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INVESTIGATION OF ANALGESIC AND NEUROPHARMACOLOGICAL ACTIVITIES OF METHANOLIC BARK EXTRACT OF *MIMUSOPS ELENGI*

Mahmuda Nasrin, Pritesh Ranjan Dash and Moni Rani Saha*

Department of Pharmacy, Stamford University, 51 Siddeswari Road, Dhaka- 1217, Bangladesh

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Correspondence to Author:

Mrs. Moni Rani Saha

Assistant Professor, Department of Pharmacy, Stamford University, 51 Siddeswari Road, Dhaka-1217, Bangladesh

ABSTRACT

The methanolic bark extract of *Mimusops elengi* was investigated for its possible analgesic and neuropharmacological activities on mice at the doses of 100mg/kg, 200mg/kg and 400mg/kg body weight. For the evaluation of analgesic activity, tail immersion and acetic acid induced writhing tests were used. The neuropharmacological activity of the extract was screened using hole cross and open field tests to investigate the central nervous system depressant (CNS) activity. In tail immersion test, the extract produced a significant ($p < 0.05$-0.001) increase of latent time to flick tail compared to control in a dose dependent manner. In acetic acid-induced writhing test, the extract, at a dose of 400 mg/kg, showed a maximum of 65.48% inhibition ($p < 0.001$) of writhing compared to the control while the reference drug Diclofenac-Na inhibited 76.36% of writhing. In CNS depressant activity tests, the extract significantly ($p < 0.05$-0.001) decreased motor activity and exploratory behavior of mice in hole cross and open field tests respectively. These results suggest that the extract possesses analgesic and CNS depressant activity.

INTRODUCTION: Each type of modern drugs currently used for the management of pain and inflammatory conditions (opioids or non-narcotics, salicylates and corticosteroids e.g. hydrocortisone) have well known adverse effects. Moreover, synthetic drugs are very expensive to develop since, for the successful development of a new product usually costs in the range of 0.5 to 5 million dollars. On the contrary, many medicinal herbs have been used as a form of therapy for the relief of pain throughout history without any adverse effects [1].

It is therefore, essential that efforts should be made to introduce new medicinal plants to develop drugs which are cheaper, safer and more effective. Plants still represent a massive untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [2].

Mimusops elengi commonly known as Bakul (Bengali name) belongs to the family Sapotaceae and is a small to large evergreen tree found all over the different parts of Bangladesh, Pakistan and India [3]. It is 5-8 m tall and is cultivated in gardens as an ornamental tree for sweet-scented flowers. It has been used in the indigenous system of medicine for the treatment of various ailments. Several therapeutic uses such as cardiotonic, alexipharmic, stomachic, anthelmintic and astringent have been ascribed to the bark of *Mimusops elengi* [4, 5].

The barks and fruits of this plant are used in the treatment of diarrhea and dysentery, and a decoction of the bark is used as a gargle. The pounded seeds

pasted with oil are used for the treatment of obstinate constipation. Pillow stuffing made from the dried flowers induces nasal discharge and relieves headache.

Several triterpenoids, steroids, steroidal glycosides, flavonoids, and alkaloids have been reported from this species [6, 7]. Phytochemical review shows the presence of taraxerol, taraxerone, ursolic acid, betulinic acid, V-spinosterol, W- sitosterol, lupeol [8, 9] alkaloid isoretronecyl tiglate [10] and mixture of triterpenoid saponins in the bark of *Mimusops elengi*. In the present study, we investigated the analgesic and neuropharmacological activities of methanolic extracts of *M. elengi.*

MATERIALS AND METHOD:

Chemicals and drugs: Diclofenac-Na and Diazepam injections were purchased from local market manufactured by Square Pharmaceuticals Ltd., Bangladesh.

Plant material: The bark of *Mimusops elengi* was collected from the district of Gazipur, Dhaka, Bangladesh. The plant was identified in Bangladesh National Herbarium Mirpur, Dhaka, and accession number was 34, 486. The plant part (bark) was thoroughly washed with water, cut into small pieces and dried in the sun.

Extraction: After drying, the bark was reduced to coarsely powder using a grinding mill. 93 gm powder was extracted with a mixture of methanol: water (8:2, v/v) by a Soxhlet's apparatus at 60˚C. The solvent was completely removed and obtained 29 gm (yield = 31.23%) dried crude extract which was used for investigation.

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 Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions (temp.: 24.0±1.0˚C, relative humidity: 55-65% and 12hrs 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 [11]. All protocols for animal experiment were approved by the Departmental Animal Research Ethical Committee.

Experimental groups: The animals were divided into control, standard and test groups containing five mice of each.

Analgesic Activity:

Tail Immersion Test: The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice [12]. The extract at the doses of 100, 200 and 400 mg/kg body weight and vehicle (1% Tween 80 in water) were administered orally to test groups and control group respectively. Diclofenac-Na (25mg/kg i.p.) was used standard drug. According to the procedure, 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 12s was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined at 0, 30, 60 and 90 min after the administration of drugs. Percentage of elongation was calculated using the following formula:

$$\textbf{Elongation (\%)} = \frac{\text{Latency (Test) - Latency (Control)}}{\text{Latency (Test)}} \times 100$$

Acetic Acid-Induced Writhing Test: Test samples at the doses of 100, 200 and 400 mg/kg body weight and vehicle (1% Tween 80 in water) were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but standard drug Diclofenac-Na at the dose of 25mg/kg was administered intraperitoneally 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min [13, 14]. Percentage inhibition of writhing was calculated using the following formula:

$$\textbf{Writhing inhibition (\%)} = \frac{\text{Mean No. of writhings (control) - Mean No. of writhings (test)}}{\text{Mean No. of writhing (control)}} \times 100$$

Neuropharmacological Activity:

Hole Cross Test: A steel partition was fixed in the middle of a cage having a size of (30×20×14) cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage [15]. The number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after the oral treatment with *M. elengi* methanolic extracts at the doses of 100, 200 and 400 mg/kg body weight. Percentage inhibition of movements was calculated using the following formula:

$$\text{Movements inhibition (\%)} = \frac{\text{Mean No. of movements (control) - Mean No. of movements (test)} \times 100}{\text{Mean No. of movements (control)}}$$

Open Field Test: The test groups received *M. elengi* methanolic extract at the doses of 100, 200 and 400 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water) and standard group received Diazepam (3mg/kg body weight i.p.). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90 and 120 min during the study period [16]. Percentage inhibition of movements was calculated using the following formula:

$$\text{Movements inhibition (\%)} = \frac{\text{Mean No. of movements (control) - Mean No. of movements (test)} \times 100}{\text{Mean No. of movements (control)}}$$

Statistical Analysis: Data obtained from pharmacological experiments are expressed as mean±SEM. Difference between the control and the treatments in these experiments were tested for significance using one-way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons using SPSS software.

RESULTS: The analgesic activities were investigated for its central and peripheral pharmacological actions using tail immersion test and acetic acid-induced writhing test respectively. Neuropharmacological activity was evaluated by using hole cross and open field tests. The tail withdrawal reflex time following administration of the extract of *M. elengi* was found to increase with increasing dose of the sample. The result of tail immersion test was statistically significant (p < 0.05-0.001) and was comparable to the control **(Table 1)**.

TABLE 1: EFFECTS OF THE METHANOLIC EXTRACT OF *M. ELENGI* BARK ON MICE IN TAIL IMMERSION TEST

Groups	Dose (mg/kg)	Mean reaction time (s) before and after drug administration (% of tail flick elongation)			
		0 min	30 min	60 min	90 min
Control		1.73±0.125	1.60±0.125	1.47±0.17	1.33±0.105
Standard	25	2.53±0.29	5.33±0.235** (69.98%)	7.39±0.07** (80.10%)	8.8±0.17** (84.88%)
Group-I	100	1.47±0.085	3.87±0.13** (58.66%)	5±0.105** (70.60%)	7.00±0.28** (81.00%)
Group-II	200	1.53±0.17	4.47±0.27** (64.20%)	6.73±0.165** (78.16%)	7.60±0.245** (82.50)
Group-III	400	1.47±0.02	5.60±0.195** (71.43%)	8.06±0.57** (81.76%)	10.60±0.43** (87.45%)

Control: animals received (1% Tween 80 in water), Standard group received Diclofenac-Na (25mg/Kg body weight i.p.), Group-I, Group-II and Group III were treated with 100, 200 and 400 mg/kg body weight of the crude extract of *M. elengi* per oral respectively. Values are mean ±SEM, (n = 5); ** p < 0.001, Dunnett's test as compared to control

The methanolic extract of *M. elengi* displayed a significant and dose dependent analgesic activity against acetic acid induced writhing test on mice. 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. The result of acetic acid induced writhing test reveals that the extract inhibited 18.2%, 45.15%, and 65.48% of writhing at the doses of 100 mg/kg, 200mg/kg and 400 mg/kg body weight respectively, whereas writhing inhibition of the standard drug Diclofenac-Na was 76.35% at 25 mg/kg body weight dose (**Table 2).** The number of hole crossed from one chamber to another by mice of the control group is increased from 30 minutes to 120 minutes. Hole cross test of *M. elengi* 100mg/kg, 200mg/kg and 400mg/kg dose showed significant (p < 0.001) decrease of movement from its initial value at 0 to 120 minutes as compared to control **(Table 3)**. The maximum decrease in movement was observed at 90 and 120 min after drug administration. In the open field test of *M. elengi* 100mg/kg, 200mg/kg and 400mg/kg dose showed significant decrease of movement from its initial value at 0 minute to 120 minutes. The maximum suppression

was exhibited at 90 and 120 min after drug administration **(Table 4)**. Initially it was observed that the number of movements of the control group overall increased from 30 minutes to 120 minutes.

TABLE 2: EFFECTS OF THE METHANOLIC EXTRACT OF *M. ELENGI* BARK ON MICE IN ACETIC ACID INDUCED WRITHING TEST.

Group	Treatment and Dose	**Writhings** (Mean ± SEM)	% of writhing	% of writhing inhibition
Control	0.7% acetic acid (10 ml/kg, i.p.)	42.3±1.32	100.00	0
Standard	Diclofenac-Na (25mg/kg i.p.)	10.0±0.42**	23.64	76.35
Group-I	Extract (100mg/kg per oral)	34.6±0.94**	81.79	18.2
Group-II	Extract (200mg/kg per oral)	23.2±1.29**	54.85	45.15
Group-III	Extract (400mg/kg per oral)	14.6±1.92**	34.51	65.48

Diclofenac- Na was administered 15 min before 0.7% acetic acid administration. Writhing was counted for 15 min, starting after 5 min of acetic acid administration. Values are mean ±SEM (n=5); **P<0.001, Dunnett's test as compared to control.

TABLE 3: EFFECTS OF THE METHANOLIC EXTRACT OF *M. ELENGI* BARK ON MICE IN HOLE CROSS TEST.

Group	Dose (mg/kg)	Number of movements (% of Number of movements inhibition)				
		0 min	30 min	60 min	90 min	120 min
Control	--	23.4±1.21	12.8±0.585	12.4±0.6	9.8±0.665	11.2±0.665
Standard	3	15.2±1.115	6.6±1.66 (48.43%)	4±1.095** (67.74%)	2.4±1.25** (75.51%)	1.6±0. 87** (84.31%)
Group-1	100	11.4±1.03	7.4±3.445 (42.18%)	4.2±1.115** (66.12%)	3.2±1.53* (67.35%)	2±0.835** (82.14)
Group-2	200	13±1.305	5.4±1.29 57.81%)	2.6±1.63** (79.03%)	3.2±1.39* (67.37%)	4.0±0.305** (64.28%)
Group-3	400	7±0.835	5±1.845* (60.94%)	1.4±0.245** (88.71%)	1.6±0.68** (83.67%)	0.8±0.375** (92.85%)

Control: animals received (1% Tween 80 in water), Standard received Diazepam (3mg/kg body weight i.p.), Group-I, Group-II and Group III were treated with 100, 200 and 400 mg/kg body weight of the crude extract of *M. elengi* per oral respectively. Values are mean ±SEM, (n = 5); * $p < 0.05$, ** $p < 0.001$, Dunnett's test as compared to control.

TABLE 4: EFFECTS OF THE METHANOLIC EXTRACT OF *M. ELENGI* BARK ON MICE IN OPEN FIELD TEST.

Group	Dose (mg/kg)	Number of movements (% of Number of movements inhibition)				
		0 min	30 min	60 min	90 min	120 min
Control	--	114 ±2.915	107.6±1.535	92.2 ±0.585	88.4 ±1.63	99±2.47
Standard	3	83.2±14.295	39.4±8.14** (63.38%)	32.6±6.225** (64.64%)	24.2±6.9** (72.62%)	11±3.115** (88.89%)
Group-1	100	43.6±16.88	43.4±8.51** (59.66%)	17±7.315** (81.56%)	12±4.145** (86.42%)	8.2±4.585** (91.71%)
Group-2	200	97±2.79	33.4±10.07** (68.96%)	13.6±7.25** (85.25%)	19.2±4.64** (78.28%)	11±2.05** (88.89%)
Group-3	400	74±8.665	20.4±4.12** (81.04%)	9.2±2.46** (90.02%)	15±8.035** (83.04%)	13.6±3.84** (86.86%)

Control: animals received (1% Tween 80 in water), Standard received Diazepam (3mg/kg body weight i.p), Group-I, Group-II and Group III were treated with 100, 200 and 400 mg/kg body weight of the crude extract of *M. elengi* per oral respectively. Values are mean ±SEM, (n = 5); ** $p < 0.001$, Dunnett's test as compared to control.

DISCUSSION: Plants are most valuable sources of biologically active products, which could provide main chemical constituents, which are of prime importance in the fight against diseases, pain, infections and ultimately death. The plant *M. elengi* has many pharmacological properties, which are directly related to the chemical constituents of the plant. Thermic painful stimuli are known to be selective to centrally active drugs [17].

Prostaglandins and bradykinins were suggested to play an important role in analgesia [18, 19]. Flavonoids and sterols are reported to inhibit prostaglandin synthesis [20]. A number of flavonoids have been reported to produce analgesic activity [21]. Phytochemical screening of the methanol extract of *M. elengi* reveals the presence of alkaloids, flavonoids, saponins, tannins, carbohydrates, glycosides, glucosides and steroids.

Presence of flavonoids and sterols in methanolic extract might suppress the formation of prostaglandins and bradykinins and exert its activity.

Pain is a sensorial modality and primarily protective in nature, but often causes discomfort. It is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause [22]. Tail immersion test is considered to be selective to examine compounds acting through opioid receptor; the extract increased mean basal latency which indicates that it may act via centrally mediated analgesic mechanism. Narcotic

analgesics inhibit both peripheral and central mechanism of pain, while NSAIDs inhibit only peripheral pain [23, 24]. Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response [25]. The response is thought to be mediated by peritoneal mast cells [26], acid sensing ion channels [27] and the prostaglandin pathways [28]. The effect of the extracts against the noxious stimulus may be an indication that it depressed the production of irritants and hereby reduction in number of writhes in the animals.

The most important step in evaluating drug action on the CNS is to observe the behavior of the test animals. Substances that have CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both. Anxiety and sedation are principally mediated in the CNS by the $GABA_A$ receptor complex, which is also involved in other physiological functions related to behavior and in various psychological and neurological disorders such as epilepsy, depression, Parkinson syndrome and Alzheimer's disease [29]. As a rule, there are three ways of increasing GABAergic activity in the brain as follows: GABA agonists, for example, diazepam and phenobarbital, directly increase inhibitory chloride conductances or upregulate the effect of synaptically released GABA on the $GABA_A$ receptor [30].

Another important step in evaluating drug action on CNS is to observe its effect on locomotors activity of the animal. The activity is a measure of the level of excitability of the CNS [31] and this decrease may be closely related to sedation resulting from depression of the central nervous system [32]. The extracts significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests. The results were also dose dependent and statistically significant.

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CONCLUSION: Finally overall results obtained from this study suggest that the extract has analgesic and neuropharmacological activity on mice. Further studies are suggested to be undertaken to pinpoint the compounds found in the methanolic bark extract of *Mimusops elengi* and to better understand the mechanism of such action scientifically.

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