A Preliminary Toxicity Study of Mitragynine, An Alkaloid from *Mitragyna Speciosa* Korth and its Effects on Locomotor Activity in Rats


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**ABSTRACT**

*Mitragyna speciosa* Korth (Rubiaceae family) is an indigenous tree found in Southeast Asia particularly in Thailand and Malaysia. The plant is popularly known as ‘kratom’ in Thailand and ‘ketum’ in Malaysia. The medicinal properties of this plant had previously reported due to its opium-like effect and cocaine-like stimulant in combating fatigue and as hard work tolerance. This study is designed to evaluate the toxicity level of mitragynine using brine shrimp lethality assay and to study the behavioral effects of mitragynine particularly locomotor activity in rats. The locomotor activity was measured using locomotor box. Male Spraque Dawley rats (*n=28*) were used in this study and administered with a single dose of mitragynine at 1.0, 5.0, 10.0 and 30.0 mg/kg. Mitragynine was found to be toxic to brine shrimp at 44µl/ml, with alkaloid extract (62 µl/ml) and aqueous extracts (98µl/ml) respectively. Interestingly, mitragynine on both lowest and highest dose was found to significantly reduce the locomotor activity in rats. Our results suggest that mitragynine may exhibit sedative effects properties in locomotor activity.

**Key words:** *Mitragyna speciosa*, mitragynine, toxicity, locomotor activity

**Introduction**

*Mitragyna speciosa* Korth, M.S, (Rubiaceae) is a tropical plant indigenous to Southeast Asia particularly in Thailand, Peninsular of Malaysia and Indonesia. This plant is endemic to Southeast Asia[1]. It is widely known as ‘Kratom’ in Thailand and ‘Ketum’ in Malaysia. The leaves have been used by natives for their opium-like effect and cocaine-like stimulant ability to combat fatigue and enhance tolerance to hard work. The alkaloid from M.S had opiate-like effects such as analgesia, antitussive and can cause hypothermia in animals[1]. Besides, the leaves of the plant had been used in treating fever, malaria, diarrhea, as tonic and as a substitution for morphine in treating addicts[2]. This plant is also used to alleviate pain, coughing and to counter fatigue[2]. Other *Mitragyna* species, *M. africanus* which is one of an African species is used in Nigeria to treat mental illness. Other African species, *M. ciliare* is used for inflammation, hypertension, headache, rheumatism, gonorrhea and broncho-pulmonary diseases. Jansen and Prast[1] found that mitragynine was obtained from the young leaves of *Mitragyna speciosa* as the major constituent (66.2% based on the crude base) together with its analogues, speciogynine (6.6%), speciociliatine (0.8%), and paynantheine (8.6%).Due to its unique medicinal properties, a number of chemical and pharmacological studies have been carried out over...
the last forty years. In order to study the effectiveness of mitragynine as potential psychostimulant, behavioral study specifically locomotor activity of lab animals is a tool to assess the behavioral effects of the drugs. The activity is useful and less robust than most behavioral test such as operant conditioning and radial arm maze. It is often used in primary evaluations of drugs. Thus, the present study was designed to evaluate the effectiveness of mitragynine as a potential psychostimulant by measuring the behavioral of the rats particularly the locomotor activity.

Materials and methods

**Brine shrimp lethality test**

The brine shrimp lethality test was used to predict the presence of cytotoxic activity in the extracts[3,4] with some modifications. Brine shrimp eggs was obtained locally and hatched in artificial sea water in a designed tank for 24 hours. The incubation process was done under provided light and warmth. The process required 7 liters of distilled water with 1.75 ml water conditioner (Sera Aqumarin). 175 gram sea salt was added into the water to maintain the pH level of the water at pH 7 to 8. The water pump was placed at the corner of the water tank. The brine shrimp eggs was added into the tank and left for 24 hours for hatching process to take place. The brine shrimp were taken out after 24 hours hatching process and put into the micro well plates. The brine shrimp had been exposed to three different levels of M.S extracts in micro well plates with a mortality endpoint after 24 hours. Then, the extract was added into the brine shrimp well according to the desired concentration of the extracts. The extracts were tested at 10-100 µl/ml. In each case, triplicates of each concentration were assayed. The mortality rate of the brine shrimp was analyzed after 24 hours under the microscope and the LC50 of the extract was determined.

**Behavioral study using locomotor box**

**Subjects**

Male Spraque Dawley rats (n=40) with the weight ranging from 250-300 grams, were used in this study. The rats were acclimatized for at least 5 days prior to the experiment with food and water were given ad libitum. All procedures described were reviewed and approved by the Animal Care and Use Committee (ACUC), Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia.

**Drugs**

Dosages of mitragynine (1.0, 5.0, 10.0 and 30.0 mg/kg) were dissolved in 20% Tween 80, and vehicle was given as control injection. Compounds were administered via intraperitoneal injection 30 to 40 minutes prior to the experiment. d-amphetamine (Lipomed Inc, USA) was dissolved in normal saline, was injected intraperitoneally at 2mg/kg to the rats for comparison between treated groups. All drugs solutions were prepared immediately prior to the experiment.

**Locomotor box**

Locomotor activity assessment in an open-topped boxed area is a procedure that has been used routinely to assess behaviour of rats[7]. Assessment of locomotor activity was carried out in a quiet and dimly room in the morning according to Ainsah et al[8] with some modifications. Temperature, sound and light conditions were maintained uniform during the course of the experiment. The apparatus consisted of square boxes (75 x 75 x 42 cm) where the floor of the box was marked into a grid and subdivided into a small square box measuring 15 x 15 cm squares.

**Procedure**

Rats were randomly assigned to three different dosages groups of mitragynine and vehicle as control group. The rats were initially accustomed to the experimental room for at least 30 minutes prior to the experiment, following 1 hour observation of locomotor activity in the box. The rats were placed in the centre of the grid and for 1 hour the number of squares entered with all four paws was recorded using a video camera mounted on the ceiling. A monitor was placed in the adjacent room, enabling assessments to be made immediately. Before the next assessment, the rat was removed and the box was cleaned with a damp cloth. The video recordings were kept to provide a permanent record.

**Results and discussion**

**Brine shrimp lethality test**

After 24 hours mortality endpoint, the toxicity assessment of three different type of extracts concentration of M.S was analyzed. The aqueous extract of M.S (Fig.1.) showed the lowest toxicity activity against brine shrimp with LC50 values at 98µl/ml. The crude alkaloid of the plant (Fig.2.) exhibited intermediate toxicity activity against brine shrimp larvae with LC50 values at 62µl/ml whilst mitragynine (Fig.3.) as a major alkaloid exhibited relatively high toxicity to the brine shrimps with LC50 at 44µl/ml.

**Locomotor activity scores**

The alkaloid of *Mitragyna speciosa* leaves, mitragynine (1.0, 5.0, 10.0 and 30.0 mg/kg, i.p)
Fig.1: LC₅₀ was determined at 98 µl/ml of dosage concentration of aqueous extract of *Mitragyna speciosa*.

Fig.2: LC₅₀ was determined at 62 µl/ml of dosage concentration of crude alkaloid of *Mitragyna speciosa*.

Fig.3: LC₅₀ was determined at 98 µl/ml of dosage concentration of mitragynine.

showed significantly changed the locomotor activity in rats which the compound reduced the activity. As shown in Table 1, the mitragynine neither highest nor lowest dose significantly changed the locomotor activity in rats, while amphetamine, a CNS stimulant, significantly increased the locomotor activity, compared with the vehicle group (saline). Fig. 4 showed the events of moving activity among the treated groups which vehicle group (Tween 80) showed the highest occurrence of moving events.

**Discussion**

**Brine shrimp lethality test**

The brine shrimp lethality assay is considered a useful tool for preliminary assessment of toxicity. In

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Locomotor activity (counts/60 mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>-</td>
<td>29.00 ± 8.96</td>
</tr>
<tr>
<td>Tween 80</td>
<td>-</td>
<td>140.0 ± 31.2293*</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>2mg/kg</td>
<td>153.8 ± 42.6854*</td>
</tr>
<tr>
<td>Mitragynine</td>
<td></td>
<td></td>
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<tr>
<td>-treatment 1</td>
<td>1mg/kg</td>
<td>38.33 ± 2.4721</td>
</tr>
<tr>
<td>-treatment 2</td>
<td>5mg/kg</td>
<td>74.66 ± 13.9634</td>
</tr>
<tr>
<td>-treatment 3</td>
<td>10mg/kg</td>
<td>72.83 ± 19.7103</td>
</tr>
<tr>
<td>-treatment 4</td>
<td>30mg/kg</td>
<td>77.83 ± 9.5653</td>
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</tbody>
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Thirty-minutes after intraperitoneal injection of drugs in rats, changes in locomotor activity were measured over 1 hour period. Each data represents the mean ± SEM the rats (n=5-6). *p<0.05, compared with control group (Dunnett’s test). Additionally, the method is rapid, simple, reproducible and economical. The test has also been used for monitoring mycotoxins, wastewater and marine
pollutants, detergents and surfactants, petroleum products, food dyes, antifouling paints for ships, heavy metals and pesticides[6]. This study was done using three different levels of Mitragyna extracts, which the extracts had been widely used in animal study in different fields. From the brine shrimp screening test, LC$_{50}$ for aqueous extract of M.S was 98 µl/ml, crude alkaloid extract of M.S (62 µl/ml) and major compound of M.S, mitragynine at 44 µl/ml. The safety level of pure compound of M.S determined at low than 40 µl/ml. Wantana et al[9] stated that the alkaloid extract exhibited higher activity than the methanol extract where the LD$_{50}$ values of orally administration of alkaloid and methanol extracts were 173.20 mg/kg and 4.90 g/kg respectively in mice. They suggested that alkaloid extract was more toxic than the methanol extract. In our findings, mitragynine was found to be toxic at 44 µl/ml (Fig. 3), which was more toxic than alkaloid extracts (Fig. 2) and aqueous extracts (Fig. 1). The screening test is a simple method in determining the LC$_{50}$ of extracts dosage. It was proved to be a convenient and rapid system for toxicity assessment of these three different levels of M.S extracts. These results were helpful in order to study the effectiveness of the different levels of extracts in any animals study. This is due to no study reported on brine shrimp screening test on M.S.

**Locomotor activity**

The results demonstrated that mitragynine as major alkaloid from Mitragyna speciosa reduced the locomotor activity of the rats treated with 1.0, 5.0, 10.0 and 30.0 mg/kg as compared with saline as control. In the other way, amphetamine as central nervous system psychostimulant significantly increased the locomotor activity, as well as Tween 80 (Table 1). As reported by Wantana et al[9], both methanol and alkaloid extracts had no significant changes on locomotor activity or pentobarbital-induced sleep in mice. It is possible that mitragynine at both lowest and highest dose had no psychostimulant effects. This result may suggest that mitragynine may exhibit sedative factor since it reduced the locomotor activity in rats treated with mitragynine.

**Conclusion**

As conclusion, brine shrimp screening test was a simple method in order to determine the lethal concentrations (LC$_{50}$) of three different level of Mitragyna speciosa extracts. The LC$_{50}$ for mitragynine as a major compound of M.S was higher than 40 µl/ml. In conclusion, the author hopes that this study will be a baseline for determining lethality dosage of Mitragyna speciosa and other species of Mitragyna. In general behavioral study, mitragynine caused no significant changes on locomotor activity in rats, so sedative properties of mitragynine could be included in this study. In conclusion, these results suggest that mitragynine possess sedative properties which may act on central nervous system of the rats.

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**References**


