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In vivo Determination of Analgesic and Neuropharmacological Activities of Methanol Extract of *Begonia thomsonii* A.DC. Roots

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Abstract: The plant roots under investigation (*Begonia thomsonii*) were a flowering plant belonging to *Begoniaceae* family. This study was designed to explore the neuropharmacological and analgesic activities of methanol extract of *Begonia thomsonii* roots. The analgesic activity was assessed by acetic acid-induced writhing test and formalin-induced paw licking test and neuropharmacological action was evaluated by the open field test, hole cross test and thiopental sodium-induced sleeping test. The SPSS program (IBM SPSS software, Version 22.0) was used to determine the statistical analysis. Results showed that in the acetic acid-induced writhing experiment the roots extract (200 and 400mg/kg body weight) exhibited significant ($P<0.05$) inhibition of writhing with 35.02 and 49.43% respectively, while pain inhibitions were 36.93 & 49.43% at acute phase and 25.6 & 43.2% at chronic stage by 200 and 400 mg/kg roots extract respectively in formalin-induced paw licking test. Open field test and hole cross test showed significant ($P<0.05$) reduction of motor activity whereas thiopental sodium-induced sleeping test showed reduced motor coordination and prolonged sleeping duration indicating the sedative effect of the roots extract. It can be concluded that the overall results indicated that the roots of *Begonia* thomsonii possess analgesic and sedative activities.

Key words: *Begonia Thomsonii* · Prostaglandins · Analgesic · Sedative · Nociception

INTRODUCTION

Medicinal plants contribute to the supportive treatment of indigenous community healthcare as well as in drug design and development in the present and future. Plants consistently provide an abundant source of structurally new compounds that can be guided to the development of novel drugs [1]. One statistics of WHO shows nearly 80% of the population of some African countries depends on plant-derived medications for some aspect of primary health care [2].

NSAIDs and opiates are widely used as analgesic agents though they have some undesirable side effects including gastric lesions caused by NSAIDs and opioids produce dependence and tolerance [3]. Therefore, scientists are searching alternatives to NSAIDs and opioids lacking those adverse effects.

Stress involves immunological, neural and complex biochemical mechanisms. These cause various disease states like anxiety and depression, endocrine disorders including male impotence, diabetes mellitus. Stress triggering the complex biochemical reaction may also be responsible for ulcerative colitis, rheumatic diseases, premature aging, carcinoma, asthma, allergies, hypertension, migraine, peptic ulcers, cognitive dysfunctions and cardiovascular disease [4]. During last two decades for the management of psychosomatic disorders: stress and anxiety; the uses of psychoactive drugs have been increased. There are much evidence that shows plants have neuroprotective actions, hemoglobin enhancing capabilities, hepatoprotective and ameliorative potentials [5-8].

Begonia thomsonii is a plant species of the family *Begoniaceae* which is found in Bangladesh and India. The scientific data of this plant on the potential central nervous system (CNS) and analgesic activity is very little. From this, we selected *Begonia thomsonii* roots to investigate the analgesic and neuropharmacological actions.

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MATERIALS AND METHODS

Plant Materials: We carefully collected the roots of *B.thomsonii* from Srimongol, Sylhet, Bangladesh in March 2016 at the mature stage which was recognized by Professor. Sheikh Bokhtear Uddin, Department of Botany, University of Chittagong, Bangladesh with a voucher specimen, Accession No.18.06.2011, Dev 10512 (HCU).The voucher specimen of this plant has been preserved for future reference in the university's herbarium. The collected roots were cut into small pieces and dried in the shade at 21-30°C for ten days. Finally, we dried the materials in an oven not at a very high temperature to facilitate grinding. Subsequently, the small pieces were crushed by a mechanical grinder followed by passing through a size of 60 mesh screen for obtaining the coarse powder. This powder was preserved in an air-tight bottle and placed into desiccators.

Preparation of Sample: The powders of roots (150 g) were taken in a clean round-bottom flask (15 L) and 1.5 L of methanol was used to soak for 15 days with occasional stirring and shaking. This process was done at room temperature. The mixture was first filtered with cotton-plugged followed by filtration with Whatman No. 1 filter paper. Then the filtrate was evaporated to dryness in Heidolph-rotary evaporator at 45°C to get the concentrated extract. Finally, the solid residue was obtained by air-drying. To prepare the test sample, the extract and the standard drug were dispersed in normal saline using DMSO (Di-methyl Sulfoxide) and tween 80 for *in vivo* test.

Chemicals and Reagents: Methanol, Formalin and Acetic acid (Merck, Germany), Diclofenac sodium, Diazepam, (Eskayef Bangladesh Ltd) and Normal saline solution (0.9% NaCl) was purchased from Orion Infusion Ltd., Bangladesh. Tween 80 was from Sigma-Aldrich and rest of the chemicals used was of analytical grade.

Experimental Animals: The authority of the animal house of Jahangirnagar University, Dhaka, Bangladesh supplied Swiss albino mice weighing 25~30gm. The mice were provided the standard lab facilities. The house was adjusted with 55-65% relative humidity, 23.0±2.0°C room temperature and 12 hours light: dark cycle. Mice were acclimatized for seven days and treated with standard diet and water. After approving the study protocol by the P&D (Planning and Development) committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh; all the experiments were performed in quiet and isolated condition.

Analgesic Activity Assay

Acetic Acid-Induced Writhing Test: We divided the mice into four groups of both sex having three of each. During writhing experiment, each mouse was injected intraperitoneally with 0.6% (v/v) acetic acid solution (10ml/kg body weight) and the number of writhing and stretching was counted for 20 min [9]. Group I served as the control receiving standard saline (10ml/kg), Group II was given Diclofenac sodium 10 mg/kg as a standard, Group III and Group IV were treated with MEBT root extract (200 and 400 mg/kg) orally 30 min before acetic acid injection.

Formalin-Induced Paw Licking Test: 20 µl of 2.5% formalin using distilled water was injected subcutaneously to a hind paw of the mice after 30 min administration of the Diclofenac sodium at the dose of 10 mg/kg and MEBT at the dose of 200 and 400 mg/kg (p.o) to the Group II, III and IV respectively. Group I mice was serving as control received 20 µl of 2.5% formalin only. For this experiment, the indicator of pain response was considered the licking and biting of injected paw. The total licking times in the early phase (0~5 min) and the late phase (15~30 min) after formalin injection were calculated [10].

Sedative Activity Test

Open Field Test: The experimental mice were divided into the test group, negative control and positive control containing five mice each. The trial groups feed the MEBT at doses of 200 and 400 mg/kg body weight whereas the negative control group received only vehicle (1% tween 80 in water) and the positive control groups were injected intraperitoneally (i.p) standard drug diazepam (1 mg/kg body weight). The design of the floor comprising of a half square meter open filed which was divided into a series of squares of which each alternatively colored black and white. The apparatus also had the wall of 40 cm height. The total numbers of squares visited by the mice were counted up for 0, 30, 60, 90 and 120 min after oral ingestion of both doses of the test sample [11]. We concurred with the same method used by Zubia Begum *et al*. [12].

Hole Cross Test: The test apparatus consisting of a steel partition which was fixed in the middle of a cage having a size of 30×20×14 cm. A three-centimeter diameter hole was made at the height of 7.5 cm in the centre of the cage. The mice were divided into different groups like positive control, negative control and test groups containing five mice for each. The test group's mice were administered MEBT at doses of 200 and 400 mg/kg (b. wt.) orally while the negative control and positive control groups mice received vehicle (1% tween 80 in water) and the standard drug diazepam (1 mg/kg body weight) respectively. The total number of passage by a mouse from one chamber to another chamber through the hole calculated at 0, 30, 60, 90 and 120 min for total 3 min after oral administration of the test sample and the standard drug [13].

Thiopental Sodium-Induced Sleeping Test: We divided the experimental mice indiscriminately with either sex into three groups containing five mice each. The positive control groups received standard drug diazepam (1 mg/kg) and the negative control groups received only vehicle (1% tween 80 in water) while the test groups treated with MEBT at doses of 400 mg/kg body weight. After thirty minutes, thiopental sodium at the dose of 40 mg/kg was administered to every mouse to persuade sleep. The mice were watched for the time between thiopental administration to loss of writhing reflex (latent period) and the time between the loss and recovery of writhing reflex (duration of sleep) [14].

Statistical Analysis: Means ± standard errors means (SEM) was used to express the results. To find out the level of significance, all the data were analyzed using ANOVA (One-Way Analysis of Variance) followed by Dunnett t-test. At the 0.05 level, the mean difference is statistically significant. The statistical analysis was done by applying the SPSS program (IBM SPSS software, Version 16.0).

RESULTS

Acetic Acid Test: Treatment with MEBT at the doses of 200 and 400 (mg/kg,) P.O (orally) considerably reduced ($^{a}P<0.05$) the total number of writhing in acetic acid-induced mice (Table 1). At the dose of 400 mg/kg, highest analgesic activity (49.43%) was observed where Diclofenac sodium (10 mg/kg) revealed69.49% inhibition against acetic acid-induced writhing in mice.

Formalin Test: The outcome of the extract of this test was given in Table 2.In case of both doses, we have observed that there was the dose-dependent decline of paw licking time in the early phase, however, the 400mg/kg dose notably($P<0.05$) lessened latency to discomfort in late period contrasted to the late stage of the control. In compare, the standard analgesic medication Diclofenac sodium at the dose of 10 mg/kg extensively reduced ($^{a}P<0.05$) the licking action against both stages of nociception.

Table 1: Effects of methanol extract of *B. thomsonii* roots on the acetic acid-induced writhing experiment.

Group	Treatment	Dose	Route	Number of Writhing per20 min	% of Inhibition
Control	1% tween solution	10ml/kg b. wt.	p. o	59 ± 2.51	--
Standard	Diclofenac-Na	10mg/kg b. wt.	p. o	18 ± 0.577^{b}	69.49
Test	MEBT	200mg/kg b. wt.	p. o	38.33± 1.45^{a}	35.02
		400mg/kg b. wt.	p. o	29.83± 0.60^{a}	49.43

The values expressing system were means ±SEM (n=3); $^{a}P<0.05$, $^{b}P<0.01$ significantly different contrasted to control. Statistical representation of this test was processed by Dunnett's test by utilizing SPSS for Windows, version 22.0.

Table 2: Effects of methanol extract of *B. thomsonii* roots on formalin-induced paw licking assessment.

Treatment	Dose, route	Early phase	% of Inhibition	Late phase	% of Inhibition
1% tween Solution	10ml/kg b. wt., p.o	58.66± 1.878	--	41.66 ± 1.763	--
Diclofenac Na	10mg/kg b. wt., p.o	25 ± 1.154^{b}	74.43	13.33± 1.452^{b}	68
MEBT	200mg/kg b. wt., p.o	37 ± 1.732^{b}	36.93	31 ± 1.154^{a}	25.6
	400mg/kg b. wt., p.o	29.66 ± 1.45^{b}	49.43	23.66± 0.667^{a}	43.2

Calculated data were articulated as means ± SEM (n=3);$^{a}P<0.05$, $^{b}P<0.01$significantly different compared to control. Statistical representation was made for the formalin-induced paw licking test preparing by Dunnett's test with the help of SPSS for Windows, version 22.0.

Table 3: The results of CNS depression action of the MEBT from the open field test.

				Number of movements				
Group	Treatment	Dose	Route	0 min	30 min	60 min	90 min	120 min
Control	1% tween Solution	10ml/kg b. wt	p.o	54.33± 0.88	53.00± 1.00	50.66± 1.20	51.33± 0.66	50.00± 1.00
Standard	Diazepam	1mg/kg b. wt	i.p	54.00±1.00	36.33± 0.88 [a]	26.33± 0.33 [a]	13.6 ± 1.20 [a]	8.3± 0.66 [a]
Test	MEBT	200mg/kg b. wt	p.o	55.33± 1.20	41.6± 1.20[a]	34.6± 0.33 [a]	17.33± 0.88 [a]	13.6± 0.88 [a]
		400mg/kg b. wt	p.o	53.3± 0.88	37.3± 1.85 [a]	31.6± 1.45 [a]	16.00± 1.52 [a]	9.66± 0.66 [a]

The obtained data were articulated as mean ± SEM (n=3); [a]P<0.001judges against control. Dunnett's test (using SPSS for Windows, version 22.0) was used to process data of this investigation.

Table 4: The CNS depressant activity of the extract was presented from hole cross test.

				Number of movements				
Group	Treatment	Dose	Route	0 min	30 min	60 min	90 min	120 min
Control	1% tween solution	10ml/kg b. wt	p.o	13.33± 0.66	13.66± 0.88	12.00± 1.15	14± 0.577	11.66± 0.33
Standard	Diazepam	1mg/kg b. wt	i.p	14.00± 1.15	08.66± 0.33	6.00± 0.57[b]	4.66 ± 0.33 [b]	2.33± 0.33 [b]
Test	MEBT	200mg/kg b. wt	p.o	13.33± 0.88	11.00± 0.57	8.66± 0.33[b]	5.6 ±0.33 [b]	3.60± 0.66 [b]
		400mg/kg b. wt	p.o	13.00± 1.00	09.30± 0.88	06.60± 0.33 [a]	05.0± 1.00[a]	2.33± 0.33[b]

The statistical representation of this test was processed by Dunnett's test with the help of SPSS for Windows, version 22.0. The assessment was demonstrated as mean ± SEM (n=3); [a]P<0.05, [b]P<0.01 considerably different compared to control.

Table 5: Actions of the extract from thiopental sodium-induced sleeping time test.

Group	Treatment	Dose	Route	Time of onset of sleep(min)	Extentof sleep(min)
Control	1% tween solution	10ml/kg b. wt	p.o	19.6 ± 1.20	30.33 ± 1.85
Standard	Diazepam	1mg/kg b. wt	i.p	6.33 ± 0.88[b]	88.60 ± 9.33[a]
Test	MEBT	400mg/kg b. wt	p.o	9.66 ± 1.20[a]	69.00 ± 4.50[a]

Here, all the data were expressed as mean ± SEM (n=3); [a]P<0.05, [b]P<0.01 much different compared to control. Statistical representation of this test in mice also processed by Dunnett's test using SPSS for Windows, version 22.0

Open Field Test: The total number of squares pass through by the experimental mice was reduced much ([a]P<0.001) in the second inspection time at both 200 and 400 (mg/kg body weight) doses of the extract from the roots of *B. thomsonii*. However, the obtained results were dose-dependent shown in Table 3.

Hole Cross Test: The number of total holes crossed from one chamber to another by experimental mice of the negative control group was almost similar from 0 to 120 min. In this test, the sample extract demonstrated a reduction in locomotion in the test mice from the second inspection period (30min) as evident by the lessening in the number of holes crossed compared to the positive control group. We used diazepam as the standard drug (Table 4).

Thiopental Sodium-Induced Sleeping Test: In this experiment, the test group treated with the extract (400 mg/kg) showed the considerable decline ([a]P< 0.05) in the onset of action and raise the length of sleep. The test sample (MEBT) showed good sedative actions regards of both beginnings of sleep and duration of sleep contrasted to the standard drug diazepam (Table 5).

DISCUSSION

It was observed from the analgesic action assessment that methanol extract of *B. thomsonii* roots demonstrated analgesic activity in both tests. While in the acetic acid-induced writhing experiment the roots extract at doses of 200 and 400mg/kg body weight showed considerable (P<0.05) inhibition of writhing with 35.02 and 49.43% respectively. On the other hand, formalin-induced paw licking test revealed that the MEBT extract could suppress both acute pain and chronic pain. Pain inhibitions were 36.93& 49.43% at acute phase and 25.6 & 43.2% at chronic phase by 200 and 400 mg/kg of MEBT respectively. These mean this extract may possess both peripheral and central analgesic activities. When an algogenic substance for example acetic acid administered intraperitoneally (i.p) of the animals damaging tissue; this tissue damage may occur owing to liberate of chemical mediators, like serotonin, histamine, bradykinin, acetylcholine and prostaglandins. These chemicals sensitize nociceptors to painful stimuli and cause late and diffuse pain [15]. Acetic acid introduces constriction response in the abdomen is supposed to be mediated by prostaglandin pathways [16]. The MEBT roots extract

may contain analgesic components that might interact with prostaglandin pathways.

In formalin test, the chronic phase (15~30 min) and acute phase (0~5 min) representing the inflammatory and neurogenic pain responses respectively. This pain in the acute phase (early phase) was because of the direct stimulation of the sensory nerve fibres (C fibre) by formalin, whereas the pain in the chronic phase (late phase) was due to the inflammatory mediators like bradykinin, prostaglandin, histamine and serotonin [17]. Therefore, by this observation, it may be suggested that methanol extract of roots of *B. thomsonii* can contain analgesic substances that suppress pain either centrally or inhibiting synthesis of prostaglandins and other pain mediators.

Stress is responsible for anxiety and depression. It portends that this types of disorders might become the second largest cause of disability within 2020 [18]. The neuropharmacological study revealed that our chosen extract significantly ($P<0.05$) decreased spontaneous motor movement in both hole cross and open field test. From the motor activity, the altitude of excitability of the central nervous system (CNS) can be measured [19]. The reduction of the motor action may be associated with sedation resulting from the depression of the CNS [20]. It reflects the integrity of the neuromuscular system as a whole and its control and regulation by the central nervous system [21].

The thiopental-induced sleeping test revealed that this plant extract abridged motor harmonization and extended thiopental-induced sleeping duration. This indicated a critical mechanism concerned in the controlling of sleep [22]. Thus it can be assumed that the extract might possess sedative action.

CONCLUSIONS

We concluded that the methanol extract of *B. thomsonii* roots might contain potent substance responsible for analgesic and sedative activity. The extract showed beneficial analgesic and sedative effect at dose 400 mg/kg body weight. So, further investigation needs to be conducted to isolate and purify the responsible compound for analgesic and sedative effects.

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