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# Dose–Response Relationship, Acute Toxicity, and Therapeutic Index between the Alkaloid Extract of *Mitragyna speciosa* and Its Main Active Compound Mitragynine in Mice

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**ABSTRACT** *Mitragyna speciosa* is a widely used medicinal plant that is commonly used for its morphine-like effect sin folklore medicine in Thailand and Malaysia due to its ability to reduce pain and ameliorate withdrawal signs after cessation of opioid abuse. The aim of the present study was to determine and compare the relative safety and therapeutic indices of *M. speciosa* alkaloid extract and its major component, mitragynine. An alkaloid extract (20–400 mg/kg) from the leaves of *M. speciosa*, as well as mitragynine (4.2–84 mg/kg), was orally administered to mice; dose–response relationship, $ED_{50}$ and $LD_{50}$ values, as well as the therapeutic index (TI), for the two substances were determined and compared with that of morphine (2.5–10 mg/kg, s.c.). The results showed a significant dose-dependent response in both extract (50 mg/kg onward) and mitragynine (10.5 mg/kg) with a higher potency of mitragynine than that of the extract. Although the $LD_{50}$ for the extract (591 mg/kg) was higher than that of mitragynine (477 mg/kg), the TI for mitragynine was wider than that of the extract (21:3). The present study indicated that mitragynine is relatively safer when compared with the alkaloid extract of *M. speciosa* in mice. Drug Dev Res 74 : 23–30, 2013. 

**Key words:** *Mitragyna speciosa*; safety; mitragynine; therapeutic index; $LD_{50}$

## INTRODUCTION

*Mitragyna speciosa*—korth or ketum—in the local language is a plant originally grown in Thailand and Malaysia [Grewal, 1932; Burkil, 1935]. For many decades, the leaves of *M. speciosa* have been chewed, smoked, or drunk in the form of a boiled–concentrated syrup by the indigenous people to alleviate pain [Jansen and Prast, 1988]. It has also been used as a natural replacement for attenuating withdrawal symptoms following abrupt cessation of opioid intake [Boyer et al., 2008], a finding confirmed in animal models [Khor et al., 2011]. However, developing physical dependence by long-term use of ketum extract was recently reported [McWhirter and Morris, 2010]. Ketum leaf is also known as a physical stimulant similar to coca for farmers in order to enhance working output by increasing physical tolerance and alertness [Suwanlert, 1975; Boyer et al., 2008].

Mitragynine, the major alkaloid of *M. speciosa*, was isolated from the leaves of samples collected from different geographical locations [Ikram, 1985]. To date, over 40 minor compounds have also been isolated from

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the leaves of *M. speciosa* grown in different regions of the world [Adkins et al., 2011]. The quantitative and qualitative differences between the minor constituents of alkaloid extract of *M. speciosa* grown in Thailand and Malaysia have been investigated [Takayama, 2004], with reports of the antinociceptive activity of mitragynine [Matsumoto et al., 1996a,b, 1997, 2005; Thongpradichote et al., 1998] or alkaloid extract of *M. speciosa* with Thai [Kumarnsit et al., 2007; Reanmongkol et al., 2007] or Malaysian origin [Mossadeq et al., 2009, Sabetghadam et al., 2010]. Studies show that mitragynine exerts its pharmacological effects as an agonist of μ- and κ-opioid receptors [Boyer et al., 2008].

The toxicity of mitragynine or its alkaloid extract in animals and cell lines has been extensively studied [Macko et al., 1972; Watanabe et al., 1992; Reanmongkol et al., 2007; Moklas et al., 2008; Saidin and Gooderham, 2008] as has that of other extracts [Harizal et al., 2010].

Although mitragynine works as μ-opioid receptor agonist, adverse effects such as respiratory depression, coma, pulmonary edema, and death were not reported following ketum use in human [Boyer et al., 2008] except for one case of death following consumption of ketum juice cocktail in Thailand [Tungtananuwat and Lawanprasert, 2010]. However, ketum consumption is associated with seizures and coma [Boyer et al., 2008; Nelsen et al., 2010] and intrahepatic cholestasis [Kapp et al., 2011].

Recent reports have documented considerable increase in ketum abuse throughout Malaysia during past years [Vicknasingam et al., 2010; Ahmad and Aziz, 2012], prompting us to determine and compare the safety margin of the alkaloid extract and mitragynine in a preclinical study by calculating the therapeutic indices of these chemical substances using mouse as suitable animal model.

## MATERIALS AND METHODS

### Animals

Male Swiss albino mice weighing 25–30 g were used for this study. Treated or control animals were kept in groups of five under room temperature (25 ± 1°C) with free access to food and water provided *ad libitum*. All the animals were acclimatized for 1 week in holding room before starting the experiment. All the experiments were carried out between 9:00 am and 5:00 pm and light was on from 7:00 am to 7 pm in order to minimize the influence of the circadian rhythm. Experimental protocols were reviewed and approved by the Animal Ethical Committee, Universiti Sains Malaysia.

### Plant Materials

The alkaloid extract of *M. speciosa* was prepared according to the method of Shellard [1974] by Professor Dato Mohd Ikram Mohd Said, Universiti Kebangsaan Malaysia. Mitragynine was isolated as previously published [Utar et al., 2011] and supplied by Dr. Mohd Ilham Adenan, Malaysian Institute of Pharmaceuticals and Nutraceuticals. The standardization of the alkaloid extract of *M. speciosa* was performed at the Centre for Drug Research, Universiti Sains Malaysia using high performance liquid chromatography (HPLC). The mitragynine content of the extract was 20–22%. The alkaloid extract of *M. speciosa* was dissolved in vehicle as a mixture of propylene glycol, Tween-80, and distilled water in the ratio of 4:1:4 (v/v/v). The final solution was administered as a single dose of 20, 50, 160, 320, 400 mg/kg p.o. body weight in a volume of 0.2 ml/10 g body weight. Mitragynine was also dissolved in 20% of Tween-20, further diluted in distilled water for better solubility, and administered with the equivalent extract dose range (4.2, 10.5, 33.6, 67.2, 84 mg/kg p.o. body weight). The extract, mitragynine, and vehicles were administered to the mice 15 min before starting the experiment.

### Drugs

Morphine sulfate concentrate (10 mg/ml) ampoules were supplied from the Hospital Universiti Sains Malaysia (Kelantan, Malaysia) and diluted in 0.9% sodium chloride and administered at doses of 2.5, 5, and 10 mg/kg s.c. body weight in volume of 0.1 ml/10 g body weight 15 min before starting the experiment to induce nociception as positive control.

### Dose–Response Relationship

The dose–response relationship was assessed by hot plate test in treated groups as reported by Wolfe and MacDonald [1944] with slight modification [Rácz and Zimmer, 2006]. The incremental hot plate (IITC Life Science Inc., Woodland Hills, CA USA) was composed of a plate (4 × 8 inches) that was contently heated at 52 ± 1°C. Shaking or licking of the hind paws of the mice was determined as a nociceptive reaction and the time elapsed since placing the animal on the hotplate until observing the nociceptive reaction (latency time) was recorded for each animal. The cutoff time was set at 60 s to prevent tissue damage. The range of baseline latency time for the animals was determined between 7 and 12 s. Different doses of the alkaloid extract, mitragynine, and morphine or vehicle were administered to each group of animals. All observations started 15 min

after the administration of test substances and were repeated every 15 min for a total duration of 105 min. The time of maximum changes in nociceptive reaction after each drug administration (drug latency time) was recorded in order to determine the time of peak effect treated and control groups [Dey et al., 2010]. The percent of maximum possible antinociceptive effect (MPE) for alkaloid, mitragynine, and morphine in the time of peak effect was calculated as follows:

$$\text{MPE} = \frac{\text{Tested latency time} - \text{baseline latency time} \times 100}{\text{Cutoff time} - \text{baseline latency time}}$$

The slopes and the $ED_{50}$ values for the extract, mitragynine, and morphine were further calculated based on MPE respected graphs.

### $LD_{50}$ Value

A preliminary orientation test of acute oral toxicity study was conducted using the limit dose test of up-and-down procedure according to the Organisation for Economic Co-operation and Development (OECD) Test Guidelines on Acute Oral Toxicity under a computer-guided statistical program—AOT425statPgm [US Environmental Protection Agency (USEPA), Washington, DC, USA], version: 1.0., (http://www.epa.gov/oppfead1/harmonization/docs/aot425manual.pdf) at a limit dose of 2,000 mg/kg body weight/p.o. and calculated sigma values (obtained from dose–response relationship study) of 0.87 and 0.91 for mitragynine and alkaloid extract, respectively. Due to the lethality of the two sequentially treated rats at 2,000 mg/kg/p.o. route of mitragynine or alkaloid extract, the main test of the up-and-down procedure was subsequently conducted, using the dose progression of 175 mg/kg/p.o. and 2,000 mg/kg/p.o. for the alkaloid extract as well as 175 mg/kg/p.o. and 1,300 mg/kg/p.o. for mitragynine. The procedure for the main test was conducted in strict compliance with the guidelines of the main test of up-and-down procedure according to OECD Test Guidelines on Acute Oral Toxicity (OECD, 2002) under a computer-guided statistical program—AOT425statPgm (Acute Oral Toxicity, 2001). The therapeutic index for alkaloid extract and mitragynine was calculated as previously reported [Vaghasiya et al., 2011].

### Data Analysis

Results were analyzed by a one-way analysis of variance (ANOVA) followed by Dunnett's test to compare the latency time for treatment groups versus that of control group for each time interval of observation. Repeated measure of one-way ANOVA followed by Dunn's post hoc test was also carried out to determine the peak time of effect in each treatment group. $ED_{50}$ values for alkaloid extract, mitragynine, and morphine with 95% confidence interval (shown in parentheses) for alkaloid extract and mitragynine were determined by nonlinear regression analysis using the software GraphPad Prism version 5.0. (GraphPad Software Inc., La Jolla, CA, USA) $LD_{50}$ values of mitragynine and alkaloid extract were estimated using the AOT425 StatPgm version 1.00. This program is based on maximum likelihood analysis with 95% confidence interval (shown in parentheses).

## RESULTS

### Dose–Response Relationship

#### Time course–response curve

As summarized in Table 1 for the alkaloid extract, all doses given prolonged the mean baseline latency time in the treatment groups. In all the treatment groups, the onset of latency was observed 15 min after administration and became significant between 30 and 60 min for an alkaloid extract dose of 50 mg/kg or greater when compared with controls. The peak time of effect was recorded 60 min after extract administration as compared with time 0 (time of administration). Tremor was observed at higher doses in some animals (1/6 animal at 320 mg/kg p.o., 2/6 animals at 400 mg/kg p.o.) around 15 min after administration. After 4–6 h, tremors were completely diminished and the animals returned to normal behavior. The animals with signs of tremor were excluded from the whole experiment.

The results of time course–latency response for ascending doses of mitragynine are shown in Table 2. Mitragynine demonstrated a dose-dependent increase in latency time in treatment groups in the hot plate test. A significant effect was observed 30–45 min above a dose of 67.2 mg/kg p.o. The peak time of effect was observed at 45 min when compared with time 0 (time of administration).

The onset of the drug latency response for morphine was observed 15 min after s.c. injection onward (Table 3) and was significantly prolonged at the 5 mg/kg and 10 mg/kg dose levels. The peak time of effect was observed at 45 min when compared with time 0 (time of administration). Furthermore, the antinociceptive effect of the highest dose (10 mg/kg) was significant during the whole experiment when compared with the control group.

#### Dose–response curves, $ED_{50}$, and relative potency

The best-fit graded curves for maximum possible effect (MPE) responses versus log dose at time of peak

**TABLE 1. Time–Latency Response (s) of Alkaloid Extract of *M. speciosa* Using Hot Plate Test in Mice**

| | | Latency of nociceptive response (s) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Drug | Dose (mg/kg) | $T_0$ | $T_{15}$ | $T_{30}$ | $T_{45}$ | $T_{60}$ | $T_{75}$ | $T_{90}$ | $T_{105}$ |
| Vehicle | – | 8.59 ± 0.59 | 9.18 ± 0.17 | 8.71 ± 0.11 | 10.48 ± 1.18 | 10.24 ± 1.30 | 9.06 ± 1.30 | 9.75 ± 0.20 | 11.03 ± 1.27 |
| Alkaloid ext. | 20 | 8.60 ± 0.85 | 9.55 ± 1.58 | 10.35 ± 1.30 | 11.09 ± 1.16* | 13.49 ± 1.20[a] | 11.91 ± 2.87 | 10.61 ± 4.09 | 8.40 ± 1.98 |
| | 50 | 7.93 ± 1.74 | 11.65 ± 1.70 | 12.57 ± 0.72* | 13.56 ± 1.70*[a] | 16.55 ± 2.54[b] | 11.94 ± 2.65 | 11.34 ± 3.53 | 10.83 ± 2.83 |
| | 160 | 8.79 ± 1.60 | 11.61 ± 1.36 | 14.31 ± 0.93[a] | 15.71 ± 0.95*[b] | 20.86 ± 2.49*[c] | 14.02 ± 1.86 | 11.97 ± 2.68 | 11.05 ± 2.42 |
| | 320 | 8.92 ± 1.92 | 11.31 ± 0.84 | 18.53 ± 1.11*[a] | 19.35 ± 1.61*[b] | 21.81 ± 4.18*[c] | 16.2 ± 1.77 | 12.83 ± 1.63 | 10.72 ± 2.84 |
| | 400 | 9.62 ± 0.44 | 16.75 ± 1.03** | 18.39 ± 1.36**[a] | 19.59 ± 1.97**[b] | 21.65 ± 5.12***[c] | 15.05 ± 2.16** | 13.03 ± 1.99 | 13.14 ± 3.48 |

Data shown as mean ± SEM ($n = 6$).
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus vehicle (one-way ANOVA, followed by Dunn's test).
[a]$P < 0.05$, [b]$P < 0.01$, and [c]$P < 0.001$ versus $T_0$ (repeated measure one-way ANOVA, followed by Dunn's test).
ANOVA, analysis of variance.

**TABLE 2. Time–Latency Response (s) of Mitragynine Using Hot Plate Test in Mice**

| | | Latency of nociceptive response (s) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Drug | Dose (mg/kg) | $T_0$ | $T_{15}$ | $T_{30}$ | $T_{45}$ | $T_{60}$ | $T_{75}$ | $T_{90}$ | $T_{105}$ |
| Vehicle | – | 7.18 ± 1.28 | 7.69 ± 1.36 | 8.72 ± 0.69 | 8.39 ± 1.21 | 8.08 ± 1.72 | 7.15 ± 0.57 | 9.30 ± 1.16 | 9.56 ± 0.97 |
| Mitragynine | 4.2 | 8.20 ± 0.89 | 11.44 ± 1.24 | 14.67 ± 1.48 | 15.39 ± 2.27[a] | 12.47 ± 0.98 | 10.36 ± 1.30 | 10.80 ± 0.95 | 10.37 ± 2.38 |
| | 10.5 | 8.23 ± 1.06 | 11.62 ± 1.88 | 17.42 ± 3.43 | 20.11 ± 2.02*[a] | 16.22 ± 4.33 | 14.38 ± 3.01 | 14.65 ± 3.82 | 11.91 ± 3.29 |
| | 33.6 | 8.22 ± 0.64 | 14.40 ± 2.01 | 19.06 ± 2.29 | 25.19 ± 4.77**[a] | 20.48 ± 3.98 | 18.83 ± 3.94 | 15.38 ± 2.03 | 11.96 ± 2.52 |
| | 67.2 | 9.86 ± 1.15 | 26.68 ± 8.14** | 27.09 ± 7.69** | 30.51 ± 4.87***[a] | 25.83 ± 4.90** | 21.91 ± 6.36* | 19.36 ± 3.89 | 17.56 ± 1.88 |
| | 84 | 9.37 ± 0.86 | 30.42 ± 6.39*** | 37.38 ± 6.05*** | 39.47 ± 5.85***[a] | 27.22 ± 3.96** | 26.23 ± 6.10** | 26.16 ± 6.88** | 24.17 ± 3.71* |

Data shown as mean ± SEM ($n = 6$).
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus vehicle (one-way ANOVA, followed by Dunnett's test). [a]$P < 0.05$ versus $T_0$ (repeated measure one-way ANOVA, followed by Dunn's test).
ANOVA, analysis of variance.

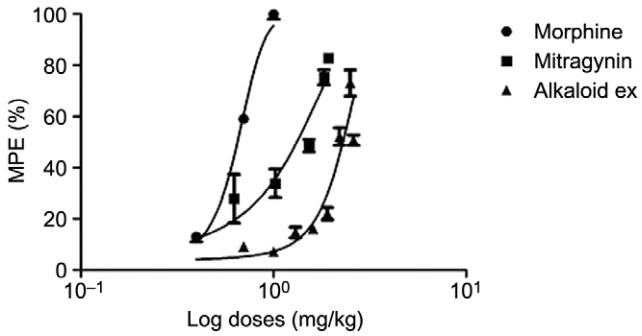


**Fig. 1.** Dose (log)–response (%) curves of mitragynine (4.2, 10.5, 33.6, 64.2, and 84 mg/kg, p.o.), alkaloid extract (20, 50, 160, 320, and 400 mg/kg, p.o.), and morphine (2.5, 5, and 10 mg/kg, s.c.) in their respected peak time of antinociceptive effect using hot plate test in mice. MPE, maximum possible antinociceptive effect.

effect for different doses of alkaloid extract of *M. speciosa*, mitragynine, and morphine using hot plate test in rats are shown in Figure 1.

The dose–response curve for mitragynine was to the left of the alkaloid extract, indicating a higher potency of mitragynine compared with the extract, whereas the mitragynine curve was on the right of the morphine curve, indicating the lesser potency of mitragynine compared with morphine. Mitragynine (4.2–84 mg/kg, p.o.) had an $ED_{50}$ = 21.96 mg/kg (16.54–29.18 mg/kg), that of the alkaloid extract (20–400 mg/kg, p.o.) was 194.4 mg/kg (161.7–233.6 mg/kg), and that of morphine (2.5–10 mg/kg, s.c.) was 3.69 mg/kg (4.24–4.64 mg/kg). The latter was comparable with previous data on morphine (5–15 mg/kg, s.c.), yielding an $ED_{50}$ value of 6.3 mg/kg (3.4–5.8) in the hot plate test for male rats [Cicero et al., 1997]. The slope ± SE of curves for morphine, mitragynine, and alkaloid extract were calculated as 3.69 ± 0.28, 0.87 ± 0.11, and 0.91 ± 0.08, respectively.

## $LD_{50}$ Value

As shown in Tables 4 and 5, acute oral treatment with either mitragynine or alkaloid extract at 2,000 mg/kg caused death in treated mice at less than 1 h posttreatment of mitragynine or alkaloid extract. Death was preceded by restlessness, perioral tremor, and generalized convulsion. Stepping down to the main test, administration of 175 mg/kg mitragynine or alkaloid extract resulted in animal survival without any toxic signs (short-term outcome). There were no delayed behavioral toxicities (long-term outcome) such as decreased feeding pattern or body weight loss. However, increasing the doses (1,300 mg/kg for mitragynine and 2,000 mg/kg for alkaloid extract) was

**TABLE 3. Time–Latency Response (s) of Morphine Using Hot Plate Test in Mice**

| Drug | Dose (mg/kg) | Latency of nociceptive response (s) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $T_0$ | $T_{15}$ | $T_{30}$ | $T_{45}$ | $T_{60}$ | $T_{75}$ | $T_{90}$ | $T_{105}$ |
| Vehicle | – | 7.54 ± 0.80 | 9.18 ± 0.08 | 8.81 ± 0.10 | 10.48 ± 0.26 | 10.24 ± 0.22 | 11.06 ± 0.16 | 9.75 ± 0.17 | 11.03 ± 0.27 |
| Morphine | 2.5 | 8.77 ± 0.22 | 10.65 ± 0.13 | 14.22 ± 0.22***[a] | 15.29 ± 0.62***[a] | 12.62 ± 1.29* | 11.79 ± 0.81 | 11.72 ± 0.66 | 11.44 ± 0.81 |
| | 5 | 7.26 ± 0.29 | 16.29 ± 0.07*** | 22.08 ± 0.61***[a] | 25.82 ± 0.57***[b] | 20.50 ± 0.62*** | 19.12 ± 0.72*** | 14.80 ± 0.54*** | 12.87 ± 0.23 |
| | 10 | 8.87 ± 0.89 | 22.75 ± 0.04***[a] | 31.65 ± 0.16***[b] | 35.00 ± 0.15***[c] | 30.03 ± 0.29*** | 27.99 ± 0.28*** | 27.51 ± 0.24*** | 19.40 ± 0.77*** |

Data shown as mean ± SEM.
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus vehicle (one-way ANOVA, followed by Dunnett's test). [a]$P < 0.05$, [b]$P < 0.01$, and [c]$P < 0.001$ versus $T_0$ (repeated measure one-way ANOVA, followed by Dunn's test).
ANOVA, analysis of variance.

**TABLE 4. Sequence and Result of the Main Test of Up-and-Down Procedure of Acute Oral Toxicity of Mitragynine in Treated Mice**

| Test sequence | Animal identity | Dose (mg/kg/oral) | Short-term outcome (the first 48 h) | Long-term outcome (the successive 12 days) |
|---|---|---|---|---|
| 1 | 1 | 175 | O | O |
| 2 | 2 | 1,300 | X | X |
| 3 | 3 | 175 | O | O |
| 4 | 4 | 1,300 | X | X |
| 5 | 5 | 175 | O | O |
| 6 | 6 | 1,300 | X | X |

Assumed sigma = 0.87; confidence interval = 95%.
O, survived; X, died.

**TABLE 5. Sequence and Result of the Main Test of Up-and-Down Procedure of Acute Oral Toxicity of *M. speciosa* Alkaloid Extract in Treated Mice**

| Test sequence | Animal identity | Dose (mg/kg/oral) | Short-term outcome (the first 48 h) | Long-term outcome (successive 12 days) |
|---|---|---|---|---|
| 1 | 1 | 175 | O | O |
| 2 | 2 | 2,000 | X | X |
| 3 | 3 | 175 | O | O |
| 4 | 4 | 2,000 | X | X |
| 5 | 5 | 175 | O | O |
| 6 | 6 | 2,000 | X | X |

Assumed sigma = 0.91; confidence interval = 95%.
O, survived; X, died.

associated with convulsions led to death for both test substances in less than 1 hr (short-term outcome). Based on the computer-generated results, the calculated $LD_{50}$ values were 477.1 and 591.6 mg/kg/p.o. for mitragynine and alkaloid extract, respectively. Consequently, the Therapeutic Index (TI) for alkaloid extract and mitragynine were calculated as follows: $TI_{alkaloid\ extract} = LD_{50}/ED_{50} = 591.6/194.4 = 3:1$; $TI_{mitragynine} = LD_{50}/ED_{50} = 477.1/21.96 = 21:1$.

## DISCUSSION

The margin of safety for alkaloid extract and mitragynine was evaluated in this study. The $ED_{50}$ values for the test substances were estimated from the respective dose–response curve. The $ED_{50}$ value of mitragynine in this study was approximately 3.5 times less than that of morphine with the alkaloid extract potency being less than those of both mitragynine and morphine. Macko et al. [1972] reported an $ED_{100} = 92$ mg/kg p.o. for mitragynine in mice. Watanabe et al. (1997) reported antinociceptive activity at a dose level of 20 mg/kg, which was 2.5-fold lower than the analgesic dose shown in this study for *M. speciosa* alkaloid extract. This could be attributed to differences in the mitragynine content between the alkaloid extract used in the present study and the one reported in literature. The mitragynine content in the crude base extract of *M. speciosa* grown in Thailand [66%; Takayama, 2004] was different from the *M. speciosa* alkaloid extract employed in this study (20–22%). In addition, the qualitative and quantitative difference in the chemical constituents (active alkaloids such as 7-hydroxymitragynine) between these two alkaloids extract of different origin could not be ruled out as well and warrants further investigation.

Initial work (limit test) was undertaken to determine the $LD_{50}$ value of mitragynine. The oral $LD_{50}$ of mitragynine in this study was 477 mg/kg, approximately twofold lower than the reported literature value of 960 mg/kg [Macko et al., 1972] for mice. Watanabe et al. [1992] reported a lower $LD_{50}$ value (126.7 mg/kg) in mice following intraperitoneal (i.p.) administration of mitragynine. These differences may reflect the different mitragynine formulations and routes of administration employed to determine the mitragynine $LD_{50}$.

In the present study, mitragynine was administered orally in mice as base in 20% Tween-20, but Macko et al. (1972) employed mitragynine salt for the similar purposes. The use of different routes of administration and mitragynine formulations could have affected the amount of compound absorbed and thus explain the discrepancies in the mitragynine $LD_{50}$ value reported.

In addition, work was also carried out to determine the $LD_{50}$ value of the alkaloid extract in mice. Tremor, as an overt sign of toxicity, was noted in some mice after administration of the alkaloid extract (320–400 mg/kg). This was reversed after 4–6 h, suggesting that the animals metabolized the test substance [Harizal et al., 2010]. In the present study, the alkaloid extract $LD_{50}$ value (591.6 mg/kg) was slightly higher than that of the pure compound mitragynine (477.1 mg/kg). This finding corresponded well with mitragynine and alkaloid extract cytotoxicity studies reported in the literature. Saidin and Gooderham [2008] also confirmed the cytotoxicity of *M. speciosa* alkaloid extract, as well as that of mitragynine, *in vitro* using somatic and cancerous cell lines, respectively. Mitragynine ($LC_{50} = 44$ µl/ml) had higher toxicity when compared with the alkaloid extract ($LC_{50} = 62$ µl/ml) in a brine shrimp test with mitragynine ($LC_{50} = 44$ µl/ml) having slightly higher toxicity as compared to the alkaloid extract [Moklas et al., 2008].

Other investigators reported an $LD_{50}$ value of 173.2 mg/kg p.o. for *M. speciosa* alkaloid extract (Reanmongkol et al., 2007), a value much lower than that for the mitragynine and alkaloid extract used in the present study. The high content of mitragynine (66%) in the base crude extract and possible discrepancy in the

chemical constituents of *M. speciosa* extracts of Thailand origin may explain these differences [Takayama, 2004]. The reported study thus warrants detailed investigation of the minor chemical constituents to generally characterize the toxicity profile of this extract.

The $LD_{50}$ and $ED_{50}$ values estimated for both mitragynine and alkaloid extract in this study were used to determine their therapeutic indices. The TI values for the alkaloid extract and mitragynine were 3:1 and 21:1, respectively. Analgesics such as remifentanyl, tetrahydrocannabinol, and morphine offer safe therapeutic indices of 33,000:1; 1,000:1, and 70:1, respectively [Stanley, 2000], while cocaine and ethyl alcohol (local anesthetic) have lesser safety margin with TIs of 15:1 and 10:1, respectively [Becker, 2007].

## CONCLUSION

Although the $LD_{50}$ value for mitragynine was lower than that of the alkaloid extract, the margin of safety for this compound is wider, implying a lesser possibility of overdose toxicity effect in mitragynine.

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