

## Pharmacological and Phytochemical Screen Activities of Roots of *Heliotropium indicum* Linn.

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### Summary

The methanol extract of the dried roots of *Heliotropium indicum* Linn. (Boraginaceae) was investigated for its possible antinociceptive, cytotoxic and diuretic activities in animal models. The extract produced significant writhing inhibition in acetic acid-induced writhing in mice at the oral doses of 250 and 500 mg/kg body weight ( $P < 0.001$ ) comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight ( $P < 0.001$ ). The crude extract produced the most prominent cytotoxic activity against brine shrimp *Artemia salina* ( $LC_{50} = 47.86 \mu\text{g/ml}$  and  $LC_{90} = 75.85 \mu\text{g/ml}$ ). Moreover, Diuretic activity was proved by the electrolyte loss ratio ( $\text{Na}^+/\text{K}^+$  excretion ratio was 1.38 and 1.45 at the doses of 200 and 400 mg/kg respectively) as that of the standard diuretic furosemide (1.37). The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

**Key words:** Antinociceptive; Cytotoxic; Diuretic; *Heliotropium indicum*; Boraginaceae

### Introduction

*Heliotropium indicum* Linn. (Family: Boraginaceae) locally known as ‘Hatishur’, is a small annual or perennial herb distributed throughout Bangladesh, India, Srilanka, Nepal, Thailand, Tropical Asia, Africa and Philippines. Its stem and roots are covered with a fine hairy layer, and its flowers are small and grow in clusters which curve in on themselves at the tips.<sup>[1]</sup> The plant is traditionally used in wound healing, bone fracture, antidote to poisoning, febrifuge, secretagogue stimulation and cure eye infections.<sup>[2-6]</sup> In Senegal the roots powder is applied to dermatitis and especially to suppurating eczema and impetigo in children.<sup>[7]</sup> In Ivory Coast the dried roots powder is taken up by the nose as decongestant in colds and sinusitis.<sup>[8]</sup> A number of research works have been performed to evaluate its biological activities as anti-inflammatory activities<sup>[9]</sup>, gastroprotective activities<sup>[4]</sup>, anti-tumour activity<sup>[10]</sup> and wound healing properties<sup>[11]</sup>. A few number of chemical investigations have been performed on this plant, as for example, pyrrolizidine alkaloids were isolated by Hartman and Ober<sup>[12]</sup> and Schoental<sup>[13]</sup>. Other chemical compounds for example Indicine-N-Oxide, Tannins, Saponins and Heliotrine were also isolated from this plant<sup>[6, 10]</sup>. In view of this evidence from the existing information show that this plant also may possess some other important biological activities. The present study was carried out to evaluate the antinociceptive, cytotoxic and diuretic activities of the methanolic extract of the roots of *Heliotropium indicum* Linn.

## Materials and Methods

### Plant material collection and extraction

The aerial parts of *Heliotropium indicum* Linn. were collected from Naogaon District, Bangladesh in June 2008, and were taxonomically identified at the Bangladesh National Herbarium, Dhaka. About 400 g of powdered sample were taken in a clean, flat-bottomed glass container and soaked in 1,300 ml of 80% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of cotton followed by a filtration through Whatmann filter paper no. grade 1 and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract.

### Drugs

Diclofenac (Square Pharmaceuticals Ltd, Bangladesh), Furosemide (Square Pharmaceuticals Ltd, Bangladesh).

### Preliminary phytochemical analysis

The crude extracts were subjected to preliminary phytochemical screening for the detection of major chemical groups. In each test 10% (w/v) solution of the extract in ethanol was used unless otherwise mentioned in individual test<sup>1</sup>

### Tests for reducing sugar

Benedict's test: 0.5 ml of the extract was placed in a test tube and then 5 ml Benedict's solution was added to it, boiled for 5 min and allowed to cool spontaneously.

Fehling's Test (Standard Test): 2 ml of the extract was added in 1 ml of a mixture of equal volumes of Fehling's solutions A and B, and was boiled for few min.

Combined Reducing Sugar test: 1 ml of the extract was boiled with 2 ml of diluted hydrochloric acid for 5 min. After cooling the mixture was neutralized with sodium hydroxide solution and then Fehling's test was performed as described above.

### Tests for tannins

Ferric Chloride Test: 5 ml of the extract was placed in a test tube and then 1 ml of 5% Ferric chloride solution was added to it.

Potassium dichromate test: 5 ml of the extract was placed in a test tube and then 1 ml of 10% potassium dichromate solution was added.

### Test for flavonoids

A few drops of concentrated hydrochloric were added to 5 ml of the extract.

### Test for saponins

1 ml of the extract was placed in a graduated cylinder and was diluted to 20 ml with distilled water and shaken gently for 15 min.

### Test for gums

5 ml of the extract was placed in a test tube and then Molish's reagent and sulphuric acid were added to it.

### Tests for steroids

Liebermann-Burchard test: 1 ml of the extract was placed in a test tube and then 2 ml Liebermann-Burchard reagent was added to it.

Sulphuric acid test: 1 ml of the extract was placed in a test tube and 1 ml sulphuric acid was added to it.

### **Tests for alkaloids**

Mayer's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube and 1 ml of Mayer's reagent was added to it.

Dragendroff's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube and then 1 ml Dragendroff's reagent was added.

Wagner's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of iodine solution (Wagner's reagent) was added.

Hager's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of picric acid solution (Hager's reagent) was added.

### **Animals**

Young Swiss-albino mice of either sex, weighing 20 - 25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for the test. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55 - 65%, room temperature  $25.0 \pm 2.0^\circ\text{C}$  and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water. The experimental met the national guidelines on the proper care and use of animals. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol.

### **Pharmacological studies**

#### **Antinociceptive activity**

Antinociceptive activity of the crude extract was tested using the model of acetic acid-induced writhing in mice.<sup>[14, 15]</sup> The experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 in water at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at dose of 25 mg/kg of body weight; group III and group IV were test groups and were treated with the extracts at dose of 250 and 500 mg/kg of body weight respectively. Control vehicle, standard drug and extracts were administered orally, 30 min prior to acetic acid (0.7%) injection. Then after an interval of 15 min, the number of writhes (squirms) was counted for 5 min.

#### **Cytotoxicity test**

The brine shrimps used for cytotoxicity test were obtained by hatching 5 mg of eggs of *Artemia salina* in natural seawater after incubation at about  $29^\circ\text{C}$  for 48h. The larvae (nauplii) were allowed another 48 h in seawater to ensure survival and maturity before use. Five doses of plant extract (10, 20, 40, 80 and 160  $\mu\text{g/ml}$ ) in 5% DMSO and/or seawater were tested. Each extract preparation was dispensed into clean test tubes in 10 ml volumes and tested in duplicates. The concentration of DMSO in the vials was kept below 10  $\mu\text{l/ml}$ . For control, same procedure was followed except test samples. After marking the test tubes properly, 10 living shrimps were added to each of the 20 vials with the help of a Pasteur pipette.<sup>[16]</sup> The test tube containing the sample and control were then incubated at  $29^\circ\text{C}$  for 24 h in a water bath, after which each tube was examined and the surviving nauplii counted. From this, the percentage of mortality was calculated at each concentration.

#### **Diuretic activity**

Diuretic activity of the extract was investigated using the method as described by Lipschitz et al.<sup>[17]</sup> The test animals were randomly chosen and divided into five groups having ten mice in each. Twenty-four hours prior to the experiment, the test animals were placed in to metabolic cages with the withdrawal of food and water. Group-1 or the control group

received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg body weight orally. Group-2 was provided with urea solution at a dose of 500 mg/kg. Group-3 was provided with standard diuretic drug furosemide at a dose of 0.5 mg/kg. Group-4 and group-5, the test groups were treated with the methanol extract of MP at the doses of 200 and 400 mg/kg respectively. From the graduated urine chamber of metabolic cage, the urinary output of each group was recorded 5 h after the above treatments. Collected urine was centrifuged and then estimated for sodium and potassium by using digital flame photometer (Elico Pvt. Ltd., model CL 22D). Chloride was estimated by the Schales and Schales method reproduced by Godkar. [18]

## Results

### Preliminary phytochemical analysis

Results of different chemical tests on the methanol extract of *Heliotropium indicum* Linn. showed the presence of alkaloids, steroids, flavonoids, saponins and tannins

**Table 1. Phytochemical properties of *Heliotropium indicum* Linn. crude roots extract**

Compound	Alkaloids	Glycosides	Steroids	Gums	Flavonoids	Saponins	Reducing sugars	Tannins
Observation	+ve	-ve	+ ve	- ve	+ ve	+ ve	- ve	+ve

Key: +ve = Presence -ve = Absence

### Antinociceptive activity

Table 2 showed the effect of the methanol extract of *Heliotropium indicum* Linn. on acetic acid-induced writhing in mice. At dose of 250 and 500 mg/kg of body weight, the extract produced about 34.76% and 64.67% writhing inhibition in test animals, respectively. The results were statistically significant ( $P < 0.001$ ) and were comparable to the standard drug diclofenac sodium, which showed about 66.67% writhing inhibition at the dose of 25 mg/kg ( $P < 0.001$ ).

**Table 2. Effects of *Heliotropium indicum* Linn. crude roots extract on writhing effect on acetic acid induced mice**

Treatment	Dose (mg/kg)	Mean writhing	% Inhibition	SD	P value (One way Anova)*
Experimental control (1% Tween80)	10	19.2.0 ± 0.73	-	1.94	-
Positive control (Diclofenac sodium)	25	4.3 ± 1.145	66.67	8.14	P<0.001
Test sample 1	250	18.7 ± 1.21	34.76	2.77	P<0.001
Test sample 2	500	17.1 ± 0.94	64.67	2.71	P<0.001

Key: \*- (VassarStats, 2009); Test sample- *Heliotropium indicum* Linn. Crude Extract. 30 minutes after treatment, 0.7% acetic acid was injected i.p. 10 minutes after injection writhing responses was recorded for 10 minutes. N=5 (No. of mice)

**Cytotoxic activity**

In brine shrimp lethality bioassay (Table 3), the extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations. From the plot of percent mortality versus log concentration on the graph paper LC<sub>50</sub> and LC<sub>90</sub> were deduced (LC<sub>50</sub>: 47.86 µg/ml; LC<sub>90</sub>: 75.85 µg/ml) (Table 3).

**Table 3: Brine shrimp lethality bioassay of *Heliotropium indicum* Linn. roots extract**

Test sample	Conc. (µg/ml)	No. of alive shrimp			% mortality	LC <sub>50</sub> (µg/ml)	LC <sub>90</sub> (µg/ml)
		Test 1	Test 2	Average			
Methanolic extract	5	10	8	9	10	47.86	75.85
	10	9	8	8.5	15		
	20	7	7	7	30		
	40	5	4	4.5	35		
	80	0	0	0	100		
	160	0	0	0	100		

**Diuretic activity**

The effect of the methanolic extract of *Heliotropium indicum* Linn. on the urination of mice was observed for 5 h which revealed that the extract has a marked diuretic effect in the test animals. This was comparable to that of standard drug furosemide and diuretic agent urea. Electrolyte loss showed similar ratio (Na<sup>+</sup>/K<sup>+</sup> excretion ratio was 1.38 and 1.45 at the doses of 200 and 400 mg/kg respectively) as that of the loop diuretic furosemide (1.37) (Table 4).

**Table 4. Effect of methanolic extract of *Heliotropium indicum* Linn. on urine excretion parameters in mice**

Treatment	Dose (mg/kg; p.o.)	Volume of urine (ml)	Concentrations of ions (m.eq.l <sup>-1</sup> )			
			N <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	Na <sup>+</sup> /K <sup>+</sup>
Group-1(Control)	-	2.43 ± 0.07 3.74 ± 0.08	76.67 ± 1.24 113.66 ±	48.75 ± 1.18	76.55 ± 1.24	1.42
Group-2(Urea)	500	1.35** 4.15 ± 0.14	122.87 ±	76.56 ± 1.27**	87.72 ± 1.38*	1.38
Group-3(Furosemide)	0.5	1.74**		85.46 ± 1.67**	92.36 ± 1.49*	1.37
Group-4(HI)	200	4.25 ± 0.08	118.51 ± 1.19**	79.35 ± 1.86**	91.74 ± 1.68*	1.38
Group-5(HI)	400	4.86 ± 0.04	132.74 ± 1.62**	92.24 ± 1.69**	97.60 ± 1.86*	1.45

ME: Methanolic extract of HI-*Heliotropium indicum* Linn.; Values are expressed as mean ± SEM (Number of animals, n = 10); \* indicates P<0.01, \*\* indicates P<0.001 vs. control;

<sup>b</sup>Collected for 5 hours after treatment.

### Discussion

Antinociceptive activity of the methanol extract of *Heliotropium indicum* Linn. was tested by acetic acid-induced writhing model in mice. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings.<sup>[19]</sup> Increased levels of PGE2 and PGF2 $\alpha$  in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid.<sup>[20, 21]</sup> The extract produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table 2). The chemical compounds present in the plant extract may be responsible for the obtained antinociceptive activity. Based on this result it can be concluded that the ethanol extract of *Heliotropium indicum* Linn. might possess antinociceptive activity.

The cytotoxic activity of the methanol extract of *Heliotropium indicum* Linn. was tested by using brine shrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc.<sup>[22]</sup> The extract was found to show potent activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds.

Diuretic activity may be very useful in a number of conditions like hypertension, hypercalciuria, cirrhosis of liver. Furosemide, used as the standard drug in this experiment belongs to the loop or high-ceiling diuretics, which act by inhibiting Na<sup>+</sup>/K<sup>+</sup>/Cl<sup>-</sup> co-transport of the luminal membrane in the ascending limb of the loop of Henle and have the highest efficacy in mobilizing Na<sup>+</sup> and Cl<sup>-</sup> from the body. The extract was able to increase the volume of urine with statistical significance along with a considerable Na<sup>+</sup> and Cl<sup>-</sup> load which was comparable to that of furosemide. The diuretic action of the extract may be due to its action on the kidney. The extract may also contain a high proportion of osmotically active compounds or their metabolites that lead to an increased urine volume. Further studies may be carried out to identify whether these actions are associated with the same agent or a number of agents that are responsible for such activities.

### Conclusion

Finally, it can be concluded from the study that the antinociceptive, cytotoxic and diuretic effects of the methanolic roots extract of *Heliotropium indicum* Linn. may be presence of different chemical compounds which works through the specific and non-specific mechanisms. However, extensive studies are needed to evaluate the precise mechanism(s), active principles, and the safety profile of the plant as a remedy for different therapeutical conditions.

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