Antinociceptive, Antidiarrhoeal and GI motility Activities of *Dillenia indica* Linn. roots.

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Summary

The crude methanol extract of the roots of *Dillenia indica* Linn. (Family: Dilleniaceae) was investigated for its possible Antinociceptive, antidiarrhoeal and GI motility tests in animal models. The extract produced significant writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 250mg/kg and 500 mg/kg body weight ($P < 0.01$) comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. The crude extract produced significant antidiarrhoeal effect at the dose of 500 mg/kg of body weight comparable to that produced by loperamide, used as standard drug. The extract also reduced significantly the charcoal induced Gastro Intestinal (GI) motility in mice; decreased the movement of GI tract in comparison to control animals. This work has found a base stone to step ahead for further researches to make them pharmaceutically useful.

Key words: Antinociceptive; Antidiarrhoeal; GI motility; *Dillenia indica*; Dilleniaceae.

Introduction

Dillenia indica Linn (commonly called elephant apple) for its pharmacognostic and pharmacological activities although it is widely used as food and for medicinal purposes [1-4]. Leaves of *Dillenia indica* Linn. are taken for investigation in the study. The fruits of this plant reported as potential anti-leukemic activities [2]. Pentacyclic triterpinoids has isolated from *Dillenia indica* [5]. Two another new compounds dihydro-isorhamnetin and dillenetin have been isolated [6]. The leaves are extracted with ethanol and the phytochemical properties of *Dillenia indica* Linn. leaves were explored. A number of chemical investigations have been performed on this plant, as for example, Parvin *et al.* (2009) [7] reported four new compounds from Dillenia indica; i.e. lupeol, betulinalhyde, betulinic acid and stigmasterol. Anti-inflammatory activity was found by the carrageenan-induced edema and acetic acid induced capillary permeability method by Yeshwante et al. (2009) [8].. Antinociceptive activity of the extracts was discovered by the acetic acid induced writhing method [9]. An important application of *Dillenia indica* Linn. in traditional medicine is its antidiarrhoeal activity. Liquid extract of the leaves are still used as herbal medication for diarrhea. This increased our interest to derive the antidiarrhoeal properties together with its influence in gastro intestinal (GI) motility. However, no significant biological activity has yet been reported for this plant. The objective of the present study was to investigate the antinociceptive, antidiarrhoeal and GI activity tests of the crude extracts of roots of *Dillenia indica* Linn.

Materials and Methods

Plant material collection and extraction

Dillenia indica Linn. plants were collected from the domestic areas around Bagerhat district, Bangladesh in September 2007 and were taxonomically identified by the experts at the Bangladesh National Herbarium (accession no: 30414). About 400 gm of dried powdered plant material was taken in a clean, flat-bottomed glass container and soaked in 1,500 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 clean cotton followed by a filtration through Whatmann filter paper grade no. 1. The filtrate then obtained and concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., UK) to get the crude extract.

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 test. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase 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. The experimental met the national guidelines on the proper care and use of animals. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol.

Drugs

Diclofenac (Square Pharmaceuticals Ltd, Bangladesh), Loperamide (Square Pharmaceuticals Ltd., Bangladesh).

Experimental

Preliminary phytochemical analysis

The crude extracts were 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 mentioned in individual test [10].

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.

Antinociceptive activity
Antinociceptive activity of the crude extract was tested using the model of acetic acid-induced writhing in mice [11, 12]. Antinociceptive activity of *Dillenia indica* Linn. extract was compared to the inhibition of writhing of a standard Antinociceptive agent (diclofenac sodium). Experimental control mice were administered with 10ml/ kg 1% Tween80 (Alpha labchem) with water; positive control mice were administered with 25 mg/kg bodyweight diclofenac-sodium (Diclofenac) solution made to 2.5mL with water and two test concentrations (250 mg/kg and 500 mg/kg body weight) of the crude extract of *Dillenia indica* Linn.was triturated by addition of small amount of Tween80 and water was slowly added to make the final volume of the test solutions to 2.5mL. Four groups (Group1, Group2, Group3 and Group4) of experimental animals were randomly selected with 5 animals in each group for each treatment. Mice were carefully administered with Tween80, diclofenac sodium and the test solutions by feeding needle. Thirty minutes interval was given to ensure proper absorption. During the time test mice were noted for any unwanted reactions. Acetic acid (0.7%) at a dose of 0.1mg/ 10gm was administered intraperitoneally to induce pain sensation. After an interval of 10 minutes which was given for the absorption of acetic acid, numbers of writhing were calculated for 10 minutes.

Antidiarrhoeal activity:
Antidiarrhoeal activity of the methanolic extract of leaves of *Aegiceras corniculatum* was tested using the model of castor oil-induced diarrhoea in mice [13]. 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.

GI motility influence:

Two groups (experimental control and sample) of five mice per group were selected on random and were starved 24 hours prior to experiment but, allowed free access to water. Control group was administered with vehicle 1% Tween80, 10 mg/kg and the test group with 500mg/kg body weight. After 30 minutes both groups received charcoal meal (3% suspension of deactivated charcoal in 0.5% aqueous methyl cellulose). 30 minutes after the administration of charcoal meal, the animals were sacrificed, abdomen was opened and distance moved by the charcoal from pylorus to caecum was determined and expressed as percentage of the total length of the small intestine [14].

Statistical analysis:

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

Results

Chemical group test

Results of different chemical tests on the methanolic extract of *Dillenia indica* Linn. showed the presence of glycoside, steroids, flavonoids, saponins and reducing sugars (Table 1).

Table 1. Phytochemical properties of *Dillenia indica* Linn. crude extract

Compound	Alkaloids	Glycosides	Steroids	Gums	Flavonoids	Saponins	Reducing sugars	Tannins
Observation	-ve	+ve	+ ve	- ve	+ ve	+ ve	+ ve	- ve

Key: +ve = Presence -ve = Absence

Antinociceptive activity test:

Table 2 showed the effect of the methanolic extract of *Dillenia indica* acetic acid induced writhing response method in mice. Healthy mice were divided into groups of 5 for each treatment and were administered with respective doses of control and test. After an absorption period of 30 minutes, the mice were administered with peritoneal injection of 0.7% acetic acid and writhing effect was counted for 10 minutes after 10 minutes. Inhibition of antinociceptive activity of the plant extract was compared against inhibition of writhing effect of standard Antinociceptive agent diclofenac sodium. Oneway Anova was used from online tool Vassarstats[15]. The ethanolic extract showed significant inhibition of writhing when compared to the control (Figure 1). At dose of 250 and 500 mg/kg of body weight, the extracts produced 48.82% and 55.88% inhibition in test animals, respectively. The results

were found to be statistically significant($P< .01$) and were comparable to the standard drug diclofenac sodium, which showed about 60% writhing inhibition at dose of 25 mg/kg ($P< .01$).

Table 2. Effects of *Dillenia indica* Linn. crude extract on writhing effect on acetic acid induced mice

Treatment	Dose (mg/kg)	Mean writhing	% Inhibition	SD	P value (One way Anova)*
Experimental control (1% Tween80)	10	34.0 ± 3.32	-	3.32	P<0.01
Positive control (Diclofenac sodium)	25	13.6 ± 3.05	60	3.05	P<0.01
DILCE	250	17.4 ± 3.97	48.82	3.98	P<0.01
DILCE	500	15.0 ± 4.40	55.88	4.36	P<0.01

Key: *- (VassarStats, 2009); DILCE - *Dillenia indica* Linn. Crude Extract. 30 minutes after treatment, 0.7% acetic acid was injected i.p. 10 minutes after injection writhing responses was recorded for 10 minutes. N=5.

Antidiarrhoeal and GI motility determination:

Antidiarrhoeal activity of the methanol extract of *Dillenia indica* Linn extract was tested by castor oil-induced diarrhea in mice. Diarrheal initiation time and the number of stools excreted by the animals in 4 hours were collected. The extract caused an increase in latent period (1.1h) i.e. delayed the onset of diarrhoeal episode of 500 mg/kg body of weight significantly ($P<.01$) which was comparable to the standard drug loperamide at the dose of 50 mg/kg body weight in which the resulted value was 1.5h ($P<.01$) (Table 3). The selected concentration of the extract also showed a good diarrheal inhibition with 65.28%. Loperamide, standard antidiarrheal agent showed an inhibition of 72.22%. The latent period for the initiation of stool excretion was noted. This was 1.104 hrs, which is 0.418 hrs earlier than loperamide treated mice but, 0.42 hrs latter than experimental control mice.

Table 3. Effects of *Dillenia indica* Linn. crude extract on inhibition of castor oil diarrhoea

Treatment	Dose (mg/kg)	Latent Period (Hrs)	Mean number of stools*[1]	% Inhibition	SD	P value (One way Anova)*
Experimental control (1% Tween80)	10	0.684 ± 0.19	3.6	-	0.91	
Positive control (Loperamide)	25	1.522 ± 0.57	1	72.22	0.67	P < 0.01
DILCE	500	1.104 ± 0.41	1.25	65.28	0.41	P < 0.01

Key: *- (VassarStats, 2009); DILCE - *Dillenia indica* Linn. Crude Extract. 40 minutes after treatment, 0.3mL castor oil was administered orally. Latent period of castor oil induced diarrhea was noted. Number of stools excreted for the next 4 hours were noted. *1 – Mean number of stools was an average number of stools for 4 hours for each treatment. % inhibition, SD and P value was also calculated with respect to the number of stools. N=5.

Antidiarrhoeal activity would be directly related to the motility of GI tract. To explore the influence of extract on the GI motility, animals treated with the test dose were sacrificed and the movement of charcoal meal from pylorus to caecum were measured which, is then compared to the experimental control animals. The movement was about 14.75 and 11.1 mm for the experimental control and for the test sample respectively. For the test group the movement was significantly reduced in compare to control group, i.e. for test group the value was (P<.01) (Table 4).

Table 4. GI track motility of experimental control and DILCE

Treatment	Dose (mg/kg)	Total GI track length mean(mm)	Charcoal meal traverse (mm)	SE	P value (One way Anova)*
Experimental control (1% Tween80)	10	43.36 ± 1.55	14.75 ± 1.52	0.91	
DILCE	500	42.70 ± 1.68	11.1 ± 2.76	0.41	P < 0.01

Key: *- (VassarStats, 2009); DILCE - *Dillenia indica* Linn. Crude Extract. 30 minutes after ingestion of charcoal, animals were treated and they were sacrificed after 30 minutes of treatment. Traverse of charcoal meal from pylorus to caecum. N=5. Treatment, 0.7% acetic acid was injected i.p. 10 minutes after injection writhing responses was recorded for 10 minutes.

Discussion

In many traditional medications, plants are the main source [16-18]. *Dillenia indica* Linn. commonly found tree plant was taken to explore the phytochemical and pharmacological properties as there was very limited work done on the plant. The roots, which are mostly used as a source of medication in traditional medicines was considered to examine the properties of the plant.

Dried roots of *Dillenia indica* Linn. were powdered and extracted with ethanol. The extract was dried and the powdered form, solubilized with solvents was used for the experiments. The foremost curiosity while working with a plant drug is to know the chemical substituent present in it. Phytochemical tests revealed the presence of reducing sugars, steroids, glycosides, flavonoids and saponins.

Antonociceptive activity was explored with two different concentrations of 250 mg kg^{-1} and 500 mg/kg body weight. Antinociceptive activity of *Dillenia indica* was tested by acetic acid-induced writhing model in mice. Acetic acid-induced writhing model causing pain sensation by tiggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes analgesia by liberation of endogeneous substances, which in turn excite the pain nerve endings [19]. Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be responsible for pain production caused by intraperitoneal administration of acetic acid 20. The methanol extract of *Dillenia indica* showed significant writhing inhibition in compare to the standard drug diclofenac sodium (Table 2). According to the basis of this result it can be concluded that the extract possesses antinociceptive activity.

Antidiarrhoeal activity of the extract of *Dillenia indica* was tested by using the model of castor oil-induced diarrhoea in mice [13]. Castor oil mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinolic acid remains in the intestine and produces its absorptive or seretory effect. The ricinolic acid thus liberated readily forms of 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. Generally ricinoleate salts stimulates the intestinal epithelial cells adenyl cyclase [21] or released prostaglandin[22]. The extract caused and increased in latent period and decreased the frequency of defecation as well as the number of total stool count. Obtained the results of castor oil-induced diarrhoea, it can be concluded that the extract contains antidiarrhoeal activity.

Antidiarrhoeal results increased the interest to further check the motility of GI track. The results explain the antidiarrhoeal action of the extract. In normal diarrhoeal condition GI motility will be less. Charcoal meal, which was used to determine GI motility, moved 14.75 mm and 11.1 mm respectively with the control mice and sample treated models. This influence on the GI motility is highly credential towards the antidiarrhoeal activity of the roots extract

Conclusion

In conclusion, it could be suggested that the methanol extract of *Dillenia indica* possesses antinociceptive nad antidiarrhoeal activities. These facts indicate the scientific basis of *Dillenia indica* Linn. being used as a traditional medicine. However, further experiments may help to determine the pharmaceutical potentialities of the plant as a medicine.

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