Mini Review

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Pharmacokinetics and pharmacodynamics of mitragynine, the principle alkaloid of *Mitragyna speciosa*: present knowledge and future directions in perspective of pain

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Abstract:
Mitragyna speciosa, commonly known as Ketum or Biak in Malaysia and Kratom in Thailand, is a native plant to Southeast Asia and has various pharmacological benefits. Mitragynine (MG) is the principal alkaloid found in the leaves of *Mitragyna speciosa* and has been reported to be responsible for the plant's therapeutic actions. Traditionally, local communities use Kratom preparations for relief from different types of pain. The potential analgesic effects of MG using rodent models have been reported in literatures. We have reviewed the published analgesic and pharmacokinetic studies and all of these findings showed the routes of drug administration, doses employed, and type of vehicles used to solubilize the drug, varied considerably; hence this posted difficulties in predicting the drug's pharmacokinetic-response relationship. A rational approach is warranted for accurate prediction of dose-response relationship; as this is essential for the development of MG as an alternative medicinal drug for pain management. PKPD modeling would serve as a better method to understand the dose-response relationship in future MG preclinical and clinical studies.

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Introduction

The *Mitragyna speciosa* plant locally known as Ketum or Biak in Malaysia [1], [2] and Kratom in Thailand, is a member of the Rubiaceae family. This plant is native to Southeast Asia. *Mitragyna speciosa* has been employed as a stimulant as well as a remedy for various physical and mental illness [3], [4]. Numerous studies have reported the traditional uses of Kratom among rural folk, peasants, and laborers in Southeast Asia [5], [6]. Its usage in US and Europe is widespread and it is accessed easily via online merchants or in physical retail stores in different forms such as dried or powdered leaves in bulk or in capsules, tablets, and other extracts [7], [8], [9], [10]. It has medicinal values to treat various ailments, but importantly to wean off opiate drug users from withdrawal symptoms [1]. This is because Kratom is believed to be a "safe" herbal drug showing medicinal importance in the West [11], [12].

Kratom preparations are sold in the United States and several European countries through online merchants or in physical retail stores in different forms as mentioned above. Fresh Kratom leaves can be consumed by chewing, smoking, or ingesting orally by brewing as a tea or juice [13] to minimize fatigue, enhance work performance, and improve physical tolerance to heavy workload [14], [15]. It is widely used to treat conditions or ailments such as muscle pain, intestinal infection, cough, diarrhea, fever, and as an appetite-suppressing agent or anti-depressant [1]. Besides its therapeutic effects, Kratom is also an affordable opiate substitute among drug users in rural areas in the northern states of Malaysia. In view of addiction, this plant is mainly used to reduce illicit drug dependency and suppression of withdrawal symptoms.

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Approximately 40 alkaloids have been discovered in *Mitragyna speciosa* leaves. The alkaloid content in the leaves depends on the geographical regions and seasons of harvest [16]. Its main active alkaloid, mitragynine (MG) was found to have a unique morphine like-effects or opioid receptor agonist effects on guinea-pig ileum [17]. Its minor constituent 7-hydroxy MG inhibited electrically induced contractions through opioid receptors in guinea-pig ileum, and its effects was about 13 times more potent than morphine. MG is the most abundant active alkaloid derived from the leaves of *Mitragyna speciosa*, which constitutes up to 66% of total alkaloid mixture.

The clinical efficacy of a drug therapy is determined by its pharmacokinetic (PK) and pharmacodynamic (PD) profiles. Although, the pharmacological effects of Kratom in human and experimental animals are well-established, the pharmacokinetics and toxicity data of pure MG and alkaloid extract still remain poorly defined. This article reviews the interplay between pharmacokinetics and pharmacodynamics of MG (i.e. dose-exposure-response relationships) with the physicochemical characterization of MG, which will provide a basis for developing a suitable formulation to further improve its solubility, stability and oral absorption for better assessment of this compound in preclinical and clinical studies.

Physicochemical properties or characterization

MG has both hydrophobic and lipophilic properties (log P 1.76) [18]. It is a weak base (pka 8.3) and poor water solubility (<100 μg/mL). It is highly soluble in acidic condition but acid-degradable. In simulated gastrointestinal fluid studies, MG showed incomplete dissolution in simulated intestinal fluid (SIF) but stable. However, the drug demonstrated good solubility in simulated gastric fluid (SGF) but acid-degradable (~20%) [18], [19]. Recent studies reported MG as a poorly soluble drug with high permeability, a dose of 40 μg/mL has high intestinal permeability drug in rats using *in situ* absorption model [20]. Studies also found that MG was not affected by P-glycoprotein efflux effect and cytochrome P3A4 which are associated with low permeability [20], [21].

Nonclinical vehicle use in studies

The use of appropriate vehicles to solubilize drug or extracts prior to oral or parenteral administration is vital in preclinical animal studies. Any drug to be absorbed must be present in the form of solution at the site of absorption; hence various vehicles were used to facilitate the solubility of MG. The vehicles commonly used to facilitate the solubility of MG were 1% cremophor in saline, 1% acetic acid (pH 4.7) or propylene glycol, 4% acacia gum, Tween 20, Tween 80 [22], [23], [24]. In most instances upon solubilizing, the drug will be either in the form of solution or suspension depending upon the dosage employed in preclinical studies. However owing to its basic sub- optimal physicochemical properties, this drug predominately exists in ionic form in gastric juice and encounter solubility issue in the intestinal fluid. Thus, there are high possibility of MG being aqueous, basic in nature and moderate lipophilic characteristic. Apart from the solubility, intestinal permeability of a drug also attributes to its bioavailability. In case of MG, it has showed a high intestinal permeability in animal model. On an average, the sub-optimal physiochemical properties of MG and difference in vehicles utilized may contribute to the higher variation and conflicting qualities in PK-parameters. PKPD modelling is essential to overcome these drawbacks.

Pharmacokinetics of MG

The pharmacokinetics of MG after oral and intravenous administration in rats was limited. As of date only one pharmacokinetic study of MG in human (Kratom users) was documented in literature [22]. In rats, investigators reported a large variation in the C_{max} (0.42–0.70 μg/mL), t_{max} (1.26–4.50 h) and elimination half-life (3.85–9.43 h^{-1}) of MG after oral administration. Both CL/F (apparent total clearance) and Vd/F (apparent volume of distribution) showed inconsistent findings, where CL/F (1.6–7.0 L/h/kg) and Vd/F (37.90–89.5 L/kg) [23], [24], [25].

Apart from studies on MG's pharmacokinetic profile in animal model, recently Trakulsrichai and colleagues reported human pharmacokinetic study with two loading doses of Kratom tea. This was the first study on the pharmacokinetic parameters from the nine existing Kratom users using non-compartmental analysis, where t_{max} was 0.83 ± 0.35 h, with terminal half-life at (23.24 ± 16.07 h), and the apparent volume of distribution (38.04

± 24.32 L/kg). The unchanged form found in the urine was 0.14% with the clearance rate of 98.1 ± 51.34 L/h/kg [22]. The overall studies have been summarized in Table 1.

Table 1: Pharmacokinetic studies of MG.

Species	MG dose, mg/kg	C_{max}, µg/mL	T_{max}, hours	t½, hours^{-1}	Clearance, L/h/kg	Volume of distribution-Vd/F, L/kg_	Authors
Rats	40 [p.o.] [Vehicle: 100% propylene glycol]	0.63 ± 0.18	1.83 ± 1.25	9.43 ± 1.74	1.60 ± 0.58	89.50 ± 30.30	Janchawee et al. [23]
	20 [p.o.] [Vehicle: 1% acetic acid]	0.42 ± 61.79	1.26 ± 0.20	3.85 ± 0.51	6.35 ± 0.43	37.90 ± 5.41	de Moraes et al. [24]
	1.5 [iv] [Vehicle: 20% Tween 20]	2.3 ± 1.2	1.20 ± 1.1	2.9 ± 2.1	0.29 ± 0.27 (absolute)	0.79 ± 0.42	Parthasarathy et al. [25]
	50 [p.o.] [Vehicle: 20% Tween 20]	0.7 ± 0.21	4.5 ± 3.6	6.6 ± 1.3	7.0 ± 3.0	64 ± 23	Parthasarathy et al. [25]
Humans	Loading dose [p.o] [vehicle: distilled water]		0.83 ± 0.35	23.24 ± 16.07	98.1 ± 51.34	38.04 ± 24.32	Trakulsirichai et al. [22]
	High: 23	0.105					
	Low: 6.25	0.0185					

Pharmacokinetic studies of MG.

Since there are limited animal studies and only one human study on PK of MG, there was a challenge in the selection of appropriate dose for further investigations. From all these pharmacokinetic studies, the range of the dose employed varied significantly. These variations were observed in both preclinical and human studies. Apart from the dosing, different routes of administration and extraction methods also contribute to the variability in pharmacokinetic data.

Pharmacodynamics of MG (anti-nociceptive or analgesic)

Opioids have been used extensively in treating pain for more than 100 years. Opioid receptors can be classified into three subtypes which are Mu (µ), kappa (κ), and delta (δ) opioid receptors [26]. With regards to MG, high affinity was found towards κ-opioid receptors followed by µ- and δ-opioid receptors [27]. It acts as a receptor agonist at µ-opioid receptors and possibly as an antagonist at κ-opioid receptors [28], [29], [30]. At cellular level, MG blocks neuronal Ca^{2+} channels [31]. It was also found to inhibit forskolin-stimulated cyclic adenosine monophosphate (cAMP) formation *in vitro* in an opiate receptor-dependent way [32], [33]. Expression of cAMP and cAMP response element-binding protein (CREB) was reported due to repeated exposure of MG [34]. Twitch contraction of the ileum of the guinea pig occurred with the aid of electrical stimulation to assess the agonistic action of the opioid receptor. Opioid agonist activity is measured as the inhibition of the twitch contraction, which is reversed by the opioid receptor antagonist naloxone. *Mitragyna speciosa* preparations, MG, and other isolated *Mitragyna speciosa* indole alkaloids as well as MG derivatives result in the inhibition of the electrically stimulated contraction. However, MG was 10 times lesser potent than morphine [17], [35].

Till date, all of the reported analgesic or anti-nociceptive studies were only performed on animal models and not on humans. Various behavioral tests and different range of doses gives a drawback mainly to compare the effective dose for analgesic or anti-nociceptive activity.

Pain assessment of Kratom was evaluated using well established behavioral test. In late 90s, Matsumoto and his colleagues discovered the anti-nociceptive properties of MG in mice using several behavioral tests such as hot plate, tail pinch and 5-Methoxy-N,N-dimethyltryptamine-inducedhead-twitch and weaving responses [36], [37].

Idid et al. reported that the anti-nociceptive action of MG at 200 mg/kg was less prominent comparable to the morphine (5 mg/kg) and more effective compared to 100 mg/kg paracetamol in acetic acid induced writhing, and the hot and cold tail-flick tests [38]. The mechanism of paracetamol is mainly focused on COX

inhibition (peripheral) and non-opioid (central) pathway which limits the comparison of the analgesic activity between MG and paracetamol [39].

Antinociceptive effects was observed after oral administration of *Mitragyna speciosa* preparations. Methanolic and alkaloid *Mitragyna speciosa* extracts extended the inactivity of a nociceptive response to noxious stimulation in the hot-plate test, but not in the tail-flick test [40]. This finding showed that *Mitragyna speciosa* extracts most likely act on the central pathway which is indicated by analgesic response in hot plate test [41]. With regards to synergism, *Mitragyna speciosa* alkaloid extract administered with caffeine and codeine improves the anti-nociceptive effect in a hot plate test with rats [42].

Following these outcomes, Shaik Mossadeq et al. conducted study where methanolic extract of *Mitragyna speciosa* yielded to anti-nociceptive responses in hot-plate, acetic-acid-induced writhing and the formalin tests in mice [43]. Thereafter, it was shown by Sabetghadam et al. and Takayama et al. that different extracts of *Mitragyna speciosa* (alkaloid, methanolic, and aqueous) and MG derivatives significantly prolonged the latency of nociceptive responses compared to the morphine in both hot-plate and tail flick tests in rats. The pre-administered-opioid antagonist naloxone abolished effects of *Mitragyna speciosa* extracts, which suggest that the extracts act through opioid-receptor [44], [45].

Recently, Criddle compared the effect of 300 mg/kg of extracts, 75 mg/kg of alkaloids fraction, and 30 mg/kg of MG of *Mitragyna speciosa* with opioid agonists; 10 mg/kg morphine and 3 mg/kg oxycodone, in rats using hot plate method. This study showed that intraperitoneal and oral administration of MG produce antinociceptive effects equivalent to the reference opioid agonists [46], [47]. The overall study has been summarized in Table 2.

Table 2: Pharmacodynamic studies of mitragynine (anti-nociceptive/analgesic).

Compound/extract	Species	Behavioural test/*In vitro* models	Authors
Mitragynine [5–30 mg/kg i.p.], [1.0–10.0 μg/mouse]	Male ddy Mice	*Antinociceptive* Hot plate test and tail pinch test	Matsumoto et al. [36]
Mitragynine [1–30 mg/kg, i.p.]	Male ddy mice	*Antinociceptive* 5-Methoxy-N,N-dimethyltryptamine-induced head-twitch and weaving responses	Matsumoto et al. [37]
Mitragynine [10 μm]	Guinea Pig	*Analgesic* Electrically stimulated contraction of ileum	Watanabe et al. [17]
– Methanolic extract – Mitragynine	Guinea Pig	*Analgesic* Electrically stimulated contraction of ileum	Horie et al. [35]
Mitragynine [200 mg/kg, p.o.]	Albino mice	*Analgesic* – Acetic acid induced writing – Hot flick test – Cold flick test	Idid et al. [38]
Methanolic extract [50, 100, 200 mg/kg, p.o.] Alkaloid extract [5, 10, 20 mg/kg, p.o.]	Mice, Rat	*Analgesic* Hot Plate test and Tail flick test	Reanmongkol et al. [40]
Methanolic extract [100, 200 mg/kg, i.p.]	Mice	*Antinociceptive* – Hot plate test – Acetic acid induced writing – Formalin test	Shaikh Mossadeq et al. [43]
Methanolic extract [50, 100, 200 mg/kg, p.o.] Alkaloid extract [5, 10, 20 mg/kg, p.o.] Aqueous extract [100, 200, 400 mg/kg, p.o.]	Rats	*Antinociceptive* Hot Plate test and Tail flick test	Sabetghadam et al. [44]
Mitragynine derivatives [icv] Intracerebroventricular	Mice	*Analgesic* – Hot Plate test – Tail flick test – Magnus assay using guinea pig ileum	Takayama et al. [45]

Methanolic extract [300 mg/kg, p.o.] Alkaloid extract [75 mg/kg, p.o.] Mitragynine [10 mg/kg, p.o. and i.p.]	Rat	*Antinociceptive* Hot Plate test	Criddle [46] Carpenter et al. [47]

Pharmacodynamic studies of MG.

Routes of administration

Important factors in the determination of PK and PD relationship in MG include the routes of administration. Till date, MG and several Kratom extracts have been administered via oral, intra peritoneal, and intravenous routes to explore MG's PK profiles and its analgesic effects individually [22], [25], [40]. These studies are insufficient to provide comprehensive relationship between PK and PD. This is because different routes affect the concentration level reaching the target tissue and subsequent analgesic activity [48]. Thus, future studies on the effects of various routes of administration on both PK and PD are recommended in order to ascertain the role of the different routes in exploring the bioavailability of MG.

PKPD modeling study

The rationale of PKPD-modeling is to link pharmacokinetics and pharmacodynamics in order to establish and evaluate dose-concentration-response relationships and subsequently describe or predict the effect-time courses resulting from a drug dose [49].

Pain perception is characterized by multiple factors, thus PKPD modeling becomes too intricate to discover the analgesic drugs. Moreover, limited responders to experimental pain model and analgesic drugs lead to difficulty in obtaining data for PKPD modelling [50].

In some cases, the analgesic effects of opioid drugs were not correlated with its plasma concentration and time-course of the effects [51]. For example, alfentanil and its derivatives show rapid transfer between plasma concentrations to its effect site while morphine and buprenorphine demonstrates contrary effects [52], [53]. On the other hand, active metabolites also have shown to contribute analgesic/anti-nociceptive activity. Morphine is the active metabolite of codeine whereas morphine-6-glucuronide for morphine and M1 metabolite for tramadol [54], [55], [56]. As for MG, the principal alkaloid of Kratom has similar effects to opioid drugs. Also, there are many factors associating with PKPD modeling of these class of analgesic drugs. The typical PK parameters of active ingredients in a single herb or complex herbal formulation could not fully describe the mechanism actions of herbal medicines which correspond to its PD. With regards to this, PKPD modeling have been used to investigate the safety and the effectiveness of the herbal medicines as well as herb-herb synergistic effects in herbal medicines [57], [58], [59]. An example, glycyrrhetinic acid with paeoniflorin is an active ingredient in liquorice decoction have been demonstrated to work synergistically as analgesic to treat dysmenorrhea using PKPD modeling [59]. Considering all the above points, development of PKPD modeling for MG with respect to dose design and the interaction of its analgesic properties are required.

Conclusions

Based on the above reports, we conclude that MG has sub-optimal physicochemical properties which could be a challenge in the development of a desirable formulation that can be used for future preclinical and clinical studies. PKPD has become an integral part of the development of designing a new formulation. However, there have not been studies reporting on the correlation between PK and PD of MG in humans as well as animal models. Because of the addiction property of Kratom, determination of safe dose is crucial for human consumption. Importantly, this is the limitation to predict the effective dose of MG with less adverse effects for the pain treatment. Therefore, PKPD modeling is essential for the development of MG as an analgesic/anti-nociceptive drug at a safe dose for human wellbeing.

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References

[1] Hassan Z, Muzaimi M, Navaratnam V, Yusoff NH, Suhaimi FW, Vadivelu R, et al. From kratom to MG and its derivatives: physiological and behavioural effects related to use, abuse, and addiction. *Neurosci Biobehav Rev*. 2013;37:138–51 .
[2] Singh D, Narayanan S, Vicknasingam B. Traditional and non-traditional uses of MG (kratom): a survey of the literature. Brain Res Bull 2016;126:41–6.
[3] Saingam D, Assanangkornchai S, Geater AF, Lerkiatbundit S. Validation of kratom (Mitragyna speciosa Korth.) dependence scale (KDS): a dependence screen for internationally emerging psychoactive substance. Subst Abuse 2014;35:276–83.
[4] Brown PN, Lund JA, Murch SJ. A botanical, phytochemical and ethnomedicinal review of the genus Mitragyna Korth: implications for products sold as kratom. J Ethnopharmacol 2017;202:302–25.
[5] Vicknasingam B, Narayanan S, Beng GT, Mansor SM. The informal use of kratom (Mitragyna speciosa) for opioid withdrawal in the northern states of peninsular Malaysia and implications for drug substitution therapy. Int J Drug Policy 2010;21:283–8.
[6] Boyer EW, Babu KM, Adkins JE, McCurdy CR, Halpern JH. Self-treatment of opioid withdrawal using kratom (Mitragyna speciosa Korth.). Addiction 2008;103:1048–50.
[7] Prozialeck WC, Jivan JK, Andurkar SV. Pharmacology of kratom: an emerging botanical agent with stimulant, analgesic and opioid-like effects. J Am Osteopath Assoc 2012;112:792–9.
[8] Cinosi E, Martinotti G, Simonato P, Singh D, Demetrovics Z, Roman-Urrestarazu A, et al. Following "the roots" of kratom (Mitragyna speciosa): the evolution of an enhancer from a traditional use to increase work and productivity in Southeast Asia to a recreational psychoactive drug in western countries. Biomed Res Int 2015;2015:968786.
[9] Swogger MT, Hart E, Erowid F, Erowid E, Trabold N, Yee K, et al. Experiences of kratom users: a qualitative analysis. J Psychoactive Drugs 2015;47:360–7.
[10] Warner ML, Kaufman NC, Grundmann O. The pharmacology and toxicology of kratom: from traditional herb to drug of abuse. Int J Legal Med 2016;130:127.
[11] Ahmad K, Aziz Z. Mitragyna speciose use in the northern states of Malaysia: cross-sectional study. J Ethnopharmacol 2012;141:446–50.
[12] Suhaimi FW, Yusoff NH, Hassan R, Mansor SM, Navaratnam V, Müller CP, et al. Neurobiology of kratom and its main alkaloid MG. Brain Res Bull 2016;126:29–40.
[13] MacLaren E. The effects of kratom use. www.drugabuse.com/library/the-effects-of-kratom-use/ (accessed 2016 Nov 17).
[14] Tanguay P. Kratom in Thailand: decriminalisation and community control? Legislative Reform of Drug Policies (Vol. 13). Amsterdam: Transnational Institute, 2011:1–16.
[15] Saingam D, Assanangkornchai S, Geater AF, Balthip Q. Pattern and consequences of kratom (Mitragyna speciosa Korth.) use among male villagers in southern Thailand: a qualitative study. Int J Drug Policy 2012;24:351–8.
[16] Shellard EJ. The alkaloids of Mitragyna with special reference to those of Mitragyna speciosa Korth. Bull Narc 1974;26:41–55.
[17] Watanabe K, Yano S, Horie S, Yamamoto LT. Inhibitory effect of mitragynine, an alkaloid with analgesic effect from Thai medicinal plant Mitragyna speciosa, on electrically stimulated contraction of isolated guinea-pig ileum through the opioid receptor. Life Sci 1997;60:933–42.
[18] Ramanathan S, Parthasarathy S, Murugaiyah V, Magosso E, Tan SC, Mansor SM. Understanding the physicochemical properties of MG, a principal alkaloid of Mitragyna speciosa, for preclinical evaluation. Molecules 2015;20:4915–27.
[19] Manda VK, Avula B, Ali Z, Khan IA, Walker LA, Khan SI. Evaluation of in vitro absorption, distribution, metabolism, and excretion (ADME) properties of MG, 7-hydroxyMG, and mitraphylline. Planta Med 2014;80:568–76.
[20] Jagabalan JD, Murugaiyah V, Zainal H, Mansor SM, Ramanathan S. Intestinal permeability of MG in rats using in situ absorption model. J Asian Nat Prod Res 2019;24:351–63.
[21] Kong WM, ChikaMohameda Zalshawsha MA. Physicochemical characterization of Mitragyna speciosa alkaloid extract and MG using in vitro high throughput assays. Comb Chem High Throughput Screen 2017;20:1–9.
[22] Trakulsrichai S, Sathirakul K, Auparakkitanon S, Krongvorakul J, Sueajai J, Noumjad N, et al. Pharmacokinetics of MG in man. Drug Des Devel Ther 2015;9:2421–9.
[23] Janchawee B, Keawpradub N, Chittrakarn S, Prasettho S, Wararatananurak P, Sawangjareon K. A high-performance liquid chromatographic method for determination of MG in serum and its application to a pharmacokinetic study in rats. Biomed Chromatogr 2007;21:176–83.
[24] de Moraes NV, Moretti RA, Furr EB III, McCurdy CR, Lanchote VL. Determination of MG in rat plasma by LC-MS/MS: application to pharmacokinetics. J Chromatogr B Analyt Technol Biomed Life Sci 2009;877:2593–7.

[25] Parthasarathy S, Ramanathan S, Ismail S, Adenan MI, Mansor SM, Murugaiyah V. Determination of MG in plasma with solid-phase extraction and rapid HPLC-UV analysis, and its application to a pharmacokinetic study in rat. Anal Bioanal Chem 2010;397:2023–30.
[26] Al-Hasani R, Bruchas MR. Molecular mechanisms of opioid receptor-dependent signaling and behavior. Anesthesiology 2011;115:1363–81.
[27] Taufik Hidayat M, Apryani E, Nabishah BM, Moklas MA, Sharida F, Farhan MA. Determination of MG bound opioid receptors. Adv Med Dent Sci 2010;3:65–70.
[28] Yamamoto LT, Horie S, Takayama H, Aimi N, Sakai S, Yano S, et al. Opioid receptor agonistic characteristics of MG pseudoindoxyl in comparison with MG derived from Thai medicinal plant Mitragyna speciosa. Gen Pharmacol 1999;33:73–81.
[29] Shamima AR, Fakurazi S, Hidayat MT, Hairuszah I, Moklas MA, Arulselvan P. Antinociceptive action of isolated MG from Mitragyna speciosa through activation of opioid receptor system. Int J Mol Sci 2012;13:11427–42.
[30] Yusoff NH, Mansor SM, Müller CP, Hassan Z. Opioid receptors mediate the acquisition, but not the expression of MG-induced conditioned place preference in rats. Behav Brain Res 2017;332:1–6.
[31] Matsumoto K, Yamamoto LT, Watanabe K, Yano S, Shan J, Pang PK, et al. Inhibitory effect of MG, an analgesic alkaloid from Thai herbal medicine, on neurogenic contraction of the vas deferens. Life Sci 2005;78:187–94.
[32] Tohda M, Thongpraditchote S, Matsumoto K, Murakami Y, Sakai S, Aimi N, et al. Effects of MG on cAMP formation mediated by delta-opiate receptors in NG108-15 cells. Biol Pharm Bull 1997;20:338–40.
[33] Jamil MF, Subki MF, Lan TM, Majid MI, Adenan MI. The effect of mitragy-nine on cAMP formation and mRNA expression of mu-opioid receptors mediated by chronic morphine treatment in SK-N-SH neuroblastoma cell. J Ethnopharmacol 2013;148:135–43.
[34] Fakurazi S, Rahman SA, Hidayat MT, Ithnin H, Moklas MA, Arulselvan P. The combination of MG and morphine prevents the development of morphine tolerance in mice. Molecules 2013;18:666–81.
[35] Horie S, Koyama F, Takayama H, Ishikawa H, Aimi N, Ponglux D, et al. Indole alkaloids of a Thai medicinal herb, Mitragyna speciosa, that has opioid agonistic effect in guinea-pig ileum. Planta Med 2005;71:231–6.
[36] Matsumoto K, Mizowaki M, Suchitra T, Takayama H, Sakai SI, Aimi N, et al. Antinociceptive action of MG in mice: evidence for the involvement of supraspinal opioid receptors. Life Sci 1996;59:1149–55.
[37] Matsumoto K, Mizowaki M, Takayama H, Sakai SI, Aimi N, Watanabe H. Suppressive effect of MG on the 5-Methoxy-N,N-dimethyltryptamineinduced head-twitch response in mice. Pharm Biochem Behav 1997;57:319–23.
[38] Idid SZ, Saad LB, Yaacob H, Shahimi MM. Evaluation of analgesia induced by MG, morphine and paracetamol in mice. In: ASEAN Review of Biodiversity and Environmental Conservation, May 1998:1–7. Available from: https://www.semanticscholar.org/paper/EVALUATION-OF-ANALGESIA-INDUCED-BY-MITRAGYNINE%2C-AND-Idid-Yaacob/77aaadbe243e107af1d6580cc816f557b3dcc43c.
[39] Jóźwiak-Bebenista M, Nowak JZ. Paracetamol: mechanism of action, applications and safety concern. Acta Pol Pharm 2014;71:11–23.
[40] Reanmongkol W, Keawpradub N, Sawangjaroen K. Effects of the extracts from Mitragyna speciosa Korth. leaves on analgesic and behavioral activities in experimental animals. Songklanakarin J Sci Tech 2007;29:39–48.
[41] Mulder GB, Kathleen P. Rodent analgesiometry: the hot plate, tail flick and Von Frey hairs. Contemp Top Lab Anim Sci 2004;43:54–5.
[42] Botpiboon O, Prutipanlai S, Janchawee B, Thainchaiwattana S. Effects of caffeine and codein on antinociceptive activity of alkaloid extract from leaves of kratom (Mitragyna speciosa Korth). In: Paper Presented at The 35th Congress on Science and Technology of Thailand, October 15–17, 2009. The Tide Resort (Bangsaen Beach), Chonburi, Thailand.
[43] Shaik Mossadeq WM, Sulaiman MR, Tengku Mohamad TA, Chiong HS, Zakaria ZA, Jabit ML, et al. Anti-inflammatory and antinociceptive effects of Mitragyna speciosa Korth methanolic extract. Med Princ Pract 2009;18:378–84.
[44] Sabetghadam A, Ramanathan S, Mansor SM. The evaluation of antinociceptive activity of alkaloid, methanolic, and aqueous extracts of Malaysian Mitragyna speciosa Korth leaves in rats. Pharmacognosy Res 2010;2:181–5.
[45] Takayama H, Ishikawa H, Kurihara M, Kitajima M, Aimi N, Ponglux D, et al. Studies on the synthesis and opioid agonistic activities of MG-related indole alkaloids: discovery of opioid agonists structurally different from other opioid ligands. J Med Chem 2002;45:1949–56.
[46] Criddle CA. A Comparison of Mitragyna speciosa and MG against Opioids on Thermal Nociception in Rats. 2015. Available from: http://thesis.honors.olemiss.edu.
[47] Carpenter JM, Criddle CA, Craig HK, Ali Z, Zhang Z, Khan IA, et al. Comparative effects of Mitragyna speciosa extract, MG, and opioid agonists on thermal nociception in rats. Fitoterapia 2016;109:87–90.
[48] Turner VP, Brabb T, Pekow C, Vasbinde MA. Administration of substances to laboratory animals: routes of administration and factors to consider. J Am Assoc Lab Anim Sci 2011;50:600–13.
[49] Meibohm B, Derendorf H. Basic concepts of pharmacokinetic/pharmacodynamics (PK/PD) modelling. Int J Clin Pharmacol Ther 1997;35:401–13.
[50] Lorenzini K, Daali Y, Dayer P, Desmeules J. Pharmacokinetic–pharmacodynamic modelling of opioids in healthy human volunteers. A minireview. Bas Clin Pharm Toxc 2012;110:219–26.
[51] Lötsch J. Pharmacokinetic–pharmacodynamic modeling of opioids. J Pain Symptom Manage 2005;29:90–103.
[52] Olofsen E, Romberg R, Bijl H, Mooren R, Engbers F, Kest B, et al. Alfentanil and placebo analgesia: no sex differences detected in models of experimental pain. Anesthesiology 2005;103:130–9.
[53] Escher M, Daali Y, Chabert J, Hopfgartner G, Dayer P, Desmeules J. Pharmacokinetic and pharmacodynamic properties of buprenorphine after a single intravenous administration in healthy volunteers: a randomized, double-blind, placebo-controlled, crossover study. Clin Ther 2007;29:1620–31.
[54] Eckhardt K, Li S, Ammon S, Schänzle G, Mikus G, Eichelbaum M. Same incidence of adverse drug events after codeine administration irrespective of the genetically determined differences in morphine formation. Pain 1998;76:27–33.
[55] Murthy BP, Pollack GM, Brouwer KL. Contribution of morphine-6-glucuronide to antinociception following intravenous administration of morphine to healthy volunteers. J Clin Pharm 2002;42:569–76.
[56] Enggaard TP, Poulsen L, Arendt-Nielsen L, Brøsen K, Ossig J, Sindrup SH. The analgesic effect of tramadol after intravenous injection in healthy volunteers in relation to CYP2D6. Anesth Anal 2006;102:146–50.

[57] Chen G, Hao B, Ju D, Liu M, Zhao H, Du Z, et al. Pharmacokinetic and pharmacodynamic study of triptolide-loaded liposome hydrogel patch under microneedles on rats with collagen-induced arthritis. Acta Pharm Sin B 2015;5:569–76.
[58] Dong LC, Fan YX, Yu Q, Ma J, Dong X, Li P, et al. Synergistic effects of rhubarb-gardenia herb pair in cholestatic rats at pharmacodynamic and pharmacokinetic levels. J Ethnopharmacol 2015;175:67–74.
[59] Ren W, Zuo R, Wang YN, Wang HJ, Yang J, Xin SK, et al. Pharmacokinetic-pharmacodynamic analysis on Inflammation rat model after oral administration of Huang Lian Jie Du decoction. PLoS One 2016;11:e0156256.