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(Research Article)

Antinociceptive and antidiarrhoeal properties of the ethanolic extract of *Brownlowia tersa* leaves

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ABSTRACT

The present study was designed to investigate the antinociceptive and antidiarrhoeal activities of ethanolic extract of the leaves of Brownlowia tersa (L.) Kosterm (Family: Tiliaceae). The ethanol extract at graded doses (250 and 500 mg/kg body weight) was investigated for antinociceptive and antidiarrhoeal activity. At the dose of 250 and 500 mg/kg body weight, the extract showed a significant antinociceptive activity in acetic acid induced writhing model in mice showing 45.61% and 63.85% writhing inhibition (P<0.001) respectively while the standard drug diclofenac was found to be 76.69% inhibition at a dose of 25 mg/kg body weight. The antidiarrhoeal activity of the extract was investigated in term of reduction in the rate of defecation and consistency of faeces in castor oil induced diarrhoea. At the dose of 500 mg/kg body weight, the extract showed moderately significant antidiarrhoeal activity showing 50.56% reduction in diarrhoea (P<0.01) comparable to that of standard drug loperamide (68.53%). Phytochemical screening of the leaf extract indicated the presence of carbohydrate (reducing sugars), glycosides, saponins, tannins and flavonoids. Therefore, the obtained results tend to suggest the antinociceptive and antidiarrhoeal activities of the crude ethanolic extract of the leaves of Brownlowia tersa, thus provide the scientific basis for the traditional uses of this plant as a pain killer and modality for diarrhea.

Key Words: *Brownlowia tersa*, Antinociceptive, Antidiarrhoeal, Phytochemical screening, Tiliaceae..

INTRODUCTION

Brownlowia tersa (*B. tersa*) (L.) Kosterm (Family: Tiliaceae) is a shrub about 1.5-2 m tall distributed widely throughout India (Orissa) to Southeast Asia, Myanmar, Cambodia, Thailand, Malaysia, Brunei, Indonesia, Coastal forests of Bay of Bengal, Andamans etc. The volatile phenolic compound 2'-hydroxyacetophenone and the lignan carinol both have been isolated for the first time from *Brownlowia tersa*[1]. (4-nitrophenyl) propandiamide and (4-methylphenyl) propandiamide have also been reported from the aerial parts of *Brownlowia tersa*[2]. *B. tersa* has long been used as a traditional folk remedy for diarrhea, dysentery, wounds and boils. *B. tersa* roots have been found to possess significant antibacterial activity[1].

Pain is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause[3]. Diarrhoeal disease is a leading cause of mortality and morbidity, especially in children in developing countries [4]. A vast majority of the people of developing countries relies on herbal drugs for the management of diarrhoea. Considering this fact the World Health Organization has constituted a diarrhoeal disease control programme, which includes studies of traditional medicinal practices, together with the elevation of health education and prevention approaches [5].

Since there is no sufficient data currently available to substantiate antinociceptive and antidiarrhoeal activities from leaf extract of *B. tersa*, therefore the present study was designed to provide scientific evidence for its use as a traditional folk remedy by investigating the antinociceptive and antioxidant activities that also confirm its use as pain killer and modality for diarrhea.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The leaves of *Brownlowia tersa* was collected from the sundarbans of Sathkhira range. The plants were mounted on paper and the sample was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession No. 34177).

Preparation of Ethanolic Extract

The leaves of *B. tersa* were freed from any of the foreign materials. Then the plant materials were chopped and air-dried under shed temperature followed by drying in an electric oven at 40º C. The dried plant materials were then ground into powder. About 100g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 400 ml of 96% ethanol. The container with its contents was sealed and kept for a period of 4 days accompanying occasional shaking and stirring. The ethanolic extract was filtered by Buchner funnel and the filtrate was concentrated with rotary evaporator at bath temperature not exceeding 40˚ to have gummy concentrate of deep greenish black extract (Yield approx. 5.60%).

Test for Different Chemical Groups

The crude ethanolic extract was tested for its different chemical groups as alkaloids, flavonoids, reducing sugars, saponins, glycosides and tannins.[6] In each test 10% (w/v) solution of the extract in ethanol was taken.

Test Animals and Drugs

Young Swiss-albino mice either sex, 3-4 weeks of age, weighing 20 -25 g, were used for in vivo pharmacological screening. Mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B). They were housed Pharmacology Laboratories, Pharmacy discipline, Khulna University, Khulna. 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 had free access to feed and water ad libitum. The cages were cleaned once daily. These studies were carried out following approval from the ethical committee comprising pharmacologist and toxicologist expert on the use and care of animals of Pharmacy discipline, Khulna University, Khulna..

The standard drug Diclofenac sodium was used for antinociceptive screening and loperamide was used as standard drug for antidiarrhoeal activity testing and both the drugs were purchased from Square Pharmaceuticals Ltd, Bangladesh.

Antinociceptive Activity

The antinociceptive activity of the crude ethanolic extract of *B. tersa* was studied using acetic acid induced writhing model in mice [7,8]. The animals were divided into control, positive control and test groups with five 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 with Diclofenac Na (standard drug) at the dose of 25 mg/kg body weight and vehicle control group was treated with 1% tween 80 in water at the dose of 10ml/kg body weight. Test samples, standard drug and control vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 min, the mice were observed writhing (constriction of abdomen, turning of trunk and extension of hind legs) for 5 min.

Antidiarrhoeal Activity

The experiment was performed according to the method described by Shoba and Thomas [9]. Briefly, mice fasted for 24 h were randomly allocated to four groups of five animals each. The animals were all screened initially by giving 0.5 ml of castor oil. Only those showing diarrhoea were selected for the final experiment. Contol group received 1% tween 80 (10 ml/kg, p.o.). Positive control group was given Loperamide (3 mg/ kg, p.o.). Test group-1 and 2 received the drug extract orally 250and 500 mg/kg respectively. After one hour each animal was given 0.5 ml of castor oil by oro-gastric polyethylene catheter and placed in separate cages, the floor of which was lined with adsorbent paper which was changed every hour, observed for 4 h and the characteristic diarrhoeal droppings were recorded.

Statistical Analysis

Data were presented as mean ± Standard Error Mean (S. E. M). 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 control group. p values < 0.05 were considered to be statistically significant (p indicates probability).

RESULTS AND DISCUSSION

Chemical Group Test

Results of different chemical tests on the ethanolic extract of *B. tersa* leaves showed the presence of reducing sugars, saponin, glycosides, tannins and flavonoids (Table-1).

Antinociceptive Activity

Table-2 showed the effect of the ethanolic extract of *B. tersa* on acetic acid induced writhing model in mice. At the dose of 250 and 500 mg/kg body weight, the extract showed a significant antinociceptive activity showing 45.61% and 63.85% writhing inhibition ($P<0.001$) respectively while the standard drug diclofenac was found to be 76.69% inhibition at a dose of 25 mg/kg body weight.

Antinociceptive activity of the ethanolic extract of ethanolic extract of *B. tersa* leaves was tested by acetic acid induced writhing model in mice. The peripheral analgesic effect of the plant's extract may be mediated via inhibition of cyclooxygenases and/or lipoxygenases (and other inflammatory mediators), while the central analgesic action of the extract may be mediated through inhibition of central pain receptors. This hypothesis is in consonance with those of Koster *et al.*[10] and Williamson *et al.*[11] who postulated that acetic acid-induced writhing and hot-plate test methods are useful techniques for the evaluation of peripherally- and centrally-acting analgesic drugs, respectively. With respect to the writhing test, the research group of Deraedt *et al.* described the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid.[12] These authors found high levels of prostaglandins PGE_2 and $PGF_{2\alpha}$ during the first 30 min after acetic acid injection. On the basis of the result of acetic acid induced writhing test, it can be concluded that the ethanolic extract of *B. tersa* might possess significant antinociceptive activity.

Test for Antidiarrhoeal Activity

The ethanol extract was found to be effective against castor oil induced diarrhoea on experimental mice at the dose of 500 mg/kg body weight (Table 3). At the dose of 500 mg/kg body weight, the extract produced a significant decrease in the severity of diarrhoea in terms of reduction in the rate of defecation and consistency of faeces in albino mice. At the same dose, the extract showed moderately significant antidiarrhoeal activity ($P<0.01$) showing 50.56% reduction in diarrhoea comparable to that of standard drug loperamide 68.53%.

Several mechanisms have been proposed to explain the diarrhoeal effect of castor oil including inhibition of intestinal Na^+,K^+-ATPase activity to reduce normal fluid absorption [13], activation of adenylate cyclase or mucosal cAMP mediated active secretion [14] , stimulation of prostaglandin formation [15], platelet activating factor and recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor oil [16]. However, it is well evident that castor oil produces diarrhoea due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [17]. Since the ethanol extract of *B. tersa* successfully inhibited the castor oil-induced diarrhoea, the extract might have exerted its antidiarrhoeal action via antisecretory mechanism which was also evident from the reduction of total number of wet faeces (not shown separately) in the test groups in the experiment. Again, flavonoids present in the plant extract are reported to inhibit release of autacoids and prostaglandins, thereby inhibit motility and secretion induced by castor oil [18]. The antidiarrhoeal activity of the extract may also be due to denature proteins forming protein tannates which make intestinal mucosa more resistant and reduce secretion.

CONCLUSION

In conclusion it can be revealed that the crude ethanolic extract of *B. tersa* leaves possess significant antinociceptive as well as antidiarrhoeal activities. The potential of the extract of *B. tersa* as antinociceptive and antidiarrhoeal activities may be due to the presence of phytoconstituents like tannins, flavonoids, phenolics etc and might be responsible for its activity and justify its use as a traditional folk remedy. However, more detailed phytochemical analysis will be necessary to isolate and characterize the active compounds which are responsible for these activities and exact mechanisms of action of these activities.

Table-1: Results of different group tests of ethanolic extract of *B. tersa* leaves

Phytoconstituents	Ethanol extract of *B. tersa*
Alkaloid	-
Reducing sugars	+
Tannins	+
Flavonoids	+
Saponins	+
Glycisides	+

+: Positive result; - : Negative result

Table-2: Effects of the ethanolic extract of *B. tersa* on acetic acid induced writhing of mice (n=5)

Group	Treatment and Dose	Number of writhes (% Writhing)	% Writhing Inhibition
Control	1% tween 80 solution 10 ml/kg, p.o.	29.6 ± 1.99 (100)	---
Positive control	Diclofenac Na 25 mg/kg, p.o.	6.90 ± 1.25 * (23.31)	76.69
Test group-1	Ethanol Extract of *B. tersa* 250 mg/kg, p.o.	16.1±1.24 * (54.39)	45.61
Test group-2	Ethanol Extract of *B. tersa* 500 mg/kg, p.o.	10.7±1.45 * (36.15)	63.85

*Values are expressed as mean±SEM (Standard Error Mean); * indicates P < 0.001, one-way ANOVA followed by Dunnet's test as compared to control; n = Number of mice; p.o.: per oral.*

Table-3: Effect of *B. tersa* leaf extract on castor oil-induced diarrhoea in mice (n=5)

Groups	Treatment	Dose (p.o)	No. of faeces in 4h	% Inhibition of defaecation
Control	1% tween 80 in water	10 ml/kg	17.8±1.19	-
Positive control	Loperamide	3 mg/kg	5.6±0.89	68.53*
Test group-1	EE of *B. tersa*	250 mg/kg	13.2±1.08	25.84
Test group-1	EE of *B. tersa*	500 mg/kg	8.8±1.22	50.56*

*Values are expressed as mean±SEM (Standard Error Mean); * indicates P < 0.01, one-way ANOVA followed by Dunnet's test as compared to control; p.o: per oral; .EE: Ethanol extract n = Number of mice.*

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