

Antipyretic Effect of Crude Methanolic Extract of *Mitragyna speciosa* in Mice

Annas Salleh, ¹Arifah Abdul Kadir & ¹Wan Mastura Shaik Mossadeq

*¹Department of Veterinary Preclinical Sciences
Faculty of Veterinary Medicine, Universiti Putra Malaysia*

Abstract

Mitragyna speciosa is a species of tropical indigenous plant that can be found mainly in Southeast Asia. This study aims to ascertain the existence of antipyretic properties of the crude methanolic extract of *Mitragynaspeciosa*, and determine its effective dose against Brewer's yeast-induced pyrexia in mice. Thirty BALB/c mice were randomly divided into three treatment groups and two control groups. Pyrexia was induced by subcutaneous injection of 30% Brewer's yeast. Rectal temperature was recorded before and 18 h after induction of pyrexia every 30 min for 5 h. All groups treated with the crude methanolic extracts of *Mitragynaspeciosa* (50 mg/kg, 100 mg/kg, 200 mg/kg) were observed to produce significant reduction of rectal temperature as compared to the negative control group at different times. Ketoprofen at the dosage of 1 mg/kg caused significant ($p < 0.001$) inhibition of fever from 0.5 to 5.0 h after treatment. In conclusion, the crude methanolic extract of *Mitragyna speciosa* possessed dose-dependent antipyretic properties in mice. The antipyretic effective dose of the crude methanolic extract of *Mitragyna speciosa* was 100 mg/kg.

Keyword: *Mitragyna speciosa*, mice, pyrexia

Introduction

Mitragyna speciosa (commonly known as “kratom”, “ketom”, “ketum”, or “biak-biak”) is a tree which is part of the family Rubiaceae. Genus *mitragyna* can usually be found in swamp and valleys in tropical and subtropical Asia such as Thailand, Laos, Cambodia, Malaysia (Burkill, 1935) and in the East and West Africa and India (Harvala and Hinou, 1988). In Malaysia, this species can be widely found in the northern half of the Peninsula (Burkill, 1935) and Selangor (Houghton and Said, 1986).

Effects of *Mitragyna speciosa* are known to be dose-dependent, where at high doses the subjects usually exhibited opioid-like effects, while at lower doses, it tends to result in stimulant-like effects. Its usage to treat pain and opium withdrawal syndrome was described as early as the nineteenth century (Shellard, 1989).

However, there is no scientific data on the antipyretic effect of the crude extract of *Mitragyna speciosa*. Therefore, the objectives of this study were to determine the antipyretic effect of crude methanolic extract of *Mitragyna speciosa* in mice and the effective dose of crude methanolic extract of *Mitragyna speciosa* against Brewer's yeast-induced pyrexia in mice.

Materials and Methods

Preparation of the crude methanolic extract of Mitragyna speciosa and ketoprofen

Ketoprofen 20 mg tablet was crushed using mortar and pestle. The ketoprofen powder or the crude methanolic extract of *Mitragyna speciosa* was then dissolved in 20% Tween 80 using a magnetic stirrer until a homogenous solution was achieved. This homogenous solution was then mixed with 0.9% NaCl, and stirred using a magnetic stirrer until a homogenous, foamy solution was produced.

Animals

Thirty BALB/c female mice weighing 20 to 25 g were used. The mice were acclimatized for at least one week and housed eight per cage under standard 12-h light: 12-h dark cycle. Food and water were available *ad libitum*. Mice were allowed to acclimatize to the laboratory environment 24 h prior to experimentation.

Antipyretic activity

The antipyretic activity was evaluated in mice according to the method described by Makonnen *et al.* (2003). A thermister probe was inserted about 1 cm into the rectum of the mice, and basal rectal temperature was recorded by a digital thermometer. Pyrexia was induced by injection of 30% (w/v) suspension of Brewer's yeast in 0.9% NaCl subcutaneously at the dosage of 10 mL/kg. The rectal temperature was recorded 18 h after the induction of pyrexia. Only mice which showed increase in the rectal temperature of $> 0.5^{\circ}\text{C}$ were subjected to the experiments.

Dosage test

The crude methanolic extract of *Mitragyna speciosa*, ketoprofen (positive control), and 20% Tween 80 in 0.9% NaCl (negative control) were administered intraperitoneally. For each dosing, six mice were used. The crude methanolic extract of *Mitragyna speciosa* was administered at the dosage of 50 mg/kg, 100

mg/kg, and 200 mg/kg. Ketoprofen was administered at the dosage of 1 mg/kg. As for the negative control group, the vehicle (20% Tween 80 in 0.9% NaCl) was administered at the dosage of 10 mL/kg. Eighteen hours after the induction of pyrexia, and immediately after dosing crude extract, the rectal temperature was measured every 30 min for 5 h.

Analysis of data

The mean change in the rectal temperature over the 5 h period was calculated for each mouse and expressed as percentage of reduction. All the data from the experiments were analysed using SPSS version 16.0. The data were analyzed using General Linear Model repeated measure ANOVA. The results were expressed as mean \pm S.E.M. and the statistical significance ($P < 0.05$, $P < 0.01$, $P < 0.001$) between the treatment group to the negative control group were analyzed using one-way ANOVA followed by Tukey's test at each point of time.

Results and Discussion

In the negative control group, the subcutaneous injection of 30% Brewer's yeast has increased the body temperature to 38°C which was maintained for 5 h, and the highest temperature was recorded at 1.0 h (38.67°C). All treated groups showed significantly lower rectal temperature as compared to the negative control group. From Figure 1, ketoprofen (1 mg/kg)-treated group showed significant reduction in rectal temperature from 0.5 to 1.0 h, and from 3.5 to 5.0 h. Ketoprofen was observed to produce the highest percentage of inhibition of fever at 5.0 h (36.65°C).

As shown in Table 1, mice treated with the methanolic extract of *Mitragyna speciosa* at the dose of 50 mg/kg had significant ($P < 0.05$) reduction in rectal temperature from 2.5 h until 5.0 h. At the dosage of 100 mg/kg, *Mitragyna speciosa* seems to produce significant ($P < 0.05$) reduction in temperature at 0.5 h and, from 2.0 to 5.0 h. The group of mice treated with the crude methanolic extract of *Mitragyna speciosa* at the dosage of 200 mg/kg resulted in significant reduction ($P < 0.001$) in rectal temperature from 0.5 to 5.0 h. The greatest percentage of reduction of temperature can be seen in the group treated at the dosage of 200 mg/kg, at 2.0 h. However, administration of methanolic extracts of *Mitragyna speciosa* at a dose of 200 mg/kg caused hypothermia in mice from 1.0 to 3.5 h.

The dose-dependent effect of the methanolic extract of *Mitragyna speciosa* also revealed findings similar to that reported by Babu *et al.* (2008), where opiate or morphine-like effects seem to predominate when high dose of *Mitragyna speciosa* extract were administered. In this study, administration of 200 mg/kg of the crude methanolic extract induced hypothermia in mice for 2.5 h. This is possibly due to the presence of other compounds in the crude extract, which may act synergistically to produce more potent hypothermic effect than that of a single compound.

Thus, it was shown that the effective antipyretic dose for the crude methanolic extract of *Mitragyna speciosa* was 100 mg/kg. The results also showed that ketoprofen, known to inhibit the cyclo-oxygenase, causes significant inhibition of pyrexia. It is assumed that the mode of action of antipyretic activities of *Mitragyna speciosa* methanolic extract might involve a mechanism possibly mediated via inhibition of cyclo-oxygenase activity. However, more studies need to be carried out to determine the actual mechanism of action of the antipyretic properties of *Mitragyna speciosa*.

Conclusion

This study revealed that the crude methanolic extract of *Mitragyna speciosa* possesses a dose-dependent antipyretic effect, where the highest dose of the crude extract resulted in opioid-like effect. The antipyretic effective dose of the crude methanolic extract of *Mitragyna speciosa* was 100 mg/kg.

References

- Babu, K.M., McCurdy, C.R. and Boyer, E.W. (2008). Opioid receptors and legal highs: *Salvia divinorum* and Kratom. *Clin Toxicol* **46**:2, 146 – 152.
- Burkill, J.H. (1935). A dictionary of economic products of the Malay Peninsula. Government of The Straits Settlements and Federated Malay States. *The Crown Agent*. **2**:1480-1483.
- Harvala, C. and Hinou, J. (1988). Flavonol derivatives from the leaves of *Mitragyna speciosa*. *Pharm. Publ* **43**: 372.
- Houghton, P.J. and Said, I.M. (1986). 3-Dehydromitragynine: an alkaloid from *Mitragyna speciosa*. *Phytochem* **25**: 2910.
- Makonnen, E., Debella, A., Zerihun, L., Abebe, D. and Teka, F. (2003). Antipyretic properties of the aqueous and ethanol extracts of the leaves of *Ocimum suave* and *Ocimumlamiifolium* in mice. *J Ethnopharmacol* **88**(1): 85-91.
- Shellard, E.J. (1989). Ethnopharmacology of kratom and the *Mitragyna* alkaloids. *J. Ethnopharmacol* **25**(1):123–124.

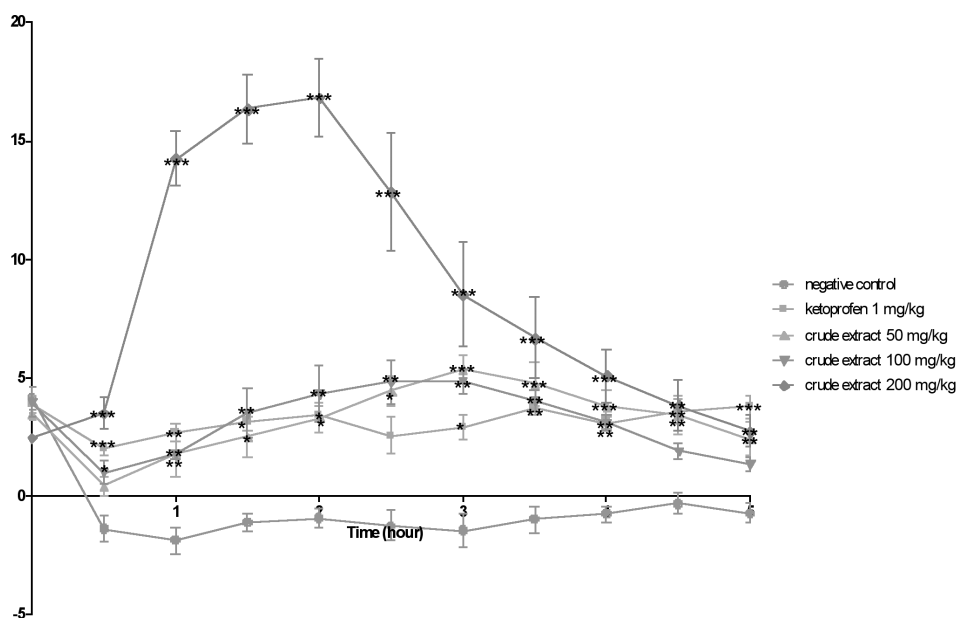


Figure 1. Percentage reduction of fever in Brewer's yeast-induced pyrexia in mice treated with the crude methanolic extract of *Mitragyna speciosa*. $n=6$, data= mean \pm S.E.M., all values marked with asterisk are statistically significant * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to negative control.

Table 1. Effect of crude methanolic extract of *Mitragyna speciosa* on yeast-induced pyrexia in mice

Treatment	Dosage (mg/kg)	Before pyrexia induction	18h after pyrexia induction	Rectal Temperature (°C)										
				Time after treatment with <i>Mitragyna speciosa</i> crude extract (h)										
				0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	
Vehicle		36.38 ± 0.145	37.97 ± 0.109	38.48 ± 0.164	38.67 ± 0.199	38.38 ± 0.168	38.32 ± 0.176	38.43 ± 0.276	38.52 ± 0.313	38.33 ± 0.235	38.25 ± 0.118	38.08 ± 0.199	38.23 ± 0.141	
Ketoprofen	1	36.62 ± 0.048	38.10 ± 0.132	37.33 ± 0.092***	37.07 ± 0.067*	36.90 ± 0.115	36.78 ± 0.111	37.12 ± 0.189	36.98 ± 0.091	36.68 ± 0.079*	36.92 ± 0.060**	36.73 ± 0.112**	36.65 ± 0.163***	
<i>M. speciosa</i>	50	36.82 ± 0.054	38.15 ± 0.159	37.98 ± 0.105	37.45 ± 0.281	37.18 ± 0.280	36.88 ± 0.220	36.45 ± 0.219*	36.10 ± 0.207**	36.33 ± 0.304**	36.70 ± 0.256**	36.83 ± 0.260**	37.22 ± 0.180**	
<i>M. speciosa</i>	100	36.48 ± 0.172	38.00 ± 0.103	37.62 ± 0.135**	37.32 ± 0.070	36.67 ± 0.384	36.35 ± 0.447*	36.17 ± 0.340*	36.15 ± 0.198**	36.47 ± 0.169**	36.82 ± 0.095**	37.27 ± 0.056	37.48 ± 0.075*	
<i>M. speciosa</i>	200	37.06 ± 0.081	38.00 ± 0.055	36.66 ± 0.238***	32.58 ± 0.418***	31.78 ± 0.530***	31.60 ± 0.585***	33.12 ± 0.936***	34.76 ± 0.829***	35.46 ± 0.661***	36.06 ± 0.432***	36.54 ± 0.421**	36.96 ± 0.268**	

Values are Mean±S.E.M. of the rectal temperature of BALB/c mice treated with crude methanolic extract of *Mitragyna speciosa* administered intraperitoneally in Brewer's yeast-induced pyrexia. n=6, all values marked with asterisk are statistically significant * P<0.05, ** P<0.01, *** P<0.001 compared to negative control.