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Pharmacological studies on roots of *Achyranthes aspera* Linn

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SUMMARY

The crude methanol extract of the roots of *Achyranthes aspera* Linn. was investigated for its possible antinociceptive, diuretic and neuropharmacological activities in animal models. At the dose of 250 and 500 mg/kg body weight, the extract showed a significant antinociceptive effect in acetic acid induced-writhing in mice comparable to that produced by diclofenac sodium, used as standard drug. The crude extract produced significant diuretic effect at the dose of 500 mg/kg of body weight comparable to that produced by furosemide, used as standard drug. The extract also potentiated significantly the pentobarbital induced sleeping time in mice; decreased the open field score in open field test, decreased the number of hole crossed from one chamber in the hole cross test and decreased the head dip responses. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key words: Achyranthes aspera; Antinociceptive activity; Diuretic activity; Neuropharmacological activity

INTRODUCTION

Achyranthes aspera Linn. (Family: Amaranthaceae) locally known as 'Apang', 'Upothlengra', is a small annual or perennial herb distributed throughout Bangladesh, India, Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America. The plant is traditionally used in piles, skin eruptions, dysentery, eye and liver diseases, in scabies and in leprosy (Kirtikar and Basu, 1987). A number of research works have been performed to evaluate its biological activities as anti-inflammatory and antiarthritic activities (Gokhale *et al*., 2002), hypoglycemic activity (Akhtar *et al*., 1991), Contraceptive and hormonal properties (Wadhwa *et al*., 1986; Tahiliani and Kar, 2000), cardiac stimulant activity (Gupta *et al*., 1972), antileprotic activity (Ojha *et al*., 1966), etc.

The main objective of this study was to evaluate the antinociceptive, diuretic and neurophamacological activities of the methanol extract of roots of *Achyranthes aspera* (*A. aspera*).

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MATERIALS AND METHODS

Plant material collection and extraction

The plants were collected in February 2003 from the district of Chapai Nawabgonj and were identified in the National Herbarium of Bangladesh (Accession no: 29761). The roots of *A. aspera* were pulverized into a fine powder. The extracts of approximately 400 g of powdered material were obtained by soxhlet apparatus with 90% methanol at 55°C. The extract was filtered and evaporated (approximate yield 14%) using vacuum rotary evaporator.

Animals

Swiss-albino mice of either sex, weighing 22 - 25 g, bred in the animal house of the Department of Pharmacy, Jahangirnagar University, Savar, Bangladesh were used for the pharmacological studies. The animals were acclimatized one week prior to the experiments and were housed under standard laboratory conditions (relative humidity 55 - 65%, room temperature 22.0 ± 2.0°C and 12 h light: dark cycle). The animals were fed with standard diet formulated by International Center for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B) and had free access to tap water.

Drugs

Diclofenac Sodium (Opsonin Chemical Industries Ltd, Bangladesh), Furosemide (Square Pharmaceuticals Ltd, Bangladesh), Pentobarbital (Sigma Chemicals, U.S.A.).

Pharmacological studies

Antinociceptive activity

The antinociceptive activity of the crude methanol extract of *A. aspera* was studied using acetic acid induced writhing model in mice (Koster *et al.*, 1959; Ahmed *et al.*, 2004). The animals were divided into control, positive control and test groups with ten mice in each group. The animals of test groups received test substance at the dose of 250 and 500 mg/kg body weight. Positive control group was administered diclofenac sodium (standard drug) at the dose of 25 mg/kg of body weight and vehicle control group was treated with 1% Tween 80 in water at the dose of 10 ml/kg body weight. Test samples, standard drug and control vehicle were administered orally 45 min before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 min, the mice were observed for specific contraction of body referred to as 'writhing' for 5 min.

Diuretic activity

Diuretic activity of the extract was investigated using the method as described by Lipschitz *et al.* (1943). The test animals were randomly chosen and divided into four groups having ten mice in each. Twenty-four hours prior to the experiment, the test animals were placed into metabolic cages with the withdrawal of food and water. Group I or the control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg body weight orally. Group II was supplied with standard diuretic drug furosemide at the dose of 0.5 mg/kg. Group III and group IV, the test groups were treated with the methanol extract of *A. aspera* roots at the doses of 250 and 500 mg/kg of body weight 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 (1994).

Neuropharmacological activity

i) Pentobarbital induced hypnosis: Pentobarbital induced sleeping time test was carried out by the method of Williamson *et al.* (1996). The test animals were divided into three groups consisting of seven mice in each. Group I was the control group and group II and III were the experimental groups. The experimental groups were administered with the methanol extract of *A. aspera* at the doses of 250 and 500 mg/kg body weight intra-peritoneally (i.p.), while the animals of group I (control) were supplied with distilled water containing 0.1% (v/v) tween-80 (i.p.) at the dose of 10 ml/kg of body weight. The total sleeping time were recorded for both control as well as for treated groups. Total sleeping time represents the time between the loss and regain of righting reflex.

ii) Exploratory behavior: This experiment was performed by (i) Open field test (Gupta *et al.*, 1971) (ii) Hole cross test (Takagi *et al.*, 1971) and (iii) Hole board test (Nakama *et al.*, 1972). The test animals were divided into three groups consisting of seven

mice in each. Group I was the control group and group II and III were the experimental groups. The experimental groups were administered with the methanol extract of *A. aspera* at the doses of 250 and 500 mg per kg body weight intraperitoneally (i.p.), while the animals of group I (control) were supplied with 0.1% (v/v) tween -80 (i.p.) at the dose of 10 ml per kg of body weight. The observations were made on 0 min before injection and 30, 60, 120 and 240 min after injections (i.p.) of the test samples and control.

Statistical analysis

Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

RESULTS

Antinociceptive activity

Table 1 showed the effect of the methanol extract of *A. aspera* on acetic acid induced writhing in mice. At the doses of 250 and 500 mg/kg, the extract produced 49.12% and 65.68% 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 68.64% writhing inhibition at the dose of 25 mg/kg.

Diuretic activity

The effect of the methanol extract of *A. aspera* roots on the urination of mice was observed for 5 h, which revealed that the extract has a marked diuretic effect in the test animals which was comparable to that produced by the standard drug furosemide (Table 2). Electrolyte loss showed similar ratio (Na^+/K^+ excretion ratio was 1.49 and 1.47 at the doses of 250 and 500 mg/kg respectively) as that of the loop diuretic furosemide (1.47).

Neuropharmacological activity

i) Pentobarbital induced hypnosis: Table 3 showed the effects of methanol extract of *A. aspera* on pentobarbital induced sleeping time. The average duration of sleep was about 34 min and 58 min at the dose of 250 and 500 mg/kg respectively where as in control group it was about 12 min. Thus the results showed that the methanol extract of *A. aspera* potentiated the pentobarbital induced sleeping

Table 1. Effect of methanol extract of *A. aspera* on acetic acid induced writhing in mice

Animal Group/Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 solution in water, p.o.	16.9 ± 0.801 (100)	-
Positive control Diclofenac sodium 25 mg/kg, p.o.	5.3 ± 0.538* (31.36)	68.64
Test group-1 Methanol extract 250mg/kg, p.o.	8.6 ± 0.635* (50.88)	49.12
Test group-2 Methanol extract 500 mg/kg, p.o.	5.8 ± 0.388* (34.32)	65.68

Values are expressed as mean ± S.E.M. (Number of animals, n = 10); * indicates $P < 0.001$ vs. control; p.o.: per oral.

Table 2. Effect of methanol extract of *A. aspera* on urine excretion parameters in mice

Treatment	Dose (mg/kg; p.o.)	Volume of urine (ml)[a]	Concentrations of ions ($m.eq.l^{-1}$)			
			Na^+	K^+	Cl^-	Na^+/K^+
Group I (Control)	-	2.75 ± 0.08	75.67 ± 1.25	49.75 ± 1.18	77.56 ± 1.24	1.52
Group II (Furosemide)	0.5	4.75 ± 0.13	125.86 ± 1.75**	85.46 ± 1.67**	94.39 ± 1.49**	1.47
Group III (ME)	250	4.00 ± 0.10	106.99 ± 1.18**	71.97 ± 1.78**	82.49 ± 1.36*	1.49
Group IV (ME)	500	4.48 ± 0.07	120.71 ± 1.65**	82.21 ± 1.89**	88.02 ± 1.67**	1.47

ME: Methanol extract of *A. cucullata*; Values are expressed as mean ± S.E.M. (Number of animals, n=10); *indicates $P < 0.05$, **indicates $P < 0.001$ vs. control; [a]Collected for 5 h after treatment.

Table 3. Effect of *A. aspera* on pentobarbital induced hypnosis

Animal group	Treatment	Total sleeping time (min)
I (Control)	0.1% Tween 80 solution	12.29 ± 1.01
II (Test group-I)	Me. Extract (250 mg/kg)	33.71 ± 1.23*
III (Test group-II)	Me. Extract (500 mg/kg)	58 ± 2.97*

Values are expressed as mean ± S.E.M; *indicates $P < 0.001$ vs. control; Me.= Methanol

Table 4. Effect of *A. aspera* on exploratory behavior in mice

Group	Response at				
	0 min	30 min	60 min	120 min	240 min
Effect on Open Field Test					
I (Control)	114.1 ± 2.36	112 ± 1.94	123.36 ± 1.24	106.36 ± 1.62	110.16 ± 1.38
II (Me. Ext.) 250 mg/kg	101.75 ± 1.37*	89.25 ± 1.54*	83.17 ± 1.96*	77.54 ± 1.47*	85.15 ± 2.85*
III (Me. Ext.) 500 mg/kg	117.33 ± 2.13*	82.45 ± 2.34*	79.83 ± 2.52*	74 ± 1.63*	80 ± 2.74*
Effect on Hole Cross Test					
I (Control)	8.42 ± 0.65	9.85 ± 0.46	10 ± 0.49	9.28 ± 0.68	8.42 ± 0.65
II (Me. Ext.) 250 mg/kg	7.42 ± 0.57*	6.07 ± 0.85**	4.89 ± 0.34 *	3.07 ± 0.50 *	2.90 ± 0.22*
III (Me. Ext.) 500 mg/kg	8.28 ± 0.39*	6.16 ± 0.73**	4.27 ± 0.62*	3.33 ± 0.18*	1.66 ± 0.13*
Effect on Hole Board Test (Head dipping)					
I (Control)	15.66 ± 1.32	17.58 ± 1.01	15.89 ± 0.98	16.50 ± 1.08	18.26 ± 1.11
II (Me. Ext.) 250 mg/kg	19.86 ± 0.54	16.02 ± 0.31****	14.24 ± 0.29****	13.31 ± 0.57***	15.04 ± 0.61***
III (Me. Ext.) 500 mg/kg	17.59 ± 0.35	14.75 ± 0.56***	11.36 ± 0.62**	9.53 ± 1.13**	10.43 ± 1.17**

Values are expressed as mean ± S.E.M. Me.; methanol. *indicates $P < 0.001$; **indicates $P < 0.01$; ***indicates $P < 0.05$; ****indicates $P < 0.2$ vs. control.

time in mice.

ii) Exploratory behavior: It was observed that the extract caused a significant decrease in the open field score (Table 4), decrease in the number of hole crossed from one chamber to another chamber (Table 4), and a significant decrease in head dip responses (Table 4) in mice at the dose of 250 mg/kg and 500 mg/kg of body weight. These results therefore further support the central depressant properties of the extract.

DISCUSSION

Antinociceptive activity of the methanol extract of *A. aspera* roots 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 (Taesotikul *et al.*, 2003). Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Derardt *et al.*, 1980). On the basis of the result of acetic acid induced writhing test, it can be concluded that the methanol extract of *A. aspera* might possess an antinociceptive activity.

Diuretic activity may be very useful in a number of conditions like hypertension, hypercalciuria, cirrhosis of liver, etc. Furosemide, used as the standard drug in this experiment belongs to the loop or high-ceiling diuretics, which act by inhibiting $Na^+/K^+/Cl^-$ co-transport of the luminal membrane in the ascending limb of the loop of Henle and have the highest efficacy in mobilizing Na^+ and Cl^- from the body. The extract was able to increase the

volume of urine with statistical significance along with a considerable Na^+ and Cl^- 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.

Neuropharmacological activity was tested by pentobarbital induced sleeping time test and the tests for exploratory behavior. Pentobarbital shorten the onset of sleep and increases sleep duration. The methanol extract of *A. aspera* potentiated the pentobarbital induced sleeping time in mice which suggests its central depressant activity (Perez *et al*. 1998), thus suggesting the probable tranquilizing action (Capasso *et al*., 1996). The extract also made mice to reduce their behavioral exploration, which further support the central sedative properties of the extract. The overall results tend to predict the central nervous system depressant action of the extract.

In conclusion, it can be suggested that the crude extract of *A. aspera* may possess antinociceptive, diuretic and CNS depressant effects, which correlate well with the traditional use of the plant. Therefore, further researches are essential to find out the active principles responsible for these activities.

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