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ANALGESIC ACTIVITIES OF METHANOL EXTRACT OF *PHYLLANTHUS SIKKIMENSIS* MUELL. ARG. LEAVES

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ABSTRACT

Objectives: The present study was to investigate analgesic action of the methanol extract of *Phyllanthus sikkimensis*. **Methods:** The animals were submitted to acetic acid-induced writhing test and Formalin induced licking test to assess analgesic activities, respectively. Two doses 400 and 200 mg/kg were administered for testing. **Results:** The methanol extract of *P. sikkimensis* (MEPS) at both doses, exhibited a significant ($p < 0.05$ - < 0.01) dose-dependent analgesic activity in acetic acid writhing test and Formalin test. In acetic acid-induced writhing test, oral administration of MEPS (200 and 400 mg/kg) also decreased the writhing significantly while compared to control. The dose 400 mg/kg showed maximum percentage of pain inhibition 45.63% and 65.73% for respectively. Diclofenac sodium (10 mg/kg) was used as reference analgesic drugs. MEPS (200 and 400 mg/kg) significantly suppressed the licking activity in both phase of the formalin-induced pain in mice in a dose dependant manner. The reference analgesic drug Diclofenac sodium (10 mg/kg) also significantly inhibited the licking activity against both phases of formalin induced pain. **Conclusions:** The leaves extract has potential analgesic activity. The present study supports the use of *P. sikkimensis* in different pain states.

KEYWORDS: *P. sikkimensis*, analgesic, Acetic acid writhing test, Formalin.

INTRODUCTION

Throughout the ages, humans have relied on Nature for their basic needs for the production of food-stuffs, shelters, clothing, means of transportation, fertilizers, flavours and fragrances, and, not the least, medicines. Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health.

Medicinal plants are one of the most important sources of active substances with therapeutic potential and these are often used to cure a variety of diseases in humans.[1][2] And man has used several therapies for the management of pain[3] and medicinal herbs are mostly used due to their availability, affordability and less side effects; example *Papaver somniferum* from which morphine, a ptototype of opiate analgesic drug was isolated.[4]

Pain is an unpleasant sensation which in many cases represents the only symptom for diagnosis of several diseases.[5] Non-steroidal anti-inflammatory drugs (NSAIDS) and opioids are used in management of mild to moderate and severe pains respectively. These drugs have serious limitations due to their side effects such as gastrointestinal irritation, tolerance and dependency.[6] There is therefore, a need to intensify research with the aim of developing efficacious agents with low toxicity profile.[7]

A number of medicinal plants are used in developing countries for the management of pain and inflammatory conditions. The validation of the folkloric claims of these medicinal plants will provide scientific basis for the development of their bioactive constituents. These could provide novel lead compounds or precursors in drug development; one of such medicinal plants with ethnomedical claims in pain and inflammatory conditions is *Phyllanthus sikkimensis*.

Phyllanthus sikkimensis Muell. Arg. is native to Bangladesh. It grows 50 to 70 centimeters tall and bears ascending herbaceous branches. The bark is smooth and light green. It bears numerous pale green flowers which are often flushed with red. The fruits are tiny, smooth capsules containing seeds. Leaves extract of *P. sikkimensis* has anthelmintic activity.[8] Leaves of *P. sikkimensis* locally used to induce diarrhea.

The purpose of this experiment was to test the analgesic activity of *P. sikkimensis* on mice using Acetic acid writhing test and Formalin test.

MATERIALS AND METHODS

Plant collection and identification

Leaves of *P. sikkimensis* were collected from hill of Chittagong region, Bangladesh. The plants were identified by Dr. Shaikh Bokhtear Uddin, Taxonomist and Professor, Department of Botany, University of Chittagong.

Preparation of extract

Leaves of the plant were dried and ground (Moulinex Blender AK-241, Moulinex, France) into powder (40-80mesh, 700 g) and soaked for 7 days with 2–3 days interval in 3.0 L of methanol at room temperature (23 ± 0.5°C). Filtrate obtained through cheesecloth and Whatman filter paper No. 1 was concentrated under reduced pressure at the temperature below 50°C using rotary evaporator (RE 200, Sterling, UK). The extracts (yield 5.2% W/W) were all placed in air tight glass tube.

Experimental animals

Swiss albino mice, weighing about 25–30 g, were collected from Jahangir Nagar University, Savar, Bangladesh. The animals were provided with standard laboratory food and distilled water ad libitum and maintained at natural day-night cycle having proper ventilation in the room. All the experiments were conducted in an isolated and noiseless condition. The study protocol was approved by the P&D Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh. The animals were acclimatized to laboratory condition for 10 days prior to experimentation

Analgesic activity

Acetic acid induced writhing test

Mice were divided into four groups of either sex containing five of each. For writhing test, 0.6% (v/v) acetic acid solution (10 mL/kg body weight) was injected intraperitoneally to each mice and the number of writhing and stretching was counted over 20 min. Group I served as control received normal saline 10ml/kg), Group II received Diclofenac sodium 10 mg/kg as a standard, Group III and Group IV treated with MEPS extract (200 and 400 mg/kg) orally 30 min before acetic acid injection.[9]

Formalin induced licking test

20 µL of 2.5% Formalin in saline was injected subcutaneously to a hind paw of the mice after 30 min administration of the Diclofenac sodium 10 mg/kg, MEPS extract 200 mg/kg and 400

mg/kg p.o to the Group II, III and IV respectively. Group I as control received only formalin (20 µL of 2.5%) during the experiment. The time spent licking and biting responses of the injected paw was taken as an indicator of pain response and the data were expressed as total licking time in the early phase (0-5 min) and the late phase (15-30 min) after formalin injection.[9]

Statistical analysis

The data was analyzed by one-way ANOVA followed by Dunnet's test to estimate significant differences between the test and control groups with GraphPad Prism Data Editor for Windows, Version 6.0 (GraphPad software Inc., San Diego, CA). Values were expressed as mean ± Standard error for mean (± SEM). $p < 0.05$ - 0.01 were considered as statistically significant.

RESULTS

Analgesic Activity

Acetic acid test

In acetic acid induced writhing test, the methanol extract of leaves of *P. sikkimensis* significantly and dose dependently suppressed the frequency of acetic acid-induced writhing in mice after oral administration. At 200 mg/kg body weight, the extract showed 45.63% writhing inhibition, at 400mg/kg body weight, the extract showed 65.73% writhing inhibition (Table 1 and Figure 1). So, the plant extract showed analgesic activity at the dose of 400 mg/kg body weight was comparable to the standard drug Diclofenac sodium that inhibited 69.66±1.38% writhing at the dose of 10 mg/kg body weight.

Table 1: Effect of *P. sikkimensis* extract on acetic acid induced writhing response in mice.

Group	No. of writhing	% inhibition
Control	59.33±1.84	-
Standard	18±0.82**	69.66
MEPS 200 mg/kg	31.67±0.85*	45.63
MEPS 400 mg/kg	20.33±0.62**	65.73

Values are expressed as mean ± S.E.M., (n = 5). MEPS=Methanol extract of *P. sikkimensis;* *P<0.05, **P<0.01 as control.

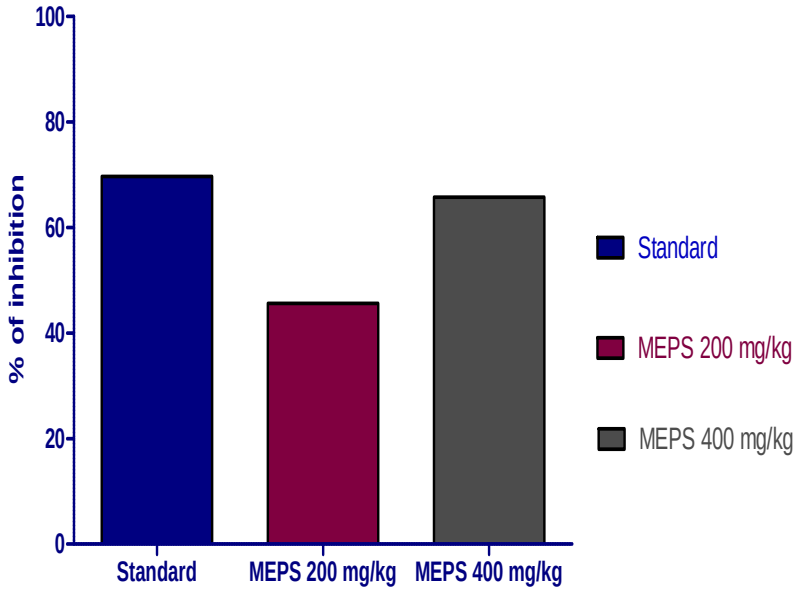

Figure 1: Effect of *P. sikkimensis* leaves extract on acetic acid induced writhing response in mice.

Formalin test

The methanol extract of leaves of *P. sikkimensis* (200 and 400 mg/kg, p.o.) significantly suppressed the licking activity in both phase of the formalin-induced pain in mice (Table 2 and Figure 2) in a dose dependant manner. The reference analgesic drug diclofenac sodium (10 mg/kg) also significantly inhibited the licking activity against both phases of formalin induced pain.

Table 2: Effect of the methanolic extract of *P. sikkimensis* on hind paw licking in the formalin test in mice.

Treatment	Early Phase (1st 5 min)	% inhibition	Late Phase (Last 15 min)	% inhibition
Control	57.31±1.06		41.74±0.60	
Standard	14.95±0.60	73.91	13.26±0.94	68.22
MEPS-200 mg/kg	44.35±0.58	22.62	26.64±0.94	36.18
MEPS-400 mg/kg	31.63±0.45	44.81	21.44±0.31	48.63

Values are expressed as mean ± S.E.M., (n = 5). MEPS=Methanol extract of *P. sikkimensis*; *P<0.05, **P<0.01 as control.

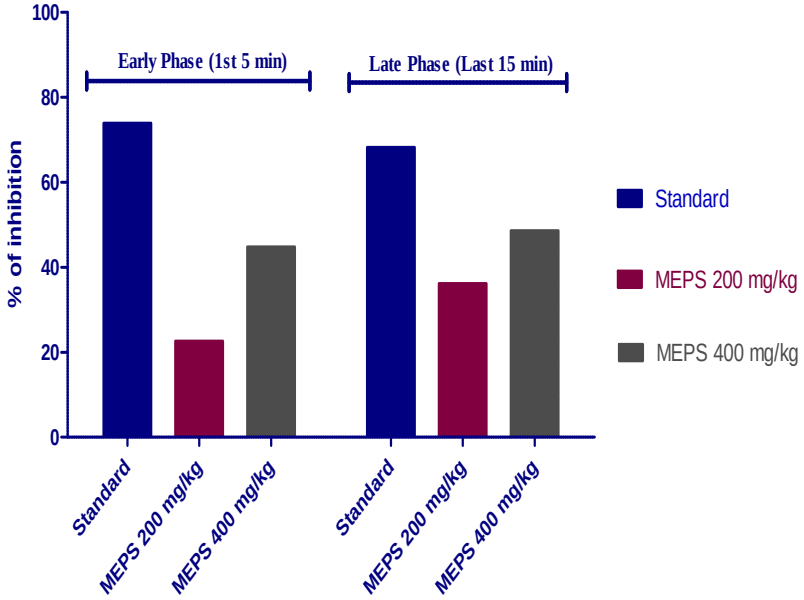

Figure 2: Effect of the methanolic extract of *P. sikkimensis* on hind paw licking in the formalin test in mice.

DISCUSSIONS

Acetic acid induced writhing test, which is the visceral pain model, was employed to evaluate the peripheral analgesic activity of the plant material. The abdominal constriction response induced by acetic acid is a sensitive procedure to determine analgesia at peripheral level. This response is thought to involve local peritoneal receptors.[10] Acetic acid is known to trigger the production of noxious substances such as prostaglandins specifically PGE2 and PGF2 as well as lipoxygenase products.[11] These prostaglandins and lipoxygenase products cause inflammation and pain by increasing capillary permeability.[12] Acetic acid may also cause release of other algesic mediators such as bradykinin, histamine and 5-hydroxytryptamine.[13] The substance inhibiting the writhings will have analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition.[14]

It was observed from the study that in both analgesic activity assay models the plant extract demonstrated analgesic effects. This means that the extract may possess both peripheral and central analgesic effects. The leaves extract of *P. sikkimensis* exhibited significant dose dependent inhibition of acetic acid–induced writhing in mice in comparison to that of the control (saline). Acetic acid induces inflammatory pain by impelling capillary permeability[15], and releasing substances that excite pain nerve endings.[16] The peripheral analgesic effect is generally mediated by the NSAIDs through inhibition of cyclooxygenase and/or lipoxygenase (and other inflammatory mediators) or inhibition of pain responses

mediated by noiceptors peripherally[17]. Therefore, it is possible that methanol extract of *P. sikkimensis* may be showing analgesic effect through these mechanisms although the exact mechanism of action is yet to be discovered. Again in hot plate test the extract also showed prominent analgesic effect against the standard drug Diclofenac Na. Moreover, activation of μ2 opioid subtype receptor leads to spinal analgesia.[18] Therefore, by considering the test report, it may be assumed that the analgesic activity of *P. sikkimensis* extract is likely to be mediated centrally although the exact mechanism is yet to be discovered. Previous studies on different plant extracts showed analgesic effect in animal models and their effects have been attributed to the presence of alkaloids, glycosides, flavonoids and saponins.[19][20] And previous studies proved that, this plant has alkaloids, glycosides, flavonoids and saponins in its leave.

CONCLUSION

The study showed that aqueous extract of *Phyllanthus sikkimensis* possesses significant analgesic activity which was validated by various pain models in this study. The results substantiate the ethnomedicinal use of *P. sikkimensis* to palliate pain disorder. The findings of present studies warrant further studies for isolation and identification of the responsible bioactive component(s) and to elucidate the mechanism(s) lying with these effects.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Kamboj VP. Herbal medicine. Curr. Sci., 2000; 78: 35–39.
2. Perumal Samy R, Gopalakrishnakone P: Therapeutic Potential of Plants as Anti-microbials for Drug Discovery. *Evid Based Complement Alternat Med.,* 2010; 7(3): 283-294.
3. Ahmadiani A, Fereidoni M, Semnanian S, Kamalinejad M, Saremi S: Antinociceptive and anti-inflammatory effects of Sambucus ebulus rhizome extract in rats. *J Ethnopharmacol,* 1998; 61(3): 229-235.

4. Bertram G K. Basic and Clinical Pharmacology. 8ed. McGraw-Hill Companies, 2001.
5. Bertram G K. Basic and Clinical Pharmacology. 8ed. McGraw-Hill Companies, 2001.
6. Howland RD, Mycek MJ. In: Lippincott's Illustration Review: Pharmacology. Harvey RA, Champe PC, editors. London: Lippincott Williams & Wilkins publishers, 2006; 157–168.
7. Howland RD, Mycek MJ. In: Lippincott's Illustration Review: Pharmacology. Harvey RA, Champe PC, editors. London: Lippincott Williams & Wilkins publishers, 2006; 157–168.
8. Kabir MSH, Ahmad S, Mahamoud MS, Al MA, Adnan M: Evaluation of Total condensed tannin content and anthelmintic activities of organic extracts of four Bangladeshi plants on Tubifex tubifex worm using in vitro method. *International Journal of Pharmacy*, 2015; 5(3): 903-910.
9. Kabir MSH, Hossain MM, Rahman MM, Ahmad S, Hasanat A, Chowdhury TA, Hoque MA, Chakrabarty N, Hossain MS: Antidepressant, anxiolytic and anti-nociceptive activities of ethanol extract of Steudnera colocasiifolia K. Koch leaves in mice model. *Journal of Coastal Life Medicine*, 2015; 3(11): 890-894.
10. Parmar Y, Chakraborthy GS. Evaluation of Cassia auriculata leaves for its potent biological activity. PhOL., 2011; 2: 128–133.
11. Ahmed A, Ilyas N, Musa KY, et al. Analgesic effects of Tacazzea apiculatao liv. Nig Journ Pharm Sci., 2007; 6(2): 134–138.
12. Muhammad N, Saeed M, Khan H. Antipyretic, analgesic and anti-inflammatory activity of Viola betonicifolia whole plant. BMC Complementary and Alternative Medicine, 2012; 12: 59.
13. Galani VJ, Patel BG. Analgesic and Anti-inflammatory Activity of Argyreia speciosa and Sphearanthus indicus in the Experimental Animals. Global J Pharmacol, 2010; 4(3): 136–141.
14. Srinivasan K, Muruganandan S, Chandra S, Tandan SK, Raviprakash V, Kumar D. Antinociceptive and Antipyretic Activities of Pongamia pinnata Leaves. Phytother Res., 2003; 17: 259–264.
15. Amico-Roxas M, Caruso A, Trombadore S, Scifo R, Scapagnini U: Gangliosides antinociceptive effects in rodents. *Arch Int Pharmacodyn Ther*, 1984; 272(1): 103-117.
16. Raj PP. Pain medicine: a comprehensive review. 1. Missouri: Mosby – year book, 1996.
17. Koster R, Anderson M, De Beer EJ. Acetic acid for analgesic screening. Fed Proc, 1959; 18: 418–420.

18. Lipman AG, Jackson RC. Principles and Practice of Pain Medicine. 2. New York: McGraw-Hill, 2004; 585–588.
19. Perazzo FF, Souza GH, Lopes W, Cardoso LG, Carvalho JC, Nanayakkara NP, Bastos JK: Anti-inflammatory and analgesic properties of water-ethanolic extract from Pothomorphe umbellata (Piperaceae) aerial parts. *J Ethnopharmacol*, 2005; 99(2): 215-220.
20. Ramaswamy S, Pillai NP, Gopalakrishnan V, Parmar NS, Ghosh MN: Analgesic effect of O-(beta-hydroxy ethyl)rutoside in mice. *Indian J Exp Biol*, 1985; 23(4): 219-220.