ORIGINAL ARTICLE

Effects of the extracts from *Mitragyna speciosa* Korth. leaves on analgesic and behavioral activities in experimental animals

Wantana Reanmongkol[1], Niwat Keawpradub[2] and Kitja Sawangjaroen[3]

Abstract

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The leaves of *Mitragyna speciosa* Korth. (*M. speciosa*) were extracted with methanol to give methanol extract. The methanol extract was made in acid and then in alkaline and extracted with chloroform to give alkaloid extract. The effects of the methanol and alkaloid extracts on analgesic activities in hot plate test in mice and tail flick test in rats and behavioral activities in locomotor activity and pentobarbital-induced sleep in mice, were examined. In acute toxicity test, the LD_{50} values of oral administration of the methanol and alkaloid extracts of *M. speciosa* leaves in mice were 4.90 g/kg and 173.20 mg/kg, respectively. Oral administration (50, 100 and 200 mg/kg) of the methanol extract of M. speciosa leaves significantly prolonged the latency of nociceptive response on hot plate test in mice. The alkaloid extract of *M. speciosa* also increased the pain response latency at the dose of 20 mg/kg but less potent than those of the methanol extract (100 mg/kg) in mice (comparing 5-10 mg/kg alkaloid extract with corresponding to approximately 200 mg/kg of

[1]Ph.D.(Pharmacology), Assoc. Prof., Department of Clinical Pharmacy, [2]Ph.D.(Pharmacognosy), Asst. Prof., Department of Pharmacognosy and Pharmaceutical Botany, [3]Ph.D.(Pharmacology), Asst. Prof., Department of Pharmacology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand.
Corresponding e-mail: wantana.r@psu.ac.th

methanol extract). The antinociceptive action of either methanol extract (100 mg/kg, p.o.) or alkaloid extract (20 mg/kg, p.o.) of *M. speciosa* leaves was blocked by naloxone (2 mg/kg, i.p.) in mice. Neither the methanol extract nor the alkaloid extract significantly prolonged latency of nociceptive response on tail flick test in rats. Both of the extracts had no significant change on spontaneous motor activity or pentobarbital-induced sleep in mice, respectively. These results suggest that the methanol and alkaloid extracts of *M. speciosa* leaves possess the analgesic activity which partly acted at opioid receptors in the supraspinal opioid system.

Key words : *Mitragyna speciosa* leaves, extract, alkaloid, analgesic, behavioral

บทคัดย่อ

วันทนา เหรียญมงคล[1] นิวัติ แก้วประดับ[2] และ กิจจา สว่างเจริญ[3]

ผลของสารสกัดจากใบกระท่อมต่อฤทธิ์แก้ปวดและพฤติกรรมในสัตว์ทดลอง

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ทำการสกัดใบกระท่อมด้วยเมทานอลได้สารสกัดเมทานอล นำสารสกัดเมทานอลมาทำให้เป็นกรดและต่อมาทำให้เป็นด่างและสกัดด้วยคลอโรฟอร์มได้สารสกัดแอลคาลอยด์ ทำการทดสอบผลของสารสกัดเมทานอล และสารสกัดแอลคาลอยด์ต่อฤทธิ์แก้ปวด ซึ่งเหนี่ยวนำให้เกิดความปวดด้วยความร้อนในหนูถีบจักร (hot plate test) และหนูขาว (tail flick test) และผลต่อพฤติกรรมโดยวัดการเคลื่อนไหว และการเหนี่ยวนำให้หลับด้วยเพนโทบาร์บิทาลในหนูถีบจักร ในการศึกษาความเป็นพิษเฉียบพลัน พบว่าค่าแอลดี 50 เป็น 4.90 กรัม/กก. และ 173.20 มก./กก. ตามลำดับจากการป้อนสารสกัดเมทานอล และสารสกัดแอลคาลอยด์ของใบกระท่อมในหนูถีบจักร การป้อนสารสกัดเมทานอล (50, 100 และ 200 มก./กก.) ของใบกระท่อม มีผลยืดเวลาการตอบสนองต่อความเจ็บปวดซึ่งเกิดจากการเหนี่ยวนำด้วยความร้อนที่เท้าหนูอย่างมีนัยสำคัญในหนูถีบจักร สารสกัดแอลคาลอยด์มีผลยืดเวลาการตอบสนองต่อความเจ็บปวดเช่นกันที่ขนาด 20 มก./กก. แต่มีความแรงน้อยกว่ากรณีของสารสกัดเมทานอล (100 มก./กก.) ในหนูถีบจักร (เปรียบเทียบสารสกัดแอลคาลอยด์ขนาด 5-10 มก./กก. เทียบเท่ากับสารสกัดเมทานอลขนาดประมาณ 200 มก./กก.) ฤทธิ์ระงับปวดของสารสกัดเมทานอล (100 มก./กก.) และสารสกัดแอลคาลอยด์ (20 มก./กก.) ของใบกระท่อมถูกต้านด้วยสารนาล็อกโซน (2 มก./กก. โดยการฉีดเข้าทางหน้าท้อง) ในหนูถีบจักร ทั้งสารสกัดเมทานอลและสารสกัดแอลคาลอยด์ไม่มีผลอย่างมีนัยสำคัญต่อการยืดเวลาการตอบสนองต่อความเจ็บปวดที่บริเวณหางหนูซึ่งเหนี่ยวนำด้วยลำแสงความร้อน สารสกัดเมทานอลและสารสกัดแอลคาลอยด์ไม่มีผลอย่างมีนัยสำคัญต่อการเคลื่อนไหวหรือการเหนี่ยวนำให้หลับด้วยเพนโทบาร์บิทาลในหนูถีบจักร จากผลการทดลองนี้เสนอว่าสารสกัดเมทานอลและสารสกัดแอลคาลอยด์ของใบกระท่อมมีฤทธิ์แก้ปวด โดยออกฤทธิ์บางส่วนที่ตัวรับโอปิออยด์ในระบบโอปิออยด์ที่อยู่เหนือไขสันหลังขึ้นไป

[1]ภาควิชาเภสัชกรรมคลินิก [2]ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ [3]ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112

Mitragyna speciosa (*M. speciosa*) Korth. (Rubiaceae) is traditionally used in Thailand and known as Kratom. It is often used as a substitute for opium when opium is unavailable, or to moderate opium addiction. In folk medicine, it is often used to treat diarrhea. A small minority of users use kratom to prolong sexual intercourse. Users of kratom tend to be peasants, laborers and farmers who use the plant to overcome the burdens of their hard work. Heavy users may chew kratom between 3-10 times a day while new users may only need a few leaves to obtain the desired effects. Some users find with time they need to increase doses to 10-30 leaves or even more per day (Anon, 2006).

Phytochemical studies of the constituents

of *M. speciosa* have been reported. Several 9-methoxy-corynanthe-type monoterpenoid indole alkaloids were isolated from *M. speciosa* leaves (Takayama *et al*., 2000).

The pharmacological activities of the compounds from *M. speciosa* leaves have been studied. Mitragynine, a major indole-alkaloid, isolated from *M. speciosa* leaves, exerts inhibitory effect on electrically stimulated contraction of isolated guinea-pig ileum (Watanabe *et al*., 1997); on forskolin-stimulated cAMP formation in NG108-15 cells (Tohda *et al*., 1997) and on 5-methoxy-N, N-dimethyltryptamine-induced head-twitch response in mice (Matsumoto *et al*., 1997) and has morphine-like action on gastric acid secretion in anesthetized rats and inhibits the vas deferens contraction of guinea-pig elicited by nerve stimulation (Tsuchiya *et al*., 2002; Matsumoto *et al*., 2005a). Mitragynine pseudoindoxyl, oxidative derivative of mitragynine possesses opioid agonist, leading to a potent inhibition of electrically stimulated contraction in guinea pig ileum through mu-receptors and in mouse vas deferens through delta-receptors (Yamamoto *et al*., 1999; Takayama *et al*., 2002). 7-hydroxymitragynine, a minor alkaloid constituent of *M. speciosa*, exhibited a potent opioid effect on the electrically-stimulated contraction in guinea-pig ileum and orally active analgesic effect based on activation of mu-opioid receptors (Horie *et al*., 2005; Takayama, 2004; Matsumoto, 2004; Matsumoto *et al*., 2005b). 9-hyhroxycorynantheidine, synthesized from mitragynine, has partial agonist properties on mu-opioid receptors in the guinea-pig ileum (Matsumoto et al., 2005c).

Although some active compounds, isolated from *M. speciosa* leaves have been reported to show antinociceptive activity, no evaluation of the analgesic activity of the methanol and alkaloid extracts of *M. speciosa* leaves has been clearly reported. In the present study, we investigated the potential analgesic activities of the methanol and alkaloid extracts obtained from *M. speciosa* leaves by using hot plate test in mice and tail flick test in rats. The general behaviors using locomotor activity measurement and pentobarbital-induced sleep in mice were also observed.

Materials and methods

Plant material

The fresh leaves of *M. speciosa* Korth. (Rubiaceae) were collected from natural sources in Songkhla and Satun Provinces during 2004-2005. Authentication of plant material was carried out at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand, where the herbarium vouchers (No. PCOG/MS001-002) have been kept.

Preparation of the methanol and alkaloid extracts from the leaves of *M. speciosa*

The fresh leaves of *M. speciosa* (5 kg) were dried in hot air oven at 45-50^{o}C, powdered and macerated with methanol for 72 hours. Then filtered and evaporated under reduced pressure at 40-45^{o}C to obtain a syrupy mass. The marc was remacerated with methanol twice, filtered and evaporated. All syrupy masses were combined to give crude methanol extract 396 g. An aliquot (300 g) of the methanol extract was dissolved in 10% acetic acid, well shaken and left to stand for 24 hours, then filtered to give the acidic filtrate, which was washed with petroleum ether, made alkaline (pH 9) with 25% ammonia solution and extracted with portions of chloroform. The combined chloroform extract was washed with distilled water, dried over anhydrous sodium sulfate and evaporated under reduced pressure at 40^{o}C to give crude alkaloid extract 9.38 g (approximately 0.25% yield of the fresh leaves weight).

The methanol and alkaloid extracts were used as the test extract. All doses were expressed in terms of the extract (mg/kg body weight).

Animals

All animals used in this study were obtained from the Animal House, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand. Male Swiss mice and Wistar rats with the weight ranging from 30-39 g and 150-230 g, respectively,

were used. The rats were handled for 5-10 min daily for several days before experiments. The animals were housed for at least one week in the laboratory animal room prior to testing. Food and water were given *ad libitum* unless otherwise specified. All procedures described were reviewed and approved by the Institutional Committee for Ethical Use of Animals, Prince of Songkla University, Thailand.

Acute toxicity

The 50% lethal dose of the methanol and alkaloid extracts of *M. speciosa* leaves were estimated by the up-and-down method in mice (Bruce, 1985). Doses were adjusted by a constant multiplicative factor; viz. 1.5, for this experiment. The dose for each successive animal was adjusted up or down depending on the previous outcome.

Antinociceptive activity

1. Hot plate test

The hot plate test was carried out according to the method described by Woolfe & MacDonald (1944). Mice were placed on a hot plate maintained at 55°C ± 1°C. Latency of nociceptive response such as licking of a hind limb or jumping was measured. Starting thirty minutes after p.o. administration of the test agents except morphine (15 min after administration), the nociceptive response was measured every 15 min over a 60 min period. Morphine sulfate was injected subcutaneously. The cut-off time was 45 sec. Only the mice that showed nociceptive responses within 15 sec were used for the experiments.

2. Tail flick test

The tail flick test was performed according to a previously described procedure (D'Amour & Smith, 1941). The tail-flick reflex latency (sec) was measured every 15 min for 1 hr period starting 30 min after oral administration of the methanol extract (50, 100, 200 mg/kg), the alkaloid extract (5, 10, 20 mg/kg) of *M. speciosa* leaves or cosolvent except morphine was subcutaneously administered 15 min (10 mg/kg). The rats whose basal responses were more than 3 sec, were discarded and a cut-off time of 10 sec was maintained throughout the experiment.

Antagonism of the antinociceptive activity of the extracts by pre-treatment with naloxone

Mice were administered with naloxone at a dose of 2 mg/kg (i.p.). After 10 min the test agents were given. The assessments were conducted by hot plate test.

General behaviors

Locomotor activity

Locomotor activity was recorded in an activity cage (Basile, Milan) using a modification of the method previously reported (Capasso *et al.*, 1996). The mice were placed in the cage for at least 10 min for acclimatization before oral administration of drugs. Temperature, sound and light conditions were maintained uniform during the course of the experiments. Measurements were performed at 5 min intervals and cumulative counts were recorded for a 1 h period. Experiments were carried out from 9 a.m. to 5 p.m.

Pentobarbital-induced sleep

Mice were injected with pentobarbital (50 mg/kg) intraperitoneally to induce sleep. The duration of sleep was measured as the period between the loss and the recovery of the righting reflex. The methanol extract (50, 100, 200 mg/kg), alkaloid extract of *M. speciosa* (5, 10 and 20 mg/kg), or cosolvent vehicle was administered orally 30 min before pentobarbital (Ferrini *et al.*, 1974).

Chemicals

The following drugs were used: morphine sulfate, pentobarbital sodium and methamphetamine hydrochloride (AR grade, Sigma Chem. Co., St. Louis, U.S.A.); methanol, petroleum ether, chloroform (AR grade, Merck, Germany); sodium chloride, sodium sulfate, ammonia (AR grade, Carlo Erba, Germanny). The methanol and alkaloid extracts of *M. speciosa* leaves were dissolved in cosolvent solution (propylene glycol : tween 80 : water = 4:1:4) and administered orally in a constant volume (10 ml/kg for mice and 5 ml/kg for rats) 30 min before the experiments. Morphine sulfate was dissolved in 0.9% sodium chloride solution

and administered subcutaneously. All drug solutions were prepared immediately before starting the experiments.

Statistical Analysis

Data are expressed as mean ± SEM and were analyzed statistically by one-way ANOVA procedures, followed by Dunnett's test. A difference was considered significant at $p<0.05$.

Results

Acute toxicity

In acute toxicity test, the signs of toxicity included lethargy, tremor, fatigue, paralysis, loss of righting reflex, apnea, tonic-clonic convulsion and death. The LD_{50} values of orally administration of the methanol and alkaloid extracts of *M. speciosa* leaves in mice were 4.90 g/kg and 173.20 mg/kg, respectively.

Effects of the methanol and alkaloid extracts of *M. speciosa* leaves and morphine on nociceptive response induced by heat in hot plate test

As shown in Table 1, oral administration (50, 100, 200 mg/kg) of the methanol extract of *M. speciosa* leaves significantly prolonged the latency of nociceptive response but less potent than those of the morphine group (10 mg/kg, s.c.) in mice. The alkaloid extract of *M. speciosa* leaves also increased the pain response latency at the dose of 20 mg/kg but had weaker analgesic activity than the methanol extract (100 mg/kg) in mice.

Effects of the naloxone on methanol and alkaloid extracts of *M. speciosa* leaves and morphine in hot plate test

The antinociceptive action of the methanol extract (100 mg/kg, p.o.) and the alkaloid extract (20 mg/kg, p.o.) of *M. speciosa* leaves was blocked by pure opioid antagonist, naloxone (2 mg/kg, i.p.). Morphine sulfate (10 mg/kg, s.c.), a centrally acting analgesic drug, was also antagonized by naloxone, as shown in Table 2.

Effects of the methanol and alkaloid extracts of *M. speciosa* leaves and morphine on nociceptive response in tail-flick test

Neither the methanol extract (50, 100, 200 mg/kg, p.o.) nor the alkaloid extract (5, 10, 20 mg/kg, p.o.) of *M. speciosa* leaves significantly prolonged the rat tail's time that subjected to heat

Table 1. Effects of the methanol and alkaloid extracts of *M. speciosa* leaves and morphine on nociceptive response in hot plate test.

Drug	Dose (mg/kg, p.o.)	Latency of nociceptive response (sec)			
		15	30	45	60 min
Cosolvent	-	9.7±0.6	11.4±1.0	10.4±1.3	11.7±1.5
Morphine	10	24.0±3.8*	28.4±3.9*	23.1±4.2*	21.9±4.0*
M. speciosa	50	13.2±2.2	11.5±0.7	17.2±3.4*	14.3±1.2
(methanol)	100	13.6±1.2	17.4±3.2*	18.5±3.4*	16.3±3.3*
	200	14.0±1.0	12.9±1.1	16.4±2.8*	13.9±1.2
Cosolvent	-	10.0±0.7	10.0±1.2	9.3±0.9	9.2±1.4
Morphine	10	17.5±2.3*	21.6±3.0*	19.7±3.2*	17.8±3.3*
M. speciosa	5	10.1±0.6	9.3±0.6	8.5±0.6	8.7±0.6
(alkaloid)	10	11.5±0.6	9.7±0.8	9.6±1.0	9.4±0.9
	20	13.5±1.1*	13.1±1.4*	12.3±1.1	10.3±0.9

Beginning 30 min after oral administration of test agents (or 15 min after morphine injection, s.c.), the nociceptive response was measured every 15 min over a 60-min period. Each datum represents the latency of nociceptive responses (sec) ± S.E.M. (n=10). * p< 0.05 compared with the control group (Dunnett's test).

Table 2. Effects of the naloxone on methanol and alkaloid extracts of *M. speciosa* and morphine in nociceptive response in hot plate test.

Drug	Dose (mg/kg, p.o.)	Latency of nociceptive response (sec)			
		15	30	45	60 min
Naloxone + Cosolvent	-	9.4±0.7	10.9±0.9	11.9±0.8	10.8±0.8
Naloxone + Morphine	10	10.8±1.2	11.5±1.2	12.8±1.3	17.3±1.7*
Naloxone + *M. speciosa* (methanol)	100	12.3±1.2	15.4±1.2	13.5±1.1	15.6±1.9
Naloxone + Cosolvent	-	9.2±0.7	9.2±0.6	8.4±0.6	7.7±0.7
Naloxone + Morphine	10	10.0±0.5	9.0±0.7	10.7±1.2	10.9±1.3
Naloxone + *M. speciosa* (alkaloid)	20	9.9±0.7	9.7±0.8	8.8±0.7	8.4±0.6

Naloxone (2 mg/kg) was intraperitoneally injected 10 min before test agents administration in mice. The nociceptive response was measured every 15 min over a 60-min period. Each datum represents the latency of nociceptive responses (sec) ± S.E.M. (n=10). *p< 0.05 compared with the control group (Dunnett's test).

generated by the tail flick apparatus while morphine (10 mg/kg, s.c.) significantly increased the latency of nociceptive response (Table 3).

Effects of the methanol and alkaloid extracts of *M. speciosa* leaves and methamphetamine on locomotor activity in mice

Neither the methanol extract (50, 100, 200 mg/kg, p.o.) nor the alkaloid extract (5, 10, 20 mg/kg, p.o.) of *M. speciosa* leaves significantly changed spontaneous motor activity in mice while methamphetamine, a CNS stimulant, significantly increased the motor activity, compared with the cosolvent group (Table 4).

Effect of the methanol and alkaloid extracts of *M. speciosa* leaves on pentobarbital-induced sleep in mice

Neither the methanol extract (50, 100, 200 mg/kg, p.o.) nor the alkaloid extract (5, 10, 20 mg/kg, p.o.) of *M. speciosa* leaves had a significant effect on pentobarbital-induced sleep in mice (Table 5).

Discussion

The results demonstrate that the methanol and alkaloid extracts obtained from the leaves of *M. speciosa* exerted the antinociceptive response to heat-induced pain in hot plate test in mice.

The methanol extract of *M. speciosa* leaves prolonged the latency of nociceptive response on heat-induced pain in hot plate test in mice. The alkaloid extract also increased the pain latency time in hot plate test but was less potent than the methanol extract. It is possible that not only the alkaloids but also some other active compounds included in the methanol extract possessed the analgesic action. Neither the methanol extract nor alkaloid extract significantly affected the pain response in tail flick test in rats. Since the analgesic action in hot plate and tail flick tests involves supraspinal (Yaksh and Rubi, 1976) and spinal components, respectively (Mayer & Liebeskind, 1974). The antinociceptive activity of the methanol and alkaloid extracts is due to action at the supraspinal system. In addition, the analgesic action of the methanol extract (100 mg/kg, p.o.) and alkaloid extract (20 mg/kg, p.o.) was also blocked by naloxone (2 mg/kg, i.p.), a pure opioid antagonist (Gutstein and Akil, 2001). These results suggest that the antinociceptive activity of the extracts partly acts at opioid receptors in the supraspinal opioid system. Furthermore, it has been reported that mitragynine, the active alkaloid in *M. speciosa* leaves, exhibited antinociceptive

Table 3. Effects of the methanol and alkaloid extracts of *M. speciosa* leaves and morphine on nociceptive response in the tail-flick test.

Drug	Dose (mg/kg, p.o.)	Latency of nociceptive response (sec)			
		15	30	45	60 min
Cosolvent	-	2.8±0.6	2.1±0.3	1.9±0.1	2.2±0.3
Morphine	10	9.5±0.4*	9.6±0.4*	9.8±0.2*	8.1±0.8*
M. speciosa	50	3.4±0.6	2.5±0.3	2.2±0.2	2.4±0.3
(methanol)	100	4.2±1.0	2.9±0.3	2.5±0.5	3.5±0.5
	200	2.6±0.4	3.0±0.3	2.2±0.3	2.9±0.6
Cosolvent	-	3.8±1.2	4.0±1.2	3.4±1.5	2.6±0.3
Morphine	10	9.5±0.3*	9.8±0.2*	10.0±0.0*	9.7±0.3*
M. speciosa	5	3.7±1.3	2.9±0.8	3.9±0.5	4.8±1.3
(alkaloid)	10	3.2±1.1	4.1±1.0	2.8±0.7	2.1±0.2
	20	4.1±1.5	4.2±1.5	2.8±0.3	3.1±0.6

Beginning 30 min after oral administration of test agents (or 15 min after morphine injection, s.c.), the nociceptive response was measured every 15 min over a 60-min period. Each datum represents the latency of nociceptive responses (sec) ± S.E.M. (n=6) * p<0.05 compared with the control group (Dunnett's test)

Table 4. Effects of the methanol and alkaloid extracts of *M. speciosa* leaves and methamphetamine on locomotor activity in mice.

Drug	Dose (mg/kg, p.o.)	Locomotor activity (counts/30 min)
Cosolvent	-	343.6±48.5
Methamphetamine (i.p.)	1	1315.9±135.7*
M. speciosa	50	233.3±93.7
(methanol)	100	163.5±63.8
	200	159.2±34.4
Cosolvent	-	507.5±179.9
Methamphetamine (i.p.)	1	1343.5±279.5*
M. speciosa	5	252.9±87.2
(alkaloid)	10	269.4±101.2
	20	258.9±93.9

Thirty min after oral administration of test agents except methamphetamine was injected intraperitoneally in mice, changes in spontaneous motor activity were measured over a 30-min period. Each datum represents the mean ± S.E.M. from 10 mice. *p< 0.05, compared with the control group (Dunnett's test).

actions by involving the descending noradrenergic and serotonergic systems of the supraspinal opioid system on the mechanical noxious stimulation (Matsumoto *et al*., 1996a, b), which is dominantly mediated by mu- and delta-oipoid receptor subtypes in mice (Thongpradichote *et al*., 1998).

In this study, the animals were orally administered the methanol extract of *M. speciosa* leaves at doses of 50, 100 and 200 mg/kg p.o., that are comparable to the peasants orally administered 10-30 leaves per day (Anon, 2006). The effective dose of the methanol extract obtained from *M.*

Table 5. Effects of the methanol and alkaloid extracts of *M. speciosa* leaves on pentobarbital- induced sleep in mice.

Drug	Dose (mg/kg, p.o.)	Duration of pentobarbital-induced sleep (min)
Cosolvent	-	62.2±4.1
M. speciosa	50	70.1±3.3
(methanol)	100	73.0±3.6
	200	75.5±4.9
Cosolvent	-	91.6±7.8
M. speciosa	5	86.8±6.6
(alkaloid)	10	90.0±5.3
	20	86.1±5.8

The methanol and alkaloid extracts of *M. speciosa* leaves were orally administered. After 30 min, pentobarbital (50 mg/kg, i.p.) was injected, and sleeping time was measured. Each datum represents the mean ± S.E.M. (n = 10).

speciosa leaves is 100 mg/kg, p.o. for antinociceptive activity. Although the alkaloid extract is expected to include more active compounds, however, it had weak analgesic activity. Taking into account the yield of 5-10 mg/kg alkaloid extract which corresponds to approximately 200 mg/kg of methanol extract of *M. speciosa* leaves, showed no significant antinociceptive effect. Furthermore, it exhibited higher toxicity than the methanol extract - 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. It is possible that some active compounds included in the methanol extract have synergistic effects on the analgesic action. Thus the preparation of *M. speciosa* leaves or kratom in the methanol extract form has more analgesic efficacy and is less toxic than the alkaloid extract.

In general behavioral study, the methanol and alkaloid extracts of *M. speciosa* leaves had no significant effect on pentobarbital-induced sleep in mice, so a sedative effect could be excluded from their antinociceptive responses in the tests used in this study. Neither of the extracts of *M. speciosa* leaves caused any significant changes in locomotor activity.

In conclusion, these results suggest that the methanol and alkaloid extracts of *M. speciosa* leaves possess analgesic activity which partly acted at opioid receptors in the supraspinal opioid system.

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References

Anon. 2006. Mitragyna speciosa - Kratom. Available from http://www.entheology.org/edoto/anmviewer.asp?a=63&z=5.

Bruce, R.D. 1985. An up- and down procedure for acute toxicity testing. Fundam. Appl. Toxicol., 5: 151-157.

Capasso, A., Di Giannuario, A., Loizzo, A., Pieretti, S. and Sorrentino, L. 1996. Dexamethasone reduced clonidine-induced hypoactivity in mice. J. Pharm. Pharmacol., 48: 615-618.

D'Amour, F.E. and Smith, D.L. 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther., 72: 74-79.

Ferrini, R., Miragoli, G. and Taccardi, B. 1974. Neuropharmacological studies on SB 5833, a new psychotherapeutic agent of the benzodiazepine class. Arzneim-Forsch., 24: 2029-2032.

Gutstein, H.B. and Akil, H. 2001. Opioid analgesics. **In**: Goodman & Gilman's The Pharmacological

basis of Therapeutics, 10th ed., Hardman, J.G. and Limbird, L.E. (eds), McGraw-Hill, New York, pp. 602-604.

Horie, S., Koyama, F., Takayama, H., Ishikawa, H., Aimi, N., Ponglux, D., Matsumoto, K. and Murayama, T. 2005. Indole alkaloids of a Thai medicinal herb, *Mitragyna speciosa*, that has opioid agonistic effect in guinea-pig ileum. Planta Med., 71: 231-6.

Matsumoto, K., Horie, S., Ishikawa, H., Takayama, H., Aimi, N., Ponglux, D. and Watanabe, K. 2004. Antinociceptive effect of 7-hydroxymitragynine in mice: Discovery of an orally active opioid analgesic from the Thai medicinal herb *Mitragyna speciosa*. Life Sci., 74: 2143-55.

Matsumoto, K., Yamamoto, L.T., Watanabe, K., Yano, S., Shan, J., Pang, P.K., Ponglux, D., Takayama, H. and Horie, S. 2005a. Inhibitory effect of mitragynine, an analgesic alkaloid from Thai herbal medicine, on neurogenic contraction of the vas deferens. Life Sci., 78: 187-94.

Matsumoto, K., Horie, S., Takayama, H., Ishikawa, H., Aimi, N, Ponglux, D., Murayama, T. and Watanabe, K. 2005b. Antinociceptive, tolerance and withdrawal symptoms induced by 7-hydroxymitragynine, an alkaloid from the Thai medicinal herb *Mitragyna speciosa*. Life Sci., 78: 2-7.

Matsumoto, K., Takayama, H., Ishikawa, H., Aimi, N, Ponglux, D., Watanabe, K. and Horie, S. 2005c. Partial agonistic effect of 9-hydroxycoryn-antheidine on mu-opioid receptor in the guinea-pig ileum. Life Sci. (in press).

Matsumoto, K., Mizowaki, M., Suchitra, T., Takayama, H., Sakai, S., Aimi, N. and Watanabe, H. 1996a. Antinociceptive action of mitragynine in mice: evidence for the involvement of supraspinal opioid receptors. Life Sci., 59: 1149-55.

Matsumoto, K., Mizowaki, M., Suchitra, T., Murakami, Y., Takayama, H., Sakai, S., Aimi, N. and Watanabe, H. 1996b. Central antinociceptive effects of mitragynine in mice: contribution of descending noradrenergic and serotonergic systems. Eur. J. Pharmacol. 317: 75-81.

Matsumoto, K., Mizowaki, M., Takayama, H., Sakai, S., Aimi, N. and Watanabe, H. 1997. Suppressive effect of mitragynine on the 5-methoxy-N, N-dimethyltryptamine-induced head-twitch response in mice. Pharmacol. Biochem. Behav. 57: 319-23.

Mayer, D.J. and Liebeskind, J.C. 1974. Pain reduction by focal electrical stimulation of the brain and anatomical and behavioral analysis. Brain Res., 68: 73-93.

Takayama, H. 2004. Chemistry and pharmacology of analgesic indole alkaloids from the Rubiaceous plant, *Mitragyna speciosa*. Chem. Pharm. Bull. (Tokyo), 52: 916-28.

Takayama, H., Aimi, N, Sakai, S. 2000. Chemical studies on the analgesic indole alkaloids from the traditional medicine (*Mitragyna speciosa*) used for opium substitute. Yakugaku Zasshi, 120: 959-67. [Abtract]

Takayama, H., Ishikawa, H., Kurihara, M., Kitajima, M., Aimi, N., Ponglux, D., Koyama, F., Matsumoto, K., Moriyama, T., Yamamoto, L.T., Watanabe, K., Murayama, T. and Horie, S. 2002. Studies on the synthesis and opioid agonistic activities of mitragynine-related indole alkaloids: discovery of opioid agonists structurally different from other opioid ligands. J. Med. Chem. 45: 1949-56.

Thongpradichote, S., Matsumoto, K., Tohda, M., Takayama, H., Aimi, N., Sakai, S. and Watanabe, H. 1998. Identification of opioid receptor subtypes in antinociceptive actions of supraspinally-administered mitragynine in mice. Life Sci., 62: 1371-8.

Tohda, M., Thongpraditchote, S., Matsumoto, K., Murakami, Y., Sakai, S., Aimi, N., Takayama, H., Tongroach, P. and Watanabe, H. 1997. Biol. Pharm. Bull. 20: 338-40.

Tsuchiya, S., Miyashita, S., Yamamoto, M., Horie, S., Sakai, S., Aimi, N., Takayama, H. and Watanabe, K. 2002. 443: 185-8.

Watanabe, K., Yano, S., Horie, S. and Yamamoto, L.T. 1997. Inhibitory effect of mitragynine, an alkaloid with analgesic effect from Thai medicinal plant *Mitragyna speciosa*, on electrically stimulated contraction of isolated guinea-pig ileum through the opioid receptor. Life Sci., 60: 933-42.

Woolfe, G. and MacDonald, A.D. 1944. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J. Pharmacol. Exp. Ther., 80: 300-307.

Yaksh, T.L. and Rubi, T.A. 1976. Analgesia mediated by a direct spinal action of narcotics. Sciences, 192: 1357-1358.

Yamamoto, L.T., Horie, S., Takayama, H., Aimi, N., Sakai, S., Yano, S., Shan, J., Pang, P.K., Ponglux, D. and Watanabe, K. 1999. Opioid receptor agonistic characteristics of mitragynine pseudoindoxyl in comparison with mitragynine derived from Thai medicinal plant *Mitragyna speciosa*. Gen. Pharmacol. 33: 73-81.