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Anti-nociceptive Activity of the Fruits of *Swietenia macrophylla* King.

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ABSTRACT

Fruits of *Swietenia macrophylla* King (Meliaceae) are used in Malay folk medicine as pain killer. The antinociceptive effect of ethanol and aqueous extracts was assessed by oral administration in acetic acid induced writhing, tail immersion and hot plate tests in mice. The ethanol extract 200 mg/kg treated group reduces the number of writhing on animal model, showed powerful anti-nociceptive effect ($P < 0.01$). Also the significant ($P < 0.01$) analgesic activity of *S. macrophylla* ethanolic extract at the dose of 200 mg/kg was further confirmed by tail-flick response and hot-plate response ($P < 0.01$).*S. macrophylla* extract produce analgesic in chemical and thermal pain models through a mechanism partially linked to either lipooxygenase and/or cyclooxygenase via the arachidonic acid cascade and/or opioid receptors. Our results corroborate the analgesic effects of *S.macrophylla*, and confirm its traditional use for treating pain.

Keywords: *Swietenia macrophylla*; analgesic; ethanol extract; folklore claim

INTRODUCTION

Swietenia macrophylla King (Meliaceae), also known as Big-leaf mahogany, has been found commonly in neotropical regions[1,2,3]. The tree can grow upto a height of 40–60 m. and is native to the tropical America, Mexico, South America, and India. This tree produces a fruit commonly called "sky fruit" because the latter seems to hang upwards from the tree. Sky fruit has been processed commercially to a wide range of health foods and healthcare products. The fruit concentrate, in particular, has been used traditionally to improve blood circulation and skin condition. In Malaysia it was traditionally used for the treatment of diabetes and also to relieve pain. Previous phytochemical investigations have revealed the presence of limonoids such as swietenine, swietenolide, 8, 30-epoxyswietenine acetate, swietenine acetate, swietenolide diacetate, swietenolidetiglate, augustineolide and 3b, 6-dihydroxydihydrocarapin in the seeds[4,5,6,7,8,9,10]. Some of these limonoids have been shown to exhibit antimalarial[11] and insect antifeedant activities[12]. The leaves have been reported to yield essential oils which contain himachalene, germacrene D, germacrene A, cadina-1,4-diene, hexadecanoic acid and ethyl hexadecanoate[13]. The fruits of S. macrophylla have been reported to have anti-inflammatory, antimutagenicity, antitumor and anti-diabetic activity. In order to scientifically substantiate the folklore claim of the plant being used to relieve pain, it has been chosen for the present study.

METHODS AND MATERIALS

Plant material

Fruits of *S. macrophylla* were collected from the district of Port Klang, Selangor, Malaysia in the month of October 2008. The fruit sample was authenticated by Dr.J.Anbu Jeba Sunilson, Pharmacognosist from Masterskill University College of Health Sciences, Cheras, Malaysia. A voucher specimen of the collected plant sample was also deposited in the Herbarium of Masterskill University College of Health Sciences, Malaysia (MUCHSPHA/M3/A 001).

Preparation of extracts

The shade dried fruits of *S. macrophylla* (1000 g) were subjected to size reduction to coarse powder. The powder was defatted with petroleum ether (60–80 °C) and then extracted with 95 % ethyl alcohol using Soxhlet apparatus[14] till exhaustion for about 32 h. The aqueous extract was also prepared by the percolation method using distilled water. Both the ethanolic and aqueous extracts were concentrated under vacuum to get the residues. The percentage yields of ethanolic extract and aqueous extracts were found to be 8.9 % (w/w) and 17.1 % (w/w), respectively. All the test suspensions were prepared in the vehicle, i.e., Tween-80.

Animals

Albino mice of either sex, weighing 18-25 g, were obtained from the Animal House of Masterskill University College of Health Sciences, Malaysia. The animals were housed in groups of six, in standard cages, at room temperature (25 ± 3 °C), with 12 h dark/12 h light cycles, food and water *ad libitum*. The experiments were approved by the Institutional Animal Ethical Committee of Masterskill University College of Health Sciences, Malaysia.

Toxicity studies

Acute toxicity study was performed for ethanolic and aqueous extracts in albino mice as per OECD guidelines. The animals were fasted overnight and provided only water, after which the extracts were administered orally at the dose of 300 mg/kg and observed for 14 days. If mortality was observed in two out of three animals, the dose administered was considered as the toxic dose. If the mortality was observed in one animal, the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was re-

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peated with a higher dose, i.e., 2000 mg/kg.

Abdominal constriction induced by acetic acid

In the acetic acid-induced writhing test[15], groups of overnight fasted mice (n = 6) were treated orally with the ethanolic and aqueous extract of *S. macrophylla* (200 mg/kg), vehicle (10 ml/kg) or the standard drug acetylsalicylic acid (200 mg/kg), 1 h before the administration of acetic acid (0.7%, 10 ml/kg, i.p.). The number of writhing was counted for each animal, starting 3 min after acetic acid injection over a period of 12 min.

Tail Immersion test

In the tail immersion test[16] the thermostat was adjusted so that a constant temperature of 54 ± 1 °C was maintained in the water bath. Pre-treatment latencies were determined three times with intervals of 15 min. Only mice showing a pretreatment reaction for less or equal to 3 s were selected for the study. Immediately after the basal latency assessment, the mice were pre-treated with the ethanolic and aqueous extract of *S. macrophylla* (200 mg/kg), or vehicle (10 ml/kg) 1 h before the measurement. A morphine (7.5 mg/kg, s.c. 30 min before the test)-treated animal group was included as a positive control. The cut-off time was 7 s in the tail-flick measurements in order to minimize tissue injuries. The animals were placed into individual restraining cages leaving the tail hanging out freely and were allowed to adapt to the cages for 30 min before testing. The lower 5 cm portion of the tail was marked. This part of the tail was immersed in a cup of freshly filled water of exactly 55 °C. Within a few seconds the rat reacted by withdrawing the tail. The reaction time was recorded in 0.5 s units by a stopwatch.

Hot-plate method

The hot-plate method[17] was carried out on groups of female mice using a hot-plate apparatus (model YLS-6B, China), maintained at 55 ± 1 °C. Pre-treatment latencies were determined three times with intervals of 20 min. Only mice that showed initial nociceptive responses between 5 and 30 s were selected for the experiment. The groups of mice were pre-treated with the the ethanolic and aqueous extract of *S. macrophylla* (200 mg/kg), or vehicle (10 ml/kg) and 1 h later the measurement started. A morphine (7.5 mg/kg, s.c.) 30 min before the test treated animal group was included as a positive control. The animals were placed on the hot plate and the time until either licking or jumping occurs was recorded by a stop-watch. The cut-off time was 60 s in the hot-plate test in order to minimize skin damages.

Statistical analysis

All data were expressed as the mean ± S.E.M. Data was subjected to ANOVA followed by Dunnett's multiple comparison test. P = 0.05 was considered significant.

RESULTS

Acute Toxicity Tests

In the acute toxicity studies, oral administration of the extracts of *Swietenia macrophylla* fruits did not produce any mortality in mice up to a dose level of 2000 mg/kg. This may be due to broad nontoxic therapeutic index of this plant. So the dose of the extracts was fixed as 200 mg/kg, i.e. 1/10th of the maximum tolerated dose.

Effect of abdominal constriction induced by acetic acid

In the acetic acid-induced writhing test, the treatment with ethanolic extract of *S. macrophylla* (200 mg/kg) and the standard drug acetyl salicylic acid (200 mg/kg) decreased significant amount of inhibition on the mean number of writhes (Table 1). The data showed that the fraction had a dose-dependent inhibition of writhing on nociception.

Effect of the tail immersion test

In the tail immersion test, the ethanolic extract of *S. macrophylla* (200 mg/kg) showed a significant effect on the duration in the hot water, when compared to vehicle-treated control group (Table 2). The positive control group treated with morphine (10 mg/kg) exhibited powerful analgesic activity.

Effect of the hot-plate method

In the hot-plate test, the mean of the durations in the test groups i.e. aqueous and ethanolic extract of *S. macrophylla* (200 mg/kg) the positive control group (morphine, 7.5 mg/kg) and control group are shown in Table 3. The results showed that the group treated with the ethanolic extract of *S. macrophylla* (200 mg/kg) and morphine had the powerful anti-nociceptive effect.

Table 1. Effects of aqueous and ethanolic extracts of *S.macrophylla* fruits on acetic acid induced writhing in mice

Groups	Dose	No. of Writhes
Vehicle: Tween 80	10 ml/kg	48.54 ± 5.62
Ethanolic Extract	200 mg/kg	24.8 ± 2.31**
Aqueous extarct	200 mg/kg	28.7 ± 3.03*
Acetyl Salicylic Acid	100 mg/kg	21.3 ± 4.22**

*Values are expressed as mean ± S.E.M. (n = 6). Asterisks indicated significant difference from control. *P < 0.05, **P < 0.01, ***P < 0.001 (ANOVA followed by Dunnett's test).*

Table 2. Effects of aqueous and ethanolic extracts of *S.macrophylla* fruits on tail immersion test in mice.

Groups	Dose	Reaction Time (s)
Vehicle: Tween 80	10 ml/kg	1.36 ± 0.25
Ethanolic Extract	200 mg/kg	3.15 ± 0.30**
Aqueous extarct	200 mg/kg	1.92 ± 0.58
Morphine	7.5 mg/kg	5.20 ± 0.46***

*Values are expressed as mean ± S.E.M. (n = 6). Asterisks indicated significant difference from control. *P < 0.05, **P < 0.01, ***P < 0.001 (ANOVA followed by Dunnett's test).*

Table 3. Effects of aqueous and ethanolic extracts of *S.macrophylla* fruits on hot plate induced thermal pain in mice.

Groups	Dose	Reaction Time (s)
Vehicle: Tween 80	10 ml/kg	14.82 ± 2.03
Ethanolic Extract	200 mg/kg	21.63 ± 3.50**
Aqueous extarct	200 mg/kg	19.22 ± 1.4*
Morphine	7.5 mg/kg	28.16 ± 2.2***

*Values are expressed as mean ± S.E.M. (n = 6). Asterisks indicated significant difference from control. *P < 0.05, **P < 0.01, ***P < 0.001 (ANOVA followed by Dunnett's test).*

DISCUSSION

In the present experiment, the analgesic activities of the aqueous and the ethanolic extract of *S. macrophylla* against chemical nociception in mice induced by intraperitoneal acetic acid and thermal nociception, was evaluated.The acetic acid-induced writhing method is widely used for the evaluation of anti-nociceptive activity. Our results indicated that the ethanolic extract of *S. macrophylla* at the dose of 200 mg/kg could reduce the number of writhing on animal model, showed powerful anti-nociceptive effects (P < 0.01) (Table 1). Furthermore, the significant effect of the ethanolic extract of *S. macrophylla* at the dose of 200 mg/kg (P < 0.01) on the tail-fick response and on the hot-plate response, provided a further confirmation of its analgesic effect (Table

1 and Table 2 respectively). In conclusion, the study demonstrated the anti-nociceptive activity of the ethanolic extract of *S. macrophylla* in the test models of nociception induced by chemical and thermal stimuli.

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