** possesses a flat trans-quinolizidine conformation in the rings of C and D as compared with a cis-quinolizine conformation in speciociliatine **(3)** (Takayama, 2004). In addition, speciociliatine **(3)** has a different spatial arrangement in comparison with mitragynine **(1)**, where both structures can be distinguished by a switch in the configuration from *R* [speciociliatine **(3)**] to *S* (mitragynine **(1)**) of the hydrogen moiety positioned at C-3. This configurational inversion from *R* to *S* will induce significant spatial change in the core skeleton (ring C and D) of speciociliatine **(3)**, where it will enhance its molecular volume while the inversion to mitragynine **(1)** will cause the *β*-methoxy acrylate moiety in the compound to adopt an axial position (Berthold et al., 2021).

Isolation of Mitragynine (1) and its Diastereomers

The first isolation of mitragynine **(1)** was reported by Field, a Scottish chemist in 1921 (Kruegel and Grundmann, 2018). Subsequently, Beckett et al. (1965) established the chemical structure of mitragynine **(1)** while the absolute configuration of the compound was later confirmed by Zacharias et al. (1965) using the X-ray crystallographic method (Gogineni et al., 2015; Flores-Bocanegra et al., 2020). Raffa (2015) reported that decades later, the diastereomers of mitragynine **(1)**, speciogynine **(2)**, and speciociliatine **(3)** were discovered and isolated by Beckett et al. (1965) and Shellard et al. (1978). Another diastereomer, mitraciliatine **(4),** was also reported from the leaves of *M. speciosa* (Flores-Bocanegra et al., 2020). The increased interest in the alkaloids of *M. speciosa* by natural products and medicinal chemists had led to an increasing amount of research conducted in isolating other phytoconstituents by using various chromatographic techniques. However, there are issues regarding the purity of the alkaloids isolated from *M. speciosa* due to the difficulty in separating and isolating isomeric alkaloids. Goh et al. (2021) found a fast and rapid method in the isolation of mitragynine **(1)** with a peak purity of 98%, which was affirmed using HPLC analysis. Meanwhile, Chear et al. (2021) reported on the isolation of speciogynine **(2)** and speciociliatine **(3)** with high purity (≥98%) using column chromatographic techniques. These studies provide an understanding in solving the purity issue of the alkaloid drugs. It also prompted researchers to develop a set of guidelines to ensure that the purity (≥95%) is within the required guidelines as it is vital for preclinical and clinical studies.

Characterization of Mitragynine (1) and Its Diastereomers

Complete characterization and elucidation of mitragynine **(1)** and its diastereomers were reported recently by Flores-Bocanegra et al. (2020) and Chear et al. (2021) using nuclear magnetic resonance (NMR) and mass spectrometry (MS) analyses. Flores-Bocanegra et al. (2020) devised a simple and comprehensive decision tree to distinguish the indole and oxindole alkaloids discovered from *M. speciosa* through the identification of important chemical shifts such as ^{1}H and ^{13}C NMR signals. Hence, **Figure 3** showed the simplified version of the decision tree chart as adapted from Flores-Bocanegra et al. (2020) where the flow of decision for the identification of mitragynine **(1)** and its diastereomers, speciogynine **(2)**, speciociliatine **(3)**, and mitraciliatine **(4)**, are comprehensively depicted. The references for spectral data of mitragynine **(1)** and its diastereomers are tabulated in **Table 2**.

PHARMACOLOGY/STRUCTURE ACTIVITY RELATIONSHIP STUDY (SARS)

The pharmacological activity of the alkaloids from *M. speciosa* has been extensively researched, focusing on the analgesic potency of the primary indole alkaloid, mitragynine **(1)**. Isomeric indole alkaloids such as mitragynine **(1)**, speciogynine **(2)**, and speciociliatine **(3)** were assessed through *in vivo* and *in vitro* approaches for their pharmacological properties, especially in assessing their analgesic and toxicological properties. Takayama (2004) accumulated evidence implicating the opioid receptor system as the primary mediator of the central nervous system effects displayed by these isomeric phytoalkaloids. To the best of our knowledge, we found very limited pharmacological evidence on mitraciliatine **(4)**. The pharmacological properties of these alkaloids are shown below. Selected pharmacological activities on mitragynine and its diastereomers are summarized in **Figure 4**.

Macko et al. (1972) first reported the pharmacological studies of mitragynine **(1)**. The analgesic potency of mitragynine **(1)** has

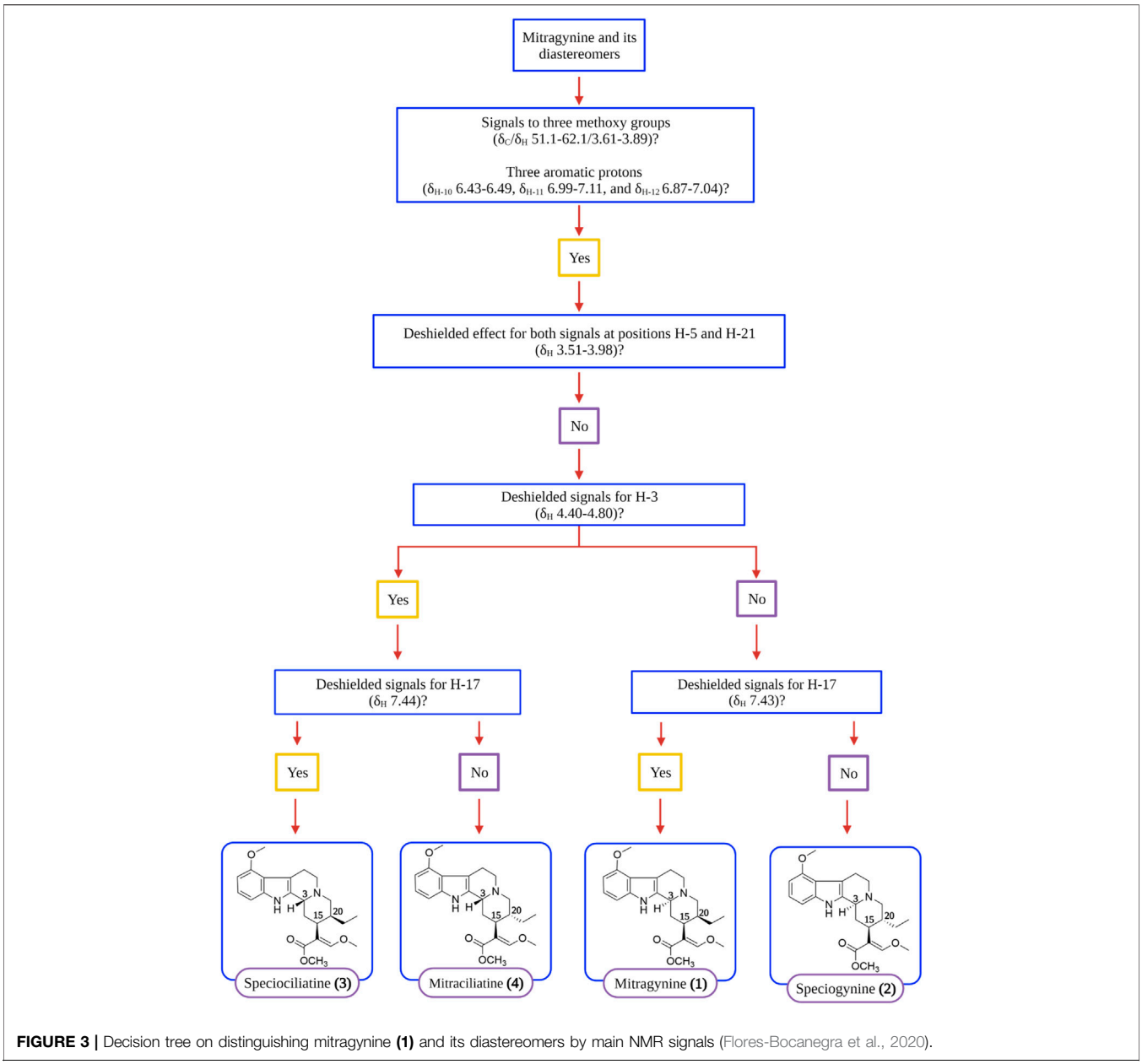


FIGURE 3 | Decision tree on distinguishing mitragynine **(1)** and its diastereomers by main NMR signals (Flores-Bocanegra et al., 2020).

TABLE 2 | Reference spectral data for mitragynine **(1)** and its diastereomers.

Compound	Spectral data
Mitragynine (1)	^{1}H (600 MHz & 400 MHz), ^{13}C (150 MHz & 100 MHz) NMR and HRESIMS data (Chear et al., 2021 and Flores-Bocanegra et al., 2020)
Speciogynine (2) Speciociliatine (3) Mitraciliatine (4)	^{1}H (400 MHz), ^{13}C (100 MHz) NMR and HRESIMS data (Flores-Bocanegra et al., 2020)

been studied mostly through tail-flick and hot plate tests. It was found to induce antinociception in the brain. It was also reported that the antinociceptive action of mitragynine in mice was at least partly involved in the supraspinal opioid systems (Matsumoto et al., 1996). Currently, detailed pharmacology studies are being conducted on mitragynine **(1)** and its diastereomers to

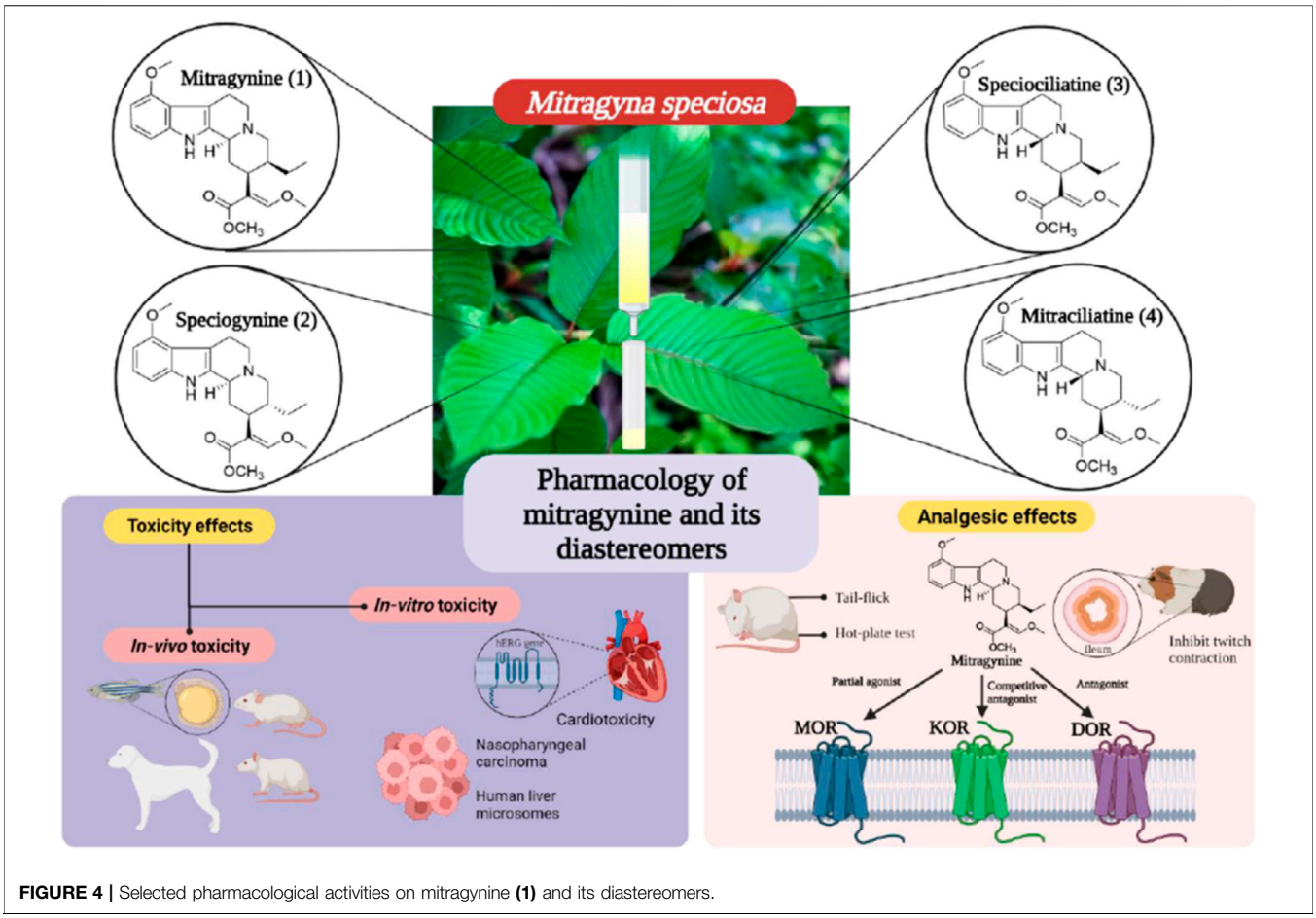


FIGURE 4 | Selected pharmacological activities on mitragynine **(1)** and its diastereomers.

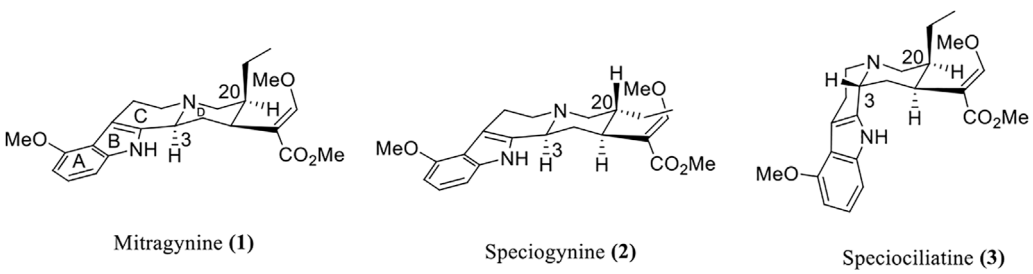


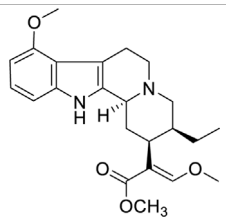
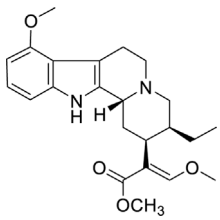
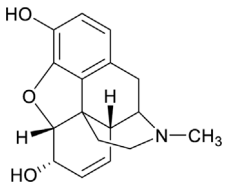
FIGURE 5 | Stereochemical conformation of mitragynine **(1)**, speciogynine **(2)**, and speciociliatine **(3)**.

understand their mechanism of action and their structure–activity relationship in pain management.

A preliminary study conducted by Takayama et al. (2002) on the biological activities of *M. speciosa* crude leave extract with several bioactive alkaloids showed promising results. The first noteworthy result was shown through the ability of mitragynine **(1)** in inhibiting the twitch contraction of guinea pig ileum, which is induced by electrical stimulation. It was reported that the opioid agonistic activities of mitragynine **(1)** and speciociliatine **(3)** were evaluated based on their ability to inhibit contraction that stimulates the guinea pig ileum, which is reversed by a classical antagonist, naloxone. Mitragynine **(1)**, as portrayed in **Figure 5** below, possesses a unique flat trans-quinolizidine conformation in the C/D ring junction, while speciociliatine **(3)**, a C-3 diastereomer of mitragynine **(1)**, assumes a folded cis-quinolizidine conformation.

Based on the opioid agonistic activities of mitragynine **(1)** and speciociliatine **(3)** in electrically stimulated guinea-pig ileum (**Table 3**), the potency of speciociliatine **(3)** toward the mu opioid receptor (MOR) was shown to be 13 times weaker than mitragynine **(1)**. The pD_2, also known as pEC_{50}, is the negative logarithm to base 10 of the EC_{50} of an agonist. The pD_2 value

TABLE 3 | Opioid Agonistic Activity of Mitragynine **(1)**, Speciociliatine **(3)**, and Morphine in Electrically Stimulated Guinea-Pig Ileum Preparation.

Compound	Structure	[a]pD_2 value	[b]Relative potency (%)	[c]Relative inhibitory activity (%)
Mitragynine **(1)**		6.95 ± 0.12	26	95
Speciociliatine **(3)**		5.40 ± 0.07	2	101
Morphine		7.17 ± 0.05	100	100

[a]*pD_2 values indicate the potency of agonist, the higher pD_2 reflects higher potency of the agonist.*
[b]*Relative potency is shown as a percentage of the pD_2 value of the compound against that of morphine.*
[c]*Relative inhibitory activity reflects to the intrinsic activity on opioid receptors, is shown as a percentage of the maximum inhibition by compounds against that by morphine. All data points represent mean ± SEM (μM) of* n ≥ 3.

TABLE 4 | Binding Affinities of Mitragynine **(1)** and its Diastereomers at Human Opioid Receptor (Obeng et al., 2021).

Compound	K_i ± SEM (μM)[a]		
	hMOR (μ)	hKOR (κ)	hDOR (δ)
Mitragynine **(1)**	0.233 ± 0.048	0.772 ± 0.207	>10
Speciogynine **(2)**	0.728 ± 0.061	3.200 ± 0.360	>10
Speciociliatine **(3)**	0.560 ± 0.168	0.329 ± 0.112	>10

[a]*All data points present mean ± SEM (μM) of* n ≥ 3.

indicates the potency of an agonist but not its efficacy. Based on the pD_2 value observed in **Table 3**, the performance of mitragynine (pD_2 = 6.95 ± 0.12) as an opioid agonist was weaker compared to morphine (pD_2 = 7.17 ± 0.05). This implies that the flat trans-quinolizine conformation form in mitragynine **(1)** was a more efficient conformation to exhibit the opioid activity than folded cis-quinolizidine.

Based on the *in vitro* studies conducted by Takayama et al. (2002), mitragynine **(1)** was reported to have a significant binding affinity on the opioid receptor. This study was conducted on the guinea pig brain homogenates. There is a total of three ligand subtypes that were used to assess the binding affinities of the compound mitragynine **(1)** and on the opioid receptor subtypes. They were MOR-selective ligand DAMGO ([D-Ala^2, *N*-MePhe^4, Gly-ol]-enkephalin), the DOR-selective ligand DPDPE (D-Pen^2-D-Pen^5-enkephalin), and the KOR-selective ligand U-69,593 (N-methyl-2-phenyl-N-[(5*R*,7*S*,8*S*)-7-(pyrrolidin-1-yl)-1-oxaspiro [4.5] dec-8-yl] acetamide). From this study, it was shown that the affinity of mitragynine **(1)** at MOR, DOR, and KOR is 7.2, 60, and >1,000 nM, respectively. It also displayed a nearly 10-fold selectivity for MOR over DOR sites and greater than 1,000-fold selectivity for MOR over KOR.

Kruegel et al. (2016) studied the activity of mitragynine **(1)** and its other analogues speciogynine **(2)** and speciociliatine **(3)**. The study was conducted on HEK cell lines expressing the human opioid receptor. The results indicate that mitragynine **(1)** acts as a partial agonist at MOR; meanwhile, speciogynine **(2)** and speciociliatine **(3)** were both weak antagonists at MOR. Additionally, it was also found that mitragynine **(1)** and its diastereomers bind significantly at MOR than KOR and DOR (Kruegel et al., 2016; Zhou et al., 2021). A deeper view on the binding affinities of mitragynine **(1)** and its diastereomer showed that mitragynine **(1)** has the highest binding affinities, followed by speciociliatine **(3)** and speciogynine **(2)** (Obeng et al., 2021). Even though mitragynine **(1)** and its diastereomer bind actively at MOR, its activity at the receptor is not identical, as shown in **Table 4**.

Based on **Table 5** above, mitragynine **(1)** act as a partial agonist at MOR with a maximal efficacy of 34%. However, at

TABLE 5 | The Functional Activity of *M. speciosa* Alkaloids at Human Opioid Receptors in G protein BRET Assays (Kruegel et al., 2016).

Compound	EC_{50} ± SEM (E_{max})[a] or [IC_{50} ± SEM (pA_2)][b]		
	hMOR	hKOR	hDOR
Mitragynine **(1)**	0.339 ± 0.178 (34%) (partial agonist)	8.5 ± 7.6 (1.4) (competitive antagonist)	>10 (antagonist)
Speciogynine **(2)**	5.7 ± 2.8 (weak antagonist effect)	>10 (weak antagonist effect)	>10 (weak antagonist effect)
Speciociliatine **(3)**	4.2 ± 1.6 (weak antagonist effect)	>10 (weak antagonist effect)	>10 (weak antagonist effect)

[a]*EC_{50} values indicate the agonist activity, (E max) relative to DAMGO, in parentheses.*
[b]*IC_{50} values indicate the inhibition of a reference agonist, (pA_2) determined from Schild analysis in parentheses.*
All data points represent mean ± SEM (μM) of n ≥ *3.*

TABLE 6 | Screening of mitragynine **(1)** and speciociliatine **(3)** at opioid receptor (Obeng et al., 2020).

Binding site	Percent displacement of bound radioligand			
	Mitragynine (1)		Speciociliatine (3)	
	100 nM	10,000 nM	100 nM	10,000 nM
MOR	29.0	93.7	64.7	98.0
KOR	25.2	88.3	61.9	98.5
DOR	0.4	18.3	0.6	69.2
Nociceptin/Orphanin FQ peptide (NOP)	4.3	40.8	−14.9	31.8

TABLE 7 | Binding affinities of mitragynine **(1)** and speciociliatine **(3)** to opioid receptor and subtype selectivity (Obeng et al., 2020).

Compound	DOP K_i ± SEM (nM)	KOP K_i ± SEM (nM)	MOP K_i ± SEM (nM)
DAMGO	ND	ND	0.41 ± 0.04
DPDPE	1.32 ± 0.004	ND	ND
U50488	ND	0.300 ± 0.002	ND
Mitragynine **(1)**	ND	198 ± 30	161 ± 10
Speciociliatine **(3)**	ND	116 ± 36	54.5 ± 4.4

KOR and DOR, the functional activity of mitragynine **(1)** alkaloid converts from partial agonist to antagonist at lower potency. Additionally, the diastereomers of mitragynine **(1)**, speciogynine **(2)**, and speciociliatine **(3)** showed null measurable agonist activity at all the opioid receptors and only revealed a weak antagonist effect. By comparing mitragynine **(1)** and speciogynine **(2)**, the ethyl group at position 20 on ring D shows a crucial point as the epimerization of this group is able to switch the agonistic activity to antagonist activity at MOR. The modification of the configuration of the ethyl group also reduced the binding affinity.

Obeng et al. (2020) carried out a pharmacological investigation on mitragynine **(1)** and speciociliatine **(3)** to evaluate its opioid binding affinities (**Table 6**). According to the results obtained in **Table 7**, the binding affinities of speciociliatine **(3)** (K_i (MOR): 116 ± 36 nM, K_i (KOR): 54.5 ± 4.4 nM) at both opioid receptors are higher than mitragynine **(1)** (K_i (MOR): 198 ± 30 nM, K_i (KOR): 161 ± 10 nM). Berthold et al. (2021) also found that the binding affinity of speciociliatine **(3)** at MOR and KOR was 3.0- and 1.7-fold higher than that of mitragynine **(1)**. Based on the two studies, it is suggested that the conversion of the configuration at position 3 from *S* [mitragynine **(1)**] to *R* [speciociliatine **(3)**] causes a significant change in terms of the binding affinities. This switch in conformation speciociliatine **(3)** will cause the molecular volume of speciociliatine **(3)** to have a larger space to bind and interact with the active sites of the opioid receptor and increase its binding affinities compared to mitragynine **(1)**. Based on molecular docking studies, the acrylate moiety will affect the interaction with the key residue, which plays an important role in binding to the opioid receptors. However, these results were contrary to what was reported by Kruegel et al. (2016), who found that speciociliatine **(3)** had no significant agonist activity at all the human opioid receptors and acted as a weak antagonist. The difference might be due to different assay types used to evaluate speciociliatine **(3)**. This result was confirmed by a study by Nickolls et al. (2011) that different types of assays used to evaluate the targeted compound will show different agonistic effects.

The ethyl group in ring D is extremely crucial in predicting the binding affinities at the opioid receptor. The ethyl group will act as a hydrophobic group that interacts with the receptor. For the binding affinities of the structure without the ethyl group, the binding affinities are diminished drastically as compared to mitragynine **(1)**. All in all, the number of different stereochemical configurations in the

diastereomers can retain to bind at MOR. Meanwhile, absolute stereochemistry is found to be crucial in agonistic activity in the opioid receptor.

In a recent report by Chakraborty et al. (2021b) on the opioid receptor function of mitraciliatine **(4)**, pharmacological evidence was reported on this phytoalkaloid. In this study, mitraciliatine **(4)** (K_i (MOR): 135.1 ± 7.7 nM) portrayed partial opioid agonism and identified it as structurally unique natural products with safer, MOR-dependent antinociception. Mitragynine **(1)** [K_i (KOR): 231 ± 21 nM] was reported to have weak KOR antagonism in contrast with mitraciliatine **(4)** (K_i (KOR): 101.2 ± 2.3 nM, K_i (MOR): 6.52 ± 0.06 nM), which showed KOR full agonism and MOR partial agonism at both human and mouse receptors. Mitraciliatine **(4)** (E_{max} (KOR): 104%) showed robust β arrestin-2 recruitment at KOR, while it does not recruit β arrestin-2 at MOR. Based on this, it appears that mitraciliatine **(4)** (Ki (KOR): 73 nM)/ (Ki (MOR): 304 nM) has a higher receptor selectivity for opioids over adrenergic receptors with fewer off-target interactions. The activity portrayed might be due to its stereochemical configuration at C-3 [*S* in mitragynine **(1)** and *R* for mitraciliatine **(4)**] and C-20 [*S* in mitragynine **(1)** and *R* for mitraciliatine **(4)**], which plays a vital role in the SAR of the respective phytoalkaloids. Chakraborty et al. (2021c) also performed an investigation on mitragynine **(1)** and its synthesized analogues, focusing on the C-9 position in the scaffold of mitragynine **(1)**. The three synthesized analogues, 9-3′-furanyl mitragynine, 9-phenyl mitragynine, and 9-methyl mitragynine, demonstrated partial agonism toward G-protein and arresting signals mediated by MORs. The synthesized analogues exhibit moderate activity and potency (EC_{50} > 50 nM) in cAMP assays and poor β-arrestin2 recruitment (E_{max} < 20%) at MOR. The semisynthetic modifications of mitragynine **(1)** at C-9 position using moieties such as 3′-furanyl, phenyl, and methyl do not enhance the potency toward the tested activities.

A previous study conducted by Matsumoto et al. (1997) had affirmed that mitragynine **(1)** displayed a suppressive effect on the central serotonin neurotransmission system. In mice, the suppression of 5-HT_{2A} agonist (5-methoxy-N, N-dimethyltryptamine)-induced head twitch response was observed due to the effect from the pre-treatment with mitragynine **(1)**, which showed that the principal kratom alkaloid acts as a competitive antagonist in blocking the stimulation of the 5-HT_{2A} receptor (Hanapi et al., 2021). León et al. (2021) investigated the *in vitro* and *in vivo* activity of kratom alkaloids, especially mitragynine **(1)**, speciogynine **(2)**, and speciociliatine **(3)** at serotonin receptors (5-HTRs). Surprisingly, speciogynine **(2)** portrayed a high affinity toward 5-HT_{1A}Rs and 5-HT_{2B}Rs, in contrast with its major diastereomer, mitragynine **(1)**. Speciogynine **(2)** exhibited antinociceptive properties in rats *via* an opioid receptor-independent mechanism. Since mitragynine **(1)** (20*S*) and speciogynine **(2)** (20*R*) are diastereomers that differ in C-20 position, the structural difference of the β-methoxyacrylate group in both diastereomers might cause the difference in the potency toward the tested activity (León et al., 2021).

Toxicity of Mitragynine (1) and Its Diastereomers

In vitro Toxicity

Several cytotoxic studies of mitragynine **(1)** and its diastereomers were conducted toward selected cancerous and non-cancerous cell lines. Lu et al. (2014) provided the first scientific evidence on cardiotoxicity of mitragynine **(1)** and its diastereomers. Based on the report, mitragynine **(1)** administered at 10 μM showed significant cardiotoxicity by inhibiting the human ether-à-go-go-related gene (hERG) current. Meanwhile, mitragynine **(1)** (10 μM) also prolongs action potential duration (APD) and induces arrhythmia. Moreover, mitragynine **(1)**, speciogynine **(2)**, and speciociliatine **(3)** were dosage-dependently (0.1, 100 μM) suppressed I_{Kr} in hiPSC-CMs at 67%~84% with IC_{50} ranging from 0.91 to 2.47 μM. The inhibition of hERG has been associated with favorable binding of drugs to open and inactivated states of hERG channels. The inward potassium currents are primarily active during phase 1 of the cardiac AP, while calcium channels are primarily active during phase 3. QT prolongation is primarily an issue arising after depolarizations.

In 2015, Saidin et al. (2015) reported on the cytotoxicity of mitragynine **(1)** tested against SH-SY5Y and MCL-5 cell lines, respectively. Mitragynine (**1**) exhibited a moderate cytotoxic effect against these reported cell lines with IC_{50} values of 75 and 80 μM, respectively. Oliveira et al. (2016) also reported on moderate cytotoxicity of mitragynine **(1)** evaluated against Caco-2 (42.5 μg/ml) and SH-SY5Y (42.6 μg/ml) cell lines. The moderate activity of mitragynine could be attributed to the absence of OH moiety in its tetracyclic monoterpenoid indole alkaloid nucleus (Rosales et al., 2020).

Kamble et al. (2020) reported on the inhibition of CYP 450 isoforms in human liver microsomes by *M. speciosa* alkaloids, mainly mitragynine **(1)**, and its diastereomers. Cytochrome P450 (CYP450) is a group of enzymes that play a predominant role in drug metabolism; therefore, an alteration in CYP450-mediated metabolism could result in drug interactions that include fatality. The findings demonstrated that mitragynine **(1)** (IC_{50}: 2.2 μM) was a potent and relatively selective inhibitor toward CYP2D6, while it possessed moderate inhibition with IC_{50} 11.4 μM toward CYP3A4/5 (an isoform of CYP450). Additionally, speciogynine **(2)** (IC_{50}: 19.5 μM) and speciociliatine **(3)** (IC_{50}: 8 μM) displayed moderate inhibition toward CYP2C19. However, there was no activity shown on mitragynine **(1)**, speciogynine **(2)**, and speciociliatine **(3)** toward the other tested CYP450 isoforms. Mitragynine **(1)** and its diastereomers have a more planar three-dimensional structure where the indoloquinazoline moiety of these compounds was completely overlapped. The planar structure of these three compounds might reflect in the strong inhibition of CYP2D6 and CYP3A4/5.

Another *in vitro* toxicity study on mitragynine **(1)** and its diastereomers was reported. Mitragynine **(1)**, speciogynine **(2)**, and speciociliatine **(3)** possessed weak-to-moderate inhibition against nasopharyngeal carcinomas NPC/HK-1 and C666-1 cell lines. In the study, mitragynine **(1)** exhibited the highest inhibition against the growth of NPC/HK-1 cells, followed by speciociliatine **(3)**. The overall SARs study revealed that the *R* and *S* orientations at positions C-3 and C-20, respectively, are the key features that determine the cytotoxicity of mitragynine **(1)** and its diastereomers. Besides, speciociliatine **(3)** was shown to possess weaker cytotoxicity than mitragynine **(1)** due to the inversion of orientation from *R* to *S* at the C-3 position. However, the cytotoxicity of speciogynine **(2)** was shown to be abolished due to the *R* orientation at the C-20 position of the respective compound (Domnic et al., 2021).

Additionally, Goh et al. (2021) found that mitragynine **(1)** possessed higher IC_{50} values against two cell lines, HEK-293 kidney cell (IC_{50}: 112.30 ± 17.59 μM) and HeLa Chang liver cell (IC_{50}: 210.04 ± 0.80 μM), being compared to all of the tested ASE *M. speciosa* extracts. Mitragynine **(1)** showed moderate toxicity toward these cell lines, which suggested that mitragynine **(1)** could have selectively exhibited a cytotoxicity effect on both cancerous and non-cancerous cell lines.

In vivo Toxicity

Initial studies on the *in vivo* toxicity of mitragynine **(1)** on rats and dogs were documented in the year 1972. A single dosing of mitragynine **(1)** (806 mg/kg) produced no toxicity in rats, and multiple oral 50 mg/kg/day also showed no observable side effects. Subsequently, the daily dosage of 16 mg/kg and two additional days of oral 32 mg/kg in dogs also showed no side effects. However, at higher doses and longer exposures, primarily, blood dyscrasias were also observed (Macko et al., 1972).

Sabetghadam et al. (2013) reported a relatively safe consumption of lower to sub-chronic amounts of mitragynine **(1)** in rats (1–10 mg/kg) but detected signs of toxicity at higher doses (100 mg/kg) when histopathological, hematological, and biochemical effects of the liver, kidney, and brain were observed. The authors suggested that the use of mitragynine **(1)** in the dose range studied is generally safe as there have been no deaths reported and no significant differences in overall behavior.

Additionally, Damodaran et al. (2021) reported on the toxicity assessment of two diastereomeric alkaloids, mitragynine **(1)** and speciociliatine **(3)**, on the zebrafish embryo model. This study aimed to assess the possible toxicity effects exhibited by the two alkaloids on the zebrafish embryo model with different toxicity parameters, namely, mortality, hatching rate, heart rate, and morphological malformations. It showed that acute embryonic exposure to mitragynine **(1)** and speciociliatine **(3)** affected the survival, hatching, and body morphology of zebrafish embryos in a concentration- and time-dependent manner, which indicates that higher compound concentrations and longer exposure times affect the development of zebrafish embryo. In the mortality assessment of zebrafish embryos, the LC_{50} value of mitragynine **(1)** was 32.01 μg/ml at 96 hpf, while the estimated LC_{50} value of speciociliatine **(3)** was slightly higher at 79.86 μg/ml, indicating that speciociliatine **(3)** is relatively safer than mitragynine **(1)**. The data obtained on the hatching parameter showed that mitragynine **(1)** and speciociliatine **(3)** at concentrations of 25 and 50 μg/ml appeared to be associated with the delayed hatching process, whereas for the morphological malformation parameter, spinal curvature (scoliosis) was observed in mitragynine **(1)** (50 μg/ml)- and speciociliatine **(3)** (25 and 50 μg/ml)-exposed groups. It was concluded that mitragynine **(1)** and speciociliatine **(3)** (≥50 μg/ml) possessed certain undesirable effects on embryonic development by affecting the survival, hatching, and body morphology of zebrafish embryos, which relays to the potential risk of kratom intake during pregnancy on the development of the fetus. This is because the early embryo developmental process of zebrafish is similar to humans.

CONCLUSION

In conclusion, the mitragynine **(1)** template and its structural information could render medicinal chemists an opportunity to develop a new analgesic that can be beneficial toward pain management and treatment. It is vital for chemists and pharmacologists to determine its maximum analgesic potency as well as alter the opioid-induced side effects through detailed preclinical and clinical studies. The alkaloidal chemistry, especially focusing on the functional activity of mitragynine **(1)** and its diastereomers toward opioid receptors such as MOR, needs to be investigated in detail. The pharmacokinetic properties of the phytoalkaloids in terms of absorptivity, distribution, and metabolism as well as its polypharmacological properties need to be studied extensively. The information obtained from the studies conducted on the chemistry of mitragynine **(1)** and its diastereomers could lead toward an efficient botanical extract development of *M. speciosa* where it can be used as an alternative botanical drug in treating pain. Based on this review, the previous preclinical and clinical studies conducted on the mitragynine **(1)** and its diastereomers could support and provide an in-depth insight on the medicinal benefits of this plant, which could lead to drug development in treating pain and addiction. Therefore, it serves as an important research point to prompt medicinal chemists and pharmacologists to conduct extensive studies on the chemistry and synergism activity of mitragynine **(1)** and its diastereomers to understand the opioid-like mechanistic activity relating to the medicinal benefits of this plant.

AUTHOR CONTRIBUTIONS

TK, KN, and AZ conceptualized and designed the review. KN, TK, AZ, VM, and MA were involved in the literature search. TK and KN wrote the first draft of the manuscript. TK, AZ, VM, and MA were involved in the revision of the manuscript and approved the submitted version.

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Searching for a Signal: Self-Reported Kratom Dose-Effect Relationships Among a Sample of US Adults With Regular Kratom Use Histories

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There is limited understanding regarding kratom use among US adults. Although motivations for use are increasingly understood, typical kratom doses, threshold of (low and high) doses for perceived effectiveness, and effects produced during cessation are not well documented. We aimed to extend prior survey work by recruiting adults with current and past kratom exposure. Our goal was to better understand kratom dosing, changes in routines, and perception of effects, including time to onset, duration, and variability of beneficial and adverse outcomes from use and cessation. Among respondents who reported experiencing acute kratom effects, we also sought to determine if effects were perceived as helpful or unhelpful in meeting daily obligations. Finally, we attempted to detect any signal of a relationship between the amount of kratom consumed weekly and weeks of regular use with ratings of beneficial effects from use and ratings of adverse effects from cessation. We conducted an online survey between April-May 2021 by re-recruiting participants from a separate study who reported lifetime kratom use. A total of 129 evaluable surveys were collected. Most (59.7%) had used kratom >100 times and reported currently or having previously used kratom >4 times per week (62 weeks on average). Under half (41.9%) reported that they considered themselves to be a current "regular kratom user." A majority (79.8%) reported experiencing acute effects from their typical kratom dose and that onset of effects began in minutes but dissipated within hours. Over a quarter reported that they had increased their kratom dose since use initiation, whereas 18.6% had decreased. Greater severity of unwanted effects from ≥1 day of kratom cessation was predicted by more weeks of regular kratom use (β = 6.74, p = 0.02). Acute kratom effects were largely reported as compatible with, and sometimes helpful in, meeting daily obligations. In the absence of human laboratory studies, survey methods must be refined to more precisely assess dose-effect relationships. These can help inform the development of controlled observational and experimental studies needed to advance the public health understanding of kratom product use.

Keywords: kratom, *Mitragyna speciosa*, dosing, use patterns, kratom withdrawal, kratom effects

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1 INTRODUCTION

1.1 History

The plant indigenous to Southeast Asia, *Mitragyna speciosa*, commonly referred to among Westerners as "kratom," has been used in the United States and other regions outside of Asia since at least 2004 (Burkill, 1935; Boyer et al., 2007; Boyer et al., 2008). In Asia, particularly Malaysia and Thailand, kratom preparations have been used for medicinal, cultural, energy-enhancing, and recreational purposes and to decrease heroin and amphetamine misuse without significant adverse effects documented to date (Singh et al., 2016; Singh et al., 2019a; Singh et al., 2020a; Leong Bin Abdullah et al., 2020; Ramanathan and McCurdy, 2020; Saref et al., 2020; Singh et al., 2021). Although there is speculation that kratom was introduced into the US contemporaneous to the Vietnam War, it is unclear when kratom use in the US began in earnest (Legislative Analysis and Public Policy Association, 2019). As far back as 1988, researchers began to note the plant's therapeutic potential as a replacement for or supplement to methadone treatment among people with opioid use disorder (OUD; Jansen and Prast, 1988) and by 2016 it was apparent that kratom was being used by persons with and without clinical disorders, including persons with opioid and other substance use disorder (SUD) histories (Boyer et al., 2008; Swogger et al., 2015; Grundmann, 2017; Smith and Lawson, 2017).

1.2 Reasons for Use

Motivations for using kratom have become the topic of numerous case reports and surveys (Griffiths et al., 2018; Agapoff and Kilaru, 2019; Aldyab et al., 2019; Coe et al., 2019; Stanciu et al., 2019; Bowe and Kerr, 2020; Covvey et al., 2020; Garcia-Romeu et al., 2020; Schmuhl et al., 2020; Smith et al., 2021a; Weiss and Douglas, 2021; Grundmann et al., 2022a). Case reports, including those of kratom-associated fatalities, are insightful but provide limited detail and generalizability beyond the clinical presentation(s) described in the report (Olsen et al., 2019; Post et al., 2019). Most do not specify motivations for kratom use and focus largely on adverse effects, given the medical context. Larger epidemiological level surveys have been conducted with samples in the US; these studies provide more definitive understandings of kratom use motivations. However, these are also somewhat limited in their use of convenience samples of current, regular kratom-using adults who self-select into kratom-specific survey participation. Regular and current use can make such respondents a good source of information, but could conceivably contribute to response bias, in that they may have favorable attitudes about kratom use compared to infrequent or remitted users. Put differently, people who have quit using kratom likely did so for a reason (which might include having found the effects unremarkable) and therefore may be less inclined to participate in a kratom survey. Conversely, some people may be regular current users due to an inability to stop.

Nevertheless, these larger surveys have been able to elucidate many broad motivations for why persons may be using kratom, such as the self-treatment for chronic pain, fatigue, psychiatric, or SUD symptoms or to improve energy, mood, and enhance recreation generally (Grundmann, 2017; Swogger and Walsh, 2018; Coe et al., 2019; Bath et al., 2020; Garcia-Romeu et al., 2020). These reports corroborate findings from Southeast Asia (Singh et al., 2016; Singh et al., 2017; Singh et al., 2019a; Singh et al., 2020b; Müller et al., 2020). Another commonly cited reason for kratom use both in the US and Asia includes reducing, substituting, or stopping licit or illicit substances, the most common being opioids, though kratom use to abstain from alcohol or amphetamine is also reported (Saingam et al., 2013; Singh et al., 2020b; Vicknasingam et al., 2020; Singh et al., 2021). These reports converge with analyses of social-media posts and online content (Smith et al., 2021b; Smith et al., 2021c; Prevete et al., 2021; Grundmann et al., 2022b). Collectively data suggest that kratom use motivations, practices, and consequences are continuing to evolve in the US, and that frequent updates are required.

1.3 Pharmacology of Kratom

Four of kratom's over 40 known bioactive alkaloids, mitragynine (MG), 7-hydroxymitragynine (7-HG), corynoxine, and speciociliatine, appear to act at μ-opioid receptors. The two most heavily studied, MG and 7-HG, seemingly act as partial opioid receptor agonists, though non-opioid actions are also observed with these and other alkaloids (Kruegel and Grundmann, 2018; Fowble and Musah, 2019; Kruegel et al., 2019; Obeng et al., 2019; King et al., 2020; Todd et al., 2020; Berthold et al., 2021; Chear et al., 2021; Kamble et al., 2021). MG and 7-HG have been found to produce a range of mostly dose-dependent acute and chronic effects (both adverse and potentially therapeutic) that are consistent with μ-opioid receptor activity in nonhuman animals, including: discriminability as opioids (with partial generalization to psychostimulants); self-administration; conditioned place preference; attenuation of opioid self-administration and opioid withdrawal; and analgesic, antinociceptive, and anxiolytic effects (Hazim et al., 2014; Harun et al., 2015; Yusoff et al., 2017; Yue et al., 2018; Hemby et al., 2019; Hiranita et al., 2019; Hassan et al., 2020; Kamble et al., 2021; Obeng et al., 2021; Suhaimi et al., 2021). The complexity of the kratom botanical and variability of its alkaloid composition is influenced by the environmental conditions in which it grows and by harvesting or post-harvest handling practices (Zhang et al., 2012; Griffin et al., 2016; Lydecker et al., 2016; Prozialeck et al., 2020).

1.4 Understanding How Kratom Dosing Corresponds to Effects

Kratom-based survey studies have rarely provided sufficient detail to determine what constitutes a "typical" or "regular" dose. Without a specific unit of measurement, it is not possible to determine the threshold at which kratom may produce specific effects. The need for specificity is supported by studies that have found associations between use patterns and outcomes (Ahmad and Aziz, 2012; Saingam et al., 2013; Singh et al., 2014; Singh et al., 2019b; Phillip, 2019; Müller et al., 2020). For instance, Grundmann (2017) found that most participants (57.5%) experienced no negative (withdrawal-like) effects if

kratom was not taken at 12-, 24-, and 48-h increments, and, among those who did experience negative effects when not using kratom, those effects were rarely characterized as severe. Coe et al. (2019) reported high variability in the prevalence of adverse effects, ranging from 0.8% (hallucinations) to 76.8% (stomach problems), though the lack of dosing information limits interpretation of these results. Garcia-Romeu et al. (2020) did collect data on dosing from persons regularly using kratom in the US and found the typical dose range was <1 g (8.6%) to >7 g (8.9%), with most respondents reporting that they consumed 1–3 g (49.0%) or 4–6 g (33.4%) per consumption. This finding was contextualized by number of doses per week, with most respondents reporting they consumed kratom daily, primarily as a prepared beverage (37.0%) or ingesting it as raw powder (43.6%) or capsule (18.9%). Most in that sample reported mild or no adverse effects, however effects as a function of dose were not examined. One notable example is Grundmann (2017) who reported odds ratios for both beneficial and adverse effects for amount/dose and doses/week finding that most beneficial effects were observed in doses of 1–3 and 3–5 g if taken 2–3 times per day; in contrast, most adverse effects required higher doses of >8 g and higher frequency of dosing between 4–5 times per day of daily use.

These data contrast and converge with some effects described in social-media posts, wherein some individuals recounted moderate to severe effects with prolonged use at higher doses (tolerance or withdrawal symptoms), and a wide-ranging beneficial effects at specified and unspecified doses (Smith et al., 2021a; Smith et al., 2021b). But the social-media data, too, lack context in that they do not quantify dosages or durations systematically. Complicating matters further is that kratom products have changed considerably since 2017, with new products available and with greater diversity and (largely unknown) variability across vendors and products in quality and alkaloid content.

1.5 Aims

We aimed to expand upon the data provided by prior studies that focused primarily on establishing the prevalence and motivations for kratom use by adding information about kratom dose conventions. This study aimed to depart from prior studies that enrolled persons with current kratom use, to include persons who had lifetime exposure to kratom but may not be using it currently. In this way, we hoped to reduce the potential for positive bias among respondents and ensure that some respondents had ceased their use; the goal of this approach was to collect a balanced perspective on the relative benefits and consequences of kratom exposure. Data were analyzed to support more precise and contextualized understanding of kratom dosing routines, changes in routines, and perception of effects—including time to onset, duration, and perceived effectiveness in terms of reasons for use. We consider the majority of findings here primarily descriptive. Among respondents who reported ever having periods of regular use, we also wanted to try to evaluate whether dosage (the amount of kratom consumed per week, and weeks of regular use) corresponded to the number of beneficial and/or aversive effects reported. Although we suspected there would be a directional association, with lower weekly doses being more likely associated with beneficial effects and higher doses more likely to be associated with adverse effects, we did not test this as a priori hypotheses, given the early state of human kratom (self-report) research and the uncertainty surrounding the statistical power we would be able to achieve when we recontacted our kratom-using respondents from a prior survey (see below). The pragmatic goal of this recontact survey was to collect descriptive data that would inform a follow-up study using ecological momentary assessment (EMA). Specifically, we needed to learn more about the time frames along which we should make multiple daily momentary random and dose-dependent assessments, as well as the types of questions we should ask (e.g., whether to assume that kratom is typically used for its acutely perceptible effects and not for chronic effects like a maintenance medication).

2 MATERIALS AND METHODS

2.1 Study Procedures

Amazon Mechanical Turk (mTurk), an online platform for crowdsourcing research participation and other online tasks requiring human interaction, was used here for recruitment, screening, and compensation (Chandler and Shapiro, 2016; Sheehan, 2018). mTurk is regularly used for obtaining national convenience samples in behavioral and substance use research and for ensuring the capacity to obtain valid data (Peer et al., 2014; Shank, 2016; Miller et al., 2017; Mortensen and Hughes, 2018; Sheehan, 2018; Strickland and Stoops, 2018; Strickland and Stoops, 2020). "Workers" are persons who register in mTurk who are then enabled to volunteer to participate in research by choosing to accept "human intelligence tasks" (HITs) that are presented to workers who meet broad study inclusion criteria or who may be eligible and are subsequently screened for eligibility. Workers can also decline to accept, or participate in, a screening HIT. No personally identifiable information was collected in our surveys (except for IP addresses, which were deleted after verification as US addresses). The studies involving human participants were reviewed and approved by the National Institutes of Health Institutional Review Board. They were given exempt status, meaning that written informed consent was not obtained from participants, just assent.

The present kratom survey study was a follow-up to a prior mTurk-based online survey study on substance use and social conditions (unrelated to kratom). That study enrolled people who met the following inclusion criteria: >18 years; US residents; a registered mTurk worker account with >100 completed HITS, and past-six-month use (≥1 day of use during the 6 months prior to screening) for one of the following two categories: 1) alcohol only (nicotine and caffeine permitted, but not illicit drug use); 2) opioids or psychostimulants. Opioid use was defined as licit opioids (prescription opioid analgesics, prescribed methadone, and/or prescribed buprenorphine), illicit opioids (heroin, fentanyl, nonmedical/diverted prescription opioids, and/or

nonmedical/diverted methadone or buprenorphine), and kratom. Psychostimulant use was defined as illicit psychostimulants [powder or crack cocaine, synthetic cathinones, "street" methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), or diverted psychostimulant medications]. Participants were admitted into that study if they endorsed using alcohol only or any of these opioid or stimulant substances, independent of whether other drugs were also endorsed.

Data collection for the larger survey study hosted on Qualtrics occurred between September 2020 and March 2021. A total of 13,608 screening questionnaires were completed. Of these, 3,414 (25.1%) met inclusion criteria; of these, 2,864 completed the full survey. To ensure data quality, four data validity checks were programmed into the screening questionnaire and 26 were programed into the full survey. Failing 1 validity check during screening or >3 during the full survey, or exceeding the 4-h survey completion time, resulted in automized unenrollment. Of the 2,864 completers, 249 cases were removed for one of the following reasons: unrealistically short completion time, discrepant screener and full survey items (e.g., drug use history, demographics), IP address outside of the US, IP address of indeterminate location, VPN or proxy IP address that made it impossible to validate respondents' US location. Thus, the final sample included 2,615 valid surveys.

2.2 Kratom Recontact Survey

Persons who endorsed lifetime use of kratom on that larger, unrelated study were recontacted and asked to provide additional information related to kratom. This strategy resulted in a convenience sample of US adults with kratom use history who had not (initially) self-selected based on their kratom use. Data from respondents who reported lifetime kratom use are the basis of the analyses presented here. A total of 289 respondents (of the 2,615) from the larger survey study endorsed lifetime kratom use and passed all data quality checks. Re-recruitment of and data collection from these 289 respondents for this kratom survey occurred during a 1-month period (April 15–May 15, 2021). We sent reminders at two time points. Those who were successfully recontacted and chose to participate were compensated $7.25.

2.3 Kratom Survey Instrument and Study Measures

We developed a kratom survey instrument based on the current literature. Given the rapid changes in kratom products available to US consumers and the ever-evolving landscape of kratom use in the US, we included items from prior surveys, but also new or refined items, based on our prior research, clinical experience, and questions needed to inform new projects. Here we focus on findings specific to relationships between dosage and effect (or some indicator of those relationships). Additional findings from the kratom recontact survey are reported elsewhere. Our survey instrument may be made available upon request.

2.3.1 Sample Characteristics

Sample characteristics included age, sex/gender, race/ethnicity, education (high school graduate, college graduate), past-year employment (part- or full-time), and past-year household income (below vs. above US Federal poverty line, for entire household).

2.3.2 Kratom-Use History

Kratom-use history was assessed first at the time of screening into the larger study survey, by asking respondents if they had used kratom during their lifetime or past year (timeframes were exclusive). On the kratom recontact survey, we collected additional details, such as age of kratom use initiation. For comparison, we also asked respondents to report the ages at which they had first used nicotine (e.g., cigarettes), alcohol, and cannabis, as these are commonly used, both in the US and among persons with kratom use histories. Lifetime kratom use disorder (KUD) was assessed using a modified DSM-5 checklist for substance use disorder (SUD) that was modified for kratom use (e.g., "I spent a great deal of time on activities necessary to get kratom, use kratom, or recovery from kratom's effects"; "I experienced cravings, strong desires, or urges for kratom"). These items were presented so as to evaluate whether respondents had ever qualified for kratom use disorder, regardless of current use [see Smith et al. (2022) for full KUD findings].

2.3.3 Kratom Dose Units

Typical dose was measured by having respondents select the formulation by which they most frequently consumed kratom: capsule, gram, spoonful, tablespoon, cups of tea, etc. These units were selected to reflect the ways in which kratom consumption has been previously reported. Respondents were asked to then indicate the amount in numerical units per dose (e.g., 2 g) and the typical number of doses per day for their preferred method of administration. Respondents then reported on the length of time that they had been using this typical daily amount in weeks, months, or years (coded in weeks here).

2.3.4 Kratom Dosing Routines

Regularity of kratom use was assessed by asking respondents to answer (yes/no) to the items: "Have you used kratom more than 100 times in your lifetime?" "Was there ever a period of time during which you used kratom at least 4 times per week?" and "What was the longest period during which you used kratom at least 4 times per week?" to which they could respond with numerical value in weeks, months, years (coded in weeks here). Respondents were also asked whether they considered themselves to currently be "a regular kratom user" (yes/no).

Daily patterns of kratom dosing were assessed, in part, using modified questions from the Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al., 1991) by asking respondents: "How soon after you wake do you use your first dose of kratom?" to which they could respond: "Within 5 min," "6–30 min," "31–60 min," or "after 60 min" Respondents could then respond to the items "Do you take kratom more during the first hours after waking than during the rest of the day?" (yes/no)

and "Which kratom dose would you hate most to give up?" ("The first one in the morning" vs. "All other times of day").

Changes in dosing routines were assessed by asking people to respond to items from the FTND found to be strongly associated with current nicotine dependence (Baker et al., 2007): "Since you first began using kratom (during times of active use), how often did you change your dosing routine?" to which they could respond on a 5-point Likert scale with an additional response option (1 = very often to 5 = never; 6 = It depends on other circumstances). Changes in frequency of kratom dosing since initiation were assessed by having respondents endorse one of the following options: "increased," "unchanged," "decreased," "never took kratom regularly," and "have quit entirely."

2.3.5 Kratom Perceived Effects as a Function of Dose

We asked all respondents: "When you take kratom, do you feel an effect pretty much every time? These could be energy-boosting effects, like those of a cup of coffee, or intoxicating effects, like those of an alcoholic beverage—or anything else you feel from each dose." The three response options were: "Yes, I feel an effect every time (or almost every time) I take kratom," "No, I never (or rarely) feel an effect when I take kratom," and "Neither of those is quite true for me (elaborate if you'd like)."

For those who reported feeling effects from each dose, we asked: "If you do feel the effects with each dose of kratom, are they primarily helpful in letting you go about your daily obligations?" The six response options were: "Yes, the effects are compatible with my daily obligations and help me achieve them," "Yes, the effects are compatible with my daily obligations, though not especially helpful for them," "No, the effects are not compatible with my daily obligations," "No, the effects are not compatible with my daily obligations and they sometimes undermine my ability to meet my daily obligations," "I don't take kratom enough to know," and "None of those are quite true for me (elaborate if you'd like)." For those who did not report feeling effects from each dose, we asked: "If you don't usually feel effects with each dose of kratom, which of these options best describes why you use it?" The three response options were: "I don't want effects with each dose: I use kratom just for its long-term effects, the way some people use antidepressants or other medications," "I feel withdrawal symptoms if I stop using it," and "Neither of those is quite true for me (elaborate if you'd like)."

Respondents were also asked to report how long it typically took them to begin feeling the effects from their usual dose of kratom (in seconds, minutes, or hours, or "I don't know"). An identical question was asked regarding how long it typically took them to stop feeling the effects of their usual dose in minutes or hours; respondents could also select the option "I'm unsure because I would take more kratom before the effects would wear off." We then assessed respondents' perceived dose effectiveness *via* the number of units (e.g., grams, capsules). Specifically, respondents reported a "too low" kratom dose that they found to be ineffective (defined for them as not producing their desired results); a lower-threshold dose (the lowest dose that was effective in producing their desired results); an upper-threshold dose (the highest dose that was effective as intended without being too high), and a "too high" dose (a dose for which the resultant effect that was "a bit too much" or produced effects that were not wanted or intended).

2.3.6 Perceived Beneficial and Adverse Effects

Motivations for use that represented beneficial or desired effects were assessed by asking respondents to select the most important factors that influenced or motivated current or past kratom use. They were then presented with 41 use-motivation categories (e.g., chronic pain management; relieve opioid withdrawal symptoms, relieve alcohol withdrawal symptoms; self-treat anxiety; for recreation). Respondents could select all applicable use indications. Respondents were then asked to rate effects on these outcomes selected using visual analogue scale (VAS) sliders (0–100), with 0 reflecting "not at all effective" and 100 reflecting "extremely effective" (see **Supplementary Table S1** for the complete list of beneficial use motivations endorsed and subsequently rated). Analyses were conducted using pooled VAS, for which the number of respondents who endorsed any effect and the mean and standard deviation for all summed VAS ratings of kratom effects were determined.

Motivations for use that represented an unwanted effect or negative reinforcement (and specifically included avoidance of withdrawal) were assessed in a similar manner. Respondents were asked "What unpleasant or unwanted side effects have you experienced when you have stopped taking kratom at least for a period of 1 day or longer? These could be considered withdrawal or withdrawal-like effects." They were then presented with a list of 23 adverse effects (e.g., depression or sadness; irritability; body aches; restless legs; stomach upset) which, when selected, were rated using a VAS slider wherein 0 reflected intensity of withdrawal-like effects as "almost nothing" and 100 reflected "severe or unbearable discomfort." (see **Supplementary Table S1**). This question, and list of response items, was based upon our prior research examining kratom withdrawal symptoms (Smith et al., 2022). Outcomes were evaluated again as independent scales and then pooled across 21 effects (those that were endorsed). Finally, respondents were provided with an open-text response option to the item, "Please describe the adverse effects you have personally experienced as a result of using kratom." All text responses were categorized for summary presentation in order to characterize adverse effects associated with kratom use.

2.4 Data Analysis

We generated means and proportions for the entire sample for all descriptive items. Demographic data include self-report from the larger survey. All other data were obtained from persons who completed the kratom recontact survey (n = 129). Our overarching goal was to provide a description of kratom doses, changes in doses, effects, and compatibility of acute kratom effects with daily life, as perceived by persons who experience acute effects and have regularly used kratom.

In addition to these characterizations we wanted to examine, to the extent possible with the sample size, the effect of dose on kratom-related outcomes that included standardized ratings of beneficial effects that respondents attributed to kratom use and

standardized ratings of unwanted or adverse effects attributed to discontinuation of kratom use (for ≥1 day).

To accommodate differences in consumption methods (e.g., capsules, grams, spoonfuls, tablespoons, or cups), we standardized reported amounts by calculating within-unit z-scores for each consumption method. Preliminary analyses included Pearson and Spearman tests for correlation or t-test (findings from preliminary analyses are provided in **Supplementary Material**). Due to the sample size, statistical significance in univariate or bivariate analyses was not what we relied on to determine variable inclusion for the final models, in part because we wanted to see how the variables interacted when all were included in the model, including covariates. Independent variables pertaining to use and dosing amounts were selected for examination as we believed they had the potential to demonstrate relation to reported kratom effects. Specifically, we assessed the relative role of dose by fitting two linear regression models that examined dose and other person-level predictors of experiencing beneficial effects of kratom or unwanted/negative effects after missing ≥1 day of use. Main predictors included in the two models were: self-reported regular kratom dose amount (kratom consumed per week), weeks of regular use, changes in use (decreased, increased, or quit versus unchanged dose), and endorsement of frequently using kratom more within the first hour after waking (yes/no). Potentially confounding demographic factors were included as covariates. For the first model, the dependent variable was pooled VAS ratings for beneficial effects attributed to as motivators for use; for the second model the dependent variable was pooled VAS ratings for unwanted effects of cessation (≥1 day). Primary outcomes of interest were to determine a relationship between each respective dependent variable and: 1) the amount of kratom consumed per week and, 2) number of weeks of regular kratom use (unstandardized). Model parameters were generated using ordinary least squares regression. Continuous explanatory variables were mean-centered and factor variables were included in the model as dummy codes. As such, model intercepts can be interpreted as the mean VAS score for people of average levels of each continuous variable and the reference level of each factor variable. Regression beta (*B*) values displayed in **Table 5** are unstandardized and represent the change in VAS scores as an explanatory variable increases by one unit, holding all other explanatory variables constant. Significance tests for individual regression betas were conducted using single degree of freedom tests represented by t-values (t) and *p*-values (p). Model error distributions were evaluated for normality and identity, and to ensure that potential assumption violations did not negatively impact model estimates; we conducted a sensitivity check with Box-Cox transformed response variables and determined that model predictions were robust to response variable transformations. Collinearity was assessed *via* variance inflation factors (VIF).

3 RESULTS

Of the 289 eligible mTurk workers who reported lifetime kratom use in our larger survey study, 6 no longer had active mTurk worker IDs and were unable to participate in our kratom recontact survey, making 283 people eligible. From those 283, we received (n = 134, 47.4%) complete responses during the 1-month data collection period. Five cases were removed due to inability to verify IP addresses, providing us with a final sample of 129. A majority of responses (59.7%) were submitted by respondents who had reported past-month kratom use on the larger survey.

3.1 Sample Characteristics and Kratom-Use History

Table 1 displays sample demographics and kratom-use history. Persons using kratom in this sample were on average 34.8 ± 8.4 years old (±indicates standard deviation), female (51.9%), white (71.9%), high school (40.3%) or college educated (59.7%), and employed at least part-time (68.2%). Just under a quarter reported an annual household income below the US poverty line.

Respondents reported first using kratom at 29.9 ± 8.8 years of age, on average. A majority had used combustible nicotine, alcohol, or cannabis, with initiation ages that were on average far younger than those for kratom (15.9, 15.0, and 16.8 years, respectively). Most (59.7%) had used kratom >100 times during their lifetime and reported currently or having previously used kratom >4 times per week, for an average of 61.9 ± 104.3 weeks (80.6%). Just under half (41.9%) considered themselves current "regular" kratom users. Nearly one-third met diagnostic criteria for lifetime KUD.

3.2 Typical Kratom Dosing Routines and Changes

As shown in **Table 1**, respondents most frequently reported consuming kratom *via* capsules (n = 47), grams (n = 37), spoonfuls (n = 25), tablespoons (n = 11), and then cups of tea (n = 8). The average amount of kratom used per unit was 5.4 ± 4.8 capsules, 4.6 ± 3.6 g, 2.5 ± 2.7 spoonfuls, 2.1 ± 1.0 tablespoons, or 1.6 ± 1.1 cups of tea. See **Figure 1** for typical kratom dosing units. On days that people used kratom, they reported dosing 2.6 ± 2.4 times and that this typical dosing routine had been stable for 65.0 ± 112.9 weeks.

A slim majority of respondents (55.8%) reported that they did not typically take kratom until they had been awake for at least an hour; 28.0% typically consumed kratom within the first 30 min after waking. Additionally, a sizeable minority (41.1%) reported consuming more kratom during the first waking hour than at other times during the day and 54.3% reported preferring their first daily kratom dose of the morning to those consumed during other times of day. When asked how often they changed their dosing routine during periods of regular use, most reported changing only "occasionally" (32.6%) or "not often" (8.7%). Some said instead that their dosing routine changes were dependent on circumstances (12.4%). Only 7.8% reported changing their dosing routine "very often." Since initiating use respondents described their dose amounts as having increased (26.3%), or remained unchanged (22.5%), though nearly as many said it had decreased (18.6%); 20.9% had quit.

TABLE 1 | Means and proportions for sample demographic characteristics, kratom use history, typical dosing routines, and changes in kratom dosing. Total sample (n = 129).

	N reporting	%	Mean	SD
Age	129		34.84	(±8.4)
Female	67	51.90		
White	102	71.90		
High School graduate	52	40.30		
College graduate	77	59.70		
Past-year employment (at least part-time)	88	68.20		
Past-year household income below Federal poverty line	28	21.70		
Age of kratom use initiation (range 16–60)	129		29.9	(±8.8)
Age of combustible nicotine use initiation (not electronic)	120		15.9	(±4.5)
Age of alcohol use initiation	128		15	(±3.3)
Age of cannabis use initiation	122		16.8	(±5.4)
Qualified for lifetime kratom use disorder	40	31.00		
Has used kratom >100 times during lifetime	77	59.70		
Has ever (regularly) used kratom >4 times per week	104	80.60		
Weeks spent using kratom >4 times per week	104		61.9	(±104.3)
Currently considers themselves a regular kratom user	54	41.90		
Kratom doses per day	104		2.68	(±1.73)
Weeks spent using typical dosing routine	129		65	(±112.9)
Typical regular kratom dose				
Capsules	47		5.38	(±4.76)
Grams	37		4.57	(±3.61)
Spoonfuls	25		2.52	(±2.71)
Tablespoons	11		2.09	(±1.04)
Cups of Teas	8		1.62	(±1.06)
Shots	1		1.00	(±0.0)
First dose after waking				
<5 min	6	4.70		
6–30 min	30	23.30		
31–60 min	21	16.30		
>60 min	72	55.80		
Uses kratom more during the first waking hour than other times	53	41.10		
The kratom dose that you would most hate to give up?				
First one of the morning	70	54.30		
All other times of day	59	45.70		
During periods of regular use, frequency of change in dosing routine				
Very often	10	7.80		
Often	12	9.30		
Occasionally	42	32.60		
Not often	37	8.70		
Never	12	9.30		
Depends on the circumstances	16	12.40		
Since kratom use initiation, the frequency of dosing has				
Increased	34	26.30		
Unchanged	29	22.50		
Decreased	24	18.60		
Never took kratom regularly enough to note a change	12	9.30		
I have quit entirely	27	20.90		
Other	3	2.30		

3.3 Kratom Perceived Effects

Table 2 displays descriptive findings pertaining to kratom perceived effects. Most (79.8%) indicated that they experienced an acute subjective effect with each dose they consumed; only 7.0% did not. Of the 13% who indicated "neither of those is quite true for me"; their free-text responses to this probe noted fluctuations in their own tolerance, effects that varied for uncertain reasons (n = 5), effects that varied by dose (n = 3), or effects that varied by product (n = 3).

Among the 103 respondents who reported experiencing acute effects from kratom, 54.4% reported that those effects were compatible with their daily life and helped them to meet daily obligations; 29.1% reported that kratom effects were compatible with, but did not necessarily help them meet, daily obligations. Only 3.9% reported that kratom effects were not compatible with their daily obligations, and 2.9% reported that kratom effects sometimes outright undermined their ability to meet daily obligations. A small number (8.7%) reported that they did not take enough kratom to know, and one chose the response option "None of these is quite true for me," explaining in free text that in her few experiences with kratom, she had sought a caffeine-like effect but had found the effect more like "two glasses of wine."

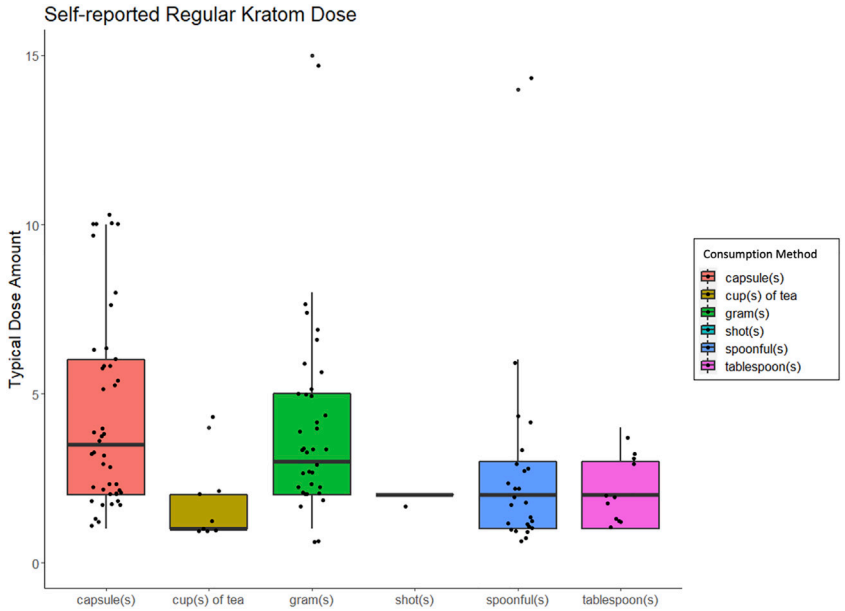

FIGURE 1 | Box and whisker plots displaing people's self-reported "regular" or "typical" kratom dose, broken down by method of kratom consumption.

Twenty-five respondents completed the item asking why they took kratom in the absence of acute effects. Of those, 6 (4.7% of the full sample) indicated they were seeking chronic benefits rather than acute effects; 3 (2.3% of the full sample) reported using kratom to avoid withdrawal, and 16 (12.4% of the full sample) felt "Neither of those is quite true for me." A follow-up free-text option revealed some were continuing to try for acute effects despite tolerance (or, as some suggested, "bad product," n = 4), using kratom to prevent withdrawal from other opioids (n = 2), using kratom for help with energy or pain relief (n = 2), and that cessation caused rebound pain and continued dosing therefore seemed beneficial (n = 1).

No respondent reported feeling kratom's subjective effects within seconds, whereas 82.9% reported typically beginning to feel kratom's effects within minutes; with lower endorsement of hours (11.6%). Nearly all (91.5%) reported that they typically stopped feeling kratom's effects within hours, with 2 respondents reporting feeling that the effects stopped within minutes; 7.0% reported that they were unsure of the duration of effects due to the fact that they consumed more kratom prior to the effects of the prior dose fully dissipating.

As shown in **Table 2**, the mean dose of kratom that respondents felt was "too low" (e.g., unable to elicit desired effects) was 3.96 capsules (n = 50), 2.64 g (n = 45), 1.37 spoonfuls (n = 19), 2.2 tablespoons (n = 5), or 1.57 cups of tea (n = 7). The mean lower-threshold dose of kratom (the lowest effective dose) was reported as 4.13 capsules (n = 45), 3.19 g (n = 43), 2.33 spoonfuls (n = 24), 2.00 tablespoons (n = 6), or 1.3 cups of tea (n = 10). The mean upper-threshold doses (effective as intended without unwanted effects) was reported as 5.88 capsules (n = 43), 6.85 g (n = 40), 2.87 spoonfuls (n = 23), 2.5 tablespoons (n = 10), or 2.25 cups of tea (n = 8). The mean "too high" dose (perceived to be "a bit too much") was reported as 7.25 capsules (n = 40), 8.68 g (n = 37), 3.39 spoonfuls (n = 27), 3.57 tablespoons (n = 7), or 3.44 cups of tea (n = 9). **Figure 2** shows these data by reported dose amount and type. See **Supplementary Material** for figures displaying self-reported effects for grams, capsules, spoonful's, tablespoons, and cups of tea, respectively.

Column 1, **Supplementary Table S1** in supplementary material lists the 41 items that respondents could endorse and rate (for perceived effectiveness) as the most important factors that motivated their past or current kratom use. These included: "self-treating anxiety symptoms," "reliving withdrawal from nonprescribed opioids or heroin," and to "boost energy, stamina and/or endurance (for work, exercise)." Ratings of the effectiveness of these beneficial kratom effects were used to calculate the average perceived effectiveness of kratom for all use indications. The average perceived effectiveness of kratom across all reported use indications, was 72.8/100 (±16.7.) Column 2, **Supplementary Table S1** in supplementary material lists the items (e.g., nausea, hot flashes, running nose) that respondents could endorse and rate as being experienced when they stopped taking kratom ≥1 day. For these unwanted effects of cessation (≥1 day), the average pooled severity rating was 53.0/100 (±24.1).

3.4 Perceived Adverse Effects From Kratom Reported *via* Open Text Responses

Table 3 lists unwanted effects that respondents reported *via* open-ended questions that they had perceived resulting from their kratom use. Those included gastrointestinal upset (nausea, vomiting, constipation, cramping), low mood (dysphoria, difficulty concentrating, anxiety) and a variety of somatic symptoms (increased urination, dehydration, dry

TABLE 2 | Means and proportions for kratom perceived acute effects, compatibility of effects with daily obligations, and effectiveness and ineffectiveness by dose. Total sample (n = 129).

	N	%	
Acute Effects			
Felt an effect every time (or almost every time) kratom was dosed	103	79.80%	
Never or rarely felt an effect when kratom was dosed	9	7.00%	
Neither of these is quite true for me	17	13.20%	
"Typically, how long would it take for you to *begin* to feel the effects of your typical dose of kratom?"			
Seconds	0	0.00%	
Minutes	107	82.90%	
Hours	15	11.60%	
I don't know	7	5.40%	
"Typically, how long would it take for you to *stop* feeling the effects of your usual dose of kratom?"			
Minutes	2	1.60%	
Hours	118	91.50%	
I'm unsure because I would take more kratom before the effects would wear off	9	7.00%	
Among those who reported feeling the effects from each dose (n = 103)			
The kratom effects are compatible with *and* help me meet my daily obligations	56	54.40%	
The kratom effects are compatible with, but do *not* help me meet my daily obligations	30	29.10%	
The kratom effects are *not* compatible with my daily obligations	4	3.90%	
No, the effects are not compatible with my daily obligations, *and* they sometimes undermine my ability to meet daily obligations	3	2.90%	
I don't use kratom enough to know if effects are compatible or helpful daily	9	8.70%	
None of those are quite true for me	1	1.00%	
	N	**Mean**	**SD**
"Too low" dose (at which kratom was *ineffective)*			
Capsules	50	3.96	(±4.95)
Grams	45	2.64	(±2.44)
Spoonfuls	19	1.37	(±0.96)
Tablespoons	5	2.2	(±2.17)
Cups of Teas	7	1.57	(±0.98)
Lower-threshold dose at which kratom was *effective*			
Capsules	45	4.13	(±3.31)
Grams	43	3.19	(±2.25)
Spoonfuls	24	2.33	(±2.2)
Tablespoons	6	2	(±0.89)
Cups of Teas	10	1.3	(±0.67)
Upper-threshold dose (highest dose at which kratom was *effective as intended)*			
Capsules	43	5.88	(±4.02)
Grams	40	6.85	(±4.58)
Spoonfuls	23	2.87	(±1.58)
Tablespoons	10	2.5	(±1.58)
Cups of Teas	8	2.25	(±1.16)
*"Too high" d*ose (at which the effect "a bit too much", or produced results that were not wanted, intended, or effective)			
Capsules	40	7.25	(±4.24)
Grams	37	8.68	(±4.38)
Spoonfuls	27	3.93	(±1.66)
Tablespoons	7	3.57	(±1.72)
Cups of Teas	9	3.44	(±2.01)
Pooled "positive" (beneficial or therapeutic) kratom effects (VAS 0–100)	104	72.84	(±16.73)
Pooled "negative" (adverse or unwanted) kratom effects (VAS 0–100)	104	52.98	(±24.12)

mouth, rash). **Table 4** provides direct quotes from some open-ended responses to contextualize findings (full text data available upon request).

3.5 Dose-Related Associations With Pooled VAS Effects Ratings

Table 5 displays results from regression analyses using only data from respondents who reported currently being or having previously been regular kratom users (n = 104, 80.6%).

The linear regression analyses of kratom dose and other related variables on pooled VAS ratings of beneficial kratom effects was significant [$F(13,90) = 2.18$, $p = 0.02$], with $R2 = 0.21$ and adjusted $R2 = 0.11$, however only having quit kratom entirely was significantly associated with lower positive ratings of beneficial motivating effects ($\beta = -16.45$, 95% CI = −25.9, −6.90; $p < 0.001$). Predictors that reflected dosing changes (e.g., self-reported amount of kratom consumed per week, weeks of regular use) were not significant.

The linear regression analyses of pooled VAS ratings of unwanted effects of ≥1 day cessation was also significant

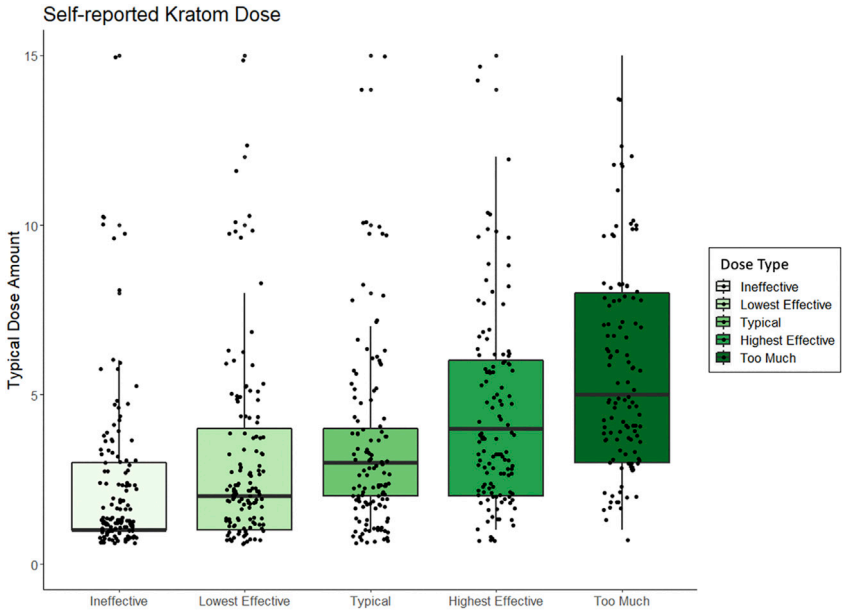

FIGURE 2 | Box and whisker plots displaying peoples self-reported kratom doses, standardized across method of kratom consumption, and which range from amounts that people perceived to be "ineffective" to what dose they perceived to be "too much."

[F(13,90) = 4.22, $p < 0.001$], with $R^2 = 0.39$ and adjusted $R^2 = 0.26$. In this model, greater severity of unwanted effects was predicted by more weeks of regular kratom use ($\beta = 6.74$, 95% CI = 0.88, 12.60; $p = 0.02$), and having decreased kratom doses after initiation ($\beta = 20.88$, 95% CI = 5.75, 35.99; $p < 0.001$). The variable of greater self-reported amount of kratom consumed per week closely approached, but did not fully achieve, significance ($\beta = 5.24$, 95% CI = −0.52, 11.0; $p = 0.07$). Moreover, likelihood of experiencing negative effects from ≥1 day of kratom cessation was also higher in women ($\beta = -10.54$, 95% CI = −21.21, 0.14; $p = 0.05$) and for people reporting annual household income below the poverty line ($\beta = 20.06$, 95% CI = 5.21, 34.91; $p < 0.001$).

4 DISCUSSION

We enrolled a diverse sample of US adults with reported lifetime kratom use to sensitively characterize patterns of kratom use and associated outcomes. These data add to our existing knowledge base through the inclusion of persons who have kratom-use histories but may not be currently consuming kratom: under half of the sample considered themselves to be current "regular" kratom users and 20.9% had quit kratom. The fact that most respondents had used kratom >100 times since initiation, over 80% had ever used kratom ≥4 times per week, and fewer than 10% had *never* taken kratom regularly indicates that the respondent sample had extensive and highly differentiated experience with kratom. Thus, the data represented here extend beyond prior studies that sampled persons with current kratom use to provide an updated perspective on kratom experiences.

4.1 Kratom Use as Another Routine of Daily Living or a Form of Drug Misuse?

Here noteworthy observations on kratom-use histories and patterns of (and changes in) use were observed. Consistent with prior research (Garcia-Romeu et al., 2020), persons in this study reported initiating kratom use at an older age and a minority had ever met criteria for KUD (Smith et al., 2022). This study also administered measures that have been associated with dependence severity for other substances in a modified format in order to assess dependence severity for kratom in our sample. The results suggest that a subset of respondents endorsed several behaviors indicative of greater dependence severity. Yet the nature of kratom use seems different from other substances and makes the interpretation of these data challenging. For instance, we observed that 60% of our respondents had used kratom >100 times during their lifetime. This value is widely accepted as evidence of someone being a verified "cigarette smoker" and would probably raise clinical concerns in reference to a drug such as heroin or cocaine. However, such an exposure would generally be considered normative for drugs such as alcohol, caffeine, or most psychiatric medications (such as SSRIs). Functionally and socially, these classes of drugs are distinguished from each other in terms of a major component of DSM criteria for SUDs: the extent to which their acute and chronic effects tend to be concordant with the goals and obligations of everyday life. We asked our respondents to characterize kratom in exactly that way: first, whether it had acute effects with each dose (like caffeine or alcohol, but unlike most psychiatric medications), and second, whether such acute effects were compatible with daily goals and obligations (perhaps like the effects of caffeine, and probably not like the effects of alcohol). Only 7% of our respondents stated categorically that

TABLE 3 | Adverse or unwanted side effects reported by participants as being directly caused by kratom use *via* open text response quantified in raw number and percent frequency (n = 129).

	N	%
Nausea	36	27.9
None	16	12.4
Vomiting	14	10.9
Constipation	14	10.9
Headaches	13	10.1
Increased feelings of anxiety/nervousness	12	9.3
Withdrawal symptoms	8	6.2
GI upset	7	5.4
Bad taste	7	5.4
Tiredness	7	5.4
Dizziness	6	4.7
Bad mood	5	3.9
Addictive/developed dependence	5	3.9
Dehydration	4	3.1
Inconsistent (wobbly) eye movement	4	3.1
Trouble sleeping	4	3.1
Stomach cramping	4	3.1
Increased feelings of depression	3	2.3
Jittery/restless	3	2.3
Tolerance	2	1.6
Irritated skin/rash/itch	2	1.6
Dry mouth	2	1.6
Increased heart rate	2	1.6
Increased perspiration	2	1.6
Cramps/body aches	1	0.8
Weight gain	1	0.8
Trouble focusing	1	0.8
Fluctuating mood	1	0.8
Speech issues	1	0.8
Restless leg syndrome	1	0.8
Craving for tobacco	1	0.8
Increased urination	1	0.8
Decreased motivation	1	0.8
Light headedness	1	0.8
Decreased appetite	1	0.8

they never felt acute effects from kratom, and—even with the broadest interpretation of the free-text responses—it appears that no more than 9% of respondents (n = 12) used kratom for chronic effects only (the way they might have used a psychiatric medication or other maintenance medication). Thus, for most respondents, the clinically and functionally relevant question about kratom is whether its acute effects were compatible with their daily goals and obligations.

The answer to that question was usually yes (for 86/120 respondents who ever experienced acute effects: 70%), with only three reporting that the acute effects outright *undermined* their daily goals or obligations (and nine more saying that they did not take enough kratom to judge). For those 86 respondents, a history of >100 exposures to kratom might be functionally comparable to a history of >100 exposures to caffeine—though we hasten to add that the comparison is not a straightforward one. Apart from the obvious fact that the two substances represent different pharmacological classes (with kratom having opioid activity), there is a host of unknowns specific to kratom. Kratom is a relatively recent introduction to US markets, and its complex and variable pharmacology are only beginning to be understood. That understanding is hampered further by a lack of standardization of kratom products. We suggest caffeine as a point of comparison in only the following functional ways: both substances are available without a prescription in a variety of dosage forms; both usually produce acute effects with each dose (despite also producing substantial tolerance), and the acute effects are usually described as helpful toward meeting daily goals or obligations; both may lead to withdrawal symptoms on cessation (Juliano and Griffiths, 2004; Bowe and Kerr, 2020; Stanciu et al., 2021); and both are occasionally used to excess, with adverse effects (Reissig et al., 2009; Schmuhl, et al., 2020), though the use of either substance for purely euphoriant purposes is more the exception than the rule (McCarthy et al., 2008; Smith et al., 2021b).

The similarities may end there, both for worse and for better. Kratom seems to have potential for instrumental "self-treatment" of chronic pain, a variety of psychological or psychiatric symptoms, and SUDs (Smith et al., 2021b; Smith et al., 2021c). For most respondents, during periods of regular kratom use, the frequency of changes in dosing routine was either "not often" or "never," suggesting arrival at a pattern of use helpful for daily functioning. Even so, some of our respondents did *increase* their dosages, and a few specified that they were continuing to use kratom despite tolerance because they hoped once again to feel acute effects. Other respondents had *decreased* their dosages, or quit, citing unwanted effects. Variability in dose-effect relationships is further underscored by our respondents' varied patterns of dosage timing: the first dose of the day occurred within an hour of waking for about 45% of our respondents, but later in the day for the other 55%. This suggests that *proximal* motivations for use of kratom need to be assessed and understood at the individual and momentary level, because there may be considerable differences in whether the effects are perceived (and under what contexts) as mostly energizing, mostly calming, or some combination of the two.

4.2 Effects and Effectiveness

Among the most intriguing findings here is how close average doses were in terms of being reported as ineffective, effective, or "a bit too much." The ranges were similar for all those ratings of effectiveness based on prior findings (Garcia-Romeu et al., 2020; Smith et al., 2021b). The highest average dose for any dose type that was considered "a bit too much" was 8.68 g, compared to 6.85 g, which was reported as effective. This was followed by 7.25 capsules ("too much") versus 5.88 capsules (effective); 3.93 spoonfuls ("too much") versus 2.87 (effective); and 3.44 cups of tea ("too much") versus 2.25 (effective). These ranges are in keeping with the typical regular doses reported in other surveys. There are several takeaways from this, the first being that most in this sample were not typically using extremely high doses of kratom. The other takeaway is that the average difference between effective kratom doses and doses that were perceived as "too much" (and unwanted) is not large, meaning that people using kratom, particularly those unfamiliar with kratom, may inadvertently dose too much. Strong conclusions cannot be

TABLE 4 | Direct quotes from participants who provided open text responses about adverse or unwanted kratom side effects.

"I felt nauseous one time while experimenting with dosages in the first 2 weeks of regular use (I think I took around 15 g which I never do anymore, but I could probably handle it now)."
"Sometimes it hits different and don't produce the same effect and can be frustrating but that could be a number of factors."
"Nausea, the awful taste, gagging from having to put so much of the powder form in a tea, the smell. Light headaches but manageable."
"Headaches happen sometimes especially if I dose too early, nausea on occasion, constipation."
"With too much kratom on an empty stomach I've gotten increased heart rate and nervousness."
"The times after I was over withdrawal from heroin and clean i used kratom to get over meth. It didn't work at all. Made me high like opioids then ill. And every time after I have ever used it any color I just get sick. Like mentally and physically."
"It often makes me very tired after it wears off or if I take repeated doses over consecutive days. It dries my mouth out a lot."
"Sometimes I would take my regular dose and I would get so freaking sick it's not even funny. The world would spin. My stomach would crap and feel like I needed to throw up so badly. Horrible headache to the point my eyes were sore."
"Too much kratom would make me feel agitated and anxious. I also wouldn't be hungry."
"I've puked before if my dose was too big or my stomach to empty; its rather rare at this point more frequent when I was new to it and figuring out dosage."
"Only thing was constipation when I first started "using" kratom, but it is long gone. I can get restless leg syndrome if I don't have any before bed, but it's not terrible."
"After the high wears off, I actually get unmotivated. I don't like how addictive it is for a plant."
"The only adverse effects I have experienced from kratom has been constipation at times. If I don't drink enough water or eat enough fiber I end up needing to take a laxative. The only other adverse effect is that after a year of being on it every day, if I don't take it I feel pretty bad but it doesn't compare to heroin or methadone withdrawal. Also sometimes if I take too much by accident or intentionally I get the wobbles. The wobbles are what kratom users refer to as nausea and dizziness from taking too much. When that happens you need to lay down."
"Never OD'd. I don't think you can. If you take too much you get shaky in your eyes that's called the wobbles. Also if you already eat fiber, this stuff will clog you up it's so fibrous. On the other hand if you are like me and eat protein bars and air for all her meals, kratom also saved my digestive system because it firms up your stools!"
"Have experienced withdrawal on a few occasions after periods of extended use; am aware that I will definitely need to taper off slowly when I eventually quit. Overall negative effects are fairly minimal; I notice Kratom does tend to cause me to urinate more frequently (I usually consume it as tea which obviously adds to that issue), and on occasions this has been a real problem when drinking tea before bed. Taking Kratom (especially at higher doses) at night before bed definitely affects the quality of my sleep, so I'm trying to cut back/avoid doing that as much as possible. I do keep track of how much I take on a day-to-day basis to avoid increasing my average daily dose."

Note: Aside from the adding quotation marks, open text responses from participants have been kept in their original form.

made, in part, due to the variability of kratom products and batches of product likely used among the sample. For example, most capsules appear to contain about 0.5 g among what is primarily being sold for kratom powders. This would roughly translate as the powder being about half as much as the number of capsules. However, this may not always be true if larger capsules are used. Presently capsules not self-prepared by those who consume kratom can be purchased in "regular" or "jumbo" sized.

Overall, the pooled VAS ratings for therapeutic or beneficial effects of kratom were higher on average than were severity ratings for withdrawal-like effects upon 1 day's cessation, among people who had ever regularly used kratom (including among people who had quit kratom but whom had once used regularly). These findings again provide a complicated picture insofar as there is reported benefit, but clearly also adverse effects, both when kratom is used (as indicated in open text responses) and when use is paused for at least a day. Many of the cessation symptoms, along with the direct adverse effects described, were similar to what would be expected from opioids.

We were unable to find strong person-level predictors of proneness to beneficial effects, withdrawal-like effects, or adverse effects. Our regression models showed mostly that respondents who did not note a preponderance of benefits were those who had quit. Amount of use per week and duration of use were not associated in either direction with beneficial effects, though they were associated with higher severity of withdrawal-like effects. As our goal was to detect any signal of a dose-effects relationship, these findings should be taken as exploratory and as a starting point for refining methods. They do generally comport with prior findings from the US and Asia that kratom withdrawal may be dependent on both dose and duration of use, but is typically mild to moderate, and severe among a minority (Ahmad and Aziz, 2012; Singh et al., 2014; Singh et al., 2018; Singh et al., 2019b; Stanciu et al., 2019; Garcia-Romeu et al., 2020; Smith et al., 2021b). Ambiguity is attributable in part to the sample size, but also again a likely artifact of the variability of kratom product types. Extracts are far more potent in terms of their alkaloid concentration for MG and 7-HG (and typically more expensive) but may have greater alkaloid purity. Conversely, pulverized plant matter may be less potent, but also may be of poorer quality or, if purchased from a less reputable vendor who does not comport to the Good Manufacturing Standards Program guidelines, may be adulterated. Moreover, it is important to underscore that this is a US sample, meaning that it is unlikely that any respondents had access to fresh, raw kratom leaves.

Ultimately, findings here support the possibility that regular kratom dosing and longer duration of regular use in weeks is associated with higher ratings of adverse effects when kratom is not used for a day or more, but that they are mild to moderate. Although we did not detect clear relationships between kratom dose and direct beneficial effects from use, that could be due to methodological and statistical power limitations. However, that pooled VAS ratings were higher for beneficial effects from use than for ratings for adverse effects from 1 day of cessation does partially support the narrative that has emerged from prior self-report among current kratom-using adults, namely that many who have regularly used kratom tend to experience beneficial effects but without the seeming severity of adverse outcomes associated with illicit psychoactive drugs.

Like all findings, these constitute provisional takeaways. The patterns of and changes in dosing here clearly show the variability of kratom use experiences. Perhaps the most important finding is

TABLE 5 | Model 1 displays results from a multiple regression that examines pooled VAS ratings of beneficial effects from kratom use. Model 2 examines pooled VAS ratings of adverse or unwanted effects when kratom is not used for ≥1 day.

Model 1: Beneficial or positive effects from kratom use indications	B	95% CI	t	p	VIF
Intercept	76.95	[68.83, 87.1]	15.09	>0.01	
Age	−1.91	[−5.31, 1.49]	−1.11	0.27	1.04
Gender (male vs. female)	−0.35	[−7.44, 6.75]	−0.1	0.92	1.14
Race/Ethnicity (minority vs. white)	2.16	[−5.59, 9.90]	0.55	0.58	1.1
Education (high school vs. college graduate)	1.99	[−5.72, 9.70]	0.51	0.61	1.28
Employment (unemployed vs. employed)	−2.77	[−11.1, 5.51]	−0.66	0.51	1.3
Below US Federal poverty line for past−year annual income	4.34	[−4.86, 13.54]	0.93	0.35	1.32
Currently "regular" kratom user	−6.76	[−14.84, 1.32]	−1.66	0.1	1.32
Kratom dose consumed per week	2.19	[−1.52, 5.89]	1.17	0.25	1.19
Weeks of regular kratom use	0.9	[−2.98, 4.78]	0.46	0.65	1.11
Decreasing kratom dose (vs. unchanged dose)	0.27	[−9.39, 9.93]	0.06	0.96	1.66
Increased kratom dose (vs. unchanged dose)	−1.3	[−11.1, −8.52]	0.26	0.79	1.66
Quit kratom (vs. unchanged dose)	−16.45	[−25.9, −6.90]	3.42	0.01	1.66
Using kratom more outside of first waking hour	−2.65	[−1.00, 4.69]	−0.72	0.48	1.18
$F(13,90) = 2.18$, $p = 0.02$; $R^2 = 0.21$; Adj $R^2 = 0.11$					
Model 2: Adverse effects when kratom is not used for a period of >1 day					
Intercept	50.88	[35.87, 65.87]	6.78	>0.01	
Age	2.31	[−3.14, 7.76]	0.85	0.4	1.04
Gender (male vs. female)	−10.54	[−21.21, 0.14]	−1.97	0.05	1.14
Race/Ethnicity (minority vs. white)	−2.88	[−15.06, 9.30]	−0.47	0.64	1.1
Education (high school vs. college graduate)	−3.22	[−14.83, 8.40]	−0.55	0.58	1.28
Employment (unemployed vs. employed)	−2.63	[−16.21, 10.94]	−0.39	0.7	1.3
Below US Federal poverty line for past-year annual income	20.06	[5.21, 34.91]	2.7	0.01	1.32
Currently "regular" kratom user	−1.29	[−13.86, 11.28]	−0.2	0.84	1.32
Kratom dose consumed per week	5.24	[−0.52, 11.00]	1.82	0.07	1.19
Weeks of regular kratom use	6.74	[0.88, 12.60]	2.3	0.02	1.66
Decreasing kratom dose (vs. unchanged dose)	20.88	[5.75, 35.99]	2.76	0.01	1.66
Increased kratom dose (vs. unchanged dose)	11.4	[−2.18, 24.98]	1.68	0.1	1.66
Quit kratom vs. unchanged dose	−5.05	[22.48, 12.38]	−0.58	0.56	1.11
Using kratom more outside of first waking hour	6.1	[−5.75, 17.94]	−1.03	0.31	1.18
$F(13,90) = 4.22$, $p < 0.001$; $R^2 = 0.39$; Adj $R^2 = 0.26$					

Note: All models use responses from those who reported being, or previously having been, a regular kratom user (n = 104).
VIF, variance inflation factor.

among the simplest: not everyone who has used kratom regularly or irregularly continues to use it. The reasons for continued regular kratom use, primarily for self-treating pain, psychiatric, or SUD symptoms are increasingly established (Grundmann, 2017; Coe et al., 2019; Bath et al., 2020; Garcia-Romeu et al., 2020). The reasons for *ir*regular or discontinued use remain less clear.

5 LIMITATIONS

One of the main limitations of this study is the cross-sectional design of the survey and the small sample size. The use of a large crowdsourcing platform is a strength in some ways (such as wide geographical coverage in a short time), but also means that findings are not generalizable to all people with kratom use histories, including those who engage with other platforms. Similar to large US online surveys that recruited current kratom-using adults, there is the possibility that those who choose to participate in this kratom survey had particular attitudes toward kratom. As we were able to recruit persons who still used kratom regularly as well as those who had stopped use, the sample is diverse, but may still reflect different biases regarding kratom. Among those who had stopped using kratom, or who had used it only ever intermittently, recall bias may be a concern. Additionally, the small sample size did not permit for precise estimates. Likewise, the kratom products that people reported dosing must be presumed to reflect products varied in both alkaloid content and quality; and we cannot know if any were adulterated. Concomitant use of kratom with other substances and dietary habits that could potentiate or attenuate kratom effects may also have occurred, thus limiting conclusions that might be drawn, including kratom's beneficial or adverse effects and effectiveness when used alone. Lastly, it is critical to keep in mind that heterogeneity of kratom products within the US is considerable, but also that commercialized and processed kratom products consumed in the US likely differ from fresh kratom preparations available and used in Southeast Asia (Saref et al., 2019; Charoenratana et al., 2021; Kamble et al., 2021).

6 CONCLUSION

The regular use of kratom in the US continues to grow at unknown rates, making it incumbent upon researchers and healthcare providers to include kratom use history items on broader surveys related to substance use or clinical assessments. Here, by using a survey sample not selected in advance for current kratom use, we uncovered an important issue of potential survivor bias in currently available data. Our findings, while preliminary, also show the diversity among kratom users in terms of dosing methods. Although a strong relationship between dose and pooled effects could not be found for beneficial effects directly associated with kratom use, and with limited precision for adverse effect ratings, it is likely that such relationships can be achieved through improved survey methods and increased sample size. Still, cross-sectional methods will be insufficient for attaining a scientific understanding of kratom dose-effect relationships, particularly when samples of kratom product types used by respondents are not also assessed. There is no peer-reviewed research about safe or effective dosing of kratom, and this paucity of information plays out in variability in doses taken (resulting in either potential increased risk or subtherapuetic dose). The variability in kratom leaves and products means that dose-effect relationships in everyday settings may be difficult to discern without also pairing self-report with assay of the kratom product being used. Although we intend to improve such methods, we anticipate similar limitations in assessment without objective data. One take-away from this exploratory characterization is that controlled human laboratory studies are critical to advancing this area further. Direct observation and assessment using validated measures of both subjective and objective acute effects and withdrawal are needed.

In the interim, we can say that most persons who use kratom experience at least some acute effects with every dose, and that those acute effects are usually seen as compatible with, or even helpful for, daily obligations (i.e., kratom is seemingly not typically used like prescribed medications that confer perceived quality-of-life benefits only chronically, but perhaps perceived more similiarly to coffee or even alcohol). We found indications that higher weekly kratom dose amounts and longer periods of regular use were associated with greater severity ratings for unwanted effects when kratom was not used for at least a day. The clinical relevance is that persons who use kratom at higher doses regularly may expect greater odds of feeling unwanted or adverse effects when use is paused. Ultimately, that pooled ratings of beneficial effects from kratom use were higher than pooled ratings of adverse effects from discontinuation for at least a day, present us with yet another layer of complexity in understanding the *perceived* benefits of kratom use versus harm or risk. The *actual* therapeutic benefits and risks of kratom, which will necessarily include subjective assessments of effectiveness or ineffectiveness of kratom at various doses among human subjects, remains to be elucidated. The public health message regarding kratom thus remains incomplete and will likely remain so until controlled human laboratory experiments are underway. Presently, persons who use kratom, and clinicians who encounter kratom-using patients, should remain cognizant of dosing regimens and dosing changes and work to document observed effects. The margin between effective doses and doses that were perceived as "too much" appears narrow enough to warrant careful attention to kratom doses, irrespective of a given dosing unit.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the National Institutes of Health Institutional Review Board. They were given exempt status, meaning that written informed consent was not obtained from participants, just assent.

AUTHOR CONTRIBUTIONS

KS, DE, JR, and KD helped conceptualize the study and designed the survey instrument. KS, KD, and DE were primary authors of the manuscript with substantial input in the interpretation of findings and with critical feedback and assistance in editing from CM and OG. JR managed data collection with the assistance of DS. JR conducted statistical analyses. All authors contributed to the creation of the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.765917/full#supplementary-material

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REVIEW
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Understanding Kratom Use: A Guide for Healthcare Providers

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Kratom (*Mitragyna speciosa* Korth., Rubiaceae) is a plant native to Southeast Asia, where it has been used for centuries as a mild stimulant and as medicine for various ailments. More recently, as kratom has gained popularity in the West, United States federal agencies have raised concerns over its safety leading to criminalization in some states and cities. Some of these safety concerns have echoed across media and broad-based health websites and, in the absence of clinical trials to test kratom's efficacy and safety, considerable confusion has arisen among healthcare providers. There is, however, a growing literature of peer-reviewed science that can inform healthcare providers so that they are better equipped to discuss kratom use with consumers and people considering kratom use within the context of their overall health and safety, while recognizing that neither kratom nor any of its constituent substances or metabolites have been approved as safe and effective for any disease. An especially important gap in safety-related science is the use of kratom in combination with physiologically active substances and medicines. With these caveats in mind we provide a comprehensive overview of the available science on kratom that has the potential to i clarity for healthcare providers and patients. We conclude by making recommendations for best practices in working with people who use kratom.

Keywords: kratom (*Mitragyna speciosa* Korth), emerging therapeutic agents, pain, mood and anxiety, substance use and misuse

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INTRODUCTION

Kratom (*Mitragyna speciosa* Korth., Rubiaceae; also known as ketum) is made from the leaves of a tropical tree in the coffee family indigenous to Southeast (SE) Asia, where it has been used for centuries as medicine for various ailments, including hypertension, diarrhea, cough, and fever (Tanguay, 2011; Cinosi et al., 2015; Singh et al., 2016). Despite such traditional medicinal use, it is important to recognize that neither kratom, nor its constituents (e.g., "alkaloids"), nor metabolites have been approved as safe and effective medicines for any therapeutic use. Nonetheless, widespread use for health and well-being include diverse uses reported by consumers as reasons for their use. For example, at low doses, kratom has long been consumed orally as a stimulant to enhance stamina and productivity, making it particularly popular among field laborers working long days in arduous conditions (Tanguay, 2011; Prozialeck et al., 2012; Hassan et al., 2013; Warner et al., 2016). Consumption remains widespread in kratom's native lands, where people commonly chew raw

kratom leaves or boil leaves to make tea (Swogger and Walsh, 2018). Kratom can also be smoked, vaporized, or consumed as a powder. Because of its purported analgesic properties, kratom is used to treat pain and, notably, as a means to alleviate opioid withdrawal or as an opioid replacement among people with opioid use disorder (OUD) (Smith and Lawson, 2017; Henningfield et al., 2018; Bath et al., 2020). In addition to analgesia produced at higher doses, kratom is reported to have relaxing, anxiolytic effects. Over the past 2 decades, kratom has gained popularity beyond Asian borders, particularly in North America and Europe (Boyer et al., 2007; Grundmann, 2017).

An estimated 10–16 million people in the United States take kratom, though current prevalence ranges of 1.3%–6.1% from national representative surveys may underestimate regular kratom users (Henningfield et al., 2019; Covvey et al., 2020). Whereas in Southeast Asia users typically buy kratom leaves directly from a grower, Westerners often purchase capsules, powders, or extracts via the internet, specialty smoke shops, and gas stations (Prozialeck et al., 2012; Singh et al., 2016). Kratom is currently not recognized as a dietary supplement in the United States, and the Food and Drug Administration (FDA) has not issued guidance or regulatory standards on kratom regarding allowable product contents, alkaloid concentrations, packaging, labeling, or marketing of kratom products that is usually provided for dietary ingredients (Coe et al., 2019). This gap in regulatory policy prompted the American Kratom Association (AKA) to develop voluntary industry guidelines through a Good Manufacturing Practice (GMP) Standards Program that tests for purity and contaminants (American Kratom Association, 2019). Due to the potential for adulteration of kratom products the unregulated status of kratom in most United States remains a concern.

Although the rise of kratom use in the West has been an opportunity for increased scientific study, the resultant publication of a great deal of research of limited rigor has created confusion for health practitioners attempting to understand the benefits and risks of the plant and the heterogeneity of kratom products. Case studies, poison control center briefings, and tallied coroner and medical examiners' reports have disproportionately emphasized, as these forms of inquiry often do, extreme and rare events, including seizure, liver damage, and death (e.g., Nelsen et al., 2010; Sheleg and Collins, 2011; Kapp et al., 2011; Neerman et al., 2013; Anwar et al., 2016; Wang and Walker, 2018; Post et al., 2019; Afzal et al., 2020), even as some have elucidated that adverse health outcomes from kratom exposure have been mild to moderate and resolved quickly (Anwar et al., 2016). Still, there remains considerable ambiguity on the potential harms from kratom use. In February 2018, the FDA cited 44 cases of kratom-associated deaths based upon coroner or forensic toxicologist reports. However, at the current level of scientific knowledge, several factors make it *impossible* to determine whether kratom contributed to lethal outcomes. Almost all of the cases cited involved adulterated kratom products and/or the co-ingestion of substances with fatal overdose potential, including heroin and synthetic opioids (Babin, 2018). For instance, nine deaths were from an herbal mix, Krypton, containing a metabolite of the opioid tramadol (Bäckstrom et al., 2010). Additionally, the mere presence of mitragynine (one of kratom's primary alkaloids believed to be responsible for analgesia) in decedents' plasma or evidence of presumed kratom consumption (e.g., kratom product packages) does not implicate the plant's role in toxicity, especially given the large variability of mitragynine serum levels of decedents, ranging from 5.6 to 29,000 ng/ml (Papsun et al., 2019). Finally, there is no clear mechanism by which kratom alone and taken even at high doses would directly cause death. Unlike classical opioids, which act as full agonists at mu opioid receptors, kratom's two primary and best understood bioactive alkaloids, mitragynine and 7-hydroxymitragynine, act at mu opioid receptors as partial putatively "biased" agonists, meaning that they do not contribute to significant respiratory depression in pre-clinical animal studies (as discussed more below), making "poisoning", when kratom alone is used, a highly questionable cause of death. Importantly, there are no reports of deaths due to kratom use in SE Asia for over a century (Veltri and Grundmann, 2019). Despite insufficient evidence for kratom's role in harm, media headlines misleadingly insinuate that kratom has been established as a cause of death (e.g., "kratom deaths" and "kratom overdose deaths; " Galvin, 2019; Kaur, 2019; Miller, 2019).

Conclusions by many negative, sensationalized, or otherwise decontextualized media reports on kratom have been questionably drawn from case studies and toxicology reports which, at best, provide low levels of evidence due to unknown internal validity and generalizability and over-representation of extreme events (Merriam, 2009). Unfortunately, warnings regarding kratom exhibit features of *drug hysteria* (Hart, 2013), which involves the promulgation of sensational and biased information and the pursuit of legislative approaches that are disproportionate to apparent public health risks. At the public health level, drug hysteria is not only scientifically unfounded, but dangerous. In the case of kratom, misinformation can lead to dehumanization of kratom users, disinclination for people with OUD to try kratom as a substitute for opioids that are causing them harm, and the continued promotion of ineffective, draconian, and punitive policies with the potential to contribute to mass incarceration, a serious public health threat in its own right. Simultaneously, drug hysteria can contribute to the inhibition of rigorous scientific study and thereby deprive the public of scientifically-informed pharmacotherapeutic interventions (PR Newswire, 2016). Banning or criminalizing kratom, as six United States have done at the time of this writing, has the potential to create a new illicit market for kratom products, increasing the likelihood of adulteration and the use of dangerous substances as kratom substitutes. All of this results in harm to people who regularly use kratom to address pain, psychiatric problems, and SUD symptoms (Grundmann, 2017; Swogger and Walsh, 2018; Coe et al., 2019; Smith et al., 2021a; Smith et al., 2021b). Moreover, sensationalized and negative reports lead some patients to fear revealing kratom use to their healthcare providers (Smith et al., 2021b) and misinform those providers about the risks of kratom use.

Subsequent to increased kratom use in the United States, an eight-factor analysis (8 FA) normally required prior to scheduling decisions was performed by the FDA, and another by an

independent agency (Pinney Associates). The former has been criticized by kratom researchers for omission of important scientific studies and pertinent data, as well as inappropriate use of a computer simulation model (PHASE) that provided data that the FDA used to deem kratom an "opioid" (Grundmann et al., 2018), linking it to more dangerous classical opioids without providing information on differences between kratom and these drugs. Meanwhile, Pinney Associates concluded that kratom is distinct from classical opioids and poses no more of a public health risk than many commonly-used substances, thereby warranting product oversight rather than a ban. Nonetheless, recent publications in medical journals espouse kratom use as "highly problematic" and its effects "contributors to the growing opioid crisis" (e.g., Goldin et al., 2019), without adequate supporting data. This rhetoric can stigmatize users and mislead well-intentioned healthcare professionals into an anti-kratom stance that could negatively impact their patients and the patient-provider relationship.

A balanced examination of what can be drawn from the existing literature directed to healthcare providers and clinicians is warranted. This is particularly true given that the study of kratom is in its infancy: there is only one published clinical trial of kratom's effects in humans. There is, however, a growing body of observational literature that represents a higher level of evidence than case reports or forensic toxicologists' and medical examiners' reports. Here, we first review research on the pharmacology of kratom and then summarize the available observational science on human kratom use in order to provide the most nuanced, accurate, and comprehensive review of kratom's potential benefits and risks possible at this early stage of kratom research. We acknowledge that information provided here will inevitably change as more data are collected on kratom and kratom use. Like all things in science, our understanding of this plant and its use is provisional. Here we provide the most up-to-date information in an accessible manner. Based on the review, we conclude with recommendations to health practitioners for conceptualizing kratom use and working with patients who use kratom.

PHARMACOLOGY AND ANIMAL STUDIES

Of the dozens of alkaloids identified in kratom, mitragynine is the most prominent (comprising approximately 60 percent; Hassan et al., 2013) and appears, along with 7-hydroxymitragynine, to be primarily responsible for the plant's unique psychoactive properties, which include opioid and non-opioid activities (Adkins et al., 2011; Kruegel and Grundmann, 2018; Raffa et al., 2018) that are dose dependent. In relatively low doses (<5 g), kratom has stimulant properties similar to its coffee relative, while larger quantities may produce sedating and analgesic effects (Kruegel and Grundmann, 2018; Coe et al., 2019; Kruegel et al., 2019; Todd et al., 2020). 7-hydroxymitragynine, while more potent than mitragynine, is unlikely to contribute to pharmacological effects due to its low natural presence in kratom leaves (Kruegel and Grundmann, 2018; Todd, et al., 2020; Obeng et al., 2021). Mitragynine is metabolized by humans via CYP enzymes into 7-hydroxymitragynine but the amount generated via metabolism is not sufficient to explain the analgesic effects of kratom products as a whole (Kamble et al., 2019; Maxwell et al., 2021).

In vitro studies reveal that biochemical pathways responsible for the analgesic and sedating effects of kratom do not carry risk of overdose comparable to classical opioids. Specifically, mitragynine and 7-hydroxymitragynine have partial affinity for the *mu* opioid receptor (Kapp et al., 2011; Prozialeck et al., 2012), whereas morphine is a full agonist. Binding of kratom alkaloids to this receptor largely activate G-protein coupled pathways, as opposed to the beta-arrestin pathway responsible for classical opioids' common deadly side effect of respiratory depression (Kruegel et al., 2016; Váradi et al., 2016; White, 2018; Kruegel et al., 2019; Basiliere and Kerrigan, 2020; Behnood-Rod et al., 2020). Mitragynine also exerts non-opioid receptor pain-relieving effects by stimulating alpha-2 adrenoceptors and inhibiting cyclooxygenase-2 messenger RNA (mRNA) and protein expression (Matsumoto et al., 1996).

The distinct affinity for and activation of opioid receptors, as well as non-opioid analgesic effects that clearly distinguish kratom from classical opioids (Raffa et al., 2018), may explain why there are relatively few kratom-related safety issues given its widespread use. It is likewise important to keep in mind that while many effects of kratom are mediated by opioid receptors, kratom's pharmacology indicates additional non-opioid mechanisms of action, including for mitragynine, again underscoring the complexity of the plant and our limited knowledge of its pharmacology (Hiranita et al., 2019). In addition to limitations in understanding the mechanisms of action and toxicity of kratom, is the limitation in data addressing use of kratom in combination with approved medicines, illicit drugs, and other herbal products. By way of example, the partial opioid agonist buprenorphine, which is approved by many regulatory agencies globally for the treatment of opioid withdrawal and use disorder, as well as pain, carries a far lower risk of lethal respiratory depression when used alone, but has been identified as contributing to overdose deaths when used in combination with benzodiazepines and other sedatives (Kumar et al., 2021). There has not been sufficient study to determine if kratom in combination with benzodiazepines and other sedatives, carries similar, greater, or lessor risks as compared to buprenorphine, so it would seem prudent for health care providers and kratom consumers to be aware of such limitations in the evidence and avoid such combinations and to minimize intake levels when combination consumption occurs because risks with most substances tend to be dose-related. The same cautions apply to use in combination with other substances.

The pharmacokinetics of mitragynine have been established in rodents, primarily rats, following oral administration (Ramachandram et al., 2019). Depending on the vehicle preparation, maximum plasma concentration, c_{max} (0.42–0.70 μg/ml), time to reach c_{max}, t_{max} (1.26–4.50 h), and elimination half-life, $t_{1/2}$, (3.85–9.43 h) indicated that mitragynine was highly variable in its absorption and/or metabolism. A study using traditionally prepared kratom tea

and a hydroalcoholic kratom extract given orally to rats resulted in a c_{max} of 63.8 and 111.9 ng/ml and t_{max} of 1.3 and 3.1 h, respectively, while the $t_{1/2}$ was not determined (Kamble et al., 2021). This supports the conclusion that the absorption of mitragynine is influenced by the presence of other kratom leaf compounds. Only one human study to date evaluated the pharmacokinetics of mitragynine following oral administration of a traditionally prepared kratom tea in 10 male volunteers (Trakulsrichai et al., 2015). The pharmacokinetic parameters for mitragynine were an average t_{max} of 0.83 h, c_{max} ranging from 0.0185 to 0.105 μg/ml, and an average terminal $t_{1/2}$ of 23.2 h. The maximum plasma concentration depends largely on the dose administered and thus needs to be interpreted within that context. However, both the time to reach maximum plasma concentration and half-life are usually comparable, at least within the same species. Because there is only one human study reporting mitragynine pharmacokinetics and substantial variability was found in rat studies, it is too early to conclude how well animal data can predict mitragynine pharmacokinetics in humans. Furthermore, the kratom preparation may impact the absorption and pre-systemic metabolism of mitragynine and other active principles.

Animal research provides further evidence of kratom's relative safety compared to classical opioids. Studies aimed to establish lethal kratom doses have not induced any acute deaths with symptoms similar to morphine. Instead, at doses of mitragynine equivalent to hundreds or more times the typical human dose range, some animals died within days or weeks from a variety of causes unrelated to respiratory depression (Henningfield et al., 2018; Prozialeck et al., 2019; Henningfield et al., 2022). Kratom doses of up to 807 mg/kg in rats or 920 mg/kg in dogs did not indicate signs of toxicity (Macko et al., 1972). Animal studies evaluating reinforcing effects through intravenous self-administration reveal that, unlike morphine, mitragynine does not serve as a reinforcer in rats (Hemby et al., 2018; Yue et al., 2018) and therefore has lower abuse potential. Mitragynine was also found to reduce rodent morphine (Hemby et al., 2018) and heroin self-administration (Yue et al., 2018). See Henningfield et al. (2022) in this special issue for an update of many more studies related to the abuse potential of kratom. Furthermore, administration of 7-hydroxymitragynine takes the equivalent of 100 times more than what humans consume to display reinforcing effects (Hemby et al., 2018). Rodent studies also demonstrate that prodigious amounts of mitragynine (not ingestible at a human equivalent) may be needed to produce severe and sustained withdrawal effects that rival those produced by classical opioids (Harun et al., 2015; Henningfield et al., 2018). Rather, kratom has been found to attenuate opioid withdrawal symptoms in animals, albeit with its own milder withdrawal effects after cessation of long-term use (Hassan et al., 2013; Sabetghadam et al., 2013; Yusoff et al., 2016). Rodent studies confirm physical withdrawal from kratom that occurs after injection with the opioid inhibitor naloxone (e.g., Matsumoto et al., 2005), as well as with cessation of repeated mitragynine administration (Yusoff et al., 2016). Symptoms include somatic withdrawal within 12 h and increased anxiety, evident after 24 h. Across studies, dose-dependent indicators of both toxicity and withdrawal related to isolated kratom alkaloids have been found to resolve after discontinuation or a short duration of time has passed, respectively.

OBSERVATIONAL RESEARCH

Our current understanding of kratom's effects in humans are based primarily on observational studies, including those using surveys, online experience reports, and/or validated self-report measures. With the increasing popularity of kratom in the West, online surveys (assessing tens of thousands of kratom consumers) have been conducted by United States researchers revealing that unlike SE Asia, where kratom appears to be predominantly consumed by males, almost half of United States consumers are female. Also, a majority of Western consumers are middle-aged, middle-income, Caucasian, and college-educated with private insurance. Most discovered kratom through the Internet or social media, about 25% from an acquaintance/friend, and a mere 3% from a healthcare provider. Only approximately 40% informed their healthcare providers about their use (Grundmann, 2017). Generally, motives for use in the West mirror those in SE Asia and include improvements in health, well-being, and productivity. Survey respondents overwhelmingly indicate that regular kratom consumption produces desired effects, including relief of various symptoms such as pain or anxiety, allowing them to live functional lives and meet daily obligations (Grundmann, 2017; Coe et al., 2019; Smith et al., 2021b).

Energy and Focus

A longstanding use for kratom in SE Asia is to increase productivity. In a survey of over a million kratom users in this region, a primary motive was to enhance physical performance (Tanguay, 2011). This may explain why a preponderance of traditional consumers are male agricultural laborers. Chewing kratom leaves while working the fields has been embedded in the culture for centuries. A recent analysis of 293 male Malaysian daily consumers revealed their main reason for ingestion was to work longer hours with less fatigue and pain (Singh et al., 2014). In another survey of 136 kratom users (predominantly male) in Malaysia, most reported the motive of increased work capacity and enhanced energy. It appears that Westerners are also increasingly using kratom to improve occupational functioning, much like the common use of coffee. Three Western online surveys (over 16,000 respondents combined) revealed increased energy and improved focus as main reasons for kratom consumption (Grundmann, 2017; Pain News Network, 2017; Coe et al., 2019). Social media analyses of kratom users also found these self-reported motivations and benefits of kratom use (Smith et al., 2021b; Smith KE. et al., 2021). In fact, clinical, scientific, and ethnographic reports spanning from 1930 to 2017 consistently reveal kratom's role in enhancing, sustaining, or making it feasible to meet work demands (Henningfield et al., 2018).

Mood and Mental Health

A substantial portion of kratom users report using the plant to improve mood or manage symptoms associated with a mental health diagnosis. In the largest scale survey in SE Asia, many revealed using kratom to "feel better" and "cope with problems" (Tanguay, 2011). In a Western survey of 2,867 current and 157 former kratom users (Coe et al., 2019), 22% reported using kratom to alleviate symptoms of anxiety, post-traumatic stress disorder (PTSD), or depression. In another US-based survey of 8,049 users (Grundmann, 2017), 66% used kratom to treat emotional or mental conditions. Additionally, a survey of 6,150 kratom consumers by Pain News Network (2017) revealed 14.5% of respondents used kratom to treat anxiety, 8.83% to treat depression, and 1.40% to treat insomnia. The most recent survey of kratom users (n = 2,798; Garcia-Romeu et al., 2020) indicated that depression and anxiety were the motivation for kratom use for 67% and 65%, respectively. A literature review of 13 peer-reviewed studies in SE Asia and the United States examining kratom use and mental health provided further support that many individuals use kratom as a mood enhancer or anxiolytic (Swogger and Walsh, 2018). Specifically, the stimulant effect at low doses reportedly acts as a mood booster, while higher doses induce relaxation that may alleviate anxiety. There is also preliminary evidence that kratom has empathogenic effects, leading the authors to hypothesize that kratom may enhance sociability beyond what would be accomplished with anxiety reduction alone (Swogger et al., 2015).

Pain Management

While kratom's longstanding medicinal use in SE Asia applies to a variety of ailments, alleviation of pain is among the most common. A study in which 562 kratom users were interviewed in Malaysia revealed pain relief as a main reason for consumption (Ahmad and Aziz, 2012). Results of large-scale United States online surveys reveal that pain relief is the most common reason for kratom use (Grundmann, 2017; Pain News Network, 2017; Coe et al., 2019; Garcia-Romeu et al., 2020). The Pain News Network (2017) survey queried pain conditions that people were managing with kratom. The most common identified were back/spine pain, followed by acute pain from injury, fibromyalgia, migraine or headache, and rheumatoid arthritis. Other pain conditions people reported treating with kratom included multiple sclerosis, neuropathy, osteoarthritis, inflammatory bowel disease, lupus or other autoimmune diseases, complex regional pain syndrome, Ehlers-Danlos syndrome, trigeminal neuralgia, and cancer. Over 90% of the respondents indicated that kratom is "very effective" in treating their pain or medical condition, while approximately 7% reported it to be "somewhat effective" (Pain News Network, 2017). Adding to data from observational studies, results from a recent randomized, double-blind, placebo-controlled trial indicated that kratom significantly increased acute pain tolerance, as measured in the laboratory using the cold-pressor task (see Brown et al., 2003), in a sample of 26 male kratom users. (Vicknasingam et al., 2020).

Harm Reduction

The value of substitution (replacing an undesirable substance with a less harmful one) is evidenced by cannabis as a successful substitute for alcohol, opioids, and cocaine (Bachhuber et al., 2014; Socías et al., 2017) or treating OUD by replacing opioids with high potential for dependence (e.g., heroin, oxycodone) with those with less potential for dependence (e.g., methadone or buprenorphine). Consistent with descriptions of kratom use in SE Asia dating back to the 19th century (Hassan et al., 2013), current research indicates that kratom is being successfully used as a harm-reduction method or self-treatment for opioid withdrawal, including as a short- or long-term opioid substitute (Ward et al., 2011; PinneyAssociates, 2016; Smith and Lawson, 2017; Smith et al., 2021b; Smith KE. et al., 2021). Notably, kratom has the advantage of being available to individuals who cannot access medical treatment due to barriers in the system or will not access it due to mistrust of health care professionals, thus providing a potential self-treatment for OUD to a wide swath of people who would otherwise receive none.

A convenience study sample of 136 kratom users (99% male; mean age = 38.7) in an area of Malaysia known for heavy kratom use revealed that 90% were using kratom as a substitute for opioids and 84% indicated that kratom helped with their opioid withdrawal symptoms (Vicknasingam et al., 2010). In another Malaysian survey (Singh et al., 2015) of 293 adult male regular kratom users (mostly manual laborers; mean age = 28), 15% indicated that they had used kratom in an effort to reduce or eliminate addictions to illicit substances (e.g., opioids, cannabis) and/or to ameliorate opioid withdrawal symptoms. Kratom has also been used in SE Asia as a substitute or self-treatment for amphetamines and alcohol (Vicknasingam, et al., 2010; Singh et al., 2021) and in the United States to self-treat alcohol dependence (Smith et al., 2021b). These self-report data converge with preliminary signals in the pharmacology literature suggesting the therapeutic potential of kratom alkaloids for harmful alcohol use (Gutridge et al., 2019; Gutridge et al., 2020).

In the West, five United States.-based internet surveys of over 20,000 kratom users, as well as over 20,000 comments to the Drug Enforcement Administration (DEA), and a survey of more than 500 people in treatment for opioid use disorder, indicate that many are using kratom as an alternative to opioids (Grundmann, 2017; Smith and Lawson, 2017; Henningfield et al., 2018; Coe et al., 2019; Garcia-Romeu et al., 2020). In one of those surveys (Grundmann, 2017), nearly half of 8,049 respondents indicated that kratom enabled them to reduce or discontinue the use of opioids. Ten percent of 3,017 respondents to another survey (Coe et al., 2019) were taking kratom to cut down on opioid use and/or relieve withdrawal. Of those using kratom in place of opioids, 90% indicated that it was helpful to relieve pain, reduce opioid use, and relieve withdrawal. An analysis of 170 kratom threads during a 12-month period (2004–2005) on a Western online pharmacy indicated that a vast majority purchased kratom to treat opioid withdrawal (Boyer et al., 2007). Furthermore, in a study of 161 respondents to a United States.-based internet forum, over 10% reported using kratom to successfully decrease or abstain from a substance that was unwanted or

considered to be causing harm (Swogger et al., 2015). Similar social media analyses indicate that kratom use as an opioid substitute is widespread (Smith et al., 2021b; Smith KE. et al., 2021).

Use of kratom as an effective substitute is supported by preclinical research, which is of particular interest in light of the current United States opioid crisis of rising dependence rates, emergency room visits, and overdose deaths. A recent study by Saref et al. (2019) provided evidence of kratom's potential as a harm-reduction agent and Yue et al. (2018) found that rodents pre-treated with mitragynine self-administered less heroin. Using a convenience sample of 260 illicit drug users, Saref et al. (2020) found association was found between self-reported initiation of kratom and reduction in both the consumption of various illicit drugs and frequency of HIV risk behaviors related to sexual practice and injection drug use. These findings are promising, given that estimated calculations put morphine-like opioids at an overdose risk of *a thousand or more times* that of kratom (Henningfield et al., 2019).

Kratom as a substitute to opioids also has the potential to improve social, family and occupational outcomes and behavior (Swogger et al., 2015; Henningfield et al., 2018; Swogger and Walsh, 2018). Like coffee drinkers, regular kratom users often consume this herbal supplement as a beverage in the company of others, enhancing social connection. In contrast, long-term daily opioid use can lead to self-isolation since this drug, unlike kratom, is conducive to the quick intense euphoria attained through snorting, injecting, and inhaling (Henningfield et al., 2018). In fact, only 2% of 6,135 kratom-users in the Pain News Network (2017) online survey responded "yes" to the question "Can you get high from kratom?" In addition to survey data, thousands of public comments to DEA and FDA attested to the successful substitution of kratom for opioids (Prozialeck et al., 2019).

Adverse Kratom Effects and Kratom Withdrawal

Despite centuries of kratom use in SE Asia, there have been few reports of serious adverse events associated with its use, and kratom overdose has not been identified as a direct cause of death in fatalities coincident with kratom use (PinneyAssociates, 2016). Among a sample of 293 SE Asian dependent kratom users, none reported having to obtain medical treatment related to kratom use (Singh et al., 2014). Recent research in the West confirms that adverse effects appear to be rare and dose dependent. In Grundmann's (2017) survey of 8,049 kratom users, less than 1% sought medical or mental health treatment related to kratom consumption, similar to low rates of adverse effects or healthcare treatment utilization for kratom found by Garcia-Romeu et al. Dosages of at least 5 g and frequency of 22 or more times per week were more likely to be associated with side effects (occurring in approximately 20% of 3,024 respondents), which were primarily gastrointestinal in nature (nausea, constipation, etc.). Other reported side effects of kratom use include vomiting, drowsiness, irritability, agitation, headache, runny nose, watery eyes, weight loss, insomnia, dehydration, and excessive thirst (Vicknasingam et al., 2010; Adkins et al., 2011; Anwar et al., 2016; Lydecker et al., 2016; Singh et al., 2016; Grundmann, 2017). These predominantly self-managed side effects occurred in about 13% of 3,024 respondents in the Coe et al. (2019) survey.

Kratom tolerance, dependence, and withdrawal have been reported with daily and heavy use, though these symptoms are generally milder and of shorter duration than those of classical opioids (Ahmad and Aziz, 2012; Singh et al., 2014; Singh et al., 2015; Swogger et al., 2015; Grundmann, 2017; Swogger and Walsh, 2018; Smith et al., 2021b). Physical dependence that can develop over time has been described as similar to that of coffee or mild opioid dependence (Brown et al., 2017). A study on dependent users (three or more daily servings) indicated that withdrawal symptoms (including insomnia, nausea, vomiting, diarrhea, muscle pain or spasms, shakiness, runny eyes or nose, and hot flashes) resolved within one to 3 days for most (Singh et al., 2014). Longer duration of use and higher average dose may extend the duration and increase the severity of withdrawal, however, and a small number of individuals may find kratom very difficult to quit (Smith et al., 2021b).

Overall, there appears to be minimal, short-term risk to the majority of people using kratom with the intention of self-treating a variety of conditions. While these findings warrant validation in controlled clinical studies, they reveal that for many people, kratom enhances their health in ways they report as unachievable, or with fewer side effects, than by other means, including pharmaceuticals. For this reason, it is important that healthcare practitioners are prepared and willing to have conversations with their patients about the use of kratom products pending greater scientific understanding of this plant and experimental validation of its traditional and (growing) conventional uses.

BEST PRACTICES IN THE CLINICAL CARE OF PEOPLE WHO USE KRATOM

Up to 60% of patients turn to non-medical modalities for treatment (Alwhaibi et al., 2015) and over 30% use herbal-based remedies, especially for conditions involving chronic pain (Barnes et al., 2008; Pain News Network, 2017). Although the majority of kratom consumers do not reveal their use to healthcare providers (Grundmann, 2017; Coe et al., 2019), popular use of natural remedies is evident on "pharmacy watch" websites, such as *drugs.com,* that disseminate information regarding alternatives for pain management, including kratom (Boyer et al., 2007). Healthcare practitioners must therefore be knowledgeable about the implications of using these substances. Indeed, the National Institute on Drug Abuse (NIDA) advises that "people should check with their healthcare providers about the safety of mixing kratom with other medicines" (NIDA, 2019), suggesting that patients do turn to their providers for information. Meanwhile, providers and patients alike have been put in unnecessarily difficult positions as they attempt to sort out contradictions between United States government agency-fueled headlines (e.g., the CDC warning that kratom may cause psychosis or death; Anwar et al., 2016) and more reasonable interpretations of the existing scientific data on

kratom. To aid in this process, and with the earlier stated caveats that kratom has not been approved as safe and effective for any medical disorder and we are not encouraging or endorsing such use, we offer *best practices* for assessing and treating people who use kratom, pending further study in controlled experiments.

Assessment

It is important to contextualize kratom assessment for the patient in a way that feels consistent with the non-judgmental and routine nature of a competent medical or mental health evaluation. Embedding questions about the use of "herbal medicines, like Valerian root or kratom" in an assessment of pharmaceuticals and supplements acknowledges kratom's place among other treatments that people choose to use. The stance is non-stigmatizing and respectful and may increase the likelihood of honest patient disclosure. Initiating a discussion with open-ended questions about patients' experiences with kratom, desired outcomes, and concerns enables practitioners to assess gaps in knowledge or false beliefs, areas for patient education. Moreover, apprehending patients' motivations for use also enables clinicians to provide education around other, FDA-approved treatments such as cognitive-behavioral therapy for anxiety and buprenorphine for opioid replacement. Kratom does not appear in standard drug screens (White, 2018). Regardless, we do not recommend routine screening for illicit substances without consideration of the potential impact on patient-provider trust, which may be necessary for honesty and positive outcomes.

Patient-Centered Conceptualization

It is helpful for providers to explicitly state that they are unable to recommend or condone the use of kratom or any substance that is not approved by the FDA, but that they can provide education and work to understand the patient's kratom use. The non-judgmental approach to assessment recommended above leads naturally to a conceptualization of kratom use within the context of individual patient values and goals. The evidence-based treatment *process* specifically highlights the need for clinical engagement and shared decision-making with patient values and goals inherent in that process (Gambrill, 2006; Melnyk et al., 2010; Hoffmann et al., 2014). Treatment around substance use that involves patient values has evidence for efficacy (Osaji et al., 2020). Someone who, for example, is unsuccessfully using kratom to self-treat anxiety may be surprised to learn that there are evidence-based psychotherapies (e.g., cognitive-behavioral therapy) that are effective for doing the same. Someone who is self-treating OUD may wish to switch to buprenorphine, or other medication-assisted therapy if they are unhappy with their response to kratom or wish to use an FDA-approved product. For people without histories of opioid dependence, however, switching from kratom to buprenorphine or methadone should be considered judiciously on a patient-by-patient basis. Assessment of patient values and goals can lead to rich, meaningful discussions of the potential risks and benefits of a number of treatments and can contribute to trust, and thus effectiveness, in the clinician-provider relationship.

Understand Patient Motives for Use

With the explicit acknowledgment that data from randomized controlled trials is lacking, the available data reviewed above indicate that people across the world consistently report that kratom is useful for increasing energy, improving mood and alleviating anxiety, and for decreasing and/or ceasing opioid use and alleviating related withdrawal symptoms. Some people have reported that kratom has been a helpful substitute for other substances that are causing harm (e.g., alcohol). Finally, the observational literature indicates that people consistently report the utility of kratom for pain relief, and this is corroborated by the only clinical trial to examine kratom and analgesia in humans.

Dosing

The literature on dosing is consistent, but imprecise. In general, lower doses up to approximately 5 g of raw plant material are reported to exert stimulant effects and have been compared to caffeine. Doses between approximately 5 and 15 g are reported to lead to relaxation and analgesic effects. These higher doses may be necessary for successful opioid substitution. Side effects are more likely at higher doses. Limited data show that there is variability in terms of lowest and highest perceived dose as being ineffective or effective, and that feelings of discomfort may arise from higher doses (Smith et al.). Although no dose or dosing range can be clinically recommended, but patients can be informed that some average doses are approximately 2.5 g (Smith et al.). The development of kratom dependence, including tolerance and withdrawal symptoms upon cessation or reduction, is more likely at higher doses and with frequent, recurring use. For this reason, patients should be encouraged to use as little as needed for therapeutic effects. People who have decided to initiate kratom use should begin with a minute amount to test for adverse reactions before slowly increasing. It is important to convey that the potency of the plant can vary based on factors such as geographical source, the season, age of the sample, and post-harvest handling (Adkins et al., 2011; Griffin and Webb, 2018; Pearson et al., 2018; Zhang et al., 2020), as well as strain, which is commonly referred to as vein type (red, green, or white) and likely corresponds to the age of the leaf. Evidence suggests that the red vein variety may be more potent than the older, green vein (Braley and Hondrogiannis, 2020). Patients should be advised to purchase kratom from the same manufacturer to be as consistent as possible in the product (see further discussion below).

Duration of Action

Generally, the effects of kratom last for approximately five to 7 hours, with the strongest effect within two to 4 hours of ingestion, though aftereffects (e.g., fatigue) can be felt as late as the following day (Maruyama et al., 2009; Prozialeck et al., 2012; Rosenbaum et al., 2012; Scott et al., 2014). One should not assume, however, that effects of kratom will not "kick in" sooner, especially if taken on an empty stomach, or last longer due to individual variation.

TABLE 1 | Legality of kratom in United States.

State	Legality
Alabama Arkansas Indiana Vermont Wisconsin Rhode Island	Illegal in all areas for use, possession, and purchase
Illinois	Legal for use, with exception of Jerseyville, Alton, and Edwardsville, to people over the age of 18
New Hampshire	Legal to use for individuals over the age of 18, except for Franklin City
California	Legal for use but banned in the city of San Diego
Florida	Legal for use but banned in Sarasota County
Mississippi	Banned in 33 counties and towns but remains legal in the rest of the State
Colorado	Legal in Colorado, with exceptions in Parker and Monument towns. Denver is illegal for human consumption
Tennessee	Legal to sell as long as it's labeled and in its natural botanical form (Pure). Legal to use for individuals over the age of 21
Arizona Georgia Nevada Utah	Kratom Consumer Protection Act passed and enacted, kratom products need to follow GMP manufacturing guidelines and labeling standards set by the state legislature
Remaining states	Legal to consume, purchase and sell. In many cases, you must be an adult over 18

Side Effects and Adverse Effects

Unwanted effects associated with kratom use have been systematically studied. Large scale surveys and other observational studies of tens of thousands of users reveal that a minority experience dose-dependent side effects that are mostly mild and self-resolve. The most common of these are constipation, nausea, vomiting, other stomach irritation, and drowsiness (Grundmann, 2017; Swogger and Walsh, 2018). Dizziness and sedation are possible, so patients should avoid driving or any other activities one would avoid after drinking alcohol. Adverse effects, while rare, cannot be ruled out. These include the potential for liver problems, seizures, and dependence (discussed below). Individuals with compromised liver, kidney, or cardiac function may be at increased risk for harm (Harizal et al., 2010). While a recent study found no QTc interval prolongation differences between kratom users and controls (Leong Abdullah et al., 2021), it is worth noting that a small number of cases allude to this possibility and cardiotoxicity in humans cannot be ruled out (see Lu et al., 2014). Clinical trials may reveal additional long-term and/or rare side effects and adverse effects.

Kratom-Drug Interactions

As discussed earlier, it is important to note the possibility of risky drug interactions between kratom and alcohol, opioids, and benzodiazepines for instance, which may induce respiratory depression, as well as use in combination with stimulants because kratom also as stimulant effects. In the absence of evidence of more detailed safety data, there should be the presumption of some level of risk of kratom use in combination with other pharmacologically active substances and that such risks are more likely to be increased by higher levels of consumption. Thus, patients taking kratom should be made aware that interaction effects with other substances have not been studied. It is therefore highly advisable to refrain from mixing kratom with other substances including herbal products. Additionally, mitragynine's observed mechanisms of action can guide warnings issued to kratom consumers taking certain medications. The alkaloid's stimulation of postsynaptic alpha-2 adrenergic receptors (Prozialeck et al., 2012) suggests that it can accentuate and therefore be dangerous with other sedative, hypnotic, and analgesic drugs (White, 2018). Additionally, a recent *in vitro* study revealed the possibility that kratom ingestion mixed with drugs that are P-gp substrates (e.g., erythromycin, loperamide, protease inhibitors) could lead to clinically significant toxicity (Rusli et al., 2019). Finally, there is evidence that kratom cytochrome P450 enzyme activities, thus raising the possibility of herb-drug interactions when administered along with agents that use the same metabolic pathway (Hanapi et al., 2013).

Dependence

Kratom dependence occurs for a minority of users, especially at high doses with frequent dosing. Individuals with substance dependence histories who are considering initiating kratom use should be informed that their risk of dependence is theoretically higher than for people who have never had harmful substance use. Solid therapeutic relationships enable patients to be honest about their kratom use, allowing for close monitoring of increased signs of dependence (e.g., tolerance, cravings, and withdrawal symptoms upon cessation). Patients looking to decrease or

discontinue kratom use can be assured that this is often done without medical intervention (Saingam et al., 2016) and that a gradual taper is recommended. Mild withdrawal effects occur in some people and usually resolve within days. These may include physical symptoms (muscle spasms or soreness, diarrhea, muscle aches, lack of appetite, fever, and runny eyes and nose) and psychological symptoms (mood swings, irritability, nervousness restlessness, disturbed sleep, tension and sadness; Singh et al., 2014). Some people who have ceased use report substituting coffee or energy drinks to help with withdrawal symptoms (Saingam et al., 2016). Patients should be encouraged to call their provider if concerns arise regarding the severity or duration of their withdrawal symptoms, as more severe and long-lasting withdrawal has been reported. In such cases, initiation of an opioid substitute (e.g., buprenorphine) and/or supportive psychotherapy may be beneficial. Patients who experience more severe withdrawal symptoms should be cautioned against making important decisions until they are feeling better and should be encouraged to contact their provider if they begin having thoughts of suicide due to mood changes occurring during the withdrawal process.

Additional Considerations

In order to purchase kratom that is of consistent potency and is unadulterated, patients should be informed that vendors who use Good Manufacturing Practices (GMP) are identifiable. It is also potentially helpful to inform patients about whether they risk arrest for use of kratom in a particular jurisdiction (see **Table 1**). Finally, it may be helpful to clarify that FDA non-approval of kratom as a treatment, despite the significant observational evidence for utility, reflects the fact that clinical trials to substantiate users' reported effects have yet to be conducted. In conclusion, we present here the most up-to-date information regarding kratom to inform healthcare providers with the necessary data to have honest and straightforward discussions with their patients concerning kratom use, which is currently on the rise in the United States, and could have important health ramifications.

AUTHOR CONTRIBUTIONS

LB and MS conceptualized the paper and wrote preliminary drafts. All other authors contributed to the significant editing, scientific review, and writing of subsequent drafts.

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Conflict of Interest: Through PinneyAssociates, JH provides scientific and regulatory consulting to support new drug applications (NDAs) and risk management programs for a broad range of CNS active substances and drug products including psychedelic substances, new chemical entities, and alternative formulations and routes of delivery, as well as dietary ingredient notifications, cannabinoid assessment, and noncombustible tobacco/nicotine products for FDA regulation. This includes advising the American Kratom Association and its affiliate, the Center for Plant Science and Health, on kratom science and regulation.

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 reviewer MFILBA declared a past collaboration with several of the authors OG, JH to the handling editor.

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