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Antidiarrhoeal activity of the leaf and fruit extracts of *Dillenia indica*

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Abstract

Plants are playing an important role in therapy of many diseases. Here we have studied on a plant which is *Dillenia indica*. In this study, ethanolic extract of the leaf and fruit of *Dillenia indica* was the experimental sample where antidiarrhoeal activity was analyzed. The plant parts showed antidiarrhoeal activity which was measured by castor oil induced method and charcoal plug method. In case of castor oil induced method the percent of inhibition of wet feces was measured and significantly decreased the total number of feces produced by the administration of castor oil; in 2nd hour (for leaf extract, 6 for 200 mg/kg dose and 4 at dose of 400 mg/kg and for fruit extract, 5.33 for 200 mg/kg dose and 4 at dose of 400 mg/kg) and 3rd hour (for leaf extract,3 for 200 mg/kg dose and 2 at dose of 400 mg/kg and for fruit extract 4 for 200 mg/kg dose and 3 at dose of 400 mg/kg). In charcoal plug method the activity was measured by distance traveled by the charcoal plug. The result was slightly lower than the control and the standard loperamide showed the maximum antidiarrhoeal effect. The average distance traveled by leaf was 28.67 cm and fruit was 22.67 cm which was lower than the control 41 cm where as the standard loperamide showed the average traveled length of 16 cm.

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Introduction

Plants continue to be a major source of medicines, as they have been throughout human history. Drug discovery, ethnobotany, and traditional and indigenous medicines have long been basic to medicinal plant research. As new uses for medicinal plants have been discovered and popularized, sustainability has become increasingly an issue; concern over the growth in biopiracy goes hand in hand with the critical need for conservation of both species and habitat.

In this research work we worked on a medicinal plant named by *Dillenia indica,* commonly known as Chalta in Bangladesh. Our experiment was to search the antidiarrhoeal activity of the plants on mice which can be established as a beneficial drug in medicine world.

This plant originally indigenous to Indonesia, this evergreen tropical tree is a medium sized tree, height up to 50 ft in some regions. The flowers are highly ornamental and fragranced. The genus Dillenia has 60 species, of which *Dillenia indica* Linnaeus (Family: Dilleniaceae) is the most common edible species. Originally from Indonesia, this evergreen tropical tree is now found from India to China. The common names include Chalta (Bengali, Hindi), Bhavya (Sanskrit) and Elephant apple (English). It is a spreading tree and has beautiful white fragrant flowers, toothed leaves, and globose fruits with small brown seeds. (Janick *et al.*, 2008) The leaf, bark and fruit of this plant are used as traditional medicine. The juice of *D. indica* leaves, bark and fruits are mixed and given orally (5-15 ml, two to five times daily) in the treatment of cancer and diarrhoea. (Sharma *et al.*, 2001) The fruit juice of this plant has cardiotonic effect, used as cooling beverage in fever and also employed in cough mixture. (Shome *et al.*, 1980) The solvent extracts of fruits of *D. indica* are reported to have antioxidant activity. (Abdille MH et.al. 2005) The fruits of this plant were reported as potential anti-leukemic activities (Kumar *et al.*, 2009). Pentacyclic triterpinoids were isolated from *Dillenia indica* (Banerji *et al.*, 1975). Two another new compounds dihydro-isorhamnetin and dillenetin have been isolated by Haque *et al.* (2008). A number of chemical investigations have been performed on this plant, as for example, Parvin *et al.* (2009) 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 (Yeshwante *et al.*, 2009). Antinociceptive activity of the extracts was discovered by the acetic acid induced writhing method (Koster *et al.*, 1959). CNS depressant activities in mice were found from the alcoholic extract of the leaves of *D. indica*. (Bhakuni *et al.*, 1969) An important application of *D. indica* in traditional medicine is its antidiarrhoeal activity. Liquid extract of the leaves are still used as herbal medication for diarrhoea. This increased our interest to derive the antidiarrhoeal properties together with its influence in Gastro Intestinal (GI) motility. The objective of the present study was to investigate the antidiarrhoeal and GI activity tests of the crude extracts of leaves and fruits of *D. indica*. Considering the traditional uses of *D. indica* the plant parts, leaves can be the source of many modern medicines. Intestinal diseases are one of the main causes of death of infants particularly in developing countries (WHO, 1994). It thus becomes important to identify and evaluate commonly available natural drugs as an alternative to currently used antidiarrhoeal drugs.

Materials and methods

Preparation of the plant extract

The plant sample of *Dillenia indica* was collected from Mirpur, Dhaka, Bangladesh, in the month of February 2011. The freshly separated different parts (leaves and fruits) of the plant were cut into small pieces, and sun dried as require to grind these into coarse powder. About 150 gm of powdered leaves and 300 gm of powdered fruits was taken in a flat bottom glass container and was extracted with ethanol. The extract was filtered through fresh cotton plug followed by

Whatman No.1 filter paper. The filtrate was then concentrated and dried at low temperature (39°C).

Study of antidiarrhoeal activity

This experiment was carried out by the slightly modified procedure described by Uddin *et al.* (2005) and Awouters et al (1978). The objective of this study was to investigate the effect of crude extracts on castor oil induced diarrhoea.

In this method Castor oil is used to induce diarrhoea to all the experimental groups. The defecation is the primer to measure the antidiarrhoeal effect. Each animal was constantly observed for consistency if faecal matter and frequency of defecation. The faeces were collected with an absorbent sheet of paper placed beneath the transparent cages (Mukherjee *et al.*, 1998). The wet faeces were read at the end of the experiment by lifting up the upper part of the cage containing the sheet of paper and animals. The percentage (%) inhibition of defection was measured using the following formula.

$$\% \text{ Inhibition of defecation} = \left[\frac{(A-B)}{A}\right] \times 100$$

A=Mean number of defecation by castor oil
B=Mean number of defecation by drug or extract

Experimental animal

Swiss Albino Mice of either sex, aged 3-4 weeks, obtained from the Animal Resource Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B) were used for the experiment. Twenty four adult Swiss Albino Mice weighing between 30-40 g were selected and housed in polypropiline cages (30x20x13 cm) in standard conditions for (21± 1°c with a 12 h light and dark cycle) for 7 days before experiment. There was free access of water and normal commercial laboratory diet (ICDDR, B, Dhaka).

Experimental design

Twenty four experimental animals were randomly selected and divided into six groups denoted as group-I, group-II, group-III, group- IV, Group V and group VI consisting of 4 mice in each group. Each group received a particular treatment i.e. control, standard and 2 different dose of the extract of different fraction of the ethanolic extract of the plant respectively. Prior to any treatment, each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Each group consisted of four mice. As it was difficult to observe the biologic response of four mice at a time receiving same treatment, it was quite necessary to identify individual animal of a group during the treatment. The animals were individualized in the following way and marked as M-1=Mice 1, M-2=Mice 2, M-3=Mice 3 and M-4=Mice 4.

Preparation of test materials

In order to administer the crude extract at doses of 400 mg/kg body weight and 200 mg/kg body weight of rat, 65 mg and 36 mg of the extract were measured respectively and were triturated unidirectional way by the addition of small amount of suspending agents Tween-80. After proper mixing of extract and suspending agent, normal saline was slowly added. The final volume of the suspension was adjusted. To stabilize the suspension, it was stirred well by vortex mixture. For the preparation of Loperamide at the dose of 5-mg/kg-body weight, calculated amount of Loperamide was taken and a suspension was made.

Procedure

In the day of the test the animals were divided into 6 groups of four mice each. They were weighted for accurate administration of drug. Group I received distilled water while Group III - Group VI with their subgroups A and B received *D. indica* extract and dose is respectively of table 1. Group II acts as a positive control where any antidiarrhoeal drug may be used. In this model we used Loperamide as standard. Thirty

minutes after the administration of the extracts, 1 ml of castor oil was administered to each animal orally. The animals were placed in transparent cages to observe for consistency of facial matter and frequency of defection for three hours. Feces were collected with an absorbent sheet of paper placed beneath the transparent cages (Mukherjee et al. 1998). The wet faces were read at the end of the experiment by lifting the upper part of the cage containing the sheet of paper and animals.

Table 1. Test samples used in the evaluation of antidiarrhoeal activity of *Dillenia indica* by castor oil induced method.

Test samples	Group	Purpose	Dose (mg/kg)	Route of administration
1%Tween 80 In saline solution	I	Control Group	0.1 ml/10 gm of body weight	Oral
Loperamide	II	Standard Group	5	Oral
DILEx	III (A)	Test sample	200	Oral
DIFEx	III (B)	Test sample	200	Oral
DILEx	IV (A)	Test sample	400	Oral
DIFEx	IV (B)	Test sample	400	Oral
Castor oil	--	Induce diarrhea	1 mL/mice	Oral

DILEx= *Dillenia indica* Leaves ethanolic extract, DIFEx = *Dillenia indica* Fruits ethanolic extract.

Calculation of percentage of defecation

Percentage of defecation was measured by the following equation.

$$\% \text{ Inhibition of defecation} = \left[\frac{(A-B)}{A}\right] \times 100$$

A=Mean number of defecation by castor oil

B=Mean number of defecation by drug or extract

Each rat of all groups was observed for consistency of facial matter and frequency of defecation.

Test of antimotility effect

This experiment was carried out by the slightly modified procedure previously described by Uddin *et al.* (2005) and Awouters *et al.* (1978). The objective of this study was to investigate the effect, of crude extracts on castor oil induced diarrhoea. In this method castor oil is used to induce diarrhoea to all the experimental groups. The defecation is the primer to measure the antidiarrhoeal effect. Each animal was constantly observed for consistency if faecal matter and frequency of defecation. The faeces were collected with an absorbent sheet of paper placed beneath the transparent cages (Mukherjee *et at*., 1998). The wet faeces were read at the end of the experiment by lifting up the upper part of the cage containing the sheet of paper and animals. The percentage (%) inhibition of defecation was measured using the following formula.

$$\% \text{ Inhibition of defecation} = \left[\frac{(A-B)}{A}\right] \times 100$$

A=Mean number of defecation by castor oil

B=Mean number of defecation by drug or extract

Experimental animal

Sixteen adult Swiss Albino Mice weighing between 30-40 g were selected and housed in polypropiline cages (30x20x13 cm) in standard conditions for (21± 1°c with a 12 h light and dark cycle) for 7 days before experiment. There was free access of water and normal commercial laboratory diet (ICDDR,B, Dhaka).

Experimental design

Sixteen experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III(A) and group- III(B) consisting of 4 mice in each group. Each group received a particular treatment i.e. control, standard and dose of the extract of different fractions of the ethanolic extract of the plant respectively. Prior to any treatment, each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly.

Method of identification of animals

Each group consisted of four mice. As it was difficult to observe the biologic response of four mice at a time receiving same treatment, it was quite necessary to identify individual animal of a group during the treatment. The animals were individualized in the following way and marked as M-1=Mice 1, M-2=Mice 2, M-3=Mice 3 and M-4=Mice 4.

Preparation of test materials

In order to administer the crude extract at doses of 200 mg/kg body weight of mice, 25.6 mg and 28 mg of the leaves and fruits extract were measured respectively and were triturated unidirectional way by the addition of small amount of suspending agents Tween-80. After proper mixing of extract and suspending agent, normal saline was slowly added. The final volume of the suspension was adjusted. To stabilize the suspension, it was stirred well by vortex mixture. For the preparation of loperamide at the dose of 5-mg/kg-body weight, 0.82 mg of loperamide was taken and a suspension of 5 ml was made.

Procedure

In the day of the test the animals were divided into four groups of four mice each. They were weighted and deprived of food. Three hours of food deprivation the animals in treated groups received (A and B) orally *Dillenia indica* extract (200mg/kg) by gavage. While controls groups received 0.9% NaCl sterile solution. (Wong:Way, 1981, Olajide et. al. 1999) and positive control group was given loperamide (5mg/kg) as an reference to antidiarrhoeal drug. Ninety minutes after the administration of the extracts, 0.3 ml of a 5% charcoal suspension in 10% aqueous suspension in charcoal powdered was administered to each animal orally. The animals were sacrificed 45 minutes later and abdomen was opened. The percentage distance of the small intestine (from pylorus to the ceccum) traveled by the charcoal plug were determined.

Table 2. Test samples used in the evaluation of antidiarrhoeal activity of *Dillenia indica* by charcoal plug method.

Test samples	Group	Purpose	Dose (mg/kg)	Route of administration
1%Tween 80 In saline solution	I	Control Group	0.1 ml/10 gm of body weight	Oral
Loperamide	II	Standard Group	5	Oral
DILEx	III (A)	Test sample	200	Oral
DIFEx	III (B)	Test sample	200	Oral
Castor oil	--	Motility marker	0.3 mL/mice (a 5% charcoal suspension of 10% aqueous suspension of charcoal powder)	Oral

DILEx = *Dillenia indica* Leaves ethanolic extract, DIFEx = *Dillenia indica* Fruits ethanolic extract.

Counting of distance traveled by charcoal plug

The distance traversed by Charcoal plug was measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileoceccal junction). The percent deviation with the control group was calculated with the following formula:

$$\text{Percent deviation} = \frac{\text{Drug - Control}}{\text{Control}} \times 100$$

Each mouse of all groups was observed individually for counting the distance traveled by then in 45 minutes after administration of charcoal plug.

Results

Antidiarrhoeal test by castor oil induced method

A study was undertaken to evaluate the effect of ethanolic plant extracts of *Dillenia indica* leaf and fruit for their antidiarrhoeal potential against castor oil induced diarrhoea in mice. The ethanolic plant extracts were more effective than aqueous plant extracts against castor oil induced diarrhoea. The ethanolic plant extracts significantly reduced induction time of diarrhoea and total weight of the faeces. The results obtained establish the efficacy of this plant extracts as antidiarrhoeal agents. (Shoba *et al.*, 2001).

Table 3. Effect of ethanolic extract of *Dillenia indica* on the mice of castor oil-induced diarrhoea.

Group	Treatment	Number of Faces at first Hour	Number of Feces at second Hour	Number of Feces at third Hour
Group I (Control)	Castor Oil (10mg/Kg)	13±1	11.33±.57	7±1
Group II (Standard)	Loperamide (5 mg/Kg)	9.33 ±.57	3 ±1	1±0
Group IIIA	Castor Oil + DILEx (200 mg/Kg)	12.33 ±.57	6±1	3±1
Group III B	Castor Oil + DIFEx (200 mg/Kg)	10±0	5.33 ±.57	4 ±0
Group IVA	Castor Oil + DILEx (400 mg/Kg)	10.33±.57	4±0	2±0
Group IVB	Castor Oil + DIFEx (400 mg/Kg)	8 ±1	4 ±1	3.33±.57

DILEx = *Dillenia indica* Leaves ethanolic extract, DIFEx = *Dillenia indica* Fruits ethanolic extract. n=3, Results are given here as mean± SD.

Table 4. Inhibition of wet faces at different hours.

Group	Treatment	1st Hour (% of inhibition of wet feces)	2nd hour (% of inhibition of wet feces)	3rd Hour (% of inhibition of wet feces)	
Group I (Control)	Castor Oil (10mg/Kg)	--	--	--	
Group II (Standard)	Loperamide (5 mg/Kg)	100±1	72.67±.57	85.67±.57	
Group IIIA	Castor Oil + DILEx	33.33±.57	45.33±.57	57±1	(200 mg/Kg)
Group III B	Castor Oil + DIFEx	33.33 ±.57	36.33±.57	42.67±.57	(200 mg/Kg)
Group IVA	Castor Oil + DILEx	66.67±.57	63.67±.57	71.33±.57	(400 mg/Kg)
Group IVB	Castor Oil + DIFEx	66.67±.57	54.67±.57	57±1	(400 mg/Kg)

DILEx = *Dillenia indica* Leaves ethanolic extract, DIFEx = *Dillenia indica* Fruits ethanolic extract. n=3, Results are given here as mean± SD.

Discussion

Crude ethanolic extract of leaves (both 200 mg/kg and 400 mg/kg dose) significantly decreased the total number of feces produced by the administration of castor oil in 2nd hour (6 for 200 mg/kg dose and 4 at dose of 400 mg/kg) and 3rd hour (3 for 200 mg/kg dose and 2 at dose of 400 mg/kg) which was comparable to the standard drug Loperamide (% of inhibition was 72.67 at 2nd hour and 85.67 at 3rd hour).

Table 5. Mean distance traveled by charcoal plug and % of deviation from control.

Group	Treatment	Mean distance traveled (cm)	% of deviation from control
I	Control(0.1 ml/10 g)	41±1	--
II	Standard(5 mg/kg)	16±0	-60.67±.57
IIIA	DILEx	28.67±.57	-30.67±.57
IIIB	DIFEx	22.67±.57	-44.33±.57

DILEx = *Dillenia indica* Leaves ethanolic extract, DIFEx = *Dillenia indica* Fruits ethanolic extract. n=3, Results are given here as mean± SD.

Crude ethanolic extract of fruits (both 200 mg/kg and 400 mg/kg dose) significantly decreased the total number of feces produced by the administration of castor oil in 2nd hour (5.33 for 200 mg/kg dose and 4 for 400 mg/kg dose) and 3rd hour (4 for 200 mg/kg dose and 3.33 for 400 mg/kg dose) which was comparable to the standard drug Loperamide (% of inhibition was 72.67 at 2nd hour and 85.67 at 3rd hour). Further investigation is necessary to isolate the active principles from this plant.

Antidiarrhoeal activity by charcoal plug method

The antidiarrhoeal activity of the extracts may be related to an inhibition of muscle contractility and motility, as observed by the decrease in intestinal transit by charcoal meal and consequently, in a reduction in intestinal propulsion. Mices were fasted for 18 h. They were divided into four groups. The first group which served as control was. The second group receives standard drug loperamide. The extract was administered orally at 200 mg/kg (leaf) to third group and 200 mg/kg (fruit) to fourth group as suspension. The animals were given 0.3 mL of a 5% charcoal suspension in 10% aqueous suspension of charcoal powder orally. 45 minutes after treatment the abdomen was cut off and the small intestine carefully removed. The distance traveled by charcoal plug from pylorus to caecum was measured, and expressed as percentage of the distance traveled by charcoal plug for each of animal.

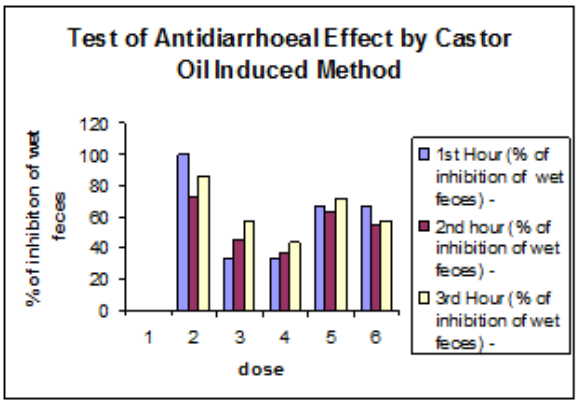

Fig. 1. % of inhibition of wet faces at different hours by different groups.

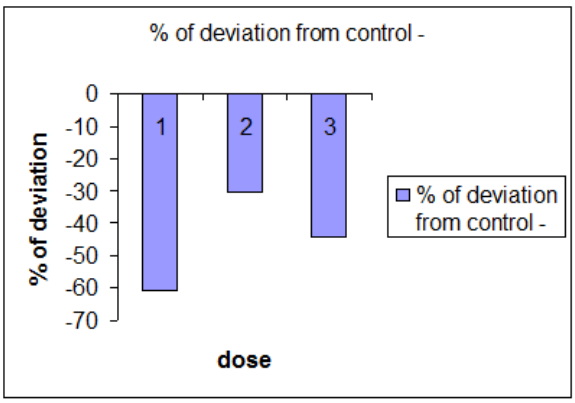

Fig. 2. % of deviation of motility in different groups (the more the deviation from control the better the action of drug as motility inhibitor).

The average distance traveled by *Dillenia indica* leaf (mean 28.67 cm) and fruit (mean 22.67 cm) extract was lower than the control (mean 41 cm) where as the standard loperamide showed the average traveled length of 16 cm.

Crude ethanolic extract of leaves at 200 mg/kg body weight fraction showed significant activity on reducing the motility of Gastro intestinal tract of mice with -

30.67% deviation and fruits at 200 mg/kg body weight fraction showed -44.33% deviation from control which was comparable to the percentage of deviation of standard Loperamide drug (percentage of deviation was -60.67%).

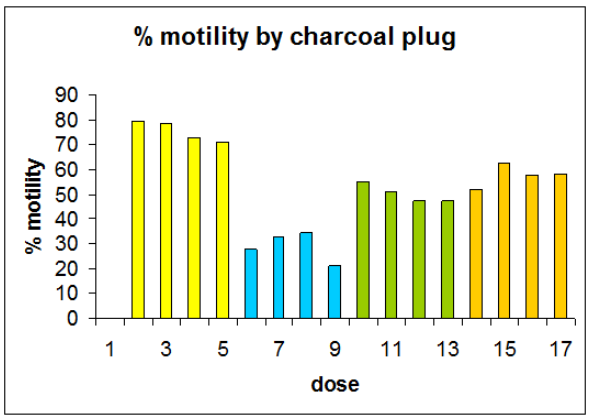

Fig. 3. % of deviation of motility in different groups (the more the deviation from control the better the action of drug as motility inhibitor).

Further investigation is necessary to isolate the active principles responsible for antidiarrhoeal activity from this plant.

Conclusion

The study was carried out to determine the antidiarrhoeal activity of *Dillenia indica* leaf and fruit extract. It showed significant activity on reducing the motility of Gastro intestinal tract of mice and significantly decreased the total number of feces produced by the administration of castor oil. The average distance traveled by *Dillenia indica* leaf and fruit extract was lower than the control where as the standard loperamide showed the average traveled length. Therefore, those might be utilized for the development of traditional medicines and further investigation should be necessary for the development of novel compound.

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