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Antinociceptive and antidiarrhoeal activities of *Bruguiera gymnorrhiza*

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SUMMARY

The methanol extract of leaves of *Bruguiera gymnorrhiza* (L.) Lam. (Rhizophoraceae) was screened for its antinociceptive and antidiarrhoeal activities. The extract produced significant inhibition in acetic acid-induced writhing in mice at dose 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 ($P < 0.001$). When tested for its antidiarrhoeal effects on castor oil-induced diarrhea in mice, it increased mean latent period ($P < 0.02$) and decreased the frequency of defecation ($P < 0.01$) significantly at the dose of 500 mg/kg body weight, comparable to the standard drug loperamide at the dose of 50 mg/kg of body weight. The overall results tend to suggest the antinociceptive and antidiarrhoeal activities of the extract.

Key words: Antinociceptive; Antidiarrhoeal; *Bruguiera gymnorrhiza*; Rhizophoraceae

INTRODUCTION

Bruguiera gymnorrhiza (L.) Lam. (Rhizophoraceae), locally known as Kankra, is a large tree distributed in the Coast forests of Bengal, Madras, Asam, Burma, Malay and Andamans. Its fruits are used for the treatment of eye diseases (Rollet, 1981; Balasooriya *et al*., 1982). A number of chemical investigations have been performed on this plant, as for example, Homhual *et al*. (2006) isolated bruguiesulfurol, a new sulfur compound from *Bruguiera gymnorrhiza*. Han *et al*. (2005) reported three new pimaren diterpenoids from *Bruguiera gymnorrhiza*. Hogg and Gillan (1984) reported fatty acids, sterols and hydrocarbons derivatives in *Bruguiera gymnorhiza*. Ghosh *et al*. (1985) isolated pentacyclic triterpenoids and sterols in *Bruguiera gymnorhiza*. Achmadi *et al*. (1994) isolated Catechin-3-*O*-rhamnoside from *Bruguiera gymnorhiza* (L.). However, no biological activity has yet been reported. Due to the presence of chemical compounds like sterols, triterpenoids, flavonoids, hydrocarbons and fatty acids, the plant may possess some pharmacological activities. That is why antinociceptive and antidiarrhoeal activities of the crude extract of leaves of *B. gymnorrhiza* were investigated in the present study.

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MATERIALS AND METHODS

Plant material collection and extraction

Leaves of *Bruguiera gymnorrhiza* were collected from the Sundarbans' Mangrove Forests, Bangladesh in September 2003 and were taxonomically identified by experts at the Bangladesh National Herbarium (accession no.: 30555). About 400 g of dried powdered plant material was taken in a clean, flat-bottomed glass container and soaked in 1,300 ml of 80% methanol. 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 clean cotton followed by a filtration through Whatmann filter paper. The filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., UK) to get the crude extract.

Drugs

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

Preliminary phytochemical analysis

The methanol extract of leaves of *B. gymnorrhiza* was subjected to a preliminary phytochemical screening for 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).

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

Libermann-Burchard test: 1 ml of the extract was placed in a test tube and then 2 ml Libermann-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 (ICDDRB) 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 (ICDDRB formulated) and had free access to tap water.

Pharmacological studies

Antinociceptive activity

Antinociceptive activity of the methanol extract of leaves of *B. gymnorrhiza* 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 five animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 in water (p.o.) 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 writhings (squirms) was counted for 5 min.

Antidiarrhoeal activity

Antidiarrhoeal activity of the methanol extract of leaves of *B. gymnorrhiza* was tested using the model of 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 a dose of 50 mg/kg of body weight; group III was test group and was treated with the extract at a 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. The latent period of each mouse was also counted. At the beginning of each hour old papers were replaced by the new ones.

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 methanol extract of *B. gymnorrhiza* showed the presence of flavonoids, reducing sugars, gums, saponins and tannins (Table 1).

Antinociceptive activity

Table 2 showed the effect of the methanol extract of *B. gymnorrhiza* on acetic acid-induced writhing in mice. At dose of 250 and 500 mg/kg of body weight, the extract produced about 46 and 59% 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 63% writhing inhibition at the dose of 25 mg/kg ($P < 0.001$).

Antidiarrhoeal activity

Antidiarrhoeal activity of the methanol extract of *B. gymnorrhiza* was tested by castor oil-induced diarrhoea in mice. The extract caused an increase

Table 1. Chemical groups present in methanol extract of *B. gymnorrhiza*

Chemical group	Test solution	Observation	Inference
Alkaloids	0.1 ml of Mayer's reagent	Yellowish buff colored precipitate was not obtaine	Alkaloids were absent
	0.1 ml of Dragendroff's reagent	Orange brown precipitate was not observed	
	0.1 ml of iodine solution (Wagner's reagent)	Reddish brown precipitate was not obtained	
	0.1 ml of picric acid solution (Hager's reagent)	Yellowish precipitate was not obtained	
Steroids	2 ml Libermann-Burchard reagent	Reddish purple color was obtained	Steroids were absent
	1 ml sulfuric acid	Chloroform layer had not acquired reddish brown color and acid layer had not showed green fluorescence	
Flavonoids	a) 1 ml dilute Ammonia solution	Greenish yellow color was obtained	Flavonoids were present
	b) 1 ml dilute sodium carbonate solution	Pale yellow color was obtained	
	c) 1 ml dilute sodium hydroxide solution	Yellow color was obtained	
Saponins	Shaken in a graduated cylinder for 15 min	One centimeter layer of foam	Saponins were present
Reducing sugars	5 ml Fehling's solution	Brick red colored precipitate	Reducing sugars were present
	5 ml Benedict's reagent	Brick red colored precipitate	
	2 drops of 5% alpha naphthol solution and 1 ml of sulfuric acid	Violet colored ring was formed at the junction of two liquids	
Tannins	1 ml of 5%Ferric chloride solution	Greenish black precipitate	Tannins were present
	1 ml of 10% Lead acetate solution	Yellow precipitate	
	1 ml of 10% potassium dichromate solution	Yellowish brown precipitate	
	1 ml of aqueous bromine solution	Brown precipitate	
Gums	Molish reagent and sulfuric acid	Red-violet ring was produced at the junction of two liquids	Gums were present
Amino acids	Ninhydrin; pH adjusted between 4 and 8	Purple color was not obtained	Amino acids were absent

Table 2. Effect of methanol extract of *B. gymnorrhiza* on acetic acid-induced writhing in mice

Animal Group/Treatment	Number of writhes (% writhing)	Inhibition (%)
Group-I (Control) 1% tween-80 solution in water, p.o.	11.80 ± 0.37 (100)	-
Group-II (Positive control) Diclofenac sodium 25 mg/kg, p.o.	4.40 ± 0.60* (37.28)	62.72
Group-III Methanol extract 250mg/kg, p.o.	6.40 ± 0.67* (54.24)	45.76
Group-IV Methanol extract 500 mg/kg, p.o.	4.80 ± 0.66* (40.68)	59.32

Values are expressed as mean ± S.E.M. (n = 5). *indicates $P < 0.001$, vs. control.

in latent period (1.67 h) i.e. delayed the onset of diarrhoeal episode at the dose of 500 mg/kg body of weight significantly ($P < 0.02$) which was comparable to the standard drug loperamide at the dose of

Table 3a. Effect of methanol extract of *B. gymnorrhiza* 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 in water)	10 ml	0.766 ± 0.128
Group-II (Positive control) Loperamide.	50 mg	1.71 ± 0.145**
Group - III Methanol extract	500 mg	1.67 ± 0.163*

Values are expressed as mean ± S.E.M. (n = 5). **indicates $P < 0.01$; *indicates $P < 0.02$ vs. control.

Table 3b. Effect of *B. gymnorrhiza* 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	1.8 ± 0.374
		2	2.8 ± 0.200
		3	3.0 ± 0.316
		4	2.6 ± 0.244
		5	1.8 ± 0.374
Group-II (Positive control) Loperamide	50 mg	1	0.4 ± 0.244*
		2	1.6 ± 0.244**
		3	0.8 ± 0.200***
		4	1.6 ± 0.244*
		5	0.4 ± 0.244*
Group -III Methanol extract	500 mg	1	0.6 ± 0.244*
		2	0.8 ± 0.200***
		3	0.4 ± 0.204***
		4	0.8 ± 0.200***
		5	0.2 ± 0.200**

Values are expressed as mean ± S.E.M. (n = 5). ***indicates $P < 0.01$, **indicates $P < 0.02$, *indicates $P < 0.05$ vs. control.

50 mg/kg body weight in which the value was 1.71 h ($P < 0.01$) (Table 3a). 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 0.6, 0.8, 0.4, 0.8 and 0.2 respectively and for loperamide the values were 0.4, 1.6, 0.8, 1.6 and 0.4 respectively (Table 3b).

DISCUSSION

Since *Bruguiera gymnorrhiza* belongs to the coastal forests, part of the plant constituents may be polar in nature. Methanol 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 extract of *B. gymnorrhiza* 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 methanol extract of *B. gymnorrhiza* 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 extract possesses antinociceptive activity.

Antidiarrhoeal activity of the extract of *B. gymnorrhiza* was tested by using the model of castor oil-induced diarrhoea in mice (Chatterjee, 1993). Castor oil 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 *et al.,* 1979) or release prostaglandin (Beubler *et al.,* 1979). The extract caused an increase in latent period 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 extract possesses antidiarrhoeal activity.

In conclusion, it could be suggested that the methanol extract of *Bruguiera gymnorrhiza* possesses antinociceptive and antidiarrhoeal activities. However, further studies are necessary to find out the active principles responsible for these activities.

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