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Acute and long-term effects of alkaloid extract of *Mitragyna speciosa* on food and water intake and body weight in rats

Ekkasit Kumarnsit [a,*], Niwat Keawpradub [b], Watcharin Nuankaew [c]

[a] *Department of Physiology, Faculty of Science, Prince of Songkla University (PSU), Hat-Yai, Songkhla 90112, Thailand*
[b] *Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, PSU, Hat-Yai, Songkhla 90112, Thailand*
[c] *Southern Botanical Garden in Classical Thai Literature, Chalung, Hat-Yai, Songkhla 90110, Thailand*

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Abstract

Acute administration of *Mitragyna speciosa* (MS) extract (45 and 50 mg/kg) significantly resulted in dose-dependent decreases in food and water intakes ($P<0.05$) in rats. Prolonged suppressing effects were observed following administration of the MS extract (40 mg/kg) for 60 consecutive days. Moreover, the long-term administration also significantly suppressed weight gaining.
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Keywords: *Mitragyna speciosa*; Anorectic activity; Antidepressant; Food intake

1. Introduction

In Thailand, *Mitragyna speciosa* is known as "Kratom". Young leaves of *M. speciosa* are recognized as a rich source of alkaloids. The plant is used in Thai traditional medicine for alleviating pain from cutting wounds, reducing coughing, and stopping diarrhea. Its anti-fatigue effects have been well known and used to enhance physical performances [1]. Still, *M. speciosa* is not approved for these therapeutic purposes in Thailand. It is also widely mentioned as a potential substitute drug for treating drug addiction due to its opium-like action [2]. There was the idea that this indigenous plant might hold the key to reduce withdrawal symptoms, following enforced drug cessation, but up to now, such an effect has not been reported.

The mechanism of action of *M. speciosa* remains largely unknown. MS extract contains at least 10 alkaloids which may be biologically active [3–5]. Mitragynine is a major alkaloid which accounts for two-thirds of the total alkaloid extracted [6]. Most studies have focused on pure mitragynine, for its antinociceptive actions on the central nervous system (CNS) mediated by opioid receptors [7,8]. However, one should not exclude that the effect of the extract may be due to the presence of some other alkaloids. Recently, it has been suggested that *M. speciosa* has therapeutic potential for the treatment of diabetes mellitus. The interest to use this plant on diabetic patients has grown with the increase of the disease in the population, although a direct hypoglycemic activity has not yet been confirmed. Until now, no research has been conducted whether it has such a regulatory action on the blood glucose level. It might have just an anorectic effect, leading to a loss of appetite and therefore reducing food intake and as a secondary effect lowering the blood glucose level. This

* Corresponding author. Tel.: +66 74 288216; fax: +66 74 446680.
E-mail address: ekkasit.k@psu.ac.th (E. Kumarnsit).

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seems to be a likely mechanism, as persons who consume this plant show anorexia and weight loss [9]. These findings suggest that the presumed hypoglycemic activity of the plant might not be true.

The present study, in which the whole alkaloid extract of the plant was used, was designed to determine the effects of *M. speciosa* on food consumption and body weight in rats. This was because several previous studies have been unable to provide any information on which particular component of *M. speciosa* would be responsible for the action. Then there is the possibility that the anorectic effect is caused by two or more of the alkaloids found in the MS extract of the plant, and that each on its own is insufficient for the observed activity.

2. Experimental

2.1. Plant material

Young leaves of *M. speciosa* Korth (Rubiaceae) were collected from natural sources in Songkla Province, Thailand. Plant material was identified by Dr. Niwat Kaewpradub, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, PSU. The use of plant material was approved by the Ministry of Agriculture of Thailand and was restricted to research purpose only.

2.2. Preparation of the extract

Extraction and isolation of alkaloids from the plant have been described in a previous study [4], with the following modifications. Briefly, young leaves were dried at 45–50 °C, powdered and macerated with MeOH. The filtrate was evaporated in vacuo. The residue was dissolved in 10% aqueous acetic acid, filtrated and washed with petroleum ether, then made into alkaline (pH 9) with 25% ammonia solution and extracted with chloroform. The combined chloroform extracts that were washed, dried over anhydrous sodium sulfate and evaporated gave 0.25% of dry crude alkaloid extract. The major alkaloid, isolated by Silica-gel CC eluting with 5:95 MeOH:$CHCl_3$, was identified as mitragynine with the standard spectroscopic methods (MS, ^{1}H NMR and ^{13}C NMR). According to the TLC analysis, mitragynine was found to constitute about 60% of the content of crude alkaloid extract.

2.3. Animals

Male Wistar rats weighing 180–200 g were used. They were housed in standard environmental conditions individually in wire-mesh cages (20 × 25 × 35 cm) and fed with standard commercial food pellets and water ad libitum. Animals were acclimatized to this condition for at least one week prior to the experimental use, provided they showed relatively stable food consumption. The experimental protocols for care and use of experimental animals described in the present study were approved and guided by the Animals Ethical Committee of the PSU.

2.4. Effect on food, water consumption and body weight gain

2.4.1. Acute administration

Rats were given a single intraperitoneal injection (i.p.) of either saline or one of the doses of *M. speciosa* (MS) extract (15, 30, 45 and 50 mg/kg) at 09:00 a.m. Imipramine [10] (40 mg/kg) was used as positive control. Food and water intakes were recorded at 24 h after the injection. Follow-up injections were performed after five or more drug-free days.

2.4.2. Chronic administration

Rats were divided into two groups (*N* 10), receiving either saline or MS extract (40 mg/kg, i.p.) once a day for 60 consecutive days. Food, water intakes and body weight were recorded once a day at 24 h after the daily injection.

2.5. Statistical analysis

Food and water intakes in gram per kilogram of body weight were presented as mean ± SEM. Statistical differences between the dose-treated and the control groups were determined by using repeated measures one-way ANOVA

followed by Dunnett's test using the SIGMA-Stat computer program. Differences between the saline- and the MS-treated groups in the chronic experiment were determined by using Student's *t*-test. A significance level of $P<0.05$ was used for all statistical analyses.

3. Results and discussion

3.1. Acute effects of MS extract administration on food and water intakes

The effects of acute administration of MS extracts on food intake are reported in Fig. 1. In ad libitum fed rats, the saline-treated group consumed 88.4±4 g/kg/24 h compared to 44.7±10 consumed by the imipramine group. A single injection of MS extract at 45 and 50 mg/kg also induced a significant reduction of food intake (47.8±7 and 45.0±8 respectively, $P<0.01$) compared to saline control, whereas the lower doses (15 and 30 mg/kg) did not have such an effect. Thereafter, average food intake per day of imipramine- and 45- and 50-treated groups gradually increased to control level on day 3 (data not shown). However, no overeating of these groups was observed on the following days after the first 24-h period.

The effects of acute administration of MS extracts on water intake were also evaluated and are shown in Fig. 1. The saline-treated group had the average water intake at 117.7±8 g/kg/24 h/rat whereas the imipramine-treated group had only 32.4±7 during the first 24 h after administration. Significant decreases were also observed in the 45- and 50-treated groups (81.4±16 and 57.3±15, $P<0.05$ and $P<0.01$ respectively) but not in the groups treated with lower (15 and 30 mg/kg) doses.

3.2. Chronic effects of MS extract administration on food intake

The effect of intraperitoneal administration of MS extract at a dose of 40 mg/kg day^{-1} for 60 days on daily food consumptions is shown in Fig. 2 (A). Significantly different amounts of food consumed by control- and MS extract-treated groups are seen from the beginning until the end of the experiment. Rats given MS extract show a significantly lower food intake throughout the experiment than saline-treated rats ($P<0.05$). The average food intakes of control- and MS-treated groups were 22.7±0.1 and 18.4±0.3 g/24 h/rat, respectively. The amounts of food intake were also analyzed as cumulative food intake throughout the chronic period and are shown in Fig. 2 (B). Up to day 2, the MS-treated group ate 8.9 g/rat less than that eaten by the control group. Thereafter, the significant difference between cumulative food intake of these two groups increased until the final day ($P<0.01$). For over 60 days, the MS-treated group ate 1133±44 g/rat, whereas the control group ate 1382±30 g/rat.

When expressed as the amount per body weight, significant decreases of food intakes in the MS-treated group are seen in Fig. 2 (C). The amount of food consumed by rats given MS extract in g/24 h per b.w. is lower than the control group

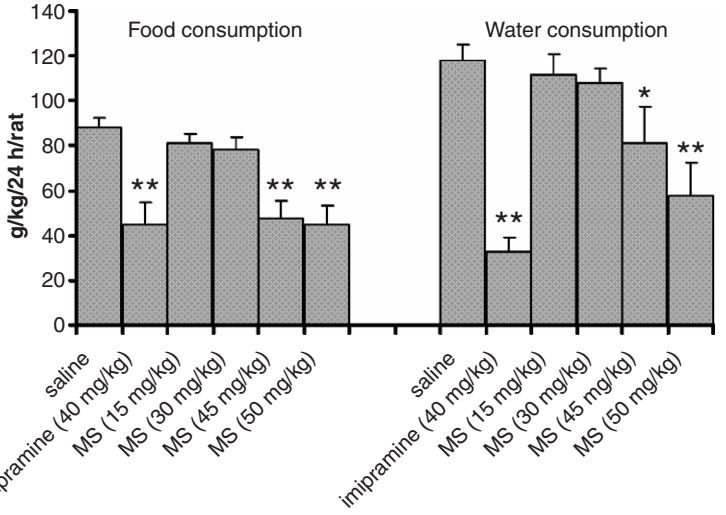

Fig. 1. Effects of acute intraperitoneal administration of *M. speciosa* (MS) extract on food and water intake in rats. Data are mean±S.E.M., *N* 10. *$P<0.05$ and **$P<0.01$ vs controls.

especially during the first 20 days of the experiment. Later in the experiment, the difference between these two groups is smaller. This is probably due to weight reduction induced by the MS extract in the MS-treated group. Anyway, the suppressive effects of the extract on food intake were clearly seen throughout the whole treatment period.

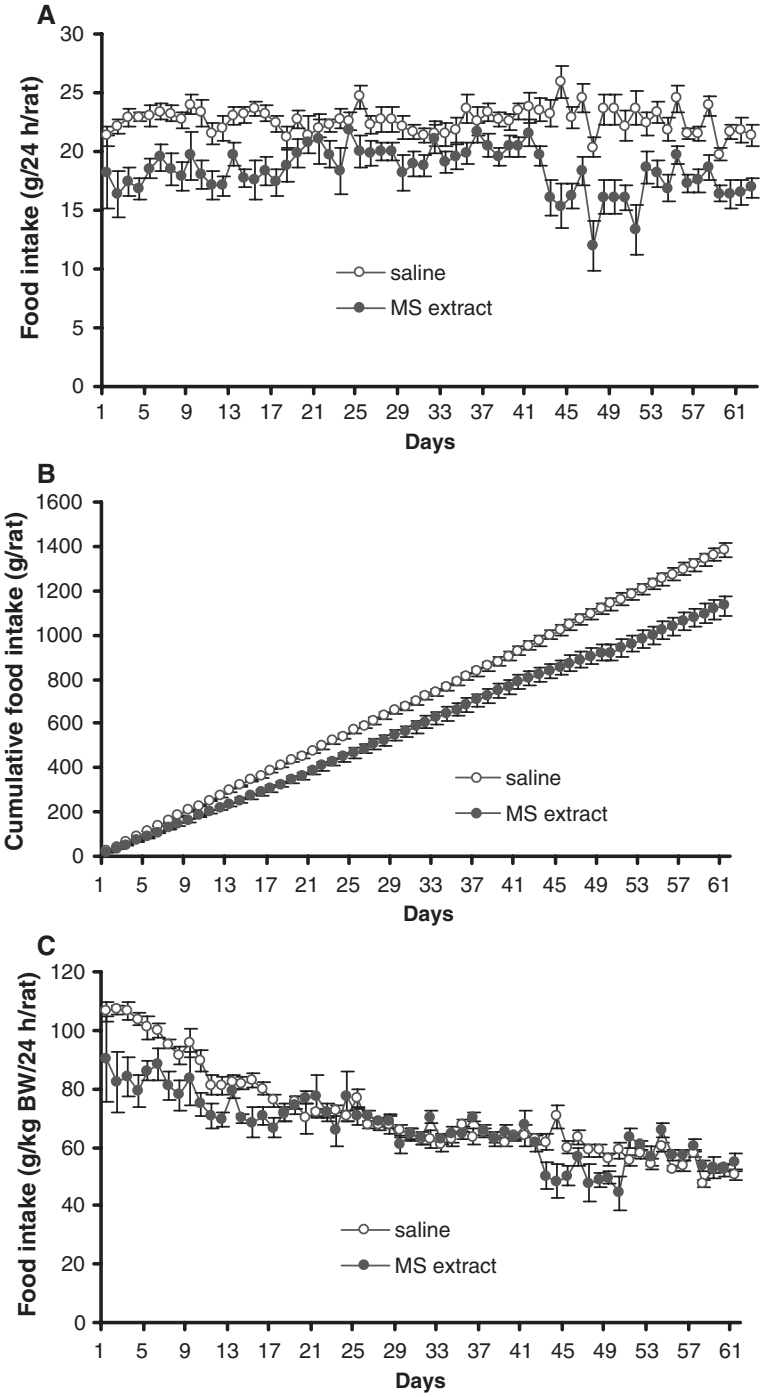

Fig. 2. Effect of intraperitoneal administration of *M. speciosa* (MS) extract at a dose of 40 mg/kg day^{-1} for 60 days on daily, (A), cumulative (B), per kilogram body weight (C), on food intake in rats. *N* 10. $^{**}P<0.05$.

3.3. Chronic effects of MS extract administration on water intake

Like food intake, rats given MS extract exhibited a lower water intake ($P<0.05$) throughout the chronic period of the experiment compared to saline-treated rats as shown in Fig. 3 (A). The average levels on day 1 were 27.9±2 and 16.6±3 g/24 h rat^{-1} for control and MS-treated groups respectively. The difference between water intakes of the two

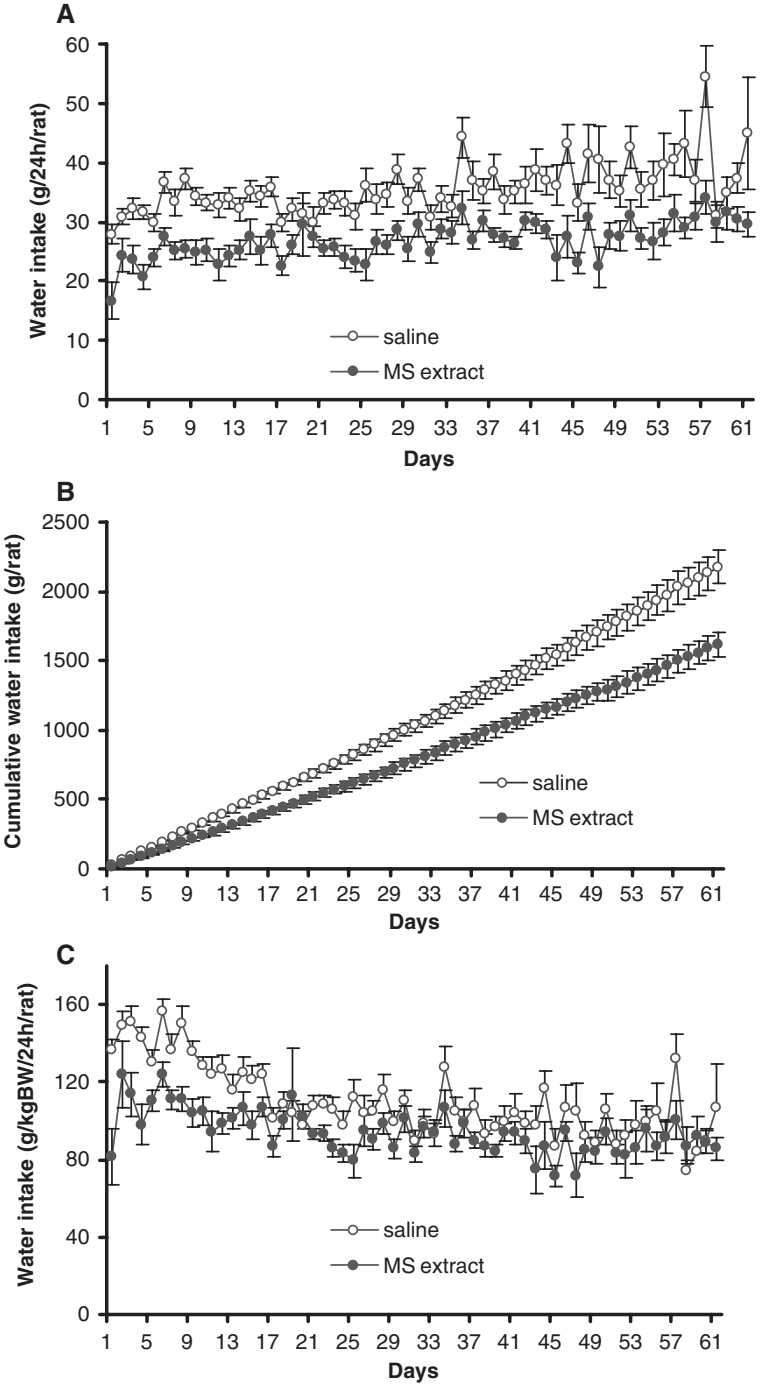

Fig. 3. Effect of intraperitoneal administration of *M. speciosa* (MS) extract at a dose of 40 mg/kg day^{-1} for 60 days on daily, (A), cumulative (B), per kilogram body weight (C), on water intake in rats. *N* 10. **P*<0.05.

groups is seen all throughout the experiment. Cumulative water intake was also analyzed and is shown in Fig. 3 (B). The cumulative water intake over 60 days is 25.6% significantly lower in the MS-treated rats than in the saline-treated rats (Student's *t*-test, $P<0.01$). Water intakes per body weight are shown in Fig. 3 (C). Average water intake per body weight of the MS-treated group is relatively lower than the saline-treated group especially during the first 20 days. Thereafter, the difference between the two groups gradually lessens and becomes more equal.

3.4. Chronic effects of MS extract administration on body weight gain

Chronic MS administration showed an average body weight gain 3.5 g day^{-1} compared to 5.8 g day^{-1} of the control group during the first 10 days as shown in Fig. 4. On the final day, the average body weight of the MS-treated group was 346±15 g compared to 420±9 g of the control group. The weight gain of the MS-treated group is 17.6% significantly less than that of the saline-treated group ($P<0.01$). It means that chronic administration of the MS extract significantly suppressed body weight gaining.

The results of this study demonstrated that both acute and chronic intraperitoneal administration of alkaloid extract of *M. speciosa* strongly suppressed voluntary food consumption in rats. Suppressions of food intake and weight gaining confirmed the anorectic action of the MS extract which was similar to that observed for imipramine used as reference [10]. Interestingly, food intake suppression seen on the first day following an acute injection of MS extract (40 mg/kg) did not result in overeating to restore energy balance on the following days. This pattern was similar to that with imipramine. Moreover, the long-term suppressing effect on food intake during the 60-day period suggests that no clear tolerance had yet been developed.

The 40-mg/kg dose of the MS extract was selected for chronic experiment since acute experiment showed that the 45-mg/kg dose was the lowest dose that suppressed food intake significantly. Higher dose would exhibit stronger effect but it could also affect locomotor activity and induce toxicity.

It would be of interest to know the neural pathways that mediate such an anorectic effect. Previous studies revealed that central opioid [11], adrenergic [7], and serotonergic [12] systems were involved in central effects of mitragynine, a pure major alkaloid of *M. speciosa*. Administrations of various types of opiate consistently reduced food consumption [13,14]. Gue and colleagues found that one of the opiate mechanisms on food consumption is gastric emptying [15]. Another possible mechanism for the anorectic effects of the MS extract could be an interaction with the serotonergic and noradrenergic systems that substances that promote central serotonergic transmission such as fenfluramine [16] or desmethylimipramine [17] are regularly found to reduce food intake and body weight. Activation of serotonin receptors might be the factor that contributes to early satiety and loss of body weight [18,19]. In our study, imipramine, a dual serotonin and noradrenalin reuptake inhibitor was also used as positive control. It significantly suppressed food intake similarly to that observed in a previous study [10].

Still, other minor compounds of the plant cannot be excluded but it is likely that mitragynine plays a major role in the effects. It accounts for 66% based on crude base of total alkaloid from young leaves of the plant and its central

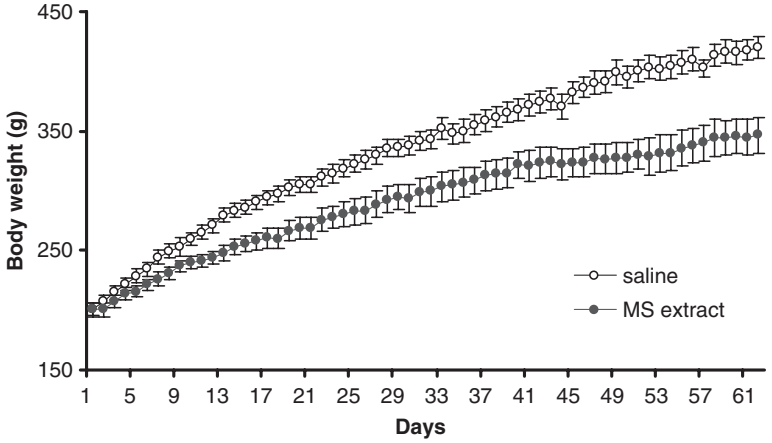

Fig. 4. Effect of intraperitoneal administration of *M. speciosa* (MS) extract at a dose of 40 mg/kg/day for 60 days on body weight gain in rats. *N* 10. *$P<0.05$.

serotonergic action has been documented. The inhibitory effects of mitragynine on gastric secretion were confirmed in rats [8]. Altogether, our results support the findings of Jansen and Prast [9] that persons who take *M. speciosa* show anorexia and weight loss.

The present study also shows the inhibitory effect of the MS extract on water intake. Tolerance to this effect of the extract was not observed even after the 60-day administration in rats. Where feeding control of central serotonin has been well documented, its effect on water intake has still to be explained. Though inhibitory patterns of the MS extract on food and water intakes were similar, their mechanism was shown to be dissociated from each other [20]. Water intake is inhibited by activation of serotonin receptor [21]. Serotonin 2A/2C receptor subtypes were found to mediate inhibitory effects on water intake [22]. Additionally, injection of clonidine, α2-adrenergic agonist, also resulted in inhibition of water intake [23]. However, activation of the α2-adrenergic and serotonergic mechanisms seemed to act independently to inhibit water intake [24]. These findings suggest that the MS extract may act on the central serotonergic or adrenergic systems to suppress water intake.

In summary, we have observed that the alkaloid extract of *M. speciosa* suppresses food and water intakes and slows weight gaining in rats. These effects persist for a prolonged period without clear tolerance. Mitragynine is a potential candidate for these effects. Opioidergic, serotonergic and adrenergic systems are thought to mediate the actions of the extract. The present study suggests that suppressing effects of MS extract on food consumption might be an indirect mechanism that reduces the blood glucose level. Further studies are needed to investigate central mechanism and long-term toxicity of the extract on bodily functions.

Acknowledgements

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