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In *Vitro* Evaluation of Analgesic Activity of the Methanol Extract of *Pilea microphylla* (L.) Liebm. in *Swiss Albino* Mice

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Abstract

Due to the availability of the plant Pilea microphylla (L.) Liemb in Bangladesh, the present study was aim to investigate in vitro analgesic activity of the methanol extract of Pileamicrophylla (L.) Liebm. analgesic activity was conducted at the doses of 200 and 400 mg/kg body weight using Swiss albino mice as animal model by two thermal (hot plate and tail immersion test) methods. Overall, the extract showed significant analgesic activity compared to standard drug (Diclofenac-Na, 5 mg/kg body weight). Thus, the obtained findings of this present work provide a rationale for the use of this plant for medicinal purposes and encourage us for further investigations to obtain more fruitful results.

Keywords: *Pilea microphylla, Urticaceae, analgesic activity, Swiss albino mice, Diclofenac-Na*

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INTRODUCTION

Pilea microphylla (L.) Liebm. an annual herb, belonging to Urticaceae family, commonly known as gun powder or artillery plant is acclimatized to grow in extreme and diverse habitats [1]. In Bangladesh, the plant grows in shady and moist places of all over the country. Literature review suggested that, the entire plant infusion is used as diuretic. The plant is used for labour pains in women. It is used in infertility, inflammation and urinary problems. It is also used for treatment of diarrhea through decoction of stem. The entire plant has antibacterial activity and used for stomach and intestinal trouble. The leaves are applied to sores and bruises [2]. *P. microphylla* is being used as folk medicine to treat several allergies/wounds. It is reported to possess antibacterial activity [3], moderate antioxidant activity, [4] and also used for infertility, inflammations, and womb cleanser [5]. There is no enough report about the analgesic activity of *Pilea microphylla*. Thus, the purpose of this study was to evaluate this plant as new potential sources of natural analgesics.

MATERIALS AND METHODS

Chemicals

The drugs and chemicals used in the current study were Diclofenac-Na (Square Pharmaceuticals Ltd.), Methanol (Merck, Germany), DMSO (Merck, Germany).

Plant Material and Extraction

The whole plant of *P. microphylla* was collected from Dhaka, Bangladesh in October, 2016. Later, the plant was identified by respective person of Bangladesh National Herbarium Institute, Mirpur, Dhaka. An accession number was given from there and a voucher specimen (DACB: 43063) has been deposited in the herbarium for future reference. The powdered plant (120 g) of *P. microphylla* was soaked in 300 mL of methanol for 10 days and then the extract was filtered through a cotton plug followed by Whatman filter paper number 1 and then concentrated by using a rotary evaporator at low temperature (40–50°C) and reduced pressure to have greenish color extract (1.3 g). The yield value of the extract was 1.083 %.

Animal

For the experiment, *Swiss Albino* mice of both sex having 3–4 weeks of age, weighing between 20–25 g. were collected from the animal Research Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). Soft wood shavings were used as bedding of cages. Animals were

maintained under Standard Environmental conditions: Temperature (24.0±1.0°C), Relative humidity (55–65%) and 12 hrs. light /12 hrs. dark cycle. Husk and excreta were removed from the cages every day [6].

Analgesic Activity

The study of analgesic activity of *P. microphylla* was performed in animal models for central mechanism of pain. For the screening of analgesic activity centrally, hot plate and tail immersion tests were used.

Hot Plate Test

The analgesic activity tests were carried out in the laboratory on five mice in each group by Hot Plate method. The paws of mice are very sensitive to temperature at 55 ± 0.5 °C, which are not damaging to the skin. The animals were placed on Eddy's hot plate kept at a temperature of 55 ± 0.5 °C. A cut-off period of 15 s [7] was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped at 0, 30, 60 and 90, 120 min after oral administration of the samples [8]. The animals of test group received test samples at the doses of 200 and 400 mg/kg body weight. Positive control group and vehicle control group were treated with Diclofenac-Na (5 mg/kg b.w.) and in distilled water (0.1 mL/mouse), respectively [9].

Tail Immersion Test

In this procedure, the animals of the control, positive control and test groups were treated with Diclofenac-Na (5 mg/kg body weight), water (10 mL/kg body weight) and test samples at the doses of 200 and 400 mg/kg body weight, respectively. 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C.

The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 s was defined as complete analgesia and the measurement was stopped when the latency period exceeded to avoid injury to mice. The latent period of the tail-flick response was determined at 0, 30, 60, 90 and 120 min after the administration of the test sample and standard [6].

RESULTS AND DISCUSSION

Hot Plate Test

Based on the findings of two thermal pain models employed in this study, *P. microphylla* was found to possess analgesic activity. The hot plate test is widely used for assessing analgesic activities. In this experiment, *P. microphylla* exhibited significant analgesic activity in hot plate test (Table 1 and Figure 1).

Tail Immersion Test

The analgesic activity tests were carried out in the laboratory on five mice in each group by tail- flick method. Time interval for the test was 30 min. The tail withdrawal reflex time after administration of *P. microphylla* was found to increase with increasing dose of the extract. The tail immersion test is also widely used for assessing analgesic activity. In this experiment, *P. microphylla* exhibited significant analgesic activity in tail immersion test (Table 2 and Figure 2).

Table 1: *Results of Hot Plate Test for Plant Extract of P. microphylla.*

Treatment	Dose (mg/kg) b.w.	Number of Movements				
		0 min	30 min	60 min	90 min	120 min
Control	0.1ml/mice	3.292±0.699	4.092±0.586	6.376±2.258	6.284±1.011	6.26±0.851
Positive control	5 mg/kg	1.756±0.076	4.846±1.544	9.29±2.921	9.44±1.913	5.54±0.901
Group-l	200 mg//kg	8.96±2.25	15.92±1.579	15.28±1.884	19.18±4.366	11.38±1.408
Group-ll	400 mg/kg	13.82±1.959	11.08±2.268	12.82±1.996	12.26±3.279	12.16±1.767

Values are expressed as Mean ± SEM, (n=5). Control: DMSO+Water, Positive Control: Diclofenac-Na (5 mg/kg), Group: Plant extract (100 mg/kg 200 mg/kg, 400 mg/kg).

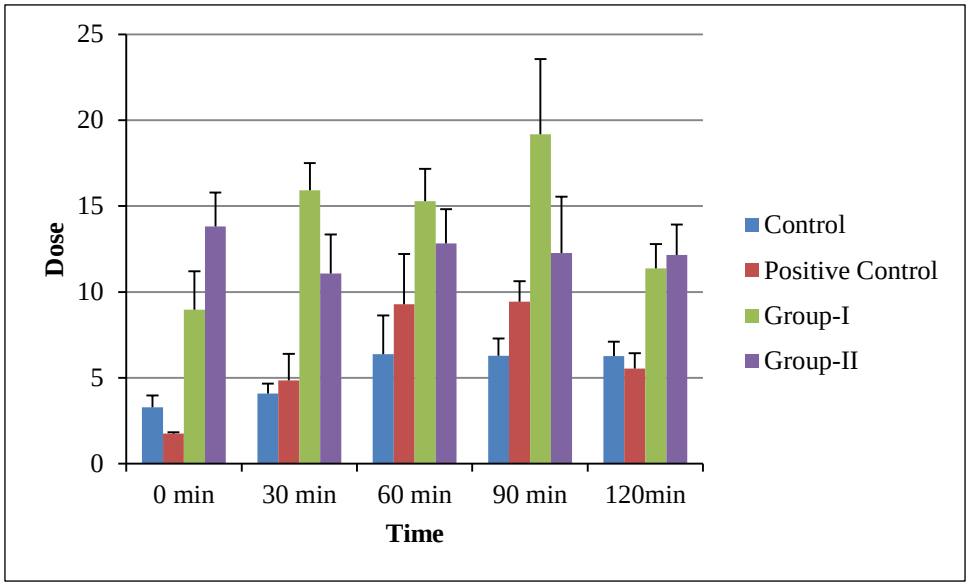

Fig. 1: *Results of Hot Plate Test for Plant Extract of P. microphylla.*

Table 2: *Results of Tail Immersion Test for Plant Extract of P. microphylla.*

Treatment	Dose (mg/kg) b.w.	Number of movements				
		0 min	30 min	60 min	90 min	120 min
Control	0.1 ml/mice	1.404±0.086	2.23±0.165	2.204±0.112	2.209±0.088	1.785±0.177
Positive control	5 mg/kg	8.0±1.225	4.8±0.860	4.0±1.0	4.2±1.113	1.0±0.447
Group-l	200 mg//kg	2.426±0.144	2.559±0.267	3.219±0.403	3.266±0.258	2.766±0.248
Group-ll	400 mg/kg	3.173±0.333	2.947±0.373	3.039±0.238	2.533±0.336	2.139±0.236

Values are expressed as Mean ± SEM, (n=5). Control: DMSO+Water, Positive Control: Diclofenac-Na (5 mg/kg), Group: Plant Extract (200 mg/kg, 400 mg/kg).

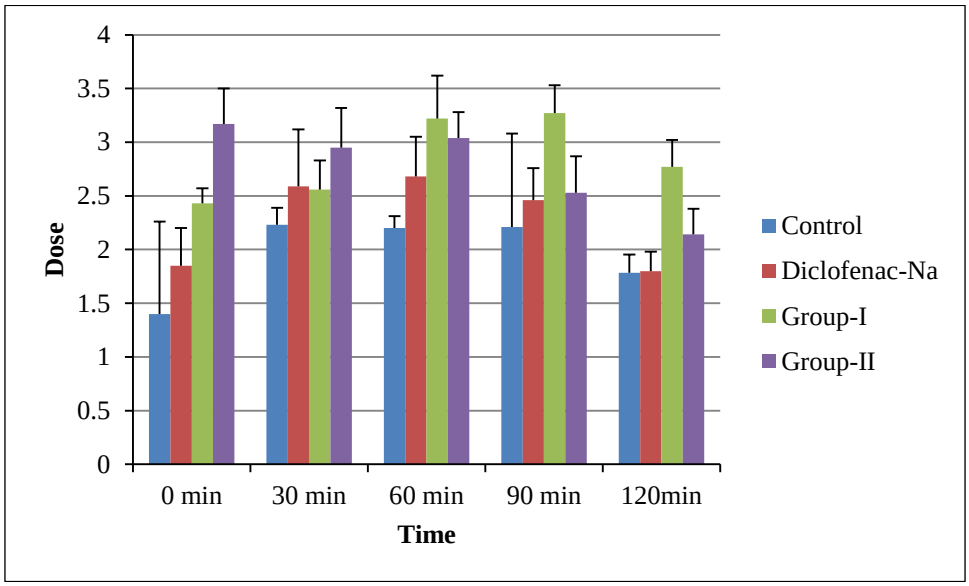

Fig. 2: *Results of Tail Immersion Test for Plant Extract of P. microphylla.*

Statistical Analysis

All experiments were performed thrice and the results averaged. Data were expressed as Mean ± SD.

CONCLUSION

Based on the result of the present study, it can be concluded that the methanol extract of *P. microphylla* possesses analgesic potential. Notable analgesic activity was observed in both hot plate and tail immersion test. Therefore, the investigation provides the rationality of this plant for traditional use. These findings also help to isolate novel bioactive compound(s).

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