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EVALUATION OF ANALGESIC AND ANTHELMINTIC ACTIVITY OF ETHANOLIC FLOWER EXTRACT OF *MICROCOS PANICULATA*

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ABSTRACT: The present study was to investigate the analgesic and anthelmintic activities of ethanolic flower extract of *Microcos paniculata*, a member of the Malvaceae family. The analgesic activity was evaluated for its peripheral pharmacological activity using acetic acid induced writhing method in swiss albino mice at the doses of 200 and 400 mg/kg body weight and displayed significant analgesic action in a dose dependent manner. In acetic acid writhing method, 400 mg/kg body weight showed the most significant analgesic action with writhing inhibition of 74.46% where the standard drug Diclofenac-Na (25 mg/kg) showed 70% inhibition. Anthelmintic activity was screened against adult Bangladeshi earthworms (*Pheretima posthuma*). Three concentrations (25, 50 and 100 mg/ml) of plant extracts were tested in bioassay. Albendazole (10 mg/ml) is used as standard reference drug whereas distilled water is used as a control. The paralysis and death times and death time of the worms were recorded. Dose dependent activity was observed in the plant extract. This result suggests the extract possess potential analgesic and anthelmintic activity.

INTRODUCTION: Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain acts as a warning signal against disturbances of the body and has a proactive function. However, it is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause ¹.

Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this respect, new compounds with improved pain management capacity and fewer side effects are being searched every nook and corner of the world.

Microcos paniculata L. is a herbaceous plant or small tree which belongs to the tiliacece family. It is widely distributed throughout Bangladesh and also grown in India, Sri Lanka, China, Cambodia, Myanmar, Thailand, Vietnam, Indonesia and Malaysia. In Bangladesh, its local name is Kathgua or Fattashi ². Traditionally it is used to treat hepatitis, diarrhea, dyspepsia, typhoid fever, small pox, eczema and itches. Important bioactive phytochemicals like alkaloids, flavonoids, resins,



saponons, steroids and carbohydrates have been reported in it ². *Microcos paniculata* (family: Euphorbiaceae) is a shrub that is abundant in secondary forests and also grown as hedges. *Microcos* is tall semi-deciduous tree, sometimes shrubby. Leaves 10-15 cm long, ellipticoblong, acuminate, entire or slightly and irregularly toothed ³. Flowers small, yellow, in terminal panicles. Fruits globose or slightly obovoid, about 10 mm across. The plant is used in indigestion, eczema, itch, small-pox, typhoid fever, dysentery and syphilitic ulceration of the mouth. Tripuras in Chittagong Hill Tracts use leaves of this plant along with turmeric and shell of snail for the treatment of jaundice ⁴. A decoction of the roots is used to treat coughs. A drink prepared from the roasted and boiled leaves is given to children as a vermifuge. It has been used traditionally to prepare herbal medicines and traditional teas, while a limited number of reports concerning the chemical constituents and biological activities of *M. paniculata* have appeared in the literature ^{5,6}.

In present study, we investigated the analgesic and anthelmintic activities of methanolic extracts of *Microcos paniculata* L.

MATERIALS AND METHODS:

Drugs and Chemicals: Diclofenac-Na, Pyrantel pamoate and Normal saline water were used during the experiment. Methanolic extract of *Microcos paniculata* L. was tested in various doses in each group. Normal saline water was used as control. Diclofenac-Na was purchased from local market manufactured by Square Pharmaceuticals Ltd., Bangladesh. Pyrantel pamoate collected from Beximco Pharmaceuticals Ltd. was used as the standard drug for comparative study in the in vitro anthelmintic activity.

Plant Material: Flowers of *Microcos paniculata* L. was collected from the local area of Kaliganj, Gazipur, during the first week of November, 2022 and was identified (Accession No. 87,251) by Bangladesh National Herbarium, Mirpur, Dhaka. Collected flowers, after cutting into small pieces, were dried and pulverized into a coarse powder and stored into an air-tight container.

Extraction and Sample Preparation: The pulverized coarse powder of flowers of *Microcos*

paniculata L. (150 gm) was extracted with methanol by successive cold extraction. The extracts obtained, were filtered off and evaporated to dryness in an oven at low temperature. The extracts rendered concentrate of reddish color.

Animal: For the experiment both male and female Swiss albino mice, 3-4 weeks of age, weighing between 20-25 gm, were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions (temperature: 24.0 ± 1 °C), relative humidity: 55-65% and 12 hrs light/12 hrs dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experimentation.

Selection of Worm: The assay was performed on adult earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Because of easy availability, earthworms have been widely used for the initial evaluation of anthelmintic compounds. Adult earthworms (*Pheretima posthuma*) were collected from moist soil of Savar area, of Dhaka in Bangladesh and washed with normal saline to remove all faecal matters. Then the worms were used for anthelmintic study. The earthworms of 3-5cm in lengths and 0.1-0.2cm in width were used for all the experimental protocol.

Anthelmintic Activity: For the preliminary evaluation of anthelmintic activity, the assay was carried out *in-vitro* using adult Bangladeshi earthworms (*Pheretima posthuma*). These worms were chosen because of their close morphological and physiological resemblance to human intestinal nematodes. Extract solutions were freshly prepared in normal saline at concentrations of 25, 50, and 100 mg/ml. For each concentration, six worms of nearly equal size were placed in a beaker containing 50 ml of the respective solution. The positive control was albendazole, a well-known benzimidazole derivative that has been demonstrated to be efficient in paralyzing and killing helminths by blocking microtubule polymerisation ⁷.

Normal saline was utilised as the negative control to ensure that any action was due to the extract or standard medication and not osmotic effects. The worms were observed carefully throughout the experiment. Paralysis was recorded at the point when the worms ceased movement, except for occasional responses upon vigorous shaking⁸. Death was confirmed when the worms failed to respond even after being exposed to gentle agitation or immersion in warm water (50°C).

Analgesic Activity:

Acetic Acid-Induced Writhing Test: The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. Test samples and Control were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac- Na was intraperitoneally administered 15 min before injection of acetic acid and Diclofenac- Na (25mg/kg) was used as standard drug. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min 6-7¹.

Percentage inhibition of writhing was calculated using the following formula:

Writhing Inhibition (%) =

$$\frac{\text{Mean No. of writhing (control)} - \text{Mean No. of writhing (test)}}{\text{Mean No. of writhing (control)}} \times 100$$

Statistical Analysis: Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the vehicle control group. p values < 0.05, 0.001 were considered to be statistically significant.

RESULTS:

Analgesic Activity: The analgesic activity of the methanolic flower extract of *Microcos paniculata* L. was evaluated in acetic acid-induced writhing methods. The doses of the extract significantly (p < 0.001) inhibited writhing response induced by acetic acid in a dose dependent manner as compared to control. At 100 mg/kg body weight the extract showed 26.15% inhibition, at 200 mg/kg body weight the extract showed 47.94% inhibition and at 400 mg/kg body weight showed 73.12% inhibition of writhing compared to the standard drug Diclofenac-Na which showed 75.78% inhibition of writhing at 25 mg/kg body weight dose.

TABLE 1: EFFECT OF MICROCOS PANICULATA ON ACETIC ACID INDUCED WRITHING METHOD

Group	Treatment and Dose	No. of Writhing	% of writhing	% of writhing inhibition
Control	0.7% acetic acid (10ml/kg, i.p.)	41.3±1.32	100.00	0
Standard	Diclofenac sodium (25mg/kg i.p.)	10.0±0.42	24.21	75.78
Group-I	Extract (200mg/kg, p.o)	21.5±0.995	52.06	47.94
Group-II	Extract (400mg/kg, p.o)	11.1±2.88	26.87	73.12

Anthelmintic Activity: At 25, 50, and 100 mg/mL, the extracts of *Microcos paniculata* was tested against adult earthworms (*Pheretima posthuma*). Maximum effects was typically seen at the highest concentration. The worms was paralyzed by *M. paniculata* at 100 mg/mL in about 30 minutes, and

they died in about 45 minutes. During the tests, the plant extracts showed better anthelmintic efficacy than the conventional reference medicine, albendazole (10 mg/ml), which resulted in paralysis at around 75 minutes and death at around 237 minutes.

TABLE 2: EFFECTS OF MICROCOS PANICULATA ON PHERETIMA POSTHUMA

Animal group	No. of worms	Concentration	Time taken for Paralysis (min)	Time taken for death (min)
Control Group	6	----	----	----
Standard Group	6	10 mg/ml	75 ± 19	237 ± 19
	6	100 mg/ml	30 ± 10	45 ± 8
<i>M. paniculata</i>	6	50 mg/ml	45 ± 13	55 ± 13
	6	25 mg/ml	65 ± 17	80 ± 15

DISCUSSION: Acetic acid induced writhing test is suitable for detecting both central and peripheral analgesia. Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic

mediators like PGE₂ and PGF_{2α} and their levels increase in the peritoneal fluid of the acetic acid induced mice⁵. The abdominal constrictions produced after administration of acetic acid is

related to sensitization of nociceptive receptors to prostaglandins. *M. paniculata* demonstrated robust and quick anthelmintic action at 100 mg/ml concentration, causing paralysis at about 30 minutes and death at about 45 minutes.

Albendazole (10 mg/mL), the reference medication, on the other hand, took significantly longer roughly 75 minutes to cause paralysis and 237 minutes to cause death. This study highlights the potential of this medicinal plants, particularly *M. paniculata*, as quick and efficient natural substitutes for traditional anthelmintic medications like as albendazole⁹. Isolating phytochemicals and validating these compounds *in-vivo* should be the main goals of future studies.

CONCLUSION: In light of the results of the present study, it can be concluded that the plant extract possesses remarkable analgesic which may be support to the traditional use of the plant in pain and inflammatory disorders. However, further studies are needed to be conducted to understand the exact mechanisms of anthelmintic and analgesic activity isolating the compound(s) responsible for UCH actions. Also this study showed that aqueous extracts of *M. paniculata* is capable of producing considerable anthelmintic effects when tested against *Pheretima posthuma*.

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CONFLICT OF INTEREST: Nil

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