Antinociceptive and CNS Depressant Activities of *Xanthium indicum* Koen. in Mice

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Hydromethanol extract of the leaves of *Xanthium indicum* was investigated for possible analgesic and CNS depressant actions. The analgesic activity was evaluated by using acetic acid-induced writhing test in mice, where the extract, at the dose of 400 mg/kg, showed a maximum of 64.1 % inhibition (p <0.001) of writhing response which is comparable to the reference drug Diclofenac-Na (77.40%). The extract was also examined for its depressive action on CNS using hole-cross and open field test. The extract displayed dose dependent suppression of motor activity and exploratory behaviour in mice.

Xanthium indicum (Family: Compositae), locally known as Ghagra or Bichphal (Beng.), is a coarse unarmed annual herb which grows as a gregarious weed in paddy fields and by the canal or ditch banks in all areas of Bangladesh. The plant is reported to have diaphoretic, diuretic, sudorific, CNS depressant and styptic properties. Decoction of the plant is used in urinary and renal complaints, gleet, leucorrhoea and menorrhagia. Seeds are used to resolve inflammatory swellings while the root is useful against scrofulous tumours and cancer. The plant is reported to contain alpha and gamma- tocopherols, polyphenols, glucoside, xanthostrumarin and xanthonolides as the principal constituents.[1] As a part of our ongoing investigation on medicinal plants of Bangladesh, we report here the analgesic and central nervous system depressant activities of the leaf extract of *X. indicum*, which is traditionally used in folkloric remedies for various disorders involving pain and inflammation.

The leaves of *X. indicum* were collected from Boteshahor, Khadim Nagar, Sylhet, Bangladesh in February, 2008 and was identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka where a voucher specimen (no:32785) has been deposited for reference. Then the leaves were dried in hot air oven at 55ºC for 5 days, coarsely powdered and extracted in a Soxhlet apparuatus with a mixture of methanol: water (7:3, v/v). The solvents were completely removed and the dried crude extract thus obtained was used for investigation.

For the experiment, male 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±1.0°C), relative humidity: 55-65% and 12hrs light/12 hrs dark cycle) and were given free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experimentation.

The freshly prepared crude extract was qualitatively tested for the presence of chemical

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constituents. These were identified by characteristic color changes using standard procedures.[1]

The analgesic activity of the samples was studied using acetic acid-induced writhing test in mice. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac-Na was administered 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 minutes.[2]

Table 1. Result of chemical group test of the hydromethanolic extract of *Xanthium indicum*

Extract	Steroid	Alkaloid	Reducing sugar	Tannin	Gum	Flavonoid	Saponin
X. indicum	-	++	++	++	+	++	-

(+): Present; (-): Absent

Table 2. Effect of the hydromethanolic extract of *X. indicum* on acetic acid-induced writhing in mice

Groups	Treatment	Dose, route	No. of writhing	% Inhibition
Group-I	1% Tween 80 in water	0.4 ml/mouse, p.o.	40.7±1.057	-
Group-II	Diclofenac Na	10 mg/kg, p.o.	9.2±0.772	77.40
Group-III	*X. indicum*	200 mg/kg, p.o.	26.7±2.276	34.4
Group-IV		400 mg/kg, p.o.	14.6±1.278	64.1

Values are presented as mean ± SEM, (n = 5); ** $p < 0.001$, Dunnet test as compared to control.

Table 3. Effect of hydromethanolic extract of *X. indicum* on Hole cross test in mice

Treatment	Dose, route	Number of movements				
		0 min	30 min	60 min	90 min	120 min
Vehicle	0.4 ml/mouse, p.o.	17±1.969	17.80±1.917	17.60±2.280	17.20±1.557	18.40±0.908
Diazepam	1 mg/kg, p.o.	15.4±0.908	6.00±0.935 [a]	2.00±0.791 [a]	1.60±1.037 [a]	1.20±0.418 [a]
X. indicum	200 mg/kg, p.o.	18.4±1.15	9.40±0.758 [a]	8.00±0.791 [a]	7.00±1.275 [a]	6.60±1.483 [a]
	400 mg/kg, p.o.	18.2±1.981	7.60±1.037 [a]	5.20±1.294 [a]	4.20±1.597 [a]	3.40±1.204 [a]

Values are presented as mean ± SEM, (n = 5); a - $p < 0.001$, Dunnet test as compared to control.

Table 4. Effect of hydromethanolic extract of *X. indicum* on Open field test in mice

Treatment	Dose, route	Number of movements				
		0 min	30 min	60 min	90 min	120 min
Vehicle	0.4 ml/mouse, p.o.	121.6±5.346	117.80±3.943	116.60±2.612	110.80±7.528	117.2±4.615
Diazepam	1 mg/kg, p.o.	118.4±7.041	67.80±5.628 [a]	41.60±3.650 [a]	19.60±2.612 [a]	10.8±2.043 [a]
X. indicum	200 mg/kg, p.o.	115±6.083	94.40±5.597 [a]	74.00±7.141 [a]	53.60±6.676 [a]	47.8±4.827 [a]
	400 mg/kg, p.o.	116.6±2.414	89.00±5.788 [a]	56.80±4.574 [a]	39.60±3.701 [a]	26.2±3.305 [a]

Values are presented as mean ± SEM, (n = 5); a- $p < 0.001$, Dunnet test as compared to control.

The hole cross method, as described by Takagi *et al.*[3] was adopted for screening CNS depressant activity in mice.[3] 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. The number of passage 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 oral administration of the test drugs.

In addition to hole cross test, the open field test was carried out to determine depressive action of the test drugs on CNS in mice.[4] The animals were divided into control, positive control, and test groups containing five mice each. The test group received *X.*

indicum extract at the dose of 200 and 400 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water). The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90, and 120 minutes after oral administration of the test drugs.

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet'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.

Phytochemical analyses of the crude extract revealed the presence of alkaloid, flavonoid, tannin, gum, and reducing sugar (Table 1). The oral administration of the extract at 200 mg/kg and 400 mg/kg body weight significantly ($p < 0.001$) inhibited writhing response induced by acetic acid in a dose dependent manner (Table 2). Again, the extract at both doses produced significant ($p < 0.001$) decrease of movement from its initial value in the hole cross experiment (Table 3) and number of squares traveled by the mice in open field test (Table 4).

However, acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids.[5] Preliminary phytochemical screening revealed the presence of flavonoid, tannin, gum, and reducing sugar and alkaloid in the plant extract. So, the observed analgesic activity may be attributed to these compounds. Reports exist on the role of flavonoid in analgesic activity primarily by targeting prostaglandins [6]. There are also reports on the role of tannins in antinociceptive activity [7]. Again extract significantly decreased the locomotor activity. The locomotor activity lowering effect was evident at the 2nd observation (30 min) and continued up to 5th observation period (120 min). Both hole cross and open field tests showed that the depressing action of the extracts was evident from the 2nd observation period in the test animals at the doses of 200 and 400 mg/kg body weight. Maximum depressive effect was observed from 3rd (60 min) to 5th (120 min) observation period. The results were also dose dependent and statistically significant (Tables 3, 4).

In light of the findings of the present study, it can be concluded that the plant extract possesses remarkable analgesic and CNS depressant potential, thereby lends support to the traditional use of the plant in painful and inflammatory disorders. However, further studies are needed to be conducted to understand the exact mechanisms of such actions and to isolate the active principles responsible for the observed activity.

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