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Antinociceptive, antidiarrhoeal and cytotoxic activity of *Aegiceras corniculatum*

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SUMMARY

The ethanol extract of leaves of the mangrove *Aegiceras corniculatum* Blanco (Myrsinaceae) was screened for its antinociceptive, antidiarrhoeal and cytotoxic activities. The extract produced significant writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 250 and 500 mg/kg body weight ($P < 0.001$), which was comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. When tested for its antidiarrhoeal effects on castor oil induced diarrhoea in mice, it increased mean latent period and decreased the frequency of defecation significantly at the oral dose of 500 mg/kg body weight ($P < 0.05$; $P < 0.01$) comparable to the standard drug loperamide at the dose of 50 mg/kg of body weight. Moreover, when tested for toxicity using brine shrimp, the extract showed potent activity against the brine shrimp *Artemia salina* (LC_{50} 10 mg/ml). The overall results tend to suggest the antinociceptive, antidiarrhoeal and cytotoxic activities of the extract.

Key words: Antinociceptive; Antidiarrhoeal; Cytotoxic; *Aegiceras corniculatum*; Myrsinaceae

INTRODUCTION

Aegiceras (A.) corniculatum Blanco (Myrsinaceae), locally known as Khulshi, Kholisha, etc., is a small tree found in mangrove forests of Bangladesh, India, China, New Guinea and Australia. Its fruits and seeds are used as fish-poison; barks and leaves are used in the treatment of asthma, diabetes, rheumatism, etc. (Balasooriya *et al.*, 1982). Xu *et al.* (2004) isolated seven new compounds, namely, 2-methoxy-3-nonylresorcinol, 5-*O*-ethylembelin, 2-*O*-acetyl-5-*O*-methylembelin, 3,7-dihydroxy-2,5-diundecylnaphthoquinone, 2,7-dihydroxy-8-methoxy-3,6-diundecyldibenzofuran-1,4-dione, 2,8-dihydroxy-7-methoxy-3,9-diundecyldibenzofuran-1,4-dione, and 10-hydroxy-4-*O*-methyl-2,11-diundecylgomphilactone, together with three known compounds, 5-*O*-methylembelin, 3-undecylresorcinol, and 2-dehydroxy-5-*O*-methylembelin from the stems and twigs of this plant. Of them, 5-*O*-methylembelin and 5-*O*-ethylembelin showed *in vitro* cytotoxicity against the HL-60, Bel (7402), U937, and HeLa cell lines. Gomez *et al.* (1989) isolated 5-*O*-methylembelin from the twigs and stems of *A. corniculatum* and reported its toxicity to fish (*Tilapia nilotica*). The objective of the present study was to investigate the antinociceptive, antidiarrhoeal and cytotoxic activities (using brine shrimp) of the crude extract of leaves of *A. corniculatum*.

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MATERIALS AND METHODS

Plant material collection and extraction

The leaves of *A. corniculatum* were collected from the Sundarbans' Mangrove Forests, Bangladesh in July 2003, and were taxonomically identified by experts at the Bangladesh National Herbarium

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(accession number 29793). About 500 g of powdered plant material was taken in a clean, flat-bottomed glass container and soaked in 4 l 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 and the filtrate thus obtained was concentrated in open air to get the crude extract (Yield: 9%).

Drugs

Diclofenac sodium (Opsonin Chemical Industries Ltd., Bangladesh), Loperamide (Square Pharmaceuticals Ltd., Bangladesh).

Preliminary phytochemical analysis

The crude extract was 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 specified in individual test (Evans, 1989; Ghani, 1998).

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 tests. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation under standard laboratory conditions (relative humidity 55 - 65%, room temperature 25.0 ± 2.0°C and 12 h light/dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water.

Pharmacological studies

Antinociceptive activity

Antinociceptive activity of the extract of *A. corniculatum* was tested using the model of acetic acid-induced writhing in mice (Whittle, 1964; Ahmed *et al.*, 2004). 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 a dose of 25 mg/kg of body weight; group III and group IV were test groups and were treated with the extracts at the doses 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.

Antidiarrhoeal activity

Antidiarrhoeal activity of the extract of *A. corniculatum* was tested using the model castor oil induced diarrhoea in mice (Chatterjee, 1993). The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The test animals were randomly chosen and divided into three groups having five mice in each. Group-I was kept as control and received 1% Tween-80 at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug loperamide at dose of 50 mg/kg of body weight; group III was test group and was treated with the extract at dose of 500 mg/kg of body weight. Control vehicle, standard drug and the extract were administered orally, 1 h prior to the oral administration of castor oil at a dose of 0.5 ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhoea every hour in five hours study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment (5 h). The latent period of each mouse

was also counted. At the beginning of each hour new papers were placed for the old ones.

Cytotoxic activity

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°C for 48 h. 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 μg/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 μl/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 (Meyer *et al.*, 1982). The test tube containing the sample and control were then incubated at 29°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.

Statistical analysis

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

RESULTS

Preliminary phytochemical analysis

Results of different chemical tests on the ethanol extract of *A. corniculatum* showed the presence of steroids, alkaloids, flavonoids, gums and saponins (Table 1).

Antinociceptive activity

Table 1 showed the effect of the ethanol extract of *A. corniculatum* on acetic acid-induced writhing in mice. At the dose of 250 and 500 mg/kg of body weight, the extract produced about 70 and 82% 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 81% writhing inhibition at the dose of 25 mg/kg of body weight ($P < 0.001$).

Antidiarrhoeal activity

Antidiarrhoeal activity of the ethanol extract of *A. corniculatum* was tested by castor oil induced diarrhoea in mice. The extract caused an increase in latent period (2.02 h) i.e. delayed the onset of diarrhoeal episode at the dose of 500 mg/kg body weight significantly ($P < 0.05$; $P < 0.01$) which was comparable to the standard drug loperamide at the dose of 50 mg/kg body weight in which the value was 2.158 h ($P < 0.01$) (Table 2a). The extract also decreased the frequency of defecation at the same dose where the mean numbers of stool at the 1st, 2nd, 3rd, 4th and 5th h of study were 1.2, 0.8, 1.5, 1.6 and 0.6, respectively and in standard drug the values were 0.4, 1.6, 2.2, 0.8 and 0.4, respectively (Table 2b).

Cytotoxic activity

In brine shrimp lethality bioassay, the extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different

Table 1. Effect of *A. corniculatum* on acetic acid induced writhing in mice

Animal Group/Treatment	Number of writhes (% writhing)	Inhibition (%)
Group-I (control) 1% tween-80 10 ml/kg, p.o.	24.375 ± 1.36 (100)	-
Group-II (positive control) Diclofenac sodium 25 mg/kg, p.o.	4.75 ± 0.745* (19.48)	80.52
Group-III Ethanol extract 250mg/kg, p.o.	7.37 ± 0.839* (30.23)	69.77
Group-IV Ethanol extract 500 mg/kg, p.o.	4.5 ± 0.560* (18.46)	81.54

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

Table 2a. Effect of *A. corniculatum* on castor oil induced diarrhoea in mice (latent period)

Animal Group/Treatment	Dose (/kg, p.o.)	Latent period (h)
Group-I (control) (1% tween-80)	10 ml	1.27 ± 0.159
Group-II (positive control) Loperamide	50 mg	2.158 ± 0.176*
Group - III Ethanol extract	500 mg	2.024 ± 0.460*

Values are expressed as mean ± SEM (n = 5); *indicates $P < 0.001$ vs. control; p.o.: per oral

Table 2b. Effect of *A. corniculatum* on castor oil induced diarrhoea in mice (Number of stools)

Animal Group/Treatment	Dose (/kg, p.o.)	Period of study (h)	Total number of stool
Group-I (control) (1% tween-80 solution in water)	10 ml	1	2.2 ± 0.580
		2	2.8 ± 0.374
		3	2.6 ± 0.506
		4	3.2 ± 0.370
		5	2.4 ± 0.401
Group-II (positive control) Loperamide	50 mg	1	0.4 ± 0.401
		2	1.6 ± 0.678
		3	2.2 ± 0.969
		4	0.8 ± 0.583*
		5	0.4 ± 0.250*
Group-III Ethanol extract	500 mg	1	1.2 ± 0.580
		2	0.8 ± 0.370*
		3	1.5 ± 0.580
		4	1.6 ± 0.509**
		5	0.6 ± 0.244*

Values are expressed as mean ± SEM (n = 5); **indicates $P < 0.05$; *indicates $P < 0.01$ vs. control; p.o. :per oral

Table 3. Brine shrimp lethality bioassay of *A. corniculatum*

Test sample	Concentration (μg/ml)	Log (concentration)	Number of alive shrimp	Mortality (%)	LC_{50} (μg/ml)	LC_{90} (μg/ml)
Ethanol extract	10	1	04	50	10	39.81
	20	1.3	03	70		
	40	1.6	1	90		
	80	1.9	0	100		
	160	2.2	0	100		

concentrations. From the plot of percent mortality versus log concentration on the graph paper LC_{50} and LC_{90} were deduced (LC_{50}: 10 μg/ml; LC_{90}: 39.81 μg/ml) (Table 3).

DISCUSSION

Since *A. corniculatum* belongs to the coastal forests, part of the plant constituents may be polar in nature. Ethanol was used which has a wide range of solubility in both polar and non-polar region. To avoid any solvent effect on the experimental animals, the solvent was evaporated completely to dryness.

Antinociceptive activity of the ethanol extract of *A. corniculatum* 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). The ethanol extract of *A. corniculatum* produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table 2). On the basis of this result it can be concluded that the ethanol extract of *A. corniculatum* might possess antinociceptive activity.

Antidiarrhoeal activity of the ethanol extract of *A. corniculatum* was tested using the model of castor oil induced diarrhoea in mice (Chatterjee, 1993). Castor oil, which is used to induce diarrhoea in mice, mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces its anti absorptive or secretory effect. The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Most agreed view is that ricinoleate salts stimulates the intestinal epithelial cell's adenyl cyclase (Racusen and Binder, 1979) or release prostaglandin (Beubler and Juan, 1979). The extract caused an increase in latent period i.e. delayed the onset of diarrhoeal episode and decreased the frequency of defecation as well as the number of stool. On the basis of the result of castor oil induced diarrhoea, it can be concluded that the ethanol extract of *A. corniculatum* might possess antidiarrhoeal activity.

The cytotoxic activity of the ethanol extract of *A. corniculatum* 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. (Anderson *et al.*, 1988). 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.

In conclusion, it could be suggested that the crude ethanol extract of *A. corniculatum* might possess antinociceptive, antidiarrhoeal and cytotoxic activities. However, further studies are necessary to find out the active principles responsible for these activities.

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