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Mitragynine reduced morphine-induced conditioned place preference and withdrawal in rodents

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ABSTRACT

Introduction: Kratom (*Mitragyna speciosa* Korth) has been long used in folklore medicine in Southeast Asian countries for its analgesic effects and the treatment of opioid withdrawal. Mitragynine is a major alkaloid found in the leaves of kratom plant that is responsible for most of the kratom pharmacological effects. It exerts its effects through the activation of opioid receptors and other receptors in the central nervous system. This study aimed to provide an evaluation of abuse liability and potential of mitragynine in the treatment for opioid addictions. **Materials and Methods:** Abuse liability of mitragynine was evaluated using drug discrimination and conditioned place preference models in rats, and naloxone precipitated withdrawal model in mice. The effects of mitragynine on acquisition and expression of morphine conditioned place preference were performed in rats. Naloxone-precipitated withdrawal was performed in mice treated with morphine. **Results and Conclusions:** Mitragynine fully substituted methamphetamine in a drug discrimination model at 10 mg/kg. It also induced conditioned place preference at doses of 30 and 90 mg/kg. Acute withdrawal symptoms precipitated by naloxone by means of repeated jumping were significant in 60 mg/kg mitragynine treated group. Straub tail reaction was observable in dose-dependent from 10 mg/kg mitragynine. In chronic withdrawal, repeated jumping behavior of mitragynine was significant at 30 mg/kg. Straub tail reaction was found in both 10 and 30 mg/kg mitragynine treated group. Mitragynine attenuated acquisition and expression of morphine conditioned place preference in dose-dependent manner from 10 to 30 mg/kg. When given with morphine, 10 mg/kg mitragynine could reduce jumping behavior to the same level as a chronic treatment of 10 mg/kg mitragynine alone and 30 mg/kg mitragynine could reduce straub tail reaction. This study indicates that mitragynine had low abuse liability and could attenuate the acquisition and expression of morphine-induced conditioned place preference and precipitated withdrawal symptoms. These results support the use of kratom in traditional medicine and self-medication for opioid addiction and withdrawal.

Keywords: Addiction, conditioned place preference, kratom, mice, *Mitragyna speciosa*, mitragynine, morphine, rat, withdrawal

INTRODUCTION

Addiction is a brain disorder involving compulsive use of the drug despite negative or harmful consequences to the quality of life. United Nations Office for Drug Control and Crime[1] reported that about 5% of adult used the drug at least once in a lifetime in 2015. Opioids are one of the most harmful types of drugs. It is accountable to 70% of the global burden of disease attributable to drug use disorders.[1] Replacement therapy by opioid agonists, buprenorphine, and methadone, is recommended for pharmacotherapy of opioid use disorder based on their mechanisms of action and the need of the patient to alleviate from withdrawal symptoms.[2] Thus, better alternative pharmacotherapy for opioid agonist-replacement therapy as well as other substance used disorders is still in need.

Kratom plant (*Mitragyna speciosa* Korth.) has been long used traditionally for its pharmacological effects and its narcotic effects in Southeast Asian nations, especially Thailand and Malaysia. The leaves of kratom plant are made into preparations. Conventionally, people chew fresh or dry kratom leaves, brew the leaves with hot water and drink as tea, or smoke the resins made from kratom extract. Powdered and crushed leaves are sold over the internet. People use kratom plant to treat pain, mood swing, coughing, diarrhea, and

intestinal infection, etc.[3,4] Laborers and farmers use kratom to increase endurance, reduce fatigue, and suppress appetite, so they are able to work longer hours in unfavorable conditions.[5] Kratom is also used as a substitution of heroin and morphine when access to drugs is prevented and as treatment for drugs withdrawal.[6-8] The effects of kratom are dose-dependent. It produces stimulant effects at a low dose and sedative-narcotic effects at higher dose.[9,10] Recently, there is the emergence of cocktail preparations incorporated with kratom such as "4×100" and "Krypton"[11,12] among drug users. They self-reported euphoric feelings on consumption.[13]

More than 40 alkaloids from kratom have been isolated and characterized. Mitragynine is a major alkaloid constituent in kratom leaves. It is responsible for 66% of total alkaloid content.[14,15] It is able to bind to all three types of opioid receptors with the highest affinity to κ opioid receptor (KOR).[16] Mitragynine acts as partial agonist on human μ opioid receptor (MOR) and δ opioid receptor (DOR) while it acts as a competitive antagonist on human KOR.[17] In addition, *in vivo* test showed that antinociceptive effects of mitragynine are mediated by supraspinal MOR and DOR.[10] Antinociceptive effect of mitragynine can be blocked by administration of MOR and DOR antagonists, naloxone and natrindole, respectively.[18] Mitragynine can also bind to many receptors such as adenosine A_{2a} receptor, dopamine D_2 receptor, serotonin receptors 5-HT_{2C} and 5-HT_7, and α_2 adrenergic receptors.[19,20] Abuse liability of mitragynine has been tested by several studies. Mitragynine served as a discriminative stimulus and was able to fully substitute morphine in drug discrimination model in higher dose.[21] In conditioned place preference model, mitragynine could induce conditioned place preference at 10 mg/kg. Acquisition of mitragynine-induced place preference could be prevented by administration of naloxone pretreatment. However, naloxone was not able to block expression of mitragynine-induced place preference.[22] More recently, it is found that baclofen was able to block both the acquisition and expression of mitragynine-induced conditioned place preference in rats.[23] Together, the involvement of opioidergic and GABA-ergic pathway is suggested in mitragynine rewarding effects.

Since it is still necessary to find better alternative drugs for opioid agonist-replacement therapy and kratom leaves are used traditionally for reducing the withdrawal symptoms and the treatment of opioid use disorder, the present study was aimed to evaluate abuse liability of mitragynine, the major alkaloid in kratom leaves, and its effects on morphine-induced condition place preference and morphine withdrawal.

MATERIALS AND METHODS

Animals

Male ICR mice weighing 18–25 g and male Wistar rats weighing 180–200 and 250–300 g were used in the experiments. The animals were obtained from National Laboratory Animal Center, Mahidol University, Salaya, Nakhonpathom, Thailand. They were allowed to acclimate for 7 days in the Animal Facility, Faculty of Pharmaceutical Sciences, Chulalongkorn University before experiments. Housing room environment was controlled with 24 ± 2°C, 40–60% relative humidity and standard 12 h light/dark cycle. Food and water were available *ad libitum* unless stated otherwise. All behavioral experiments were conducted between 08:00 and 18:00. The research proposal was reviewed and approved by Institution Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Chulalongkorn University (Protocol No. 1333009). All experimental procedures were performed according to the Ethical Principles and Guidelines for the Procedures on Animals for Scientific Purposes, National Research Council of Thailand.

Drugs and Treatments

Mitragynine was extracted and isolated from the leaves of *M. speciosa* Korth using the method described by Ponglux *et al*.[24] Morphine sulfate was purchased from Temed Co. (Tehran, Iran). Other drugs and chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Morphine sulfate and naloxone hydrochloride were dissolved in 0.9% NaCl solution (saline solution). Mitragynine was dissolved in 20% TWEEN 80 in saline solution. All the drug preparations were freshly prepared and injected intraperitoneally.

Substitution of Mitragynine or Morphine in Rat Discriminating Methamphetamine

This experiment followed the protocol described by Solinas *et al*.[25] Male Wistar rats were trained to press the lever to get food pellets in the Standard Operant Conditioning Chambers (ENV-001; Med Associates, St Albans, VT, USA) connected to Med Associates Interface Model SG-503 with MED-IV software.

After they were able to press the lever to consume the food pellets, they were trained to discriminate saline (1 ml/kg) from methamphetamine (1 mg/kg). Rats were injected with either saline or methamphetamine 10 min before the start of the training session. The right lever was made an active lever for methamphetamine whereas the left lever was made an active lever for saline for half of that rats. Left lever was an active lever for methamphetamine and the right lever was active for saline for another half of the rats. This was counterbalanced to neutralized lever bias in rats. The schedule of reinforcement begins at a fixed ratio (FR) with timeout period of 10 s (TO10). House light was on at the beginning of the session. Every completion of response required on the schedule resulted in delivery of a 45 mg food pellet following by timeout period in which all the lights in the chamber were off and any lever depression, both on correct or incorrect lever, was recorded but yields no consequence. Depression of incorrect lever during the trial was recorded. It also reset the number of pressing required on correct lever. House light was turned on again after the completion of timeout period for the next trial. Methamphetamine and saline were given in double alternate schedule. For example, a 2-week schedule was MMSSM MSSMM where M represents methamphetamine and S represents saline. The schedule of reinforcement and timeout was gradually increased until the rats could reliably response to FR10 and TO45. Each session ended when 20 trials were completed or 30 min time elapses, whichever comes first. Training continues until rats could complete (1) 90% or more response occurs on the correct lever and (2) not >4 responses occurred on the incorrect lever on the first trial for 8 consecutive sessions before the testing phase begins.

In the testing phase, different doses of methamphetamine, morphine, and mitragynine were given to trained rats. Test session was similar to a training session, except that both levers were made active and 10 consecutive pressing was required on the same lever for food pellet delivery. Test sessions were conducted in between methamphetamine training and saline solution training session. Rats were given single drug each session. A 2-week schedule was MTSMT STMST (t = test). Test sessions were conducted only when the rats were still able to maintain (1) 90% accuracy on the correct lever and (2) not more than 4 responses on the incorrect lever in the first trail in the two preceding training sessions. Data were expressed as a percentage of response on the correct lever. Test drugs were saline solution, methamphetamine (0.1, 0.3, 0.6, and 1.0 mg/kg), morphine (1.0 and 2.0 mg/kg), and mitragynine (1, 5, and 10 mg/kg).

Mitragynine-induced Conditioned Place Preference

Apparatus

A rectangular chamber consisted of three compartments isolated by solid removable vertical screens. One central chamber connecting to two lateral compartments had gray walls. One lateral compartment had black and white vertical stripes walls with the black textured floor. The other lateral compartment had black and white horizontal stripes walls with black smooth floor. Close circuit camera system connecting to personal computer was positioned above the chamber for behavior observation. Subjects' behaviors were then analysed by VideoMot2 software (TSE Systems, Bad Homburg, Germany).

Protocol

Conditioned place preference method was modified from Dias *et al*.[26] and Mizoguchi *et al*.[27] The procedure contains three phases (1) pre-conditioning phase (day 1–3): Rats were placed in the middle compartment for 5 min and, then, allowed to explore the entire apparatus for 15 min. Time spent in each compartment was recorded on day 3 to be used as a baseline and to determine preferred compartment. The preferred compartment was then paired with saline solution administration (saline-paired compartment), and the other lateral compartment was paired with drug administration (drug-paired compartment). (2) Conditioning phase (day 4–11): Each group of rats received different doses of saline solution, morphine (5 mg/kg) or mitragynine (5, 10, 30, and 90 mg/kg) on day 4, 6, 8, and 10 before being confined in drug-paired compartment of conditioned place preference apparatus for 30 min. On day 5, 7, 9, and 11, rats received saline solution before being confined in saline-paired compartment for 30 min. (3) Post-conditioning phase (day 12); each rat, in drug-free state, was confined in the central compartment of the apparatus for 5 min before the doors connecting to both lateral compartments were removed allowing the rat to freely explore the apparatus for 15 min. Time spent in each compartment was collected. Data were expressed by changes in time spent in drug-paired compartment after conditioning ($time_{postcondition}$-$time_{precondition}$) and used for statistical analysis.

Mitragynine Withdrawal

Acute mitragynine withdrawal

Repeated jumping behavior was a predominant sign of opioid withdrawal syndrome in mice.[28,29] Jumping was defined by the simultaneous removal of all four paws from the horizontal surface. Mice were given a single dose of morphine or mitragynine (0, 5, 10, 30, and 60 mg/kg) 2 h before administration of naloxone (3 mg/kg). Mice were then placed in glass cylinders for assessment of jumping behavior frequency and straub tail reaction for 30 min immediately after naloxone injection.

Chronic mitragynine withdrawal

Mice were treated with mitragynine (10 and 30 mg/kg) or morphine for 7 consecutive days. Treatments were given to mice 2 times daily on day 1–day 6, and only one treatment was given on day 7. The doses of morphine each day were as follows: 10, 20, 30, 40, 50, 60, and 70 mg/kg, respectively. 2 h after treatment on day 7, mice were injected with naloxone (3 mg/kg) to precipitate withdrawal symptoms and immediately placed into the glass cylinders for behavioral observation for 30 min.

Effect of Mitragynine on Morphine-induced Conditioned Place Preference

Effects of mitragynine on acquisition of morphine-induced conditioned place preference

On the conditioning phase, rats received two treatments daily. Pre-treatment was injected 30 min before treatment injection. Rat received saline solution for both pre-treatment and treatment on day 4, 6, 8, and 10. They received a saline solution or mitragynine (5 and 10 mg/kg) as pre-treatment followed by saline solution or morphine (5 mg/kg) as a treatment on day 5, 7, 9, and 11. Rats were confined within respective compartment immediately after treatment injection for 30 min.

Effect of mitragynine on the expression of morphine-induced conditioned place preference

To observe the effect of mitragynine on the expression of morphine-induced conditioned place preference, rats were conditioned with saline solution on day 4, 6, 8, and 10 where they were conditioned with saline solution or morphine (5 mg/kg) on day 5, 7, 9, and 11. Before testing began, they were injected with either saline solution or mitragynine (5 and 10 mg/kg).

Effect of Mitragynine on Chronic Morphine Withdrawal

Treatments were given to mice 2 times daily on day 1–day 6, and only one treatment was given on day 7. The doses of morphine each day were as follows: 10, 20, 30, 40, 50, 60, and 70 mg/kg, respectively. Mitragynine (10 and 30 mg/kg) was given to mice 30 min before each morphine administration. 2 h after the last dose of morphine, naloxone (3 mg/kg) was injected into each mouse. Repeated jumping behavior and straub tail reaction were observed for 30 min.

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM) and analyze by one-way analysis of variance. Difference between control and test groups was revealed by Bonferroni's multiple comparison tests as *post hoc* analysis. Statistical significance was set at $P < 0.05$.

RESULTS

Substitution of Mitragynine or Morphine in Rat Discriminating Methamphetamine

In rats trained to discriminate methamphetamine (1 mg/kg) from saline solution, the response on methamphetamine appropriate lever increased in dose-dependent manner from 0.1 to 1.0 mg/kg with mean maximal response for 0.6 and 1.0 mg/kg at 82.54 ± 10.43% and 91.95 ± 4.971%, respectively ($n = 6$). ED_{50} was 0.34 mg/kg (0.1691–0.6754; 95% confidence interval). Mitragynine could substitute methamphetamine in dose-dependent manner. It could fully substitute methamphetamine at the dose of 10 mg/kg with mean maximal response at 80.88 ± 10.37% ($n = 6$). Morphine could also fully substitute methamphetamine at the dose of 2 mg/kg with mean maximal response at 86.09 ± 6.592% ($n = 4$) [Figure 1].

Mitragynine-induced Conditioned Place Preference

Rats showed preference to lateral compartment with vertical stripes wall and perforated floor over horizontal stripes wall and smooth floor in the preconditioning phase. Thus, the horizontal stripes wall compartment was assigned as drug-paired compartment. After 4 sessions conditioning with saline solution and 4 sessions conditioning with drugs, changes in time spent in drug-paired compartment in 5 mg/kg morphine-treated groups were 207.4 ± 29.02 s ($n = 7$) compared with saline-treated group, 8.8 ± 18.80 s ($n = 10$) [Figure 2]. The time changes increased in dose-dependent manner in mitragynine treated groups where they were statistically significant from the saline-treated group but not statistically significant from the morphine-treated group at the doses of 30 and 90 mg/kg, 143.4 ± 28.29 s ($n = 8$) and 143.0 ± 33.69 s ($n = 12$), respectively.

Mitragynine Withdrawal

Acute withdrawal

Naloxone injection-induced repeated jumping in both mice treated with morphine and mitragynine [Figure 3a]. The numbers of jumping in the 30 min period were statistically significant in mice given single dose of 100 mg/kg morphine and 60 mg/kg mitragynine, 49.17 ± 11.08 times ($n = 12$) and 43.25 ± 8.37 times ($n = 8$), respectively, compared with saline control, 0.88 ± 0.64 times ($n = 8$). Straub tail reaction observed in morphine and mitragynine treated mice except in 5 mg/kg mitragynine-treated group. Percent of mice exhibiting straub tail reaction was 83.33% in morphine group while in mitragynine treated group was 0, 8.33, 75.00, and 75.00% in 5, 10, 30, and 60 mg/kg mitragynine treated groups, respectively. No straub tail reaction could be observed in saline-treated group [Figure 3b].

Chronic withdrawal

After chronic treatment of either morphine (10–70 mg/kg) or mitragynine (10 and 30 mg/kg) for 7 days, naloxone injection could precipitate repeated jumping behavior in mice. The numbers of jumping in 30 min period in morphine and 30 mg/kg mitragynine treated groups were statistically higher than of saline-treated group, 38.25 ± 6.86 times ($n = 8$) and 42.50 ± 14.68 times ($n = 6$), respectively [Figure 3c]. The number of jumping in 30 min periods in 10 mg/kg mitragynine-treated group was 28.60 ± 11.08 times ($n = 5$). All mice (100%) in morphine and 30 mg/kg mitragynine-treated groups showed straub tail reaction while 80% of the mice in 10 mg/kg mitragynine-treated group exhibited such reaction [Figure 3d].

Effects of Mitragynine on Acquisition of Morphine-induced Conditioned Place Preference

Injection of mitragynine before morphine could suppress acquisition of morphine-induced conditioned place preference.

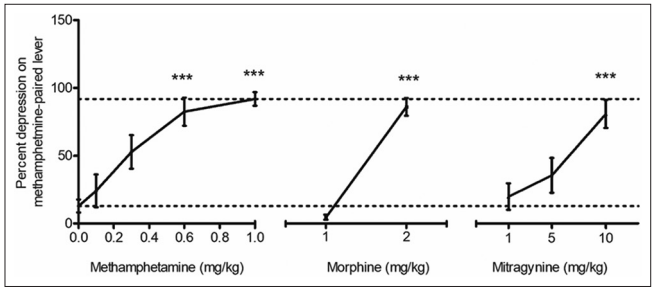

Figure 1: Effect of methamphetamine, morphine (1–2 mg/kg), and mitragynine (1, 5, and 10 mg/kg) in rats trained to discriminate 1 mg/kg methamphetamine from saline. Under FR 10 schedules, percent response on methamphetamine paired lever for each treatment is shown. The above-dotted line represents the average percent response at 1 mg/kg methamphetamine and below-dotted line represents the average percent response of saline treatment (***$P < 0.001$ vs. saline-treated group; $n = 4$–6)

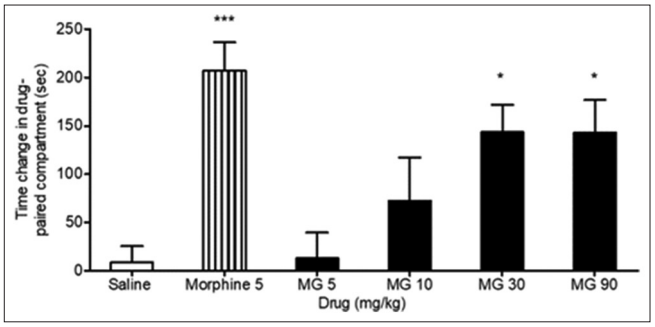

Figure 2: Mitragynine-induced conditioned place preference. The effects of mitragynine (MG; 5–90 mg/kg) and morphine (5 mg/kg) on conditioned place preference in rats. Data are expressed as mean ± standard error of the mean of time changes in drug-paired compartment between the postconditioning and preconditioning phases (*$P < 0.05$ and ***$P < 0.001$ vs. saline-treated group; $n = 7$–12)

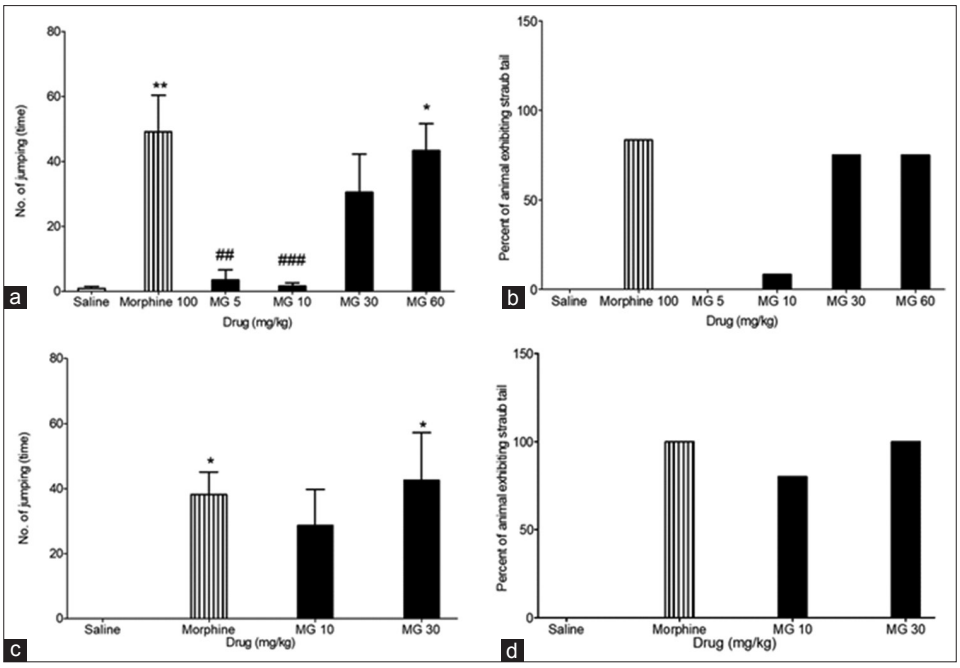

Figure 3: Mitragynine withdrawal precipitated with naloxone. Upper panel (a and b) shows acute mitragynine (5, 10, 30, and 60 mg/kg) withdrawal (a) number of repeated jumping behavior and (b) straub tail reaction. Lower panel (c and d) shows chronic mitragynine (10 and 30 mg/kg) withdrawal (c) number of repeated jumping behavior and (d) straub tail reaction. (a and c) Bars represent mean frequency of jumping in 30 min period after naloxone injection ± SEM. (b and d) Bars represent the percent of animal exhibited straub tail behavior (*$P <$ 0.05 and **$P < 0.01$ vs. saline-treated group; $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ vs. morphine-treated group; n = 8–12)

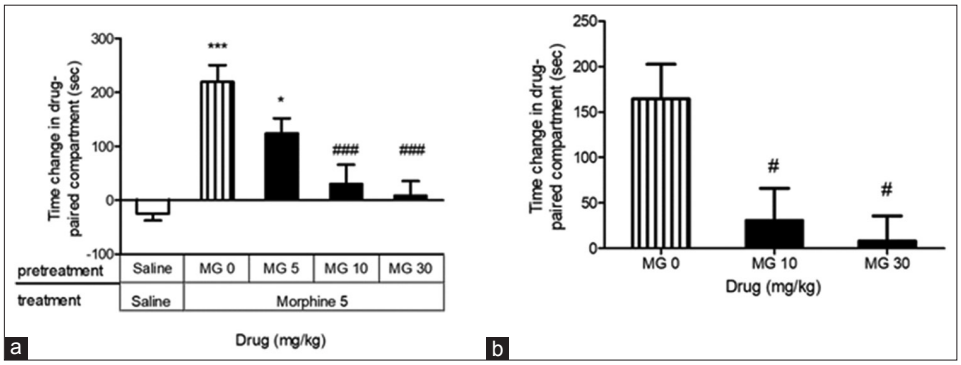

Figure 4: Effect of mitragynine on morphine-induced conditioned place preference (a) the effects of mitragynine (MG; 0, 5, 10, and 30 mg/kg) on morphine-induced conditioned place preference acquisition and (b) the effects of mitragynine 10 and 30 mg/kg on expression of morphine-induced conditioned place preference. Data are expressed as mean time changes in morphine-paired compartment between postconditioning and preconditioning phases ± standard error of the mean (*$P < 0.05$ and ***$P < 0.001$ vs. saline-treated group; $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ vs. MG 0 group; n = 6–9).

The level of suppression was dependent on the dose given. Changes in time spent in morphine paired compartment in saline/5 mg/kg morphine and 5 mg/kg mitragynine/5 mg/kg morphine-treated groups were significantly higher than of saline/saline control group, 219.6 ± 31.11 s (n = 7), 124.0 ± 28.21 s (n = 9), and −24.83 ± 13.22 s (n = 6), respectively [Figure 4a].

Effect of Mitragynine on Expression of Morphine-induced Conditioned Place Preference

Mitragynine was able to attenuate expression of morphine-induced conditioned place preference. This attenuation was statistically significant at doses of 10 and 30 mg/kg mitragynine compared with the saline group, 30.38 ± 35.72 s (n = 8), 8.38 ± 27.30 s (n = 8), and 164.30 ± 38.10 s (n = 8), respectively [Figure 4b].

Effect of Mitragynine on Chronic Morphine Withdrawal

Figure 5a showed that only mice chronically pretreated with saline solution and 30 mg/kg mitragynine followed by morphine treatment each day for 7 days showed significantly higher numbers of jumping in 30 min time period. The number of jumping in 10 mg/kg mitragynine pretreated group was lower than of saline pre-treated group, 19.83 ± 2.09 times (n = 6) and 38.25 ± 6.86 times (n = 8), respectively, to the same level as chronic treatment of 10 mg/kg mitragynine

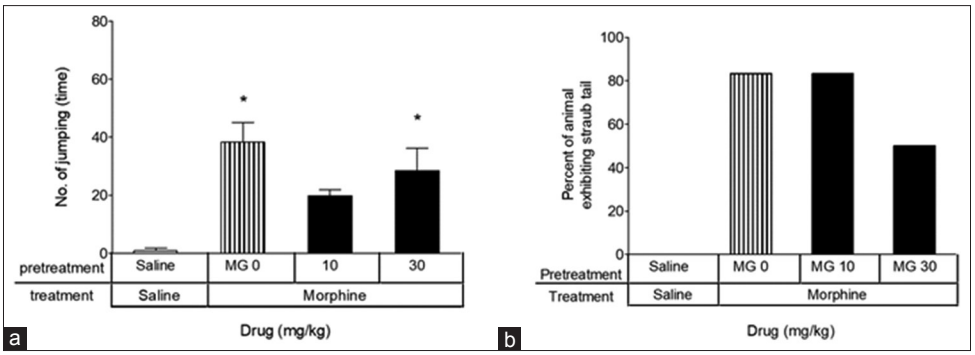

Figure 5: Effect of mitragynine (10 and 30 mg/kg) pre-treatment on chronic morphine withdrawal precipitated with naloxone (a) mean frequency of repeated jumping behavior in 30 min period after naloxone injection ± standard error of the mean and (b) percent of animal exhibited straub tail behavior (*$P < 0.05$ vs. saline-treated group; $n = 6–8$)

alone (28.60 ± 11.08 times, $n = 5$). Straub tail reaction was found in all groups treated with morphine. The percent of mice showing straub tail reaction was 83.33% and 83.33% in mice pretreated with saline solution and 10 mg/kg mitragynine [Figure 5b]. At the dose of 30 mg/kg, mitragynine reduced the straub tail reaction down to 50.00%.

DISCUSSION

Abuse liability of mitragynine in male rodents had been access by drug discrimination, conditioned place preference and physical withdrawal in the present study. Several preclinical studies have showed that female was more prone to drug addiction than its male counterpart due to differences in neurobiology between two gender and the effects of ovarian hormones.[30] In addition, the fluctuation in sex hormones along the estrous/menstrual cycle affects drug-taking behavior in clinical study.[31,32] A study by Karami and Zarrindast[33] indicated that female Wistar rats adopted conditioned place preference at a lower dose compared to male. Taken together, to avoid the effect from gender and estrous cycle on addiction and for uniformity of subjects, male rodents were used exclusively throughout the study.

In drug discrimination model, 10 mg/kg mitragynine was able to substitute methamphetamine in rats trained to discriminate 1 mg/kg methamphetamine from saline solution despite different mechanisms of action [Figure 1]. This was confirmed by the administration of morphine at 1 and 2 mg/kg. It showed that morphine could also fully substitute methamphetamine. A study by Harun *et al*.[21] demonstrated that cocaine could only partially substitute mitragynine in rats trained to discriminate 15 mg/kg mitragynine from the vehicle. This showed that mitragynine and stimulants share at least some similar interoceptive stimulus properties. In the same study, morphine fully substituted mitragynine at 5 mg/kg where mitragynine was able to fully substitute 5 mg/kg morphine at a dose of 15 mg/kg. Dose >15 mg/kg of mitragynine could partially substitute morphine.[21]

In conditioned place preference model, the present study demonstrated that only relatively high doses of mitragynine (30 and 90 mg/kg) could produce a conditioned place preference [Figure 2]. Although 10 mg/kg mitragynine also showed the tendency to induce conditioned place preference, no significant difference from control was found. Other studies reported mitragynine conditioned place preference at doses as low as 10 and 15 mg/kg.[23,34] We speculated that there was some influence from the intraspecies difference (Wistar rats vs. Sprague-Dawley rats). One study indicated that the dose of morphine used for induction of conditioned place reference in Wistar rats was significantly higher than the one used to induced Sprague-Dawley rats.[35]

In withdrawal models, naloxone successfully precipitated repeated jumping behavior in morphine-treated mice both acutely and chronically [Figure 3]. The numbers of jumping in 30 min for mitragynine treated groups were significant only at relatively high doses (30 and 60 mg/kg in acute treatments and 30 mg/kg in chronic treatments) [Figure 3]. Plus, the numbers of jumping reaction was dose-dependent in mitragynine-treated mice in both acute and chronic treatments. Morphine dependence was independent of beta-arrestin pathway as beta-arrestin knockout mice still developed morphine physical withdrawal and showing jumping reaction following naloxone precipitation comparable to those observed in wild-type mice.[36] Intracellular signaling regarding opioid receptors activation by mitragynine biased toward G proteins over beta-arrestin.[17] Taken from these studies, it was not surprising that application of naloxone would precipitate jumping behavior in mice received mitragynine in this study. It is also worth noting that the numbers of jumping behavior were significant only at the high dose in mitragynine treated mice (60 mg/kg in acute withdrawal and 30 mg/kg in chronic withdrawal).

Other behavior observed in this experiment was straub tail reaction. Straub tail reaction was defined by rigidity and erection of tail across the back of the animal in an S-shaped curve due to contraction of acrococcygeus muscle.[37] The mechanisms of this reaction were mediated by activation of μ opioid subtype two receptors (μ_2 receptors) and serotonin subtype two receptor (5-HT_2).[38,39] Straub tail reactions were observed in mice given morphine and mitragynine in both acute and chronic treatment after injection of naloxone. Considering with receptor binding assay studies,[16,20] it is suggested that mitragynine physical dependence might be due to activation of μ_2 receptors and/or 5-HT_2 receptor.

This study is the first to demonstrate that mitragynine attenuated both the acquisition and expression of morphine-induced conditioned place preference [Figure 4]. Mitragynine was a partial agonist on MOR and competitive antagonist at

KOR.[17] The affinity of mitragynine at DOR was low.[17,40] In addition, Guanosine 5′-(gamma-thio)triphosphate (^{35}S-GTPγS) binding assay showed that mitragynine had less specific effect to MOR compared with (D-Ala^2, N-Me-Phe^4, Gly-ol^5)-enkephalin (DAMGO) or morphine.[40] In functional activity at human opioid receptors in G protein bioluminescent resonance energy transfer assay showed that maximum efficacy of mitragynine at human MOR was 34% compared with DAMGO. Mitragynine could fully inhibit the effect of U-50,488, a KOR agonist, with pA_2 of 1.4.[17] The fact that mitragynine served as an antagonist at KOR may have additional effects and become a candidate for use as a treatment for opioid addiction.[41,42] Antidepressant effect of kratom had been reported in traditional use and some studies.[9,43] In mice model of depression, mitragynine reduced immobility time in forced swimming test and tail suspension test. Moreover, it was shown that mitragynine reduced corticosterone level in mice exposed to forced swimming- and tail-suspension tests.[44] These results were in line with a morphine withdrawal attenuating effect found in zebrafish model.[45] Mitragynine showed to lowered stress-related swimming behaviors, whole-body cortisol level and mRNA expression of corticotropin-releasing factor receptors and prodynorphin in zebrafish during the morphine withdrawal phase, suggesting that mitragynine interferes with corticotropin pathway in the reduction of opioid withdrawal symptoms. Anti-addictive property of KOR antagonists had been tested. KOR antagonists blocked stress-induced potentiation of drug reward, stress-induced reinstatement of drug-seeking behavior, and escalation of drug consumption in long access models.[46]

Physical dependence observed by jumping and straub tail reactions showed that, when given with morphine, 10 mg/kg mitragynine could reduce jumping behavior to the same level as chronic treatment of 10 mg/kg mitragynine alone [Figures 3c and 5a] and 30 mg/kg mitragynine could reduce Straub tail reaction [Figures 3d and 5b]. Reduction of withdrawal symptoms might be due to the fact that mitragynine exerts its actions through several mechanisms other than activation of MOR. Mitragynine blocked neuronal calcium channels.[10,47] They were found to involve in morphine withdrawal.[48,49] Many calcium channels blockers such as verapamil, diltiazem, and nifedipine were successfully able to attenuate opioid withdrawal symptoms.[50,51]

Kratom side effects and withdrawal in human were reviewed by Singh *et al*.[52] In brief, individuals faced both physical and psychological symptoms. Physical symptoms included irritability, muscle pain, cramps, and diarrhea where psychological symptoms included restlessness, tension, aggression, sadness, delusion, hallucination, and craving. People who consume high amount of kratom were more likely to develop severe dependence and withdrawal symptoms. However, those symptoms usually lasted only for 1–3 days.[53] Mitragynine withdrawal was similar to opioid withdrawal symptoms, and it could be reversed by administration of opioid agonist.[54]

From our results and other studies, it showed that mitragynine served as MOR partial agonist and KOR competitive antagonist. This is similar to buprenorphine. Buprenorphine is a partial agonist at MOR and competitive antagonist at KOR.[55] On activation of MOR, G-protein pathway mediates analgesic effect of opioid where beta-arrestin pathway mediates side effects, especially respiratory depression and constipation.[56] Like mitragynine, buprenorphine also showed bias antagonism at MOR for G-protein coupling over beta-arrestin.[17] It had low abuse liability and respiratory depression. Thus, buprenorphine has been approved for use in the treatment of opioid dependence in replacement therapy or opioid maintenance treatment.[57] All these results support the use of kratom in traditional medicine, informal use or self-medication in the treatment for opioid addiction, withdrawal, and cessation of opioid analgesics.[10,58]

CONCLUSIONS

Mitragynine showed positive reinforcing and rewarding effects in drug discrimination and conditioned place preference models with a lower potency than morphine. It was able to attenuate both acquisition and expression of morphine-induced conditioned place preference in dose-dependent manner. The receptor pharmacology profile of mitragynine was greatly similar to of buprenorphine. Mitragynine acts as the partial agonist at MOR and antagonist at KOR. It also has a functional bias toward G-protein over beta-arrestin that led to lower side effect than conventional opioids such as respiratory depression and constipation. This study provides scientific evidence supporting the use of kratom plant in traditional medicine in the treatment of opioid dependence and withdrawal. Together with other studies, mitragynine has great potential in the application for treatment of opioid and other drugs addiction. More studies on mitragynine and its derivatives are needed to clarify the effectiveness and adverse drug reaction of mitragynine in the treatment of drug addiction.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

RM performed the experiments, analyzed, interpreted the data, and prepared the manuscript (50% contribution). TS designed the experiments, analyze and interpreted the data, and prepared the manuscript (50% contribution). All authors approved the final version of the manuscript and are accountable for all aspects of the work.

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