Review

Neurobiology of Kratom and its main alkaloid mitragynine

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ABSTRACT

Kratom or its main alkaloid, mitragynine is derived from the plant *Mitragyna speciosa* Korth which is indigenous to Southeast Asian countries. This substance has become widely available in other countries like Europe and United States due to its opium- and coca-like effects. In this article, we have reviewed available reports on mitragynine and other *M. speciosa* extracts. *M. speciosa* has been proven to have a rewarding effect and is effective in alleviating the morphine and ethanol withdrawal effects. However, studies in human revealed that prolonged consumption of this plant led to dependence and tolerance while cessation caused a series of aversive withdrawal symptoms. Findings also showed that *M. speciosa* extracts possess antinociceptive, anti-inflammatory, anti-depressant, and muscle relaxant properties. Available evidence further supports the adverse effects of *M. speciosa preparations*, *mitragynine* on cognition. Pharmacological activities are mainly mediated *via* opioid receptors as well as neuronal Ca^{2+} channels, expression of cAMP and CREB protein and *via* descending monoaminergic system. Physico-chemical properties of mitragynine have been documented which may further explain the variation in pharmacological responses. In summary, current researchs on its main indole alkaloid, mitragynine suggest both therapeutic and addictive potential but further research on its molecular effects is needed.

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Contents

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1. Introduction

Mitragyna speciosa Korth (*M. speciosa*) is a medicinal herb originated from the Rubiaceae (coffee) family. It is a naturally occurring plant in tropical and sub-tropical regions of Southeast Asia and Africa. This plant is also known as *Ketum* or *biak-biak* in Malaysia and *Kratom*, *Kakuam*, *Kraton*, *Ithang* or *Thom* in Thailand (Jansen and Prast, 1988; Matsumoto et al., 1996a,b; Ponglux et al., 1994; Boyer et al., 2008; Ingsathit et al., 2009; Adkins et al., 2011; Gong et al., 2012; Hassan et al., 2013; Saingam et al., 2014). Today, it is one of several psychoactive herbal products that are widely available over the Internet and its use is being spread around the world (Adkins et al., 2011). This plant has been widely used throughout Southeast Asian countries as a herbal drug for decades, as early as the late 1800 (Nelson et al., 2014) such as for the treatment of muscle pain, diarrhea, cough, and to enhance productivity. It is used to reduce intake of more expensive opiates and as alternative to other opioid-replacement medications. It mitigates opioid withdrawal symptoms and can develop euphoric or pleasure effect (Assanangkornchai et al., 2007a,b; Chan et al., 2005; Hassan et al., 2013; Vicknasingam et al., 2010; Ahmad and Aziz, 2012). In animal models, mitragynine has shown to elicit reward behaviour (Sufka et al., 2014; Yusoff et al., 2016) and it is effective in ameliorating morphine withdrawal effects (Khor et al., 2011). However, prolonged consumption of this plant preparation may develop tolerance. Therefore, increasing dosage is required to achieve the desired effects (Hassan et al., 2013). In addition, aversive withdrawal effects upon abstaining from consumption have been documented (for review see: Hassan et al., 2013). Withdrawal symptoms include hostility, aggression, aching of muscles and bones, jerky movements of the limbs, anorexia, weight loss, insomnia, and psychosis (Hassan et al., 2013; Singh et al., 2014; Yusoff et al., 2016). Here, we aim to provide an overview about the latest findings of Kratom and its main alkaloid, mitragynine on its physicochemical properties as well as its psychological, pharmacological and behavioural activities based on published reports. The review shall contribute to a more comprehensive understanding about Kratom in regards to its potential medical application, legal status and future research needs.

2. Preparations and consumption

The fresh leaves of *M. speciosa* can be chewed. The dried leaves can be smoked or taken as tea by brewing the powder with hot water and some sugar or honey to mask the bitter taste of the brew (Tanguay, 2011; Hassan et al., 2013). Extraction of the alkaloids is facilitated by the addition of lemon juice. Other than that, the fresh leaves can be chewed alone with removal of the veins before eating, or taken together with the betel nut (*Areca catechu*) (Scholz and Eigner, 1983; Hassan et al., 2013). Sometimes, this plant is consumed as a pill made from syrup. Dried leaves are powdered and boiled in hot water until syrup is produced. Then the syrup is mixed with the finely chopped leaves of palas palm and made into pills known as 'madat' in Malaysia which are smoked in long bamboo pipes (Macmillan et al., 1991; Hassan et al., 2013). People in southern Thailand created a homemade ice-cold cocktail called '4 × 100' that are made of three basic ingredients, the *M. speciosa* leaves, caffeine-containing soft drink and codeine- or diphenhydramine-containing cough syrup (Tanguay, 2011). However, consumption of this cocktail may result in fatal outcomes due to its multidrug actions (Tungtananuwat and Lawanprasert, 2010).

3. Epidemiology and legal status

In Southern Thailand, the lifetime prevalence for *M. speciosa* use among high school students was approximately 2.3–4.9% in 2002–2004 (Assanangkornchai et al., 2007a). The prevalence among 12–65 years old in the year 2007 was 3.76% and a year before was 4.73% (Assanangkornchai et al., 2007b, 2008). However, the use of *M. speciosa* is no longer restricted to Southeast Asia. It has been reported that the use of *M. speciosa* substance has spread to Japan (Kikura-Hanajiri et al., 2011; Maruyama et al., 2009), Europe and United States as it can be easily purchased on Internet (Prozialeck et al., 2012; Hillebrand et al., 2010; Schmidt et al., 2011). *M. speciosa* has been used as an ingredient of 'legal- or herbal-high' preparations and is distributed under various names such as Krypton in which after its consumption showed the presence of mitragynine, other *M. speciosa* alkaloids and synthetic drugs in urine test (Dresen et al., 2010; Arndt et al., 2011).

Due to its abuse potential, *M. speciosa* and its preparation have been placed under Poison Act 1952 since 2003 in Malaysia (Vicknasingam et al., 2010; Chan et al., 2005). This means that any selling of *M. speciosa* and its preparations is an offence with a penalty or a jail sentence. In Thailand, Kratom is placed under Schedule 5 of the Thai Narcotic Act which makes it illegal to buy, sell, import or possess it. The law also applies to the planting of trees and led to the cutting down of existing ones. However, Kratom is legally cultivated in Indonesia and its leaves are exported to North America and Europe for processing and re-distribution (Tanguay, 2011). European countries like Denmark, Latvia, Lithuania, Romania, Poland and Sweden classified *M. speciosa* and its derivatives as a controlled drug whilst other countries including Australia and Myanmar have put them under the control of the narcotic laws. *M. speciosa* and its preparations are not controlled drugs in US, UK and Germany, but they are put under surveillance (EMCDDA, 2012). United Nation Office of Drugs and Crime had conducted a survey on natural psychoactive substances in 2012 and reported that Kratom was among the top plant-based substances used (United Nations Office on Drugs And Crime, 2013). The US Drug Enforcement Administration (DEA) has listed 'Kratom' on its Drugs and Chemicals of Concern list which suggest that there is a potential of banning the substances once more convincing data on the addictive properties and/or health hazards become available in future (Hassan et al., 2013).

4. Medicinal use

Over years, *M. speciosa* leaves have been traditionally used to treat muscle pain, intestinal infections, coughing and diarrhea (Suwanlert, 1975; Jansen and Prast, 1988; Said et al., 1991; Watanabe et al., 1997; Prozialeck et al., 2012) particularly in Malaysia and Thailand. Apart from that, *M. speciosa* may also possess analgesic, antipyretic, anti-depressant and anxiolytic effects. They can also improve the immune system, lower blood pressure, act as antiviral, antidiabetic as well as appetite-suppressing agent (Macko et al., 1972; Chan et al., 2005).

M. speciosa also has been consumed by Malay and Thai natives to enhance tolerance for hard work under scorching sun due to

its opium- and coca-like effects (Shellard, 1974; Suwanlert, 1975; Tanguay, 2011; Ramanathan et al., 2015). It has been reported that *M. speciosa* was used as a substitute in the treatment of opium addiction in Malaysia (Beckett et al., 1965; Tanguay, 2011). In Thailand, *M. speciosa* also has been used for detoxification in treatment programmes for morphine addicts (Norakanphadung, 1966). The plant was also used as a self-treatment for opiate and alcohol withdrawal as well as for chronic pain (Boyer et al., 2008; Havemann-Reinecke, 2011; Ward et al., 2011).

5. Phytochemistry

Isolation and chemical characterization of constituents from *M. speciosa* started as early as 1960s (Beckett et al., 1965, 1966; Zacharias et al., 1965). Since then, a number of alkaloids have been isolated from *M. speciosa*. Investigation of the young leaves of Thai *M. speciosa* showed the presence of mitragynine and its analogues, speciogynine, paynantheine and speciociliatine. A new alkaloid, 7α-hydroxy-7H-mitragynine (7-hydroxymitragynine) has also been isolated from the plant (Ponglux et al., 1994; Takayama et al., 2002; Takayama, 2004; León et al., 2009; Orio et al., 2012). Methanolic extraction of the mature leaves of Malaysian *M. speciosa* also yielded the same alkaloids along with other minor constituents known as mitragynaline, pinoresinol, mitralactonal, mitrasulgynine and 3,4,5,6-tetradehydromitragynine (Takayama et al., 1998; Takayama, 2004). In addition, 7-hydroxyspeciociliatine was found in the fruits of *M. speciosa* (Kitajima et al., 2007).

Mitragynine is the principle alkaloid from the leaves of *M. speciosa* with 66% (Thailand) and 12% (Malaysia) of the total alkaloid contents (Takayama et al., 1998; Chittrakarn et al., 2008; Hassan et al., 2013; Harun et al., 2015). The difference in the alkaloidal content depends on several factors, such as the particular variety and age of the plant, environmental factors, and the time of harvest (León et al., 2009). The percentage of mitragynine varies between younger and older plants, being much more abundant in older plants than the younger ones. In addition, there are minor alkaloids besides the common 9-methoxy-corynanthe-type indole alkaloids in the Malaysian *M. speciosa*, which are not present in the Thai *M. speciosa* (Takayama et al., 1998). Mitragynine has a molecular composition of $C_{23}H_{30}N_2O_4$ (Ramanathan et al., 2015). The structure of mitragynine was first determined by Zacharias et al. (1965) using X-ray crystallography and further confirmed by Liu et al. (2010) in a computational study. Takayama et al. (1995) reported the first synthesis of mitragynine. An alternative synthesis was reported later by Ma et al. (2009). The molecular structures of *M. speciosa* alkaloids were found to be either indoles with a methoxy group in the C19 position and an open E ring with substitution at C9 position, or oxindoles with a closed E ring and no substitution at C9 position (Beckett et al., 1966; Shellard and Phillipson, 1966; Takayama et al., 2002). Mitragynine is a white amorphous powder with solubility in alcohol, chloroform and acetic acid and it is chemically known as 9-methoxy-corynantheidine. There are many other alkaloids which are structurally related to mitragynine that includes 7-hydroxy-mitragynine, speciogynine, speciociliatine and paynantheine (Hassan et al., 2013; Fig. 1).

Takayama et al. (1995) reported the first total synthesis of mitragynine. The total synthesis was initiated using optically pure alcohol (R)-(3) which was prepared by enzymatic hydrolysis of the racemic acetate or through enantioselective reduction of ketone derivative. Later, alternative total synthesis of mitragynine using 4-methoxytryptophan was reported (Ma et al., 2007, 2009). Initially, 4-methoxytryptophan was prepared *via* a Mori-Ban-Hagedus indole synthesis which involved the radical-mediated regioselective bromination of indoline. Then, a more efficient route called regiospecific Larock heteroannulation was introduced to produce

the tryptophan derivative. This route utilized asymmetric Pictet-Spengler reaction and $Ni(COD)_2$—mediated cyclization steps (Ma et al., 2007, 2009). However, these total synthesis of mitragynine are laborious (18–23 steps), time-consuming and not economical. Moreover, the total synthesis of mitragynine produce low yield which is approximately 3–13% (Isabel, 2012). Therefore, semi-synthetic approach or isolation of mitragynine from plant are more cost effective than to prepare mitragynine *via* total synthesis.

6. Pharmacokinetics

A bioavailability study found that mitragynine and 7-hydroxy-mitragynine have moderate permeability across human colonic adenocarcinoma (Caco-2) and Madin Darby Canine Kidney (MDCK)-transfected with the *MDR1* gene (MDR-MDCK) monolayers with no significant efflux. However, another minor constituent, mitraphylline showed a significant efflux mediated by P-glycoprotein in both Caco-2 and MDR-MDCK monolayers (Manda et al., 2014). Using equilibrium dialysis, these compounds exhibited plasma protein binding of more than 90%. One of the earliest research done on the pharmacokinetic of mitragynine was carried by Janchawee et al. (2007). A simple high performance liquid chromatography method with ultraviolet detection (HPLC–UV) was developed to measure mitragynine in rats plasma sample. After a single oral administration of 40 mg/kg mitragynine, mitragynine was found to be rapidly absorbed. The maximum serum concentration (C_{max}) of 0.63 ± 0.18 μg/mL was achieved at 1.83 ± 1.25 h (T_{max}) with an absorption rate constant (ka) of 1.43 ± 0.90 h^{-1}. Mitragynine had a high volume of distribution (Vd/F, 89.50 ± 30.30 L/kg). This may be due to its distribution to highly perfused and lipid containing tissues, especially the brain, which is its site of action. It was slowly eliminated with an elimination rate constant (λz) of 0.07 ± 0.01 h^{-1} and a clearance (Cl/F) of 1.60 ± 0.58 L/h. The half-life of absorption $(t_{1/2}$ ab) and elimination $(t_{1/2}$ $\lambda z)$ were 0.48 ± 0.36 and 9.43 ± 1.74 h, respectively. The mean residence time (MRT0 → ∞) was 14.00 ± 2.84 h. In a separate study, de Moraes et al. (2009) had described a method to detect mitragynine in rat plasma using HPLC and tandem mass spectrometry (LC–MS/MS). In this study, an oral dose of 20 mg/kg mitragynine led to maximum serum concentration (C_{max}) of 0.42 ± 0.06 μg/mL after a T_{max} of 1.26 h. The half-life of absorption $(t_{1/2}$ ab) and elimination $(t_{1/2}$ $\lambda z)$ were 0.28 ± 0.095 and 3.85 ± 0.51 h, respectively. Total clearance was 6.35 L/h/kg. Mitragynine could still be quantified in the plasma after 24 h (de Moraes et al., 2009). A detailed pharmacokinetic profile of mitragynine after oral and intravenous administration was determined by Parthasarathy et al. (2010). The mitragynine was determined in the plasma with solid-phase extraction and rapid HPLC–UV analysis. After intravenous administration of 1.5 mg/kg, the concentration peaked at 1.2 ± 1.1 h (T_{max}) with 2.3 ± 1.2 μg/mL (C_{max}) followed by a biphasic elimination with a $t_{1/2}$ of 2.9 ± 2.1 h and a total clearance of 0.29 ± 0.27 L/h/kg. The volume of distribution (Vd/F) was relatively small 0.79 ± 0.42 L/kg indicating that mitragynine is not distributed into tissue compartments. The bioavailability of mitragynine through intravenous administration was reported to be complete. However, in contrast to intravenous application, the oral absorption of mitragynine was shown to be prolonged and incomplete with an oral bioavailability of around 3.03 ± 1.47%. After oral administration of 50 mg/kg mitragynine, C_{max} was 0.7 ± 0.21 μg/mL after T_{max} 4.5 ± 3.6 h with $t_{1/2}$ of 6.6 ± 1.3 h. The apparent total clearence was 7.0 ± 3.0 L/h/kg. The bioavailability of mitragynine through inhalation and the bioavailability of other analogues are yet to be explored.

The pharmacokinetic study of mitragynine was carried out in healthy human volunteers who are kratom chronic users. From data of nine subjects, mitragynine levels decline by exponen-
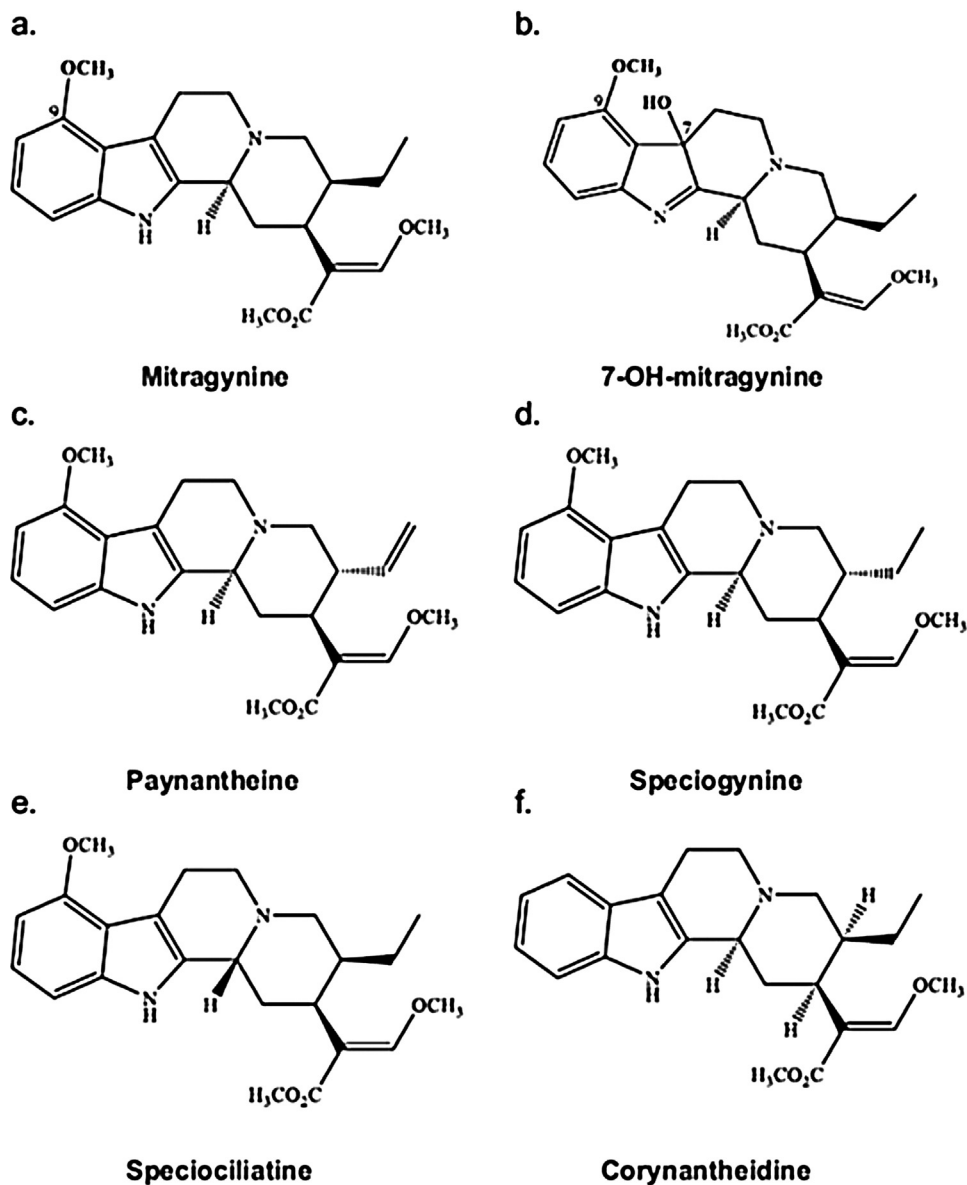
Fig. 1. Chemical structure of mitragynine and its major analogues. (Reprinted with permission from Hassan et al., 2013).

tially suggesting it followed the oral two-compartment model. The maximum plasma concentration was for T_{max} 0.83 ± 0.35 h with terminal $t_{1/2}$ (23.24 ± 16.07 h), and the apparent volume of distribution (38.04 ± 24.32 L/kg). The urine excretion of unchanged mitragynine was 0.14%. However the metabolites were not screen in this study (Trakulsrichai et al., 2015).

The physicochemical properties of mitragynine was recently documented (Ramanathan et al., 2015). The pKa of mitragynine measured by conventional ultraviolet (UV) detection at 248 nm (8.11 ± 0.11) was in agreement with the microplate reader determination (8.08 ± 0.04). Mitragynine is a lipophilic alkaloid, as indicated by a logP value of 1.73. Mitragynine had poor solubility in water and basic media, and conversely in acidic environments, but it is acid labile. In *in vitro* dissolution, the total drug release was higher for the simulated gastric fluid but was prolonged and incomplete for the simulated intestinal fluid. The hydrophobicity, poor water solubility, high variability of drug release in simulated biological fluids and acid degradable characteristics of mitragynine probably explain the large variability of its pharmacological responses reported in the literature (Ramanathan et al., 2015).

Metabolism studies of mitragynine on phase I and phase II in rat and human urine have been reported by Philipp et al. (2009). Seven of the phase I metabolites have been identified which indicates that the metabolic pathways of mitragynine for rats and humans were *via* hydrolysis of the methylester at position 16 and *O*-demethylation of the 9- and 17-methoxy group. These metabolites were from either *via* aldehyde intermediates or oxidation to carboxylic acids or reduction to alcohol metabolites. In rats, five of the phase II metabolites have been identified which are four glucuronides and one sulfate which were conjugated products of the phase I metabolites. Meanwhile, in humans, six of the phase II metabolites have been identified which are three glucuronides and three sulfates (Philipp et al., 2009).

There is a possibility of drug-drug interactions when mitragynine and 7-hydroxy-mitragynine are co-administered with drugs that are P-glycoprotein substrates. Both mitragynine and 7-hydroxy-mitragynine inhibited P-glycoprotein with EC_{50} values of 18.2 ± 3.6 μM and 32.4 ± 1.9 μM, respectively, determined by the calcein-AM fluorescent assay (Manda et al., 2014). Administration of mitragynine and its crude alkaloid extract also

hinder the metabolism of permethrin since both were able to bind to the carboxylesterase enzyme. Hence, there is an increased risk of permethrin toxicity (Srichana et al., 2015). Since mitragynine has been reported to inhibit cytochrome P450 2C9 ($IC_{50} = 9.701 \pm 4.80$ mM), 2D6 ($IC_{50} = 0.45 \pm 0.33$ mM) and 3A4 ($IC_{50} = 41.32 \pm 6.74$ mM) enzyme activities, drug interactions may occur when mitragynine and other drugs that are metabolized by the same enzymes, particularly CYP206, are given concomitantly (Hanapi et al., 2013). With respect to phase II drug metabolism, the possibility of drug-drug interaction may happen if 7-hydroxymitragynine, ketamine and buprenorphine are administered together with drugs that are UG72B7 substrates since these three drugs have been reported to exhibit significant inhibition on human UG72B7 enzyme activity(Haron and Ismail, 2015).

7. Detection of breakdown products of mitragynine

The exposure or abuse of *M. speciosa* can be confirmed by the presence of mitragynine and its metabolites in urine samples. This compound can be detected by gas chromatography coupled with mass spectrometry (GC–MS) (Kaewklum et al., 2005), liquid chromatography with linear ion trap mass-spectrometry (Philipp et al., 2009, 2010a, 2010b; Arndt et al., 2011) or with electrospray tandem mass spectrometry (Lu et al., 2009; Le et al., 2012). Another study did a comparison of three chromatographic techniques using GC with MS, supercritical fluid chromatography (SPC) with diode array detection, and HPLC with MS and diode array detection. Both HPLC and SPC method could resolve mitragynine apart from its diastereoisomers, speciogynine, and speciociliatine. GC however could not fully distinguish these three diastereoisomers as the spectra of the EI, ESI, and ESI MS/MS is nearly identical (Wang et al., 2014).

Mitragynine has been reported to be metabolized to 7-hydroxy-mitragynine, 5-desmethylmitragynine and 17-desmethylhydromitragynine in human urine (Le et al., 2012). A stability study showed that mitragynine was unstable in simulated gastric fluid with 26% degradation but stable in simulated intestinal fluid. 7-hydroxy-mitragynine degraded up to 27% in simulated gastric fluid, which could account for its conversion to mitragynine (23%), while only 6% degradation was seen in simulated intestinal fluid. Mitraphylline was stable in simulated gastric fluid but unstable in simulated intestinal fluid (13.6% degradation). Mitragynine was found to be metabolically stable in both human liver microsomes and S9 fractions. In contrast, both 7-hydroxy-mitragynine and mitraphylline were metabolized by human liver microsomes with $t_{1/2}$ of 24 and 50 min, respectively (Manda et al., 2014).

8. Toxicology

The toxicology of mitragynine and analogues have been reviewed recently (Ramanathan and Mansor, 2015). In animal models, mitragynine showed a relatively low toxicity (Macko et al., 1972; Sabetghadam et al., 2013b). Azizi et al. (2010) reported that oral doses of total alkaloid extract of *M. speciosa* at 200 mg/kg caused lethality in rats. Janchawee et al. (2007) also reported that a single dose of mitragynine (200 mg/kg) given orally caused death in rats.

Another study demonstrated that oral treatment of methanolic extract of *M. speciosa* at 100, 500 and 1000 mg/kg for 14 days caused no changes in food and water intake, behaviour, hematological status and organ weights, but increased the blood pressure after one hour of administration in rats. Biochemical studies also revealed an increase in alanine transaminase (ALT) and aspartate aminotransferase (AST), triglyceride, albumin and cholesterol, regardless of the

doses. However, only the highest dose caused acute severe hepatotoxicity and mild nephrotoxicity (Harizal et al., 2010). Sabetghadam et al. (2013a) reported an LD_{50} of 477 mg/kg for mitragynine and 591 mg/kg for alkaloid extract in mice. The therapeutic index for the alkaloid extract and for mitragynine was estimated as 3:1 and 20:1, respectively, suggesting that mitragynine is relatively safer compared to the alkaloid extract. The authors also reported that mitragynine treatment at 100 mg/kg for 28 days led to hepatotoxicity as evidenced by the increase of ALT and AST. Mild kidney toxicity with a significant increase in serum levels of urea was also observed. Histopathological examination revealed brain abnormalities as indicated by local vacuolation, necrotic and degenerating neurons in the 100 mg/kg subchronic regimen in both female and male rats (Sabetghadam et al., 2013b). No signs of toxicity, such as haemorhage and infiltration of inflammatory cells, were observed for heart, lung, and spleen.

A recent study showed that rats when orally administered with 100, 200, and 500 mg/kg of the standardized methanolic extract of *M. speciosa* (SMEMS) for 28 days, had an altered body weight compared to control group. Biochemistry findings showed that liver and kidney were affected with the abnormal values in AST, creatinine, globulin, glucose, total protein, and urea. However, SMEMS produced toxic effect more to liver, kidney, and lung than other organs as observed histopathologically. The results suggested subchronic exposure of methanolic extract is toxic to the animals (Ilmie et al., 2015). However, chronic studies of mitragynine and its extract are needed in order to understand the effects of long term exposure.

To date, there have been no reports of fatal overdose of Kratom *per se*. If there are such occurrences, they are probably the result of Kratom products contaminated with synthetic adulterants. For instance, Kroonstad et al. (2011) reported nine fatal cases involving adulterated Kratom products. The product known as "Krypton" consists of powdered Kratom leaves and mu-opioid receptor agonist, *O*-desmethyltramadol was detected in the post-mortem blood samples. Despite limited data involving fatal cases through consumption of adulterated Kratom products, serious adverse reactions have been reported in several cases (Ramanathan and Mansor, 2015). A typical case of adverse reactions related to Internet access to kratom was reported by Roche et al. (2008), where the user did not report taking it together with other drugs or substance of abuse. The patient experienced foaming at the mouth and seizure-like movements and later developed fever, aspiration, pneumonia and presented an episode of hypotension in response to intravenous fluids. Another case characterized by seizure and coma was reported by Nelsen et al. (2010). The hepatic toxicity in the form of intrahepatic cholestasis was reported after intake of powdered Kratom with escalating doses for 2 weeks in the absence of any other drugs. Mitragynine and its metabolites were detectable in the urine sample 2 weeks after cessation of drug use (Kapp et al., 2011).

Taken together, the above findings suggest that various *M. speciosa* preparations and consumption may be toxic and could be potentially lethal depending on the dose, duration and possible herb-drug interactions.

9. Pharmacology

9.1. Receptor interactions

Previous studies have suggested that mitragynine acts as an opioid receptor agonist with high affinity to μ-opioid receptors (Yamamoto et al., 1999; Watanabe et al., 1997). Mitragynine pseudoindoxyl, its derivative compound also demonstrates potent opioid agonistic properties *in vitro* (Yamamoto et al., 1999). Mitragynine exhibited its antinociceptive effects *via* supraspinal μ- and δ-opioid receptors in both *in vivo* and *in vitro* studies (Babu et al.,

2008; Thongpradichote et al., 1998; Tohda et al., 1997; Matsumoto et al., 1996a). A recent study by Shamima et al. (2012) further confirmed that mitragynine acts *via* opioid receptors as the administration of naloxone, a non- selective opioid receptor antagonist completely reversed the antinociceptive effects of mitragynine. Mitragynine also acts *via* δ opioid receptors since blockade by naltrindole, a δ-opioid antagonist yields the same result. However, this study also showed that mitragynine acts partially on κ-opioid receptors (Shamima et al., 2012).

Meanwhile, a competitive binding study has shown that mitragynine has different affinity to different opioid receptor subtypes. Mitragynine exerts the highest affinity to κ-opioid receptors followed by μ- and δ-opioid receptors. These differences in binding affinity may be due to the differences in interaction between polar structures of mitragynine with a set of N-termini and carboxyl (COOH) transmembrane 4 and extracellular loop 2 and 3 located at the membrane which differentiate between the μ-, κ- and δ-opioid receptors (Taufik Hidayat et al., 2010). In addition, central opioid receptors may also be involved in mediating the effects of mitragynine, particularly on its psychoactive effects of mitragynine.

At cellular level, mitragynine blocked neuronal Ca^{2+} channels, which partly contributes to the inhibition of neurotransmitter release from the nerve endings at the vas deferens. The neuronal Ca^{2+} channel-blocking effect of mitragynine is believed to be a general mechanism for other physiological effects (Matsumoto et al., 2005b). Mitragynine was also found to inhibit forskolin-stimulated cAMP formation *in vitro* which can be blocked by the opioid receptor antagonist, naloxone (Tohda et al., 1997; Jamil et al., 2013). Study by Fakurazi et al. (2013) demonstrated that repeated exposure to mitragynine and morphine concomitantly caused a reduction in the expression of cAMP and CREB protein level. However, previous studies mainly focused on the interactions of mitragynine with opioid receptors. Present findings do not rule out involvement of other receptors which are crucially involved in *e.g.* psychostimulant action. Thus, studies on other receptor interactions are warranted.

9.2. Pharmacological effects

Many scientific reports provide accumulating evidences that active compounds present in *M. speciosa* produce a variety of pharmacologic effects, both *in vivo* and *in vitro*. One of the pharmacologic effects include the inhibition of ileum (Watanabe et al., 1997) and vas deferens contraction (Matsumoto et al., 2005b) as well as the inhibition of gastric acid secretion (Tsuchiya et al., 2002) which is comparable to the actions of morphine.

9.3. Antidepressant activity

Chronic administration of *M. speciosa* extract induced Fos expression in the dorsal raphe nucleus, the major source of serotonergic projections in the brain. However, acute administration of *M. speciosa* extract only caused a slight increase in Fos expression (Kumarnsit et al., 2007b). Meanwhile, single administration of *M. speciosa* extract reduced the duration of immobility in forced swim test indicating that the extract has antidepressant-like activity. Thus, it seems likely that at least some of the immunoreactivity or behavioural effects of *M. speciosa* extract were mediated *via* activation of the dorsal raphe nucleus (Kumarnsit et al., 2007b).

Farah Idayu et al. (2011) further proved that mitragynine possesses antidepressant-like effect as supported by the significant reduction in corticosterone levels in mice exposed to the forced swim test and tail suspension test. The authors suggested that the antidepressant-like action of mitragynine might be mediated *via* restoration of monoamine neurotransmitter levels including serotonin, noradrenaline and dopamine, and/or *via* interaction

with neuroendocrine hypothalamic-pituitary-adrenal axis systems (Farah Idayu et al., 2011). In addition, repeated treatments of *M. speciosa* extract for seven days increased the time spent in open arm of elevated plus-maze, indicating the anxiolytic-like effects of *M. speciosa* extract (Moklas et al., 2013). In another study, anxiolytic-like effects of acute mitragynine was also observed in the open field and elevated plus maze tests (Hazim et al., 2014). Recent study further supported the anxiolytic-like effects of acute mitragynine in a light/dark box and elevated plus-maze (Yusoff et al., 2016).

δ opioid receptor agonists was found to produce antidepressant-like effects in the forced swimming test of animal model (Saitoh and Yamada, 2012). In addition, δ opioid receptor knockout mice also demonstrated increased levels of anxiety and depressive-like behaviour but not for μ- and κ- opioid receptors (Filliol et al., 2000). The effects of AZD2327, a δ opioid receptor agonist, was reported to have anxiolytic-like properties in prenatal stress rodents, but no statistically significant differences between drug and placebo groups in rating scale scores for either anxiety or depression in participants with anxious depression (Richard et al., 2016). Evidence from animal studies indicate that mitragynine acts on μ-, κ- and δ-opioid receptors (Shamima et al., 2012; Taufik Hidayat et al., 2010; Babu et al., 2008; Thongpradichote et al., 1998; Tohda et al., 1997; Matsumoto et al., 1996a). These could probably explain mitragynine exerts an antidepressant-like effects.

9.4. Gastrointestinal effects

The methanolic extract of *M. speciosa* reduced the defecation frequency and faecal weight in castor oil-induced diarrhoea in rats. However, the methanolic extract of *M. speciosa* may affect mechanisms other than opioid-receptor-mediated since naloxone pre-treatment showed no effect on the inhibition of the defecation frequency and faecal weight. A single dose of the methanolic extract of *M. speciosa* also resulted in a dose-dependent reduction of the intestinal transit, which is the time taken for the ingesta to pass through the gastrointestinal tract. Repeated treatments with this extract, however, did not cause any significant change of the intestinal transit and fluid (Chittrakarn et al., 2008). Subcutaneous 7-hydroxy-mitragynine also caused an inhibition of the gastrointestinal transit in mice (Matsumoto et al., 2006). Meanwhile, central administration of mitragynine into the lateral ventricle did not alter basal gastric acid secretion. Administration into fourth ventricle of anesthetized rats, however, caused an inhibition of 2-deoxy-ᴅ-glucose-stimulated gastric acid secretion in a dose dependent manner. This inhibition was reversed by naloxone, thus suggesting an involvement of opioid receptors.

9.5. Anorectic effect

Acute administration of the alkaloid extract of *M. speciosa* in rats significantly reduced food and water intake while chronic administration caused a prolongation in these reductions and eventually led to a suppression of body weight gain (Kumarnsit et al., 2006). The level of cholecystokinin, a peptide hormone of the gastrointestinal system which is associated with hunger suppression, was not affected by the methanolic extract of *M. speciosa*. These findings suggest that the anorectic effect of the plant extract may be attributed to other factors (Chittrakarn et al., 2008). Tsuchiya et al. (2002) suggested that the anorectic effects of *M. speciosa* is related to a direct inhibition of neurons in the lateral hypothalamus or with an addictive disorder.

9.6. Antinociceptive effects

Extensive studies have been performed to investigate the antinociceptive properties of *M. speciosa* (Matsumoto et al., 1996a,

b, 2004, 2005a, 2006; Reanmongkol et al., 2007; Shaik Mossadeq et al., 2009). Methanolic and alkaloid extracts from *M. speciosa* exert antinociceptive activity in mice. However, oral administration of both methanolic and alkaloid extracts only prolonged the latency of nociceptive responses to noxious stimulation in hot-plate test with higher potency in methanolic extracts, but not in tail-flick test. The antinociceptive action could be blocked by naloxone, thus suggesting the action *via* opioid receptors (Reanmongkol et al., 2007). In accordance with these findings was a study by Shaik Mossadeq et al. (2009) who showed that intraperitoneal administration of methanolic extract of *M. speciosa* in mice increased the latency to nociceptive responses in the hot-plate test. Acetic-acid-induced writhing test and formalin test further proved that the methanolic extract of the plant has an antinociceptive activity as it significantly inhibits the writhing responses and pain sensation in both tests (Shaik Mossadeq et al., 2009). Another study showed that oral administration of alkaloid (20 mg/kg), methanolic (200 mg/kg) and aqueous (400 mg/kg) extracts of *M. speciosa* prolonged the latency to nociceptive response in both hot-plate and tail-flick tests. The antinociceptive effects of these extracts could be blocked by pretreatment with naloxone (Sabetghadam et al., 2010).

A recent study compared *M. speciosa* and its active component, mitragynine, against the well-known and commonly abused opioids, morphine and oxycodone, on thermal nociception in rats. In this study, mitragynine exhibited antinociceptive effects similar to oxycodone when administered both intraperitoneally (i.p) and orally. *M. speciosa* exhibited a trend towards antinociceptive effects when administered both i.p and orally. This research demonstrated that *M. speciosa* possesses properties like oxycodone and raises the possibility of an abuse liability which might warrant consideration for restrictions on the consumer marketplace (Criddle, 2015; Carpenter et al., 2016).

7-Hydroxy-mitragynine, a minor constituent of *M. speciosa*, has been found to have more potent antinociceptive activity than morphine in tail-flick and hot-plate tests when administered orally or subcutaneously. The higher potency and rapid effect of 7-hydroxymitragynine might be attributed to its strong lipophilicity and easy penetration of the blood brain barrier. However, 7-hydroxymitragynine is actually more polar than mitragynine which makes it more difficult to cross the blood brain barrier (Matsumoto et al., 2004, 2006). The antinociceptive effects of 7-hydroxy-mitragynine were dose-dependent and primarily mediated through μ1-opioid receptors since blockade of this receptor completely abolished the antinociceptive effects in both tail-flick and hot-plate tests (Takayama, 2004). In addition, supraspinal δ- (Matsumoto et al., 2006) and κ-opioid receptors (Matsumoto et al., 2005a) were also considered to be partially responsible for the antinociceptive activity of 7-hydroxy-mitragynine.

A dual-acting μ- and δ-opioid agonist derived from 7-hydroxymitragynine and MGM-16 (7-hydroxy-mitragynine and (E)-methyl 2-((2S,3S,7aS,12aR,12bS)-3-ethyl-9-fluoro-7a-hydroxy-8-methoxy-1,2,3,4,6,7,7a,12,12a,12b-decahydroindolo[2,3-a]quinolizin-2-yl)-3-methoxyacrylate) showed potent anti-allodynic effect on neuropathic pain in mice. It has high affinity to both μ- and δ-opioid receptors with Ki values of 2.1 and 7.0 nm respectively (Matsumoto et al., 2014).

In general, studies have shown that *M. speciosa* and its preparations possessed various pharmacological activities with the focus on antinociception, antidepression and antiinflammation. However, mechanisms underlying the pharmacological activities of *M. speciosa* need to be elucidated.

9.7. Abuse potential effects

M. speciosa has been claimed to possess both narcotic and stimulant-like effects, which both contribute to an abuse poten-

tial (Suwanlert, 1975). The users claimed that they became happy, strong and active after five to ten minutes of *M. speciosa* consumption (Hassan et al., 2013). The psychomotor stimulant effects urged them to continue consuming the plant until it developed into a habit. Cheapness and easy access to this plant may contribute to the gradual increase of the user's daily dosage (Suwanlert, 1975; Chan et al., 2005; Vicknasingam et al., 2010).

Aziz and Latiff (2006) conducted drug discrimination procedures in rats to identify the psychoactive class of Kratom. Rats were trained to discriminate between kratom extract and saline. Thereby kratom exerted only a weak control over differential lever responding compared to more readily discriminable drugs such as D-amphetamine and pentobarbital.

A recent study in rats demonstrated that the discriminative stimulus effect of mitragynine depend on both opioid- and psychostimulant-like subjective. Rats acquired the mitragynine discrimination (15.0 mg/kg, i.p.) which was similar to the acquisition of morphine discrimination (5.0 mg/kg, i.p.) in another group of rats. Mitragynine also substituted fully to the morphine discriminative stimulus in a dose-dependent manner, suggesting pharmacological similarities between the two drugs. The administration of 7 hydroxy-mitragynine (3.0 mg/kg, i.p.) engendered full generalisation to the morphine discriminative stimulus. The mitragynine stimulus also partially generalised to a cocaine (10.0 mg/kg, i.p.) stimulus (Harun et al., 2015).

In humans, chronic consumption of *M. speciosa* preparations is usually followed by withdrawal symptoms such as hostility, aggression, excessive tearing, inability to work, aching of muscle and jerky limb movements (Hassan et al., 2013; Singh et al., 2014). It can also be accompanied by anorexia, weight loss, insomnia, skin pigmentation particularly on cheeks, dry mouth, frequent micturition and constipation with blackish stools. In some cases, psychotic symptoms such as confusion and delusion were reported (Sheleg and Collins, 2011). *M. speciosa* dependence also produced withdrawal symptoms like anxiety, restlessness, tremor, sweating and craving in an old man with a history of alcohol and anxiety disorder (McWhirter and Morris, 2010). This is in line with reports in animals that describe opioid-like somatic withdrawal, locomotor hypersensitivity after single drug stimulation and enhanced anxiety levels following withdrawal from chronic mitragynine (Yusoff et al., 2016).

Drug tolerance occurs when a dose of specific drug no longer gives the same reactions and a higher dose is required to produce the desired effects. 7-hydroxy-mitragynine and (E)-methyl 2-(3-ethyl-7a, 12a-(epoxyethanoxy)-9-fluoro-1,2,3,4,6,7,12,12b-octahydro-8-methoxyindolo[2,3-a]quinolizin-2-yl)3methoxyacrylate (MGM-9), a derivative of mitragynine, induced tolerance in mice after repeated administration for 5 consecutive days. This was shown by the significant reduction of the analgesic effect of each substance. The anti-nociceptive tolerance was mediated by μ-opioid receptors for 7-hydroxy-mitragynine, but both μ- and κ-opioid receptors for MGM-9 (Matsumoto et al., 2005a, 2008).

Accumulating evidences suggest that *M. speciosa* may be beneficial to mitigate the harshness of drug withdrawal. Kumarnsit et al. (2007a) demonstrated that aqueous extract of *M. speciosa* reduced the ethanol withdrawal-induced behaviours such as rearing and head weaving. Another study demonstrated that the alkaloid extract from *M. speciosa* alleviated ethanol withdrawal severity with no side effect on rapid eye movement (REM) sleep. The crude alkaloid extract from *M. speciosa* was found to produce anti-depressant activities. It was hypothesized that the alkaloid extract from *M. speciosa* may attenuate ethanol withdrawal without REM sleep disturbance. In this study, adult male Wistar rats implanted with electrodes over the frontal and parietal cortices were used for two separated studies. For an acute study, 10 mg/kg

fluoxetine or 60 mg/kg alkaloid extract from *M. speciosa* were administered intragastrically. EEG signals were recorded for 3 h to examine sleep profiles and EEG fingerprints. Another set of animal was used in an ethanol withdrawal study. They were rendered dependent on ethanol *via* a modified liquid diet (MLD) containing ethanol ad libitum for 28 days. On day 29, fluoxetine (10 mg/kg) or alkaloid extract from *M. speciosa* (60 mg/kg) were administered 15 min before the ethanol-containing MLD was replaced with an isocaloric ethanol-free MLD to induced ethanol withdrawal symptoms. The sleep analysis revealed that alkaloid extract from *M. speciosa* did not change any REM parameters which included average duration of each REM episode, total REM time, number of REM episode and REM latency whereas fluoxetine significantly suppressed all REM parameters and delayed REM latency. However, power spectral analysis revealed similar fingerprints for fluoxetine and alkaloid extract from *M. speciosa* characterized by decreasing powers in the slow frequency range in frontal and parietal cortical EEG. Neither treatment affected spontaneous motor activity. Finally, alkaloid extract from *M. speciosa* or fluoxetine were found to significantly attenuate ethanol withdrawal-induced hyperexcitability (increases gamma activity) in both cortices and to reduce locomotor activity. In addition, these data suggest that suppressive effects on slow frequency power, but not REM sleep may be hallmarks of effective antidepressants for ethanol withdrawal treatment (Cheaha et al., 2015).

In another study using zebrafish, the effects of mitragynine on anxiety behaviour, cortisol level and gene expression of stress pathway were assessed during the morphine withdrawal phase. Cessation of two weeks chronic treatment of adult zebrafish with morphine caused a decrease in exploratory behaviour, increased erratic movements and elevated whole-body cortisol level. However, exposure to mitragynine attenuated the stress-related swimming behaviours and reduced the whole-body cortisol level in morphine-withdrawn fish. Mitragynine was also able to reduce the mRNA expression of corticotrophin releasing factor receptors and prodynorpin in zebrafish brain, suggesting that mitragynine may be effective in ameliorating opiate withdrawal effects (Khor et al., 2011). Acute anxiolytic effect of mitragynine was also observed in two different tests, the light-dark box and elevated plus maze by Yusoff et al. (2016).

The rewarding properties of kratom metabolites and its derivatives have been elucidated in animal models by Matsumoto et al. (2008) using conditioned place preference (CPP). This associative learning procedure is based on the notion that animals prefer environments previously associated with positively reinforcing substances, such as morphine and other drugs of abuse. The rewarding properties can lead to dependence and addiction (Huston et al., 2013). From the study, 7-hydroxy-mitragynine induced a significant CPP and hyperlocomotion effects in mice, which were suggested to be mediated by μ-opioid receptors. In contrast, MGM-9 did not produce such a rewarding effect, probably due to its dual-acting μ- and κ-opioid agonist properties. Based on the previous studies, systemic administration of a μ-opioid agonist activates dopaminergic system and induces CPP as well as hyperlocomotion effects (Matthes et al., 1996) whereas κ-opioid agonist administration decreases locomotor activity and exhibits place aversion (Kuzmin et al., 2001; Narita et al., 2001).

Using extract-fraction-constituent strategy, Sufka et al. (2014) investigated the putative liabilities of kratom by concomitant screening of kratom extract, kratom alkaloid fraction and mitragynine for their rewarding properties. This approach is believed to reveal, if any, antagonistic or synergistic effects within the kratom extract and fraction, fully characterize the liabilities of mitragynine. From the findings, mitragynine exhibited a robust increase in preference score indicative of a CPP. Meanwhile, kratom extract and its fraction increased preference scores in a lesser degree compared to

mitragynine, which could be due to lower concentration of mitragynine present and/or presence of other psychoactive constituents that affect mitragynine's rewarding effects (Sufka et al., 2014).

In conjunction to the above findings, our group has also demonstrated a significant CPP effect by mitragynine at dose of 10 mg/kg after 8 conditioning trials (Yusoff et al., 2016). Like morphine, mitragynine at all doses tested did not show any increment in locomotor activity after single drug administration. However, mitragynine did not resemble morphine' responses in terms of sensitization development. Morphine increased locomotor activity after the second treatment. In contrast, mitragynine, induced locomotor sensitization only after four treatment trials and only after the highest dose tested (30 mg/kg). It seems that mitragynine may need a higher dose and longer time to develop locomotor sensitization effect compared to morphine. Furthermore, mitragynine did not produce a profound conditioned locomotor effect as elicited by morphine and methamphetamine, suggesting that the mitragynine response is less associated with psychomotor activation. From the CPP studies, it can be concluded that the rewarding properties of mitragynine support the abuse potential of kratom.

It has been established that the CPP effects induced by various classes of drugs of abuse, such as cocaine, methamphetamine and morphine, depend on activation of the mesolimbic dopaminergic system (McCreary et al., 2015). The ventral tegmental area of the midbrain contains the cell bodies of the mesolimbic dopaminergic neurons, which are under tonic inhibition of GABAergic interneurons. Activation of opioid receptors localized on the GABAergic neurons reduces GABAergic neuronal activity and consequently disinhibits dopaminergic system. Increases in the extracellular dopamine releases at particular brain areas including nucleus accumbens, result in reward effects (Sahraei et al., 2009). In our lab, research on some basic aspects of these pathways has been undertaken to study the neurobiology of mitragynine reward. Since pharmacological studies revealed that mitragynine has agonistic effects on opioid receptors (Watanabe et al., 1997; Matsumoto et al., 2005b; Taufik Hidayat et al., 2010), there is a possibility that mitragynine may shares the common reward circuit as above.

Apart from that, the mechanistic analysis of mitragynine action in the brain reward system has been studied following subchronic administration of mitragynine in mice and rats (Yusoff et al., 2016). The locomotor sensitization observed was accompanied by sensitization of the dopamine system in a brain region containing dopamine neurones (mesencephalon) but not in the target areas of their projections (ventral striatum), as reflected by an enhanced expression of dopamine transporter (DAT) and dopamine receptor-regulating factor (DRRF) mRNA. Other potential mechanisms identified for mitragynine addictive behaviours include serotonergic mechanisms, however it should be noted that they are not comprehensive and warrant further investigation (Hassan et al., 2013; Yusoff et al., 2016).

In general, data from both human reports and animal studies suggests that *M. speciosa* extracts and its psychoactive compounds may have a significant abuse and addiction potential through its narcotic and stimulant-like effects.

9.8. Cognitive effects

Emerging evidence has shown that consumption of *M. speciosa* or its psychoactive compounds can alter cognitive functions. Chronic administration of mitragynine (5, 10 and 15 mg/kg, i.p) decreased the performance in an object location task, indicating the impairment of the working memory (Apryani et al., 2010). However, a study by (Hazim et al., 2011) showed a contrasting results. Acute oral administration of an alkaloid extract of *M. speciosa* or mitragynine (20, 40 and 80 mg/kg) did not affect the short-term

memory as no difference was observed in the spontaneous alternation scores in the Y-maze task.

In a passive avoidance task, animals that were orally treated with the methanolic extract of *M. speciosa* (1000 mg/kg) were able to avoid the test environment where it previously received aversive stimulus. This finding suggested that the methanolic extract of *M. speciosa* can facilitate learning. However, the reduction in the step-through latency of a passive avoidance task indicated that memory consolidation was impaired while no changes were observed in the memory consolidation of the two-way active avoidance task (Senik et al., 2012b). Meanwhile, the acute oral ethanolic extract of *M. speciosa* impaired the acquisition of the shuttle box avoidance learning, but not the memory consolidation (Stolt et al., 2014).

Recently, a more comprehensive study was carried out by Yusoff et al. (2016). Acute administration of mitragynine (1, 5 and 10 mg/kg, i.p) impaired all phases of learning and memory, *i.e.* acquisition, consolidation, and retrieval, of the passive avoidance task. These impairments are in line with the disruption of the low-frequency rhythms (delta and theta) in the electroencephalogram of the rats treated with mitragynine. Chronic administration of mitragynine (1, 5 and 10 mg/kg, i.p, 28 days) led to the impairment of the passive avoidance learning that could be seen during early withdrawal and abstinence. However, memory impairment in a new learning task during abstinence was only observed at a dose of 10 mg/kg, suggesting a dose-dependent effect of mitragynine (Yusoff et al., 2016).

Physiological study further supported the effects of *M. speciosa* on the cognition. The methanolic extract of *M. speciosa* was found to produce an irreversible reduction of field excitatory post-synaptic potentials (fEPSP) amplitude with an IC_{50} of 0.008% in the hippocampal slices of rats. The methanolic extract of *M. speciosa* at 0.008% also inhibited long term potentiation (LTP) induction but induced a short-term potentiation. LTP is considered to underlie the learning and memory processes in the brain (Hansen and Manahan-Vaughan, 2015, Shapiro, 2001; Martin and Morris, 2002). Therefore, the finding may elucidate one of the mechanisms underlying the memory-impairing effects of *M. speciosa* (Senik et al., 2012a).

In conclusion, studies in rodents have revealed that consumption of mitragynine or other *M. speciosa* extracts can cause cognitive deficit as observed in different behavioural tasks. However, evidence for the mechanisms underlying the cognitive deficit is still missing and merits further investigations.

Authors' contributions

F.W.S., N.H.M.Y., R.H, S.M.M, C.P.M. and Z.H. wrote the paper. All authors read and approved the manuscript.

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