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# Subchronic exposure to mitragynine, the principal alkaloid of *Mitragyna speciosa*, in rats

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ABSTRACT

*Ethnopharmacological relevance: Mitragyna speciosa* is a popular medicinal plant in Southeast Asia which is commonly used for its morphine-like effects. Although the analgesic properties of *Mitragyna speciosa* and its ability to ameliorate withdrawal signs after abrupt cessation of opioid abuse are well known, information about the long-term safety of the plant's active compounds is lacking. In this work, we evaluated the effects of sub-chronic exposure to mitragynine, the principal alkaloid of *Mitragyna speciosa* leaves in rats. Materials and methods: Male and female Sprague-Dawley rats received three doses of mitragynine (1, 10, 100 mg/kg, p.o) for 28 days respectively. Food intake and relative body weight were measured during the experiment. After completion of drug treatment biochemical, hematological, and histological analyses were performed.

*Results:* No mortality was observed in any of the treatment groups. The groups of rats treated with the lower and intermediate doses showed no toxic effects during the study. However, the relative body weight of the group of female rats treated with the 100 mg/kg dose was decreased significantly. Food intake also tended to decrease in the same group. Only relative liver weight increased after treatment with the high dose of mitragynine (100 mg/ kg) in both the male and female treatment groups of rats. Biochemical and hematological parameters were also altered especially in high dose treatment group which corresponds to the histopathological changes.

*Conclusions:* The study demonstrated that mitragynine is relatively safe at lower sub-chronic doses (1–10 mg/kg) but exhibited toxicity at a highest dose (sub-chronic 28 days: 100 mg/kg). This was confirmed by liver, kidney, and brain histopathological changes, as well as hematological and biochemical changes.



## 1. Introduction

*Mitragyna speciosa* belongs to the family Rubiaceae which originates from south eastern Asia. For many decades the leaves of *Mitragyna speciosa* (MS) have been chewed, smoked or drunk as a boiled syrup, and is known as "Ketum" or "Kratom" by the indigenous people of Malaysia and Thailand. The ethnopharmacological reputation of this plant is mainly for its medicinal uses as an analgesic, its ability to reduce withdrawal symptoms in opioid addicts (Boyer et al., 2008), for treatment of diarrhea and diabetes, and for boosting immunity (Jansen and Prast, 1988). To date, over 40 compounds have been isolated from the leaves of MS grown in different regions (Adkins et al., 2010). Mitragynine, the major indole alkaloid of the plant, was consistently present a different concentrations in leaves collected from various geographical locations (Houghton et al., 1991).

Recently, the pharmacological properties of various MS extracts or mitragynine have been broadly evaluated. Many studies were performed on the antinociceptive effects of MS extracts and mitragynine in appropriate animal model (Macko et al., 1972; Matsumoto et al., 1996a, 1996b; Tohda et al.,1997; Watanabe et al., 1997; Idid et al., 1998; Thongpradichote et al., 1998; Matsumoto et al., 2005; Horie et al., 2005; Reanmongkol et al., 2007; Sabetghadam et al., 2010). MS extracts or mitragynine were also reported to have anti-inflammatory (Mossadeq et al., 2009; Utar et al., 2011) and anti-depressant-like (Kumarnsit et al., 2007b; Farah Idayu et al., 2010) properties in animal models. Impairment of cognitive function (Apryani et al., 2010), improvement in learning with no significant effect on short- and long-term memory (Hazim et al., 2011; Senik et al., 2012) and reduction of opioid withdrawal abstinence signs (Kumarnsit et al., 2007a; Khor et al., 2011) were also observed following treatment with MS extracts or mitragynine in animal models.

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In spite of the broad spectrum of pharmacological effects, few studies have focused on evaluation of the safety profile of MS extracts or mitragynine in vivo (Macko et al., 1972; Watanabe et al., 1992; Reanmongkol et al., 2007; Moklas et al., 2008) or in vitro (Saidin and Gooderham, 2007; Saidin et al., 2008). However, most of the in vivo studies were limited to the acute oral toxicity of MS extracts in animal models (Harizal et al., 2010). In addition, except for a few human case-reports regarding the toxic effect of MS extract following short or long term consumption, clinical toxicological investigations are also scarce. Respiratory depression, coma, pulmonary edema and death were not reported following MS usage although mitragynine is an agonist at mu-opioid receptors (Boyer et al., 2008). However, there was a single case of death reported following consumption of ketum juice cocktail (Tungtananuwat and Lawanprasert, 2010). Other toxicity signs such as seizure and coma (Nelsen et al., 2010) as well as intrahepatic cholestasis (Kapp et al., 2011) were also occasionally reported following MS extract consumption.

Given the therapeutic potential of mitragynine and the increasing consumption of MS extracts for recreational use especially in Malaysia (Vicknasingham et al., 2010; Ahmad and Aziz, 2012), an assessment of the preclinical toxicity study of mitragynine after long term use has become of high importance. Thus, this study has been designed to investigate the sub-chronic (28-days) oral toxicity of mitragynine in male and female Sprague-Dawley rats by evaluating the biochemical, hematological, histopathological changes and the clinical signs of systemic toxicity induced by treatment with mitragynine. Since mitragynine is an agonist at μ opioid receptors, this property is probably responsible for its opiate-like effects such as analgesia and physical dependence (Matsumoto et al., 1996a, 1996b; Tohda et al., 1997; Thongpradichote et al., 1998). Consequently, the potential for development of physical dependence after prolonged treatment with mitragynine in rats was also assessed. Withdrawal signs and their reversibility 2 weeks after a 28 days treatment period with mitragynine were also determined in rats in accordance with Organization for Economic Co-operation and Development (OECD) guideline No. 407.

## 2. Materials and methods

### 2.1. Plant material and mitragynine isolation

Fresh leaves of *Mitragyna speciosa* were collected from Kedah and Perlis and deposited in the Malaysian Institute of Pharmaceuticals and Nutraceuticals (IPHARM) under the specimen voucher number IPHARM-49-35-C1. Mitragynine was isolated by the method previously described by Utar et al. (2011). This compound was obtained from Malaysian Institute of Pharmaceuticals and Nutraceuticals (IPHARM) and had a purity of 94.5% as determined by Centre for Drug Research analytical lab. The pure compound was kept at 4 °C until the time of experiment.

### 2.2. Experimental animals

Male and female Sprague-Dawley rats (5-weeks old) were obtained from the Animal Facility of the Universiti Sains Malaysia and acclimatized in a holding room for at least 7 days before performing any experimental procedure. Female and male rats were assigned to four groups respectively in which each group consisted of 14 rats. In addition, satellite rats (two male and two female) which were treated along with the experimental rats (five male and five female) were used for assessment of physical dependence. The first three groups were treated with mitragynine 1, 10 and 100 mg/kg, respectively. The remaining control group was given the vehicle. All animals were kept in a temperature of 25 ± 1 °C in a light-controlled room (12 h light/dark cycles). Water was provided ad libitum but the animals had restricted access to food.. The amount of food supplied was calculated according to daily food intake of 5 g/100 g body weight for Sprague-Dawley rats (Harkness and Wagner, 1989) based on accumulated body weight of rats in each cage per day. All procedures were approved by the Animal Ethics Committee, Universiti Sains Malaysia.

### 2.3. Drug preparation

Mitragynine was dissolved in 20% Tween-20 solution and freshly prepared daily before starting the experiment. In the present study, mitragynine was administered orally in doses of 1, 10 and 100 mg/kg in a volume of 5 mL/kg. The lowest dose corresponded to the average daily intake of ketum users in Malaysia (Vicknasingham et al., 2010). The highest dose (100 mg/kg, p.o.) was calculated by applying a safety factor of one tenth of the lethal dose (LD50) obtained from our previous study (LD50= 1098 mg/kg, p.o.) (Sabetghadam et al., 2013) and previously reported by Macko et al. (1972) while the medium dose was established (10 mg/kg) accordingly.

### 2.4. Evaluation of sub-chronic toxicity of mitragynine

The experiments were carried out according to OECD guideline No. 407 (OECD, 2008). In order to calculate the daily dosage, both treated and control animals were weighed and general behavior was observed daily for clinical signs of toxicity. Food intake for each cage was recorded daily. On the day after the last treatment (day 29), the animals were fasted overnight before being anesthetized with $CO_2$ according to the procedure described by Deckardt et al. (2007). Animals were sacrificed by cervical dislocation and blood samples were collected for further hematological and biochemical analysis. Selected organs were removed for measurement of the relative weight and for the histopathological analysis. The remaining rats ($n$=4 per group) were kept at the same laboratory conditions for another 2 weeks (day 43) and assessed for development of physical dependence.

#### 2.4.1. Relative body weight

The Relative Body Weight (RBW) of each animal was calculated once a week for 4 weeks during the 28-days of experiment (Days 1, 7, 14, 21, and 28). The RBW was calculated according to Mukinda and Eagles (2010) which is as follows:

$$\text{Relative Body Weight (RBW) of each rat} = \frac{\text{Body weight at each time interval } (g)}{\text{Body weight on Day1 } (g)} \times 100$$

#### 2.4.2. Food intake

Daily food intake of rats in each cage was measured by weighing the left-over food from the amount provided in the last 24 h. Consequently, the food consumption of each cage per body weight during the 24 h was calculated according to the following formula:

$$\text{Food consumption of each cage}(g/\text{kg}/24\ h/\text{cage}) = \frac{\text{Daily food intake of one cage } (g)}{\text{Daily accumulated body weight of one cage (kg)}} \times 100$$

#### 2.4.3. Hematological analysis

All hematological analyses were performed by the Pathology laboratory, Lam Wah Ee Hospital, Penang, Malaysia. Blood samples were collected by cardiac puncture and transferred into heparinized