Drug and Alcohol Dependence 216 (2020) 108310

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Lyophilized Kratom Tea as a Therapeutic Option for Opioid Dependence

Lisa L. Wilson [a], Hannah M. Harris [a,1], Shainnel O. Eans [a], Ariana C. Brice-Tutt [a], Thomas J. Cirino [a], Heather M. Stacy [a], Chloe A. Simons [a], Francisco León [b], Abhisheak Sharma [c], Edward W. Boyer [d], Bonnie A. Avery [c], Jay P. McLaughlin [a,*], Christopher R. McCurdy [b,**]

[a] Department of Pharmacodynamics, University of Florida, Gainesville, FL, United States
[b] Department of Medicinal Chemistry, University of Florida, Gainesville, FL, United States
[c] Department of Pharmaceutics, University of Florida, Gainesville, FL, United States
[d] Department of Emergency Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States

ARTICLE INFO

Keywords:
Antinociception
Dependence
Kratom
Opioid
Withdrawal

ABSTRACT

Background: Made as a tea, the Thai traditional drug "kratom" reportedly possesses pharmacological actions that include both a coca-like stimulant effect and opium-like depressant effect. Kratom has been used as a substitute for opium in physically-dependent subjects. The objective of this study was to evaluate the antinociception, somatic and physical dependence produced by kratom tea, and then assess if the tea ameliorated withdrawal in opioid physically-dependent subjects.

Methods: Lyophilized kratom tea (LKT) was evaluated in C57BL/6J and opioid receptor knockout mice after oral administration. Antinociceptive activity was measured in the 55 °C warm-water tail-withdrawal assay. Potential locomotor impairment, respiratory depression and locomotor hyperlocomotion, and place preference induced by oral LKT were assessed in the rotarod, Comprehensive Lab Animal Monitoring System, and conditioned place preference assays, respectively. Naloxone-precipitated withdrawal was used to determine potential physical dependence in mice repeatedly treated with saline or escalating doses of morphine or LKT, and LKT amelioration of morphine withdrawal. Data were analyzed using one- and two-way ANOVA.

Results: Oral administration of LKT resulted in dose-dependent antinociception (≥1 g/kg, p.o.) absent in mice lacking the mu-opioid receptor (MOR) and reduced in mice lacking the kappa-opioid receptor. These doses of LKT did not alter coordinated locomotion or induce conditioned place preference, and only briefly reduced respiration. Repeated administration of LKT did not produce physical dependence, but significantly decreased naloxone-precipitated withdrawal in morphine dependent mice.

Conclusions: The present study confirms the MOR agonist activity and therapeutic effect of LKT for the treatment of pain and opioid physical dependence.

1. Introduction

Agonists of the mu-opioid receptor (MOR) such as morphine are effective analgesics, but demonstrate adverse effects such as constipation, respiratory depression, physical dependence, and addiction (Li and Zhang, 2012). In 2018, these effects claimed an average of 130 lives daily in the United States from opioid overdose (CDC/NCHS, 2018).

Withdrawal symptoms in subjects physically-dependent on morphine are severe and include increased blood pressure and heart rate, pronounced diarrhea and vomiting, and dysphoria (Ballantyne and LaForge, 2007). The severity of these effects contibutes to the high rate of relapse in subjects abusing opioids (Kreek and Koob, 1998). Current pharmacological treatments for opioid withdrawal include substitution

Abbreviations: ANOVA, Analysis of Variance; β-FNA, β-funaltrexamine; CLAMS, Comprehensive Lab Animal Monitoring system; CPA, conditioned place aversion; CPP, conditioned place preference; DOR, Delta-opioid receptor; KOR, Kappa-opioid receptor; KO, Knockout; LKT, Lyophilized Kratom Tea; MOR, Mu-opioid receptor; nor-BNI, nor-binaltorphimine; p.o., *per os*; RM, Repeated Measures.

* Corresponding author at: Jay P. McLaughlin, Department of Pharmacodynamics, 1345 Center Drive, Gainesville, FL 32610, United States.
** Corresponding author at: Christopher R. McCurdy, Department of Medicinal Chemistry, 1345 Center Drive, Gainesville, FL 32610, United States.
E-mail addresses: jmclaughlin@cop.ufl.edu (J.P. McLaughlin), cmccurdy@cop.ufl.edu (C.R. McCurdy).
[1] Current address: Department of BioMolecular Sciences, University of Mississippi, University, MS.

https://doi.org/10.1016/j.drugalcdep.2020.108310
Received 15 June 2020; Received in revised form 13 September 2020; Accepted 15 September 2020
Available online 22 September 2020

with the MOR agonist methadone or the multifunctional opioid/nociception-OFQ agonist, buprenorphine (Kreek et al., 2002). These therapeutics produce liabilities of abuse and physical dependence that complicates treatment for opioid dependence, highlighting an urgent need for improved therapeutics for pain and opioid withdrawal.

Case studies and surveys suggest a tea traditionally prepared from the fresh leaves of *Mitragyna speciosa* (Korth.) Havil., kratom, may transition physically-dependent individuals off opioids with fewer symptoms of withdrawal (Boyer et al., 2008; Swogger et al., 2015). Kratom tea is documented to decrease fatigue and treat other ailments such as pain, cough, and inflammation (Swogger et al., 2015; Warner et al., 2016). It is speculated that kratom effects are mediated by two of its constituents, mitragynine and 7-hydroxymitragynine (approximately 66% and 1-2% of the plant's alkaloid content, respectively) (Kamble et al., 2019; Kruegel et al., 2019), both of which have shown affinity for, and agonistic effects at, the MOR (Takayama et al., 2002; Boyer et al., 2008; Hassan et al., 2013; Váradi et al., 2016).

Most kratom users partake of the natural product orally as a tea extract (Avery et al., 2018; Boyer et al., 2008). Despite this anecdotal use of kratom to treat pain and opioid withdrawal (Garcia-Romeu et al., 2020), few behavioral pharmacology studies have assessed the analgesic efficacy, liabilities, or the potential of kratom tea to treat opioid withdrawal under controlled conditions. The present study utilized mice to evaluate antinociception and liabilities of relevant doses of orally ingested lyophilized kratom tea (LKT), where the constituents were previously characterized (Sharma et al., 2019) and constant herein. Additionally, the therapeutic potential of LKT to prevent naloxone-precipitated withdrawal in mice physically-dependent on morphine was assessed.

2. Materials and Methods

Male C57BL/6J and gene-disrupted (knock-out, or KO) mice for the mu- (MOR), kappa- (KOR) or delta-opioid receptor (DOR) (20–35 g) were used (Jackson Laboratories, Bar Harbor, Maine, USA). Mice were housed five per cage on a 12:12 h light/dark cycle (lights off at 7 pm). Access to water and food was *ad libitum*. Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny et al., 2014). Sample sizes were approximated by Power analysis, with animals assigned to groups randomly and double blinded treatment groups. Animal studies were approved and conducted in agreement with the Institutional Animal Care and Use Committees at the Universities of Mississippi, Florida and in accordance to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.1. Chemicals

Dried leaves of *Mitragyna speciosa* (kratom) were purchased from Pure Land Ethnobotanicals in February of 2009 and authenticated by Dr. Rita Moraes from the National Center for Natural Products Research at the University of Mississippi. All other chemicals were obtained commercially from Sigma-Aldrich.

2.2. Preparation of Lyophilized Kratom Tea (LKT)

The plant material was prepared as previously described (Avery et al., 2018; Kamble et al., 2019). As reported previously, 200 g of the processed leaf material was added to a finum tea filter, and boiled in 2 L of water for 20 min. The filtered aqueous layer was evaporated to yield LKT. All LKT used were from the same preparation, analyzed for the content of 8 kratom alkaloids using an ultra-performance liquid chromatography tandem mass spectrometer (UPLC-MS/MS) method (see Table 1, reproduced from Sharma et al., 2019).

Table 1
Alkaloid content of LKT.

	mg/g
Alkaloid	
Mitragynine	7.4
Speciociliatine	3.5
Paynantheine	2.5
Corynoxine B	0.3
Corynantheidine	0.2
Isocorynantheidine	0.2
7-hydroxymitragynine	0.1
Speciogynine	0.1

2.3. Dosing of LKT

The test dose of LKT used in the present mouse study was calculated using the FDA-recommended guidelines (listed as equation 1, below) from the normalized dose of mitragynine (56.7 mg) available in a glass of kratom juice consumed by the native kratom users from Malaysia (FDA, 2005; Singh et al., 2020). The correction factors (K_m) were derived from average body weight (kg) of species to its body surface area, with FDA recommended values of K_m as 37 and 3 used for human (60 kg) and mice (0.02 kg), respectively.

$$\text{Equivalent mice dose}\left(\frac{\text{mg}}{\text{kg}}\right) = \text{Human equivalent dose}\left(\frac{\text{mg}}{\text{kg}}\right) * \left[\frac{Km\ for\ human}{Km\ for\ mice}\right] \quad (1)$$

The calculated human equivalent dose of mitragynine in mice (MED) was 11.7 mg/kg. According to the quantitative analysis of LKT for mitragynine content (7.4 mg/g), 1.6 g/kg of LKT would be required to achieve the MED. The MED of LKT (rounded to 2 g/kg) as well as a dose equivalent to half of MED (1 g/kg) was thus used for the LKT characterization in most animal experiments.

2.4. Antinociceptive Characterization (55 °C Warm-Water Tail-Withdrawal Assay)

Mice were divided into 5 groups (n = 8, or 16 for KOR KO mice). The 55 °C warm-water tail-withdrawal assay was performed as previously described (Reilley et al., 2010). Latency to remove the tail from the water was the recorded end point. After measuring baseline responses, mice were administered either morphine (1-60 mg/kg, i.p. or p.o.), vehicle (saline (0.9%)) or LKT (45-4000 mg/kg, p.o.) and the tail withdrawal measured every 10 minutes until a return to baseline was recorded. Antinociception was calculated using the following formula:

% antinociception = 100 x ((test latency-baseline latency)/ (15-baseline latency))

Mice that failed to withdraw their tails within 15 seconds were assigned a maximum antinociceptive response (100%) to avoid tissue damage.

2.5. Respiratory Depression/Locomotor Activity Assessment

Locomotor activity and respiratory depression were assessed using the automated, closed-air Comprehensive Lab Animal Monitoring system (CLAMS) as previously described (Brice-Tutt et al., 2020; Cirino et al., 2019). Randomly grouped mice were habituated in individual chambers for 60 min, then administered morphine (30 mg/kg, p.o.), LKT (1 g/kg, p.o.) or vehicle. Animals moved freely inside the chambers for 3 hours, where respiration (breaths/minute) and ambulation (number of photobeam breaks) were counted.

2.6. Rotarod Assessment of Impaired Motor Activity/Sedation

Sedative or locomotor impairing effects of LKT were assessed as

described previously using the computer-controlled rotarod apparatus (San Diego Instruments, San Diego, CA) (Brice-Tutt et al., 2020; Cirino et al., 2019). Mice were first habituated to the rotarod over seven trials, with the last trial serving as the baseline response. Habituated mice were administered saline (p.o.), U50,488 (10 mg/kg, i.p.), morphine (10 mg/kg, i.p.) or LKT (45 mg/kg or 1 g/kg, p.o.) 15 min prior to assessment in accelerated speed trials (180 s max. latency at 0-20 rpm) performed every 10 min from 0 min over a 60 min period for a total of 14 trials (seven habituation trials + seven drug trials). Decreased latencies to fall indicate impaired motor performance and possible sedation (Cirino et al., 2019).

2.7. Conditioned Place Preference (CPP)

An automated, balanced three-compartment place conditioning apparatus (San Diego Instruments, San Diego, CA) and 2-day counterbalanced conditioning design was used (Cirino et al., 2019; Eans et al., 2015). The amount of time subjects spent in each of three compartments was measured over a 30 min testing period. Each of the next two days, mice were administered vehicle (0.9% saline) and confined in a randomly assigned outer compartment: half of each group in the right and left chambers. Four hours later, mice were administered morphine (10 mg/kg, i.p.) or LKT (100 mg/kg or 1 g/kg, p.o.) and confined to the opposite compartment for 40 min.

2.8. Naloxone Precipitated Opioid Withdrawal Assay

Mice (n = 8-10 group) were placed into six groups (Table 2): saline (i.p.), morphine (10-75 mg/kg, i.p.), escalating doses of LKT (30-125 mg/kg, p.o.), 7-day morphine + LKT (acute), 4-day morphine + LKT (100 mg/kg), 4-day morphine + LKT taper dosing (100-40 mg/kg). Morphine (i.p.) and LKT (p.o.) was dosed daily at 9:00 A.M. and 7:00 P.M., as previously reported (Kamei and Ohsawa, 1997). A final single treatment of morphine or kratom was given on the final day of testing (see Table 2). Two hours post-injection on the last day of testing, the all mice were administered naloxone (10 mg/kg, s.c.) to induce opioid withdrawal symptoms.

Opioid withdrawal behaviors (see Supplemental Table 1) were quantified from mice placed in a 16 cm × 45 cm plexiglass cylinder for 15 min after naloxone administration using established methods (Fernandes et al., 1977; Shaw-Lutchman et al., 2002).

2.9. Statistical Analysis

All data were analyzed using Prism 8.0 software (GraphPad Software, La Jolla, California, USA). Normality and equal variance were confirmed statistically and justified using parametric analysis. Nonlinear or linear regression modeling was performed to analyze ED_{50} values (dose yielding 50% effect) along with 95% confidence intervals (C.I.) using each individual data point. CLAMS data is reported as the % of matching vehicle control responses. The rotarod data are expressed as the % change from baseline performance, using within subject controls. CPP data are reported as the difference in time spent in the drug- and vehicle paired compartments between pre-conditioning and post-conditioning responses. Weight was identified as the percent of weight change each day when compared to the baseline (or naïve weight) of each mouse. Significant differences in behavioral data were analyzed by ANOVA (one-way or two-way RM) as appropriate, with significant results further analyzed with Tukey's multiple comparisons post-hoc tests for significant pairwise comparisons within and between groups. Significance was $p \leq 0.05$.

3. Results

3.1. Lyophilized kratom tea-mediated antinociception in the 55°C warm-water tail-withdrawal assay

Morphine dose-dependently produced full antinociception with an ED_{50} (and 95% C.I.) value of 3.91 (2.92-5.17) mg/kg, i.p., and 4.67 (3.47-6.21) mg/kg, p.o. (Fig. 1A) that significantly differed from vehicle ($F_{(8,144)} = 4.99$, $p < 0.0001$; two-way RM ANOVA) up to 70 min after oral (*per os*, p.o.) administration of approximately the ED_{50} dose (5 mg/kg, p.o.; green triangles, Fig. 1B). LKT also demonstrated significant time and dose-dependent antinociception after oral administration ($F_{(62,490)} = 10.31$, $p < 0.0001$), but the ED_{50} value for LKT was not determined, as a full response was not observed within the doses tested (Fig. 1A). The

Table 2
Method: Experimental Design of Treatment Administration for Naloxone Precipitated Withdrawal Assay.

		Treatment				
Treatment Day	Saline	Escalating Doses of Morphine	Escalating Doses of LKT (30-125 mg/kg)	7d Morphine + LKT-Acute	4d Morphine + LKT Continuing (100 mg/kg)	4d Morphine + LKT Tapering (100-40 mg/kg)
Day 1 am	-	10 mg/kg	30 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg
pm	-	15 mg/kg	35 mg/kg	15 mg/kg	15 mg/kg	15 mg/kg
Day 2 am	-	20 mg/kg	45 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg
pm	-	30 mg/kg	60 mg/kg	30 mg/kg	30 mg/kg	30 mg/kg
Day 3 am	-	50 mg/kg	100 mg/kg	50 mg/kg	50 mg/kg	50 mg/kg
pm	-	60 mg/kg	100 mg/kg	60 mg/kg	60 mg/kg	60 mg/kg
Day 4 am	-	70 mg/kg	125 mg/kg	70 mg/kg	70 mg/kg	70 mg/kg
pm	-	75 mg/kg	125 mg/kg	75 mg/kg	75 mg/kg	75 mg/kg
Day 5 am	-	25 mg/kg	25 mg/kg Kratom	80 mg/kg	100 mg/kg Kratom	100 mg/kg Kratom
pm	-	Morphine -	-	80 mg/kg	100 mg/kg Kratom	100 mg/kg Kratom
Day 6 am	-	-	-	80 mg/kg	100 mg/kg Kratom	80 mg/kg Kratom
pm	-	-	-	80 mg/kg	100 mg/kg Kratom	70 mg/kg Kratom
Day 7 am	-	-	-	80 mg/kg	100 mg/kg Kratom	60 mg/kg Kratom
pm	-	-	-	80 mg/kg	100 mg/kg Kratom	50 mg/kg Kratom
Day 8 am	-	-	-	40 mg/kg Kratom	40 mg/kg Kratom	40 mg/kg Kratom

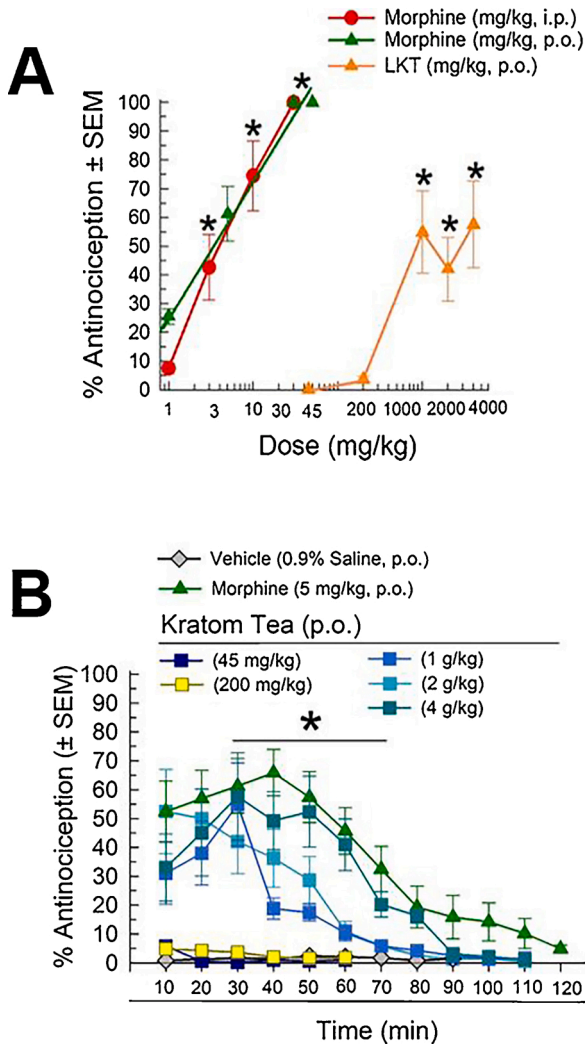

Fig. 1. Characterization of **(A)** dose– and **(B)** time-dependent antinociception of oral Lyophilized Kratom Tea (LKT) in the mouse 55 °C warm-water tail-withdrawal test. Latency to tail withdrawal was evaluated 30 min after administration of morphine (i.p. or p.o.) or LKT (p.o.) for evaluation of dose-response **(A)**, or every 10 minutes post administration for evaluation of time course **(B)**. Points represent the mean ± SEM of n = 8 mice. *$p < 0.05$ versus the pretreatment tail withdrawal response, Two-Way RM ANOVA, with Tukey's multiple comparisons post-hoc test.

peak response of antinociception from high doses of LKT (1 - 4 g/kg p.o.) from 30 to 50 min post-administration was equivalent to that produced by morphine (5 mg/kg, p.o.; Two-Way RM ANOVA w/Tukey's test, Fig. 1B), and the duration of significant LKT-mediated antinociception when given at 4 mg/kg, p.o. lasted as long as morphine (70 min; Fig. 1B).

Potential opioid receptor mediation of antinociception observed after oral dosing of LKT (1 g/kg, p.o.) was tested using mu- (MOR), kappa- (KOR), or delta-opioid receptor (DOR) knockout mice (KO) (Fig. 2). Compared to the response of wild-type mice, the antinociceptive activity was significantly decreased (factor: treatment; $F_{(4, 86)} = 16.7$, $p < 0.0001$; Two-way ANOVA) in the MOR KO animals ($p < 0.0001$, Tukey's test) and to a lesser extent KOR KO mice ($p = 0.006$, Tukey's test), but remained unaffected in DOR KO mice ($p = 0.15$, Tukey's test). Antinociception of LKT was abolished in MOR KO mice pretreated with the KOR-selective antagonist, nor-BNI (Fig. 2, rightmost bar), with a response significantly different from that of the WT mice ($p < 0.0001$, Tukey's test), but not from the baseline (untreated) response (1.29 ± 0.04 s vs 1.45 ± 0.07 s; $p > 0.99$; Tukey's test). Together, these

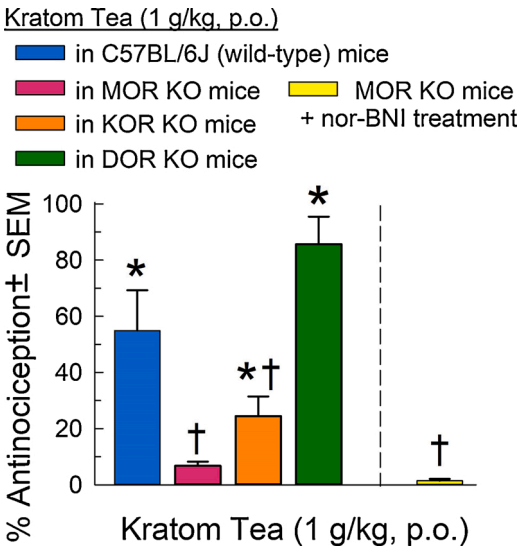

Fig. 2. Opioid receptor selectivity of Lyophilized Kratom Tea (LKT) antinociception. LKT was administered orally (1 mg/kg, p.o.) to wild-type or individual opioid receptor knock out (KO) mice, or MOR KO mice pretreated 24 h with nor-BNI (10 mg/kg, i.p.). Antinociception was evaluated using the 55 °C warm-water tail-withdrawal latency 30 min post administration of LKT. Each bar represents the mean ± SEM of n = 8 mice, with n = 16 KOR KO mice. *$p < 0.05$ versus the group's baseline tail withdrawal latency; † $p < 0.05$ versus antinociceptive response of the wild-type group, Two-Way ANOVA with Tukey's multiple comparisons post-hoc test.

results indicate that this dose of LKT-induced antinociception in mice occurs primarily through activation of the mu-opioid receptor, but with additional kappa-opioid receptor agonism as well.

3.2. Evaluation of potential liabilities of Lyophilized Kratom Tea

The effects of LKT on spontaneous locomotor activity and respiration rate were assessed in C57BL/6J mice following administration of LKT (1 g/kg, p.o.) using the Comprehensive Laboratory Animal Monitoring System (CLAMS; Fig. 3). Morphine (30 mg/kg, p.o.) or vehicle (saline, p.o.) were tested as positive and negative comparison controls, respectively. Morphine produced significant time-dependent increases in ambulation (factor: time x treatment, $F_{(16, 248)} = 9.26$, $p < 0.0001$, Two-way RM ANOVA; Fig. 3A) from 20-160 min ($p < 0.05$, Tukey's post-hoc test) and decreases in respiration rate (factor: time x treatment, $F_{(16, 248)} = 7.10$, $p < 0.0001$, Two-way RM ANOVA; Fig. 3B) up to 80 min ($p \leq 0.007$, Tukey post-hoc test). In contrast, LKT significantly increased ambulation from 60-160 min ($p = 0.002$, Tukey's test), but suppressed respiration only for the first 20 min ($p \leq 0.03$, Tukey's test). Both behavioral effects of LKT were significantly less than those of morphine at matching time points ($p \leq 0.03$, Tukey's test). Assessing opioid contributions, LKT (1 g/kg, p.o.) was further examined in MOR KO mice alone (green triangles, Fig. 3) or MOR KO mice pretreated 24 h with the KOR antagonist nor-BNI (10 mg/kg, i.p.; purple squares, Fig. 3). Compared to wild-type mice, LKT produced significant global reductions in ambulation (factor: time x treatment, $F_{(16, 248)} = 6.81$, $p < 0.0001$, Two-way RM ANOVA; Fig. 3A), suggesting opioid mediation of the limited LKT locomotor effects. Likewise, significant global differences were observed in respiration among these three groups (factor: time x treatment, $F_{(16, 248)} = 3.07$, $p < 0.0001$, Two-way RM ANOVA; Fig. 3B). Post-hoc analysis demonstrated no significant difference across time in respiration rate between LKT-treated wild-type and MOR KO mice ($p \geq 0.22$, Tukey's test), although MOR KO mice pretreated with nor-BNI displayed decreased respiration from wild-type at some intervals (†≤0.03; Tukey's test, Fig. 3B). However, the respiration rate of nor-BNI pretreated MOR KO mice administered LKT did not significantly differ

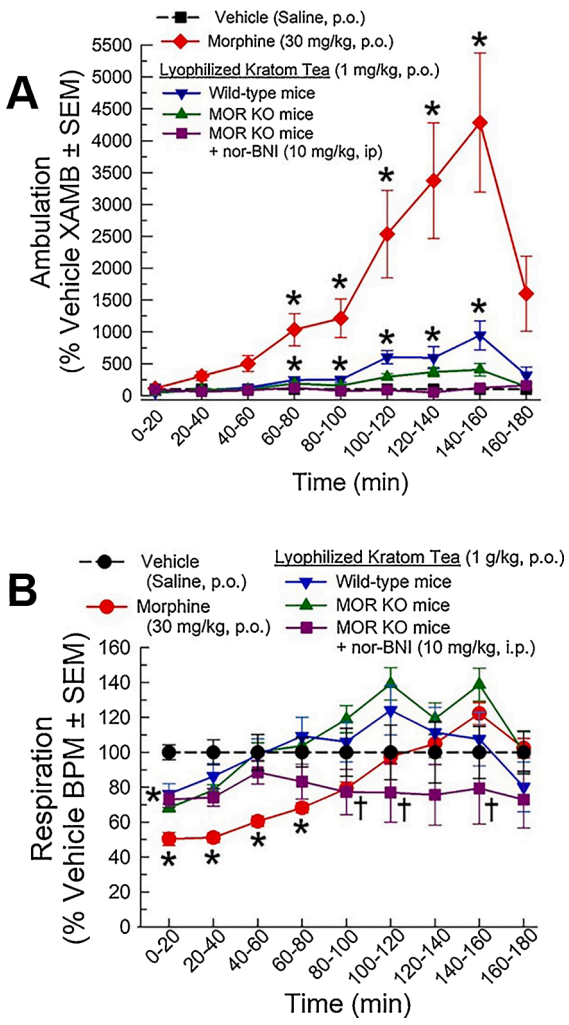

Fig. 3. Time-dependent effects of oral Lyophilized Kratom (LKT, 1 mg/kg) on **(A)** spontaneous locomotion (as ambulations) and **(B)** respiration rate were assessed in the Comprehensive Laboratory Animal Monitoring System (CLAMS) with wild type C57BL/6J mice and MOR KO mice. Morphine (30 mg/kg, p.o.) and saline (p.o.) are included as controls. * $p < 0.05$ versus saline control; † $p < 0.05$ versus the LKT wild-type group, Two-Way RM ANOVA with Tukey's multiple comparisons post-hoc test). n = 12-16/group.

from that of saline-treated control mice at any time point (factor: time x treatment, $F_{(8, 176)} = 0.20$, $p = 0.99$, Two-way RM ANOVA).

The effect of LKT on evoked locomotor activity was further examined in the mouse rotarod assay. The KOR agonist U50,488 (10 mg/kg, i.p.) was used as a positive control, producing a significant decrease in latency to fall (factor: treatment, $F_{(4,37)} = 4.01$, $p = 0.009$ and factor: time, $F_{(4.84,179)} = 11.8$, $p < 0.0001$; each Two-way RM ANOVA w/Tukey's test; Fig. 4). Morphine (10 mg/kg, i.p.) produced a trend towards increased latency to fall, although this did not significantly differ from the response of vehicle-treated control mice at any time point. Likewise, LKT (45 mg/kg or 1 g/kg, p.o.) did not significantly alter locomotor responses compared to saline controls ($p = 0.63$ and $p = 0.35$, respectively).

Morphine (10 mg/kg, i.p.) produced significant conditioned place preference (CPP) ($F_{(1,126)} = 7.73$, $p = 0.006$; Two-way ANOVA w/ Tukey's post-hoc test; Fig. 5). In contrast, LKT (0.1 or 1 g/kg, p.o.) demonstrated no significant difference from pre-conditioning responses after place conditioning ($p = 0.60$ and 0.73, respectively; Tukey's test; Fig. 5). Collectively, these results indicate that while LKT possesses MOR agonist activity, it lacks some liabilities associated with clinically-used MOR agonists such as morphine.

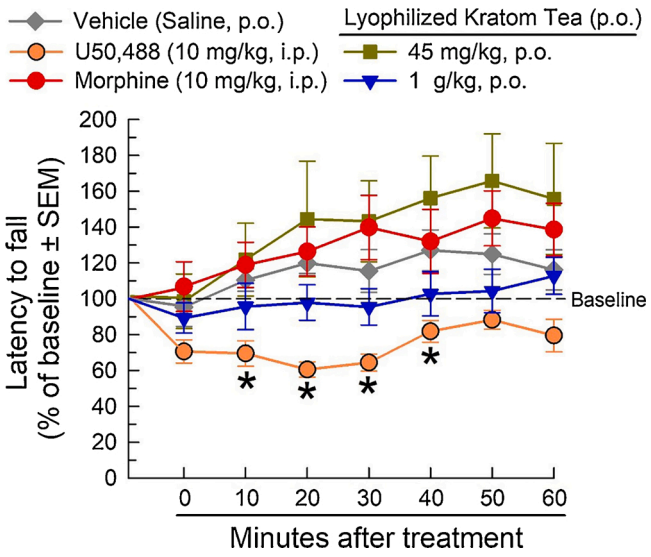

Fig. 4. Time- and dose-dependent effects of Lyophilized Kratom Tea (LKT) after a 45 mg/kg or 1 g/kg, p.o. administration in the mouse rotorod assay. Vehicle (saline, p.o.), morphine (10 mg/kg, i.p.) and U50,488 (10 mg/kg, i.p.) were tested as controls. * $p < 0.05$ versus vehicle control, Two-Way RM ANOVA with Tukey's multiple comparisons post-hoc test. n = 8-10 mice/treatment.

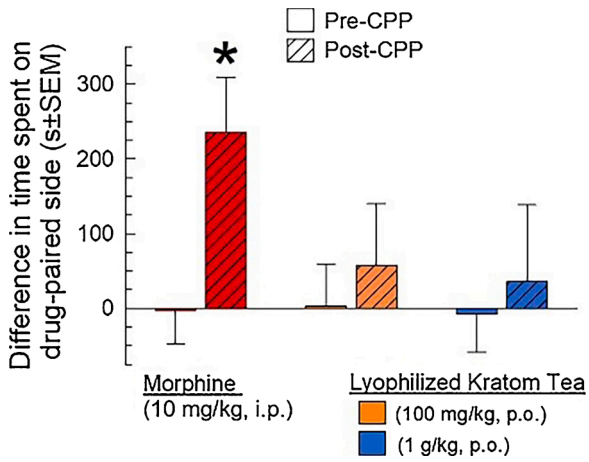

Fig. 5. Characterization of oral Lyophilized Kratom Tea (0.1 and 1 g/kg, p.o.) in the conditioned place preference assay. LKT did not display place preference or aversion following place conditioning (Post-CPP), whereas morphine place conditioning resulted in significant place preference as compared to initial (Pre-CPP) baseline responses. Each bar represents the mean ± SEM. * $p < 0.05$ versus Pre-CPP response; Two-Way RM ANOVA with Tukey's multiple comparisons post-hoc test. n = 17 (morphine) or 21 mice (LKT testing).

3.3. Evaluation of LKT physical dependence and amelioration of opioid withdrawal

Repeated treatment with escalating doses of morphine induced physical dependence, demonstrated by administration of naloxone (Table 3). Morphine produced significant increases in the frequency of jumping ($F_{(5,62)} = 20.2$, $p < 0.0001$; Fig. 6A) and teeth chattering ($F_{(5,62)} = 9.48$, $p < 0.0001$; both One-way ANOVA w/Tukey's test; Fig. 6B) compared to the saline control group. A significant reduction in rearing and forepaw licking frequency ($F_{(5,62)} = 9.53$, $p < 0.0001$ and $F_{(5,62)} = 6.18$, $p < 0.0001$, respectively) was demonstrated, confirming that this morphine treatment produced physical dependence and naloxone-precipitated withdrawal symptoms. In contrast, daily escalating dosing of LKT (30-125 mg/kg, po) followed by naloxone produced few

Table 3
Behavioral endpoints of naloxone-precipitated withdrawal in morphine-dependent mice following administration of either escalating or tapering doses of LKT over four to seven days.

			Treatment				
Withdrawal Behavior	Saline	Morphine	Escalating Doses LKT (30-125 mg/kg)	7 d Morphine + LKT-Acute	4d Morphine + LKT-high (100 mg/kg)	4d Morphine + LKT-Taper (100-40 mg/kg)	Outcome (1-Way ANOVA)
Forepaw Tremor	22.6 ± 6.45	24.9 ± 10.6	12.3 ± 3.67	30.4 ± 6.05	35.1 ± 11.05	19.3 ± 7.74	$F_{(5,62)} = 0.78$, $p = 0.57$
Wet Dog Shakes	0.867 ± 0.39	2.9 ± 1.71	0.4 ± 0.27	2.4 ± 1.06	1.3 ± 0.41	1.3 ± 0.41	$F_{(5,62)} = 0.90$, $p = 0.487$
Body Straightening	5.7 ± 2.33	3.7 ± 1.22	3.2 ± 0.81	4.3 ± 1.37	1.5 ± 0.63	4.6 ± 1.43	$F_{(5,62)} = 1.85$, $p = 0.116$
Presence of Diarrhea	1.1 ± 0.41	3.8 ± 1.02	0.7 ± 0.47	4.3 ± 0.84*	3.6 ± 0.76	3.2 ± 0.81	$F_{(5,62)} = 3.95$, $p = 0.0036$
Jumping Frequency	0 ± 0	89 ± 13.27*	2.9 ± 1.63	15.6 ± 5.46†	0.6 ± 0.47†	0 ± 0	$F_{(5,62)} = 20.24$, $p < 0.0001$
Rearing Frequency	41.6 ± 6.85	7 ± 1.94*	14.7 ± 3.41*	9.3 ± 2.48*	40.7 ± 7.28†	28.5 ± 6.59†	$F_{(5,62)} = 9.53$, $p < 0.0001$
Forepaw Licking Frequency	20.1 ± 4.26	1.4 ± 0.90*	7.2 ± 2.17	5.8 ± 1.88	9.3 ± 3.46	7.1 ± 1.53	$F_{(5,62)} = 6.18$, $p = 0.0001$
Teeth Chattering Frequency	0.8 ± 0.61	90.7 ± 50.5*	1.3 ± 0.80	61.6 ± 17.4*	15 ± 7.24†	9.6 ± 2.93†	$F_{(5,62)} = 9.48$, $p < 0.0001$

Average and Standard Error of the Mean for 5-8-day treatment with saline, morphine, or Lyophilized Kratom Tea (LKT). *$p < 0.05$ versus Saline control, †$p < 0.05$ versus Morphine control (Tukey's post-hoc test) n = 8-10/group.

significant withdrawal signs (reduced rearing behavior ($p = 0.005$)), confirming reduced LKT physical dependence.

The ability of LKT to attenuate naloxone-precipitated withdrawal in subjects physically-dependent on morphine was evaluated over three LKT treatment paradigms (as detailed in Table 2). While a single acute dose of LKT (40 mg/kg, po) significantly reduced jumping frequency ($p < 0.0001$; Table 3), acute dosing was unable to attenuate increased diarrhea ($F_{(5,62)} = 3.95$, $p = 0.011$), teeth chattering ($p = 0.015$), and decreased rearing ($p = 0.0004$) compared to the saline control group. Prolonged treatment over 4 days with LKT (100 mg/kg, p.o., twice daily) significantly ameliorated all subsequent symptoms of naloxone-precipitated opioid withdrawal (jumping frequency ($p < 0.0001$; Fig. 6A); teeth chattering ($p = 0.002$; Fig. 6B); rearing frequency ($p = 0.0003$); Table 3). Finally, tapering the doses of LKT given over 4 days (from 100 to 40 mg/kg, p.o., twice daily) still significantly reduced naloxone-precipitated measures of opioid withdrawal, just as effectively as the repeated high doses (jumping frequency ($p < 0.0001$; Fig. 6A) and teeth chattering ($p = 0.0006$; Fig. 6B; Table 3). Notably, higher doses of LKT and methadone were evaluated for innate physical dependence and efficacy against morphine dependent withdrawal symptoms with comparable effects (see supplemental data).

4. Discussion

Mitragyna speciosa (Korth.) Havil., called kratom, is anecdotally consumed both to treat pain and prevent opioid withdrawal (Hassan et al., 2020; Saref et al., 2019). Indications of rapidly increasing use prompted a warning from the U.S. FDA about the absence of scientific evidence supporting the application of kratom, and the potential for abuse liabilities (Anwar et al., 2016; O'Malley, 2018). While the upsurge of reports of kratom use has increased interest in the plant's active ingredients, there are few preclinical studies that evaluate the effects of consumption of whole, characterized plant material on pain and opioid withdrawal symptoms in a controlled manner.

The present study demonstrates characterized LKT (whole plant material) produced dose-dependent antinociception with reduced liabilities while ameliorating opioid withdrawal in physically-dependent subjects. Although clinical trials examining the potentially analgesic properties of kratom in a cold pressor task (Clinical Trials.gov, NCT03414099, 2018) and the pharmacokinetics of a well-characterized kratom product on opioid-metabolizing CYP2D6 and CYP3A4 activity (Clinical Trials.gov, NCT04392011, 2020) are underway, results are not yet available, and there exists a knowledge gap for controlled human studies with kratom. However, the current findings are generally consistent with a number of case studies and surveys of self-reported uses for kratom. For example, these results are consistent with Boyer et al. (2008), a published case study of individuals self-treating opioid addiction and withdrawal with tea brewed from the leaves of kratom. That uncontrolled study revealed that individuals self-medicating with kratom tea consumed approximately 1-4 grams per day. Doses of kratom tea studied presently were guided by the findings of Boyer et al. (2008) and reported pharmacokinetic studies (Avery et al., 2018). Consistent with present content (Sharma et al., 2019), kratom is reported to represent a botanical mixture of at least 40 alkaloids (Adkins et al., 2011). Mitragynine and 7-hydroxymitragynine are reported to represent approximately 60% and 2% of the alkaloids extracted from kratom (Warner et al., 2016).

Consistent with another user survey, LKT produced antinociception with fewer liabilities than morphine (Garcia-Romeu et al., 2020). Existing literature reports attribute most effects of kratom tea and its main alkaloids to the activation of the mu-opioid receptor (Takayama et al., 2002; Hassan et al., 2013). Presently, LKT demonstrated dose- and time-dependent antinociception characteristic of partial agonism, but the reduction of LKT antinociception in KOR KO mice and abolition of antinociception in MOR KO mice treated with the KOR antagonist nor-BNI further suggest a modest KOR agonism. This is perhaps not surprising, given reports that kratom alkaloids display affinity for the kappa opioid receptor (Obeng et al., 2020). Notably, these results contrast with findings indicating that kratom's main alkaloid, mitragynine, displayed DOR-mediated antinociception (Prozialeck et al., 2012). Recent mitragynine-mediated thermal antinociception in rats was not blocked by naltrexone, and only occurred at higher doses that disrupted

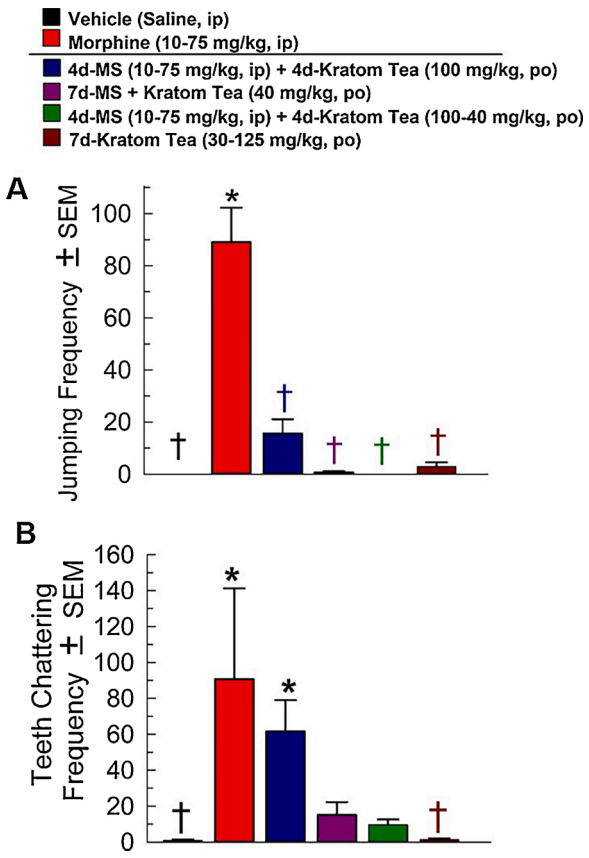

Fig. 6. Assessment of LKT's ability to produce direct treatment-induced physical dependence and reduce naloxone precipitated opioid withdrawal symptoms (i.e. jumping (A) and teeth chattering (B)) in morphine dependent mice. Vehicle (saline, i.p.), morphine (10-75 mg/kg, i.p.), MS (10-75 mg/kg, ip) + LKT (100 mg/kg, p.o.), morphine (10-80 mg/kg, i.p.) + an acute dose of LKT (40 mg/kg, p.o.), morphine (10-75 mg/kg, i.p.) + tapering doses of LKT (100-40 mg/kg, p.o.), and LKT (30-125 mg/kg, p.o.) were evaluated for opioid withdrawal symptoms after administration of naloxone (10 mg/kg, s.c.). * $p < 0.05$ versus vehicle control, One-way ANOVA with Tukey's multiple comparisons post-hoc test. n = 9-10 mice/treatment. †Mean and SEM lower than 1.

learned behavior, suggesting non-opioid mediation (Hiranita et al., 2019). The reduction of LKT-mediated antinociception by MOR KO mice confirms MOR mediation consistent with earlier reports with rodents finding mitragynine-induced antinociception to be antagonized by the opioid receptor antagonists naloxone or naltrindole (Shamima et al., 2012). Admittedly, doses that are considered low (1-5 g) in human consumption studies (Grewal et al., 1932) are also reported to interact with alpha-adrenergic and serotonin receptors that mediate analgesia (Matsumoto et al., 1996). Regardless, this study confirms that even low to medium doses of kratom tea possess analgesic effects. It should be noted that some of the antinociceptive effects of kratom may be attributed to mitragynine's metabolism to 7-hydroxymitragynine by CYP3A4 in the intestine and the liver (Kamble et al., 2019; Kruguel et al., 2019). Studies have indicated that analgesic effects of 7-hydroxymitragynine are 10-fold more potent than those of morphine (Hemby et al., 2018). While beyond the scope of the current study, future studies of the antinociceptive effects of the other individual constitutive components of kratom would be of mechanistic value.

LKT (1 g/kg, p.o.) produced less hyperlocomotion and respiratory depression than might be expected of a MOR agonist such as morphine. The cause for this difference is not clear. Historically, case studies with kratom tea users report that low doses of the tea produced stimulant-like effects (Grewal, 1932), whereas doses above 5 grams of dried leaves reportedly produced effects similar to opioids (Prozialeck et al., 2012) and sedation (Boyer et al., 2008; Grewal, 1932). Increased locomotion is a characteristic sign of MOR activation in rodents (Zhang and Kong, 2017), as is respiratory depression mediated by activation of MOR on respiratory centers in the medulla (Martin, 1983). Of interest, LKT showed increased respiration in MOR KO mice. The nor-BNI data in this assay implicates KOR mediation of the increased respiration, as suggested by earlier reports (Dosaka-Akita et al., 1993). Even modest KOR agonist activity has been shown to offset the respiratory depression induced by MOR agonism in multifunctional opioid agonists (Brice-Tutt et al., 2020). Alternatively, the changes in respiration may be due to physical inactivity or non-opioid activity. Mice treated with LKT show no significant signs of inactivity in CLAMS-measured ambulations or in the rotarod assays, potentially discounting the involvement of physical inactivity. However, the individual alkaloid mitragynine has been reported to possess relevant non-opioid activity, activating dopamine-2 (D2) and serotonin receptors (Lydecker et al., 2016), both of which modulate respiration and which warrant future examination.

Previous studies established that MOR agonists such as morphine are rewarding (Ballantyne and LaForge, 2007; Kreek and Koob, 1998). Rewarding effects are attributed to the agonist-induced suppression of inhibitory interneurons, thereby indirectly increasing the activity of dopaminergic A10 neurons in the brain reward pathway (Matthes et al., 1996). With MOR-mediated antinociception, it might be expected that LKT would be rewarding. Instead, LKT produced no rewarding effects in a CPP assay. In previous studies, mitragynine itself was sufficient to decrease self-administration of MOR agonists (Hemby et al., 2018). The possibility of an underlying multifunctional interaction is further supported by report of mitragynine dose-dependent decreases in operant responding for food in rats that was not blocked by naltrexone, but was attributed to an interaction with alpha-2 adrenergic receptors (Hiranita et al., 2019). Supporting this, recent reports confirm kratom alkaloids possess micromolar affinity for the adrenergic receptors (Obeng et al., 2020).

Although clinical surveys suggest discontinuation of kratom by regular users may result in opioid withdrawal symptoms (Warner et al., 2016), repeated administration of LKT presently demonstrated less physical dependence than morphine. Compared to methadone, a significant reduction of withdrawal symptoms was observed with repeated oral dosing of LKT (1 g/kg, see supplemental data). LKT (2 g/kg, p.o.) showed increased withdrawal liability with a reduction of locomotor activity, increased wet dog shakes, and a reduced weight gain, but these were less than demonstrated with morphine-treated mice. Admittedly, studies of prolonged LKT exposure and at higher doses may demonstrate greater adverse effects (see supplement), but these results support the anecdotal claim that kratom produces less physical dependence than MOR agonists such as morphine.

Historically, treatments ameliorating opioid withdrawal syndrome reduce opioid cravings and withdrawal signs but suffer from limitations. Both methadone and buprenorphine possess partial mu agonism thought to prevent full agonist effects such as respiratory depression (McCambridge et al., 2007). However, treatment with buprenorphine is poorly tolerated by patients due to the precipitation of a withdrawal syndrome if used too soon after ingestion of opioid agonists (Hassan et al., 2020). Likewise, methadone treatment is sometimes not well tolerated and associated with increased physical dependence over time (Dart et al., 2005; Strang and Gossop, 1990). Alpha-2 adrenergic receptor agonists such as clonidine and lofexidine alleviate some autonomic symptoms of opioid withdrawal, attributed to the suppression of withdrawal-induced locus coeruleus hyperactivity (Gold et al., 1978; Hicks and Muvvala, 2018), However, these drugs are less effective in preventing some symptoms of opioid withdrawal, including insomnia, lethargy and muscle aches (Jasinski et al., 1985), are ineffective in minimizing opioid craving (Charney et al., 1986), and produce potentially dangerous anticholinergic effects such as hypotension (Jasinski et al., 1985). Given the drawbacks of current treatments, the need for alternative treatments persists.

A major finding of the current study was that orally administered LKT at doses up to 2 g/kg were able to significantly reduce naloxone-precipitated opioid withdrawal in morphine-dependent subjects. After repeated dosing with morphine, substitution with lower oral doses of LKT attenuated symptoms of physical naloxone-precipitated withdrawal, although in the higher doses there was a lack of rebounding in weight and increased tremors. This discrepancy could be due to the persistence of reduced appetite and increased tremor that were shown in the direct measure of physical dependence in subjects treated repeatedly with LKT, an effect also suggested in the literature (Singh et al., 2014). Acute dosing with LKT also significantly reduced some withdrawal symptoms, indicating that the acute use of low doses during the initial abstinence from chronic opioid abuse may have some therapeutic relevance. Janchawee et al. (2007) reported that oral doses (40 mg/kg) of kratom tea's main constituent, mitragynine, are quickly absorbed yet have a half-life of over 9 hours. If acting as a MOR agonist, it is possible the long half-life may simply provide prolonged competition from either antagonist-precipitated or spontaneous physical withdrawal. Alternatively, it is possible that kratom tea may be acting through non-opioid means such as alpha-2-adrenergic receptor activity to ameliorate opioid withdrawal. Supporting this, selected kratom alkaloids were recently reported to possess micromolar affinity for the adrenergic receptors (Obeng et al., 2020). The negative effects of alpha-2-adrenergic receptor agonists were not evaluated after LKT administration, although characteristic sedation was not observed. Overall, while more safety and mechanistic studies are needed, the present findings validate the clinical reports where consumption of kratom tea may offset opioid physical dependence.

4.1. Conclusion

Kratom tea induces antinociception predominately via mu opioid receptor agonism in mice. The optimal dose of kratom tea used in these studies did not induce respiratory depression or conditioned place preference. Moreover, acute doses of kratom tea as low as a single dose of 40 mg/kg (p.o.) effectively reduced withdrawal symptoms without sedation. While multifunctional pharmacological contributions of the individual major and minor components of *Mitragyna speciosa* (Korth.) Havil. to the overall effect of LKT remain to be investigated, this study suggests that kratom tea and/or the alkaloid components it contains could be prime candidates for the treatment of pain and opioid physical dependence.

Authorship Contributions

Participated in research design: Boyer, McCurdy, McLaughlin, Wilson.

Conducted experiments: Brice-Tutt, Cirino, Eans, Harris, Simons, Stacy, Wilson.

Contributed new reagents or analytic tools: Avery, León, Sharma

Performed data analysis: McLaughlin, Wilson

Wrote and contributed to the writing of manuscript: McCurdy, McLaughlin, Wilson.

All authors critically reviewed content and approved final version for publication.

Role of funding source

Nothing declared.

Data availability statement

All data herein is available upon request to the authors.

Declaration of Competing Interest

The authors declare no conflicts.

Acknowledgements

This research was supported by funding from a Centers of Biomedical Research Excellence (COBRE) P20GM104932 from the National Institutes of Health to CRM, a McKnight Fellowship (to LLW) and the University of Florida to JPM.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.drugalcdep.2020.108310.

References

Adkins, J.E., Boyer, E.W., McCurdy, C.R., 2011. Mitragyna speciosa, a psychoactive tree from Southeast Asia with opioid activity. Current Topics in Medicinal Chemistry 11 (9), 1165–1175. https://doi.org/10.2174/156802611795371305.

Anwar, M., Law, R., Schier, J., 2016. Notes from the field: kratom (Mitragyna speciosa) exposures reported to poison centers—United States, 2010–2015. MMWR Morb Mortal Wkly Rep 65 (29), 748–749. https://doi.org/10.15585/mmwr.mm6529a4.

Avery, B.A., Boddu, S.P., Sharma, A., Furr, E.B., Leon, F., Cutler, S.J., McCurdy, C.R., 2018. Comparative pharmacokinetics of mitragynine after oral administration of Mitragyna speciosa (Kratom) leaf extracts in rats. Planta Medica 85 (04), 340–346. https://doi.org/10.1055/a-0770-3683.

Ballantyne, J.C., LaForge, S.K., 2007. Opioid dependence and addiction during opioid treatment of chronic pain. Pain 129 (3), 235–255. https://doi.org/10.1016/j.pain.2007.03.028.

Boyer, E.W., Babu, K.M., Adkins, J.E., McCurdy, C.R., Halpern, J.H., 2008. Self-treatment of opioid withdrawal using kratom (Mitragynia speciosa korth). Addiction 103 (6), 1048–1050. https://doi.org/10.1111/j.1360-0443.2008.02209.x.

Brice-Tutt, A.C., Wilson, L.L., Eans, S.O., Stacy, H.M., Simons, C.A., Simpson, G.G., Coleman, J.S., Ferracane, M.J., Aldrich, J.V., McLaughlin, J.P., 2020. Multifunctional opioid receptor agonism and antagonism by a novel macrocyclic tetrapeptide prevents reinstatement of morphine-seeking behavior. British Journal of Pharmacology 177 (18), 4209–4222. https://doi.org/10.1111/bph.15165.

CDC/NCHS, 2018. National Vital Statistics System, Mortality. CDC WONDER. US Department of Health and Human Services, CDC, Atlanta, GA.

Charney, D.S., Heninger, G.R., Kleber, H.D., 1986. The combined use of clonidine and naltrexone as a rapid, safe, and effective treatment of abrupt withdrawal from methadone. The American Journal of Psychiatry 143 (7), 831–837. https://doi.org/10.1176/ajp.143.7.831.

Cirino, T.J., Eans, S.O., Medina, J.M., Wilson, L.L., Mottinelli, M., Intagliata, S., McLaughlin, J.P., 2019. Characterization of Sigma 1 Receptor Antagonist CM-304 and Its Analog, AZ-66: Novel Therapeutics Against Allodynia and Induced Pain. Frontiers in Pharmacology 10, 678. https://doi.org/10.3389/fphar.2019.00678.

ClinicalTrials.gov [internet], 2018. Bethesda (MD): National Library of Medicine (US). 2000 Feb 29 – Identifier NCT03414099, "Ketum and Pain Tolerance". January 29, [cited 2020 September 9]; approximately 4 screens. https://clinicaltrials.gov/ct2/show/NCT03414099?term=Kratom%2C+Mitragynine&draw=2&rank=2.

ClinicalTrials.gov [internet], 2020. Bethesda (MD): National Library of Medicine (US). 2000 Feb 29 – Identifier NCT04392011, "Assessing the Pharmacokinetics and Drug Interaction Liability of Kratom, an Opioid-like Natural Product". May 18, [cited 2020 September 9]; approximately 4 screens.https://clinicaltrials.gov/ct2/show/NCT04392011?term=NCT04392011&draw=2&rank=1.

Dart, R.C., Woody, G.E., Kleber, H.D., 2005. Prescribing methadone as an analgesic. Annals of Internal Medicine 143 (8), 620. https://doi.org/10.7326/0003-4819-143-8-200510180-00028.

Dosaka-Akita, K., Tortella, F.C., Holaday, J.W., Long, J.B., 1993. The kappa opioid agonist U-50, 488H antagonizes respiratory effects of mu opioid receptor agonists in conscious rats. J Pharmacol Exp. Ther 264 (2), 631–637. https://doi.org/10.21236/ada263043.

Eans, S.O., Ganno, M.L., Mizrachi, E., Houghten, R.A., Dooley, C.T., McLaughlin, J.P., Nefzi, A., 2015. Parallel synthesis of hexahydrodiimidazodiazepines heterocyclic peptidomimetics and their in vitro and in vivo activities at μ (MOR), δ (DOR), and κ (KOR) opioid receptors. Journal of Medicinal Chemistry 58 (12), 4905–4917. https://doi.org/10.1021/jm501637c.

Fernandes, M., Kluwe, S., Coper, H., 1977. Quantitative assessment of tolerance to and dependence on morphine in mice. Naunyn-Schmiedeberg's Archives of Pharmacology 297 (1), 53–60. https://doi.org/10.1007/bf00508810.

Food and Drug Administration, 2005. Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. Center for Drug Evaluation and Research (CDER), p. 7.

Garcia-Romeu, A., Cox, D.J., Smith, K.E., Dunn, K.E., Griffiths, R.R., 2020. Kratom (Mitragyna speciosa): User demographics, use patterns, and implications for the opioid epidemic. Drug and Alcohol Dependence 208, 107849. https://doi.org/10.1016/j.drugalcdep.2020.107849.

Gold, M., Redmond Jr, D.E., Kleber, H., 1978. Clonidine blocks acute opiate-withdrawal symptoms. The Lancet 312 (8090), 599–602. https://doi.org/10.1016/s0140-6736(78)92823-4.

Grewal, K.S., 1932. The effect of mitragynine on man. British Journal of Medical Psychology 12 (1), 41–58. https://doi.org/10.1111/j.2044-8341.1932.tb01062.x.

Hassan, Z., Muzaimi, M., Navaratnam, V., Yusoff, N.H., Suhaimi, F.W., Vadivelu, R., Jayabalan, N., 2013. From Kratom to mitragynine and its derivatives: physiological and behavioural effects related to use, abuse, and addiction. Neuroscience & Biobehavioral Reviews 37 (2), 138–151. https://doi.org/10.1016/j.neubiorev.2012.11.012.

Hassan, R., Pike See, C., Sreenivasan, S., Mansor, S.M., Müller, C.P., Hassan, Z., 2020. Mitragynine Attenuates Morphine Withdrawal Effects in Rats—A Comparison With Methadone and Buprenorphine. Frontiers in Psychiatry 11, 411. https://doi.org/10.3389/fpsyt.2020.00411.

Hemby, S.E., McIntosh, S., Leon, F., Cutler, S.J., McCurdy, C.R., 2018. Abuse liability and therapeutic potential of the Mitragyna speciosa (kratom) alkaloids mitragynine and 7-hydroxymitragynine. Addiction Biology 24 (5), 874–885. https://doi.org/10.1111/adb.12639.

Hicks, H., Muvvala, S., 2018. Methadone Maintenance versus Detoxification and Psychosocial Treatment for Opioid Dependence. 50 Studies Every Psychiatrist Should Know. Oxford University Press, pp. 309–313. https://doi.org/10.1093/med/9780190625085.001.0001.

Hiranita, T., Leon, F., Felix, J.S., Restrepo, L.F., Reeves, M.E., Pennington, A.E., Wilkerson, J.L., 2019. The effects of mitragynine and morphine on schedule-controlled responding and antinociception in rats. Psychopharmacology 236 (9), 2725–2734. https://doi.org/10.1007/s00213-019-05247-7.

Janchawee, B., Keawpradub, N., Chittrakarn, S., Prasettho, S., Wararatananurak, P., Sawangjareon, K., 2007. A high-performance liquid chromatographic method for determination of mitragynine in serum and its application to a pharmacokinetic study in rats. Biomedical Chromatography 21 (2), 176–183. https://doi.org/10.1002/bmc.731.

Jasinski, D.R., Johnson, R.E., Kocher, T.R., 1985. Clonidine in morphine withdrawal: differential effects on signs and symptoms. Archives of General Psychiatry 42 (11), 1063–1066. https://doi.org/10.1001/archpsyc.1985.01790340041006.

Kamble, S.H., Sharma, A., King, T.I., León, F., McCurdy, C.R., Avery, B.A., 2019. Metabolite profiling and identification of enzymes responsible for the metabolism of mitragynine, the major alkaloid of Mitragyna speciosa (kratom). Xenobiotica 49 (11), 1279–1288. https://doi.org/10.1080/00498254.2018.1552819.

Kamei, J., Ohsawa, M., 1997. Role of noradrenergic functions in the modification of naloxone-precipitated withdrawal jumping in morphine-dependent mice by diabetes. Life Sciences 60 (15), PL223–PL228. https://doi.org/10.1016/s0024-3205(97)00074-x.

Kilkenny, C., Browne, W., Cuthill, I., Emerson, M., Altman, D., 2014. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. Animals 4 (1), 35–44. https://doi.org/10.3390/ani4010035.

Kreek, M.J., Koob, G.F., 1998. Drug dependence: stress and dysregulation of brain reward pathways. Drug and Alcohol Dependence-Shannon 51 (1), 23–48. https://doi.org/10.1016/s0376-8716(98)00064-7.

Kreek, M.J., LaForge, K.S., Butelman, E., 2002. Pharmacotherapy of addictions. Nature Reviews Drug Discovery 1 (9), 710–726. https://doi.org/10.1038/nrd968.

Kruegel, A.C., Uprety, R., Grinnell, S.G., Langreck, C., Pekarskaya, E.A., Le Rouzic, V., Javitch, J.A., 2019. 7-Hydroxymitragynine is an active metabolite of mitragynine and a key mediator of its analgesic effects. ACS Central Science 5 (6), 992–1001. https://doi.org/10.1021/acscentsci.9b00141.

Li, J.X., Zhang, Y., 2012. Emerging drug targets for pain treatment. European Journal of Pharmacology 681 (1-3), 1–5. https://doi.org/10.1016/j.ejphar.2012.01.017.

Lydecker, A.G., Sharma, A., McCurdy, C.R., Avery, B.A., Babu, K.M., Boyer, E.W., 2016. Suspected adulteration of commercial kratom products with 7-hydroxymitragynine. Journal of Medical Toxicology 12 (4), 341–349. https://doi.org/10.1007/s13181-016-0588-y.

Martin, W.R., 1983. Pharmacology of opioids. Pharmacological Reviews 35 (4), 283–323. PMID: 6144112.

Matsumoto, K., Mizowaki, M., Suchitra, T., Murakami, Y., Takayama, H., Sakai, S., Watanabe, H., 1996. Central antinociceptive effects of mitragynine in mice: Contribution of descending noradrenergic and serotonergic systems. European Journal of Pharmacology 317 (1), 75–81. https://doi.org/10.1016/s0014-2999(96)00714-5.

Matthes, H.W., Maldonado, R., Simonin, F., Valverde, O., Slowe, S., Kitchen, I., Tzavara, E., 1996. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the μ-opioid-receptor gene. Nature 383 (6603), 819–823. https://doi.org/10.1038/383819a0.

McCambridge, J., Gossop, M., Beswick, T., Best, D., Bearn, J., Rees, S., Strang, J., 2007. In-patient detoxification procedures, treatment retention, and post-treatment opiate use: comparison of lofexidine+ naloxone, lofexidine+ placebo, and methadone. Drug and Alcohol Dependence 88 (1), 91–95. https://doi.org/10.1016/j.drugalcdep.2006.09.020.

Obeng, S., Kamble, S.H., Reeves, M.E., Restrepo, L.F., Patel, A., Behnke, M., Chear, N.J.-Y., Ramanathan, S., Sharma, A., León, F., Hiranita, T., Avery, B.A., McMahon, L.R., McCurdy, C.R., 2020. Investigation of the adrenergic and opioid binding affinities, metabolic stability, plasma protein binding properties, and functional effects of selected indole-based Kratom alkaloids. J Med Chem. 63 (1), 433–439. https://doi.org/10.1021/acs.jmedchem.9b01465.

O'Malley, P.A., 2018. Think kratom is a safe opioid substitute? Think again!: History, evidence, and possible future for Mitragyna speciosa. Clinical Nurse Specialist 32 (5), 227–230. https://doi.org/10.1097/NUR.0000000000000392.

Prozialeck, W.C., Jivan, J.K., Andurkar, S.V., 2012. Pharmacology of kratom: an emerging botanical agent with stimulant, analgesic and opioid-like effects. The Journal of the American Osteopathic Association 112 (12), 792–799. PMID: 23212430.

Reilley, K.J., Giulianotti, M., Dooley, C.T., Nefzi, A., McLaughlin, J.P., Houghten, R.A., 2010. Identification of two novel, potent, low-liability antinociceptive compounds from the direct in vivo screening of a large mixture-based combinatorial library. The AAPS Journal 12 (3), 318–329. https://doi.org/10.1208/s12248-010-9191-3.

Saref, A., Suraya, S., Singh, D., Grundmann, O., Narayanan, S., Swogger, M.T., Balasingam, V., 2019. Self-reported prevalence and severity of opioid and kratom (Mitragyna speciosa korth.) side effects. Journal of Ethnopharmacology 238, 111876. https://doi.org/10.1016/j.jep.2019.111876.

Shamima, A.R., Fakurazi, S., Hidayat, M.T., Hairuszah, I., Moklas, M.A.M., Arulselvan, P., 2012. Antinociceptive action of isolated mitragynine from Mitragyna speciosa through activation of opioid receptor system. International Journal of Molecular Sciences 13 (9), 11427–11442. https://doi.org/10.3390/ijms130911427.

Sharma, A., Kamble, S.H., León, F., Chear, N.J.Y., King, T.I., Berthold, E.C., Avery, B.A., 2019. Simultaneous quantification of ten key Kratom alkaloids in Mitragyna speciosa leaf extracts and commercial products by ultra-performance liquid chromatography− tandem mass spectrometry. Drug Testing and Analysis 11 (8), 1162–1171. https://doi.org/10.1002/dta.2604.

Shaw-Lutchman, T.Z., Barrot, M., Wallace, T., Gilden, L., Zachariou, V., Impey, S., Nestler, E.J., 2002. Regional and cellular mapping of cAMP response element-mediated transcription during naltrexone-precipitated morphine withdrawal. Journal of Neuroscience 22 (9), 3663–3672. https://doi.org/10.1523/JNEUROSCI.22-09-03663.2002.

Singh, D., Chear, N.J.Y., Narayanan, S., Leon, F., Sharma, A., McCurdy, C.R., Balasingam, V., 2020. Patterns and reasons for kratom (Mitragyna speciosa) use among current and former opioid poly-drug users. Journal of Ethnopharmacology 249, 112462. https://doi.org/10.1016/j.jep.2019.112462.

Singh, D., Müller, C.P., Vicknasingam, B.K., 2014. Kratom (Mitragyna speciosa) dependence, withdrawal symptoms and craving in regular users. Drug and Alcohol Dependence 139, 132–137. https://doi.org/10.1016/j.drugalcdep.2014.03.017.

Strang, J., Gossop, M., 1990. Comparison of linear versus inverse exponential methadone reduction curves in the detoxification of opiate addicts. Addictive Behaviors 15 (6), 541–547. https://doi.org/10.1016/0306-4603(90)90054-2.

Swogger, M.T., Hart, E., Erowid, F., Erowid, E., Trabold, N., Yee, K., Walsh, Z., 2015. Experiences of kratom users: a qualitative analysis. Journal of Psychoactive Drugs 47 (5), 360–367. https://doi.org/10.1080/02791072.2015.1096434.

Takayama, H., Ishikawa, H., Kurihara, M., Kitajima, M., Aimi, N., Ponglux, D., Horie, S., 2002. Studies on the Synthesis and Opioid Agonistic Activities of Mitragynine-Related Indole Alkaloids: Discovery of Opioid Agonists Structurally Different from Other Opioid Ligands. Journal of Medicinal Chemistry 45 (9), 1949–1956. https://doi.org/10.1021/jm010576e.

Váradi, A., Marrone, G.F., Palmer, T.C., Narayan, A., Szabó, M.R., Le Rouzic, V., Hunkele, A., 2016. Mitragynine/corynantheidine pseudoindoxyls as opioid analgesics with mu agonism and delta antagonism, which do not recruit β-arrestin-2. Journal of Medicinal Chemistry 59 (18), 8381–8397. https://doi.org/10.1021/acs.jmedchem.6b00748.

Warner, M.L., Kaufman, N.C., Grundmann, O., 2016. The pharmacology and toxicology of kratom: from traditional herb to drug of abuse. International Journal of Legal Medicine 130 (1), 127–138. https://doi.org/10.1007/s00414-015-1279-y.

Zhang, J.J., Kong, Q., 2017. Locomotor activity: a distinctive index in morphine self-administration in rats. PLoS one 12 (4), e0174272. https://doi.org/10.1371/journal.pone.0174272.