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Studies on the anti-diarrheal properties of leaf extract of *Desmodium puchellum*

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PEER REVIEW

Peer reviewer

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Comments

This is an interesting study that describes a functional characterization of *D. puchellum* leaves extracts. Study presented is scientifically sound. The results are explained in a constructive way by providing the suitable references. Overall, the methanol extract of leaves showed good anti-diarrhoeal activity than petroleum ether extract. So this will be helpful for new anti-diarrhoeal drugs exploration in the future.
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ABSTRACT

Objective: To evaluate the pharmacological activity against diarrhea of methanol and petroleum ether extract of *Desmodium puchellum* (Family: Fabaceae) leaves.
Methods: The extract was evaluated for castor oil-induced diarrhea and enteropooling as well as intestinal motility in rats. Both of the extracts were given to the rats at 200 mg/kg orally. Loperamide was used as a standard drug for diarrhea.
Results: The diarrheal severity was reduced significantly ($P<0.05$) by methanol and petroleum ether extracts by 31.95% and 28.33%, respectively, whereas 54.75% inhibition was found for standard drug loperamide at 5 mg/kg. The two extracts also significantly ($P<0.05$) reduced the intestinal volume in case of castor oil induced enteropooling.
Conclusions: It is concluded that both fractions contain some biologically active ingredients that are active for anti-diarrheal actions whereas methanol fraction has better potential.

KEYWORDS
Antidiarrhea, Thrombolysis, *Desmodium puchellum*, Loperamide

1. Introduction

Among the death causing diseases, diarrhea is the leading one, especially in developing countries. Every year, millions of people die of diarrhea in third world's countries, so this is the most concerning issue for these countries. Children are more susceptible to this disease which is accounted as the second leading causes of death of children under five years old[1]. Diarrhea is gastrointestinal disorder, characterized by an increase in stool frequency and change consistency[2]. To combat this problem, the World Health Organization (WHO) has initiated a diarrhea disease control program to study traditional medicine practices and prevention approaches[3,4]. From a long time ago, plant kingdom played an important role for discovering new drug source. Number of therapeutics drug isolated from plant species. For the treatment of diarrhea, medicinal plants are a potential source of anti-diarrheal drugs[3]. Drug from plant sources has negligible

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adverse effect and for their potential activities against diarrhea. Many international organizations have encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices[4-6].

Plant kingdom provides an enormous reservoir of biologically active compounds with distinctive chemical properties which can prevent or cure diseases. Traditionally, decoction of dried leaves of *Desmodium pulchellum* (*D. pulchellum*) is used in cold fever, malaria, enlargement of liver and spleen, rheumatism bone pains and swelling due to contusion or sprain. Decoction of charred roots used to reduce excessive menstrual flow. Leaves are also applied to ulcers and skin sores in hemorrhages. Decoction of bark is used in diarrhea, poisoning, and eye diseases. The whole plant is used in Chinese medicine to treat rheumatic fever, infant convulsions, toothache, and also to dissolve internal blood clots, and to aid digestion[7]. As there is no scientific report on the biological activity of the plant, the present investigation was done to evaluate its anti-diarrheal and thrombolytic actions.

2. Material and methods

2.1. Drugs and chemicals

Methanol was bought from SIGMA® (Sigma-Aldrich®, St Louis, USA), while loperamide manufactured by Square Pharmaceuticals Ltd., Bangladesh, was bought from local pharmacy. Castor oil was purchased from WELL's Heath Care, Spain. All the chemicals and reagents were analytical grade.

2.2. Plant materials

Fresh leaves of *D. pulchellum* were collected from the local area (Ispahani hill) of Chittagong district, Bangladesh and identified by the experts of Bangladesh Forest Research Institution. A voucher specimen has been deposited in Bangladesh National Herbarium, Dhaka, Bangladesh under the accession number DACB-32182.

2.3. Preparation of extract

The mature leaves were collected and dried in room temperature and pulverized into coarse powder using a pulverizer. Ground leaves were dissolved in sufficient amount of methanol for one week at room temperature with occasional shaking. The extract was filtered through a cotton plug followed by Whatman No. 1 filter paper. The filtrate was then evaporated under reduced pressure to give a dark green viscous mass. The methanol crude extract was further extracted with petroleum ether and then separated using separating funnels. This petroleum ether fraction was later concentrated and afforded the petroleum ether extract. Both extracts were then preserved for anti-diarrheal activity.

2.4. Animals

Healthy, adult rats of either sex (90-100 g) were collected from International Center for Diarrheal Diseases Research, Bangladesh and were acclimatized to normal laboratory conditions for one week prior to study and given pellet diet and water *ad libitum*.

2.5. Ethical clearance

This study was given ethical clearance by the Animal Husbandry section of Rajshahi University, Bangladesh. The animals were handled according to the guideline of National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85-23 revised 1985).

2.6. Anti-diarrheal activity

2.6.1. Castor oil-induced diarrhea in rats

Castor oil-induced diarrhea was done according to the method of Soba *et al.*[8] and Uddin *et al*[9]. Rats of either sex were divided into four groups of five rats each. The animals were fasted for 18 h prior to the test. Group I was treated with normal saline (2 mL/kg), which served as control; while Group II received loperamide (5 mg/kg). Groups III and IV received methanol and petroleum ether extracts (200 mg/kg). All doses were administered orally. After 1 h, all groups received 1 mL of castor oil orally. Then animals were placed in cages lined with adsorbent papers and observed for 4 h for the presence of diarrhea defined as watery (wet), unformed stool. The control group result was considered as 100%. The activity of each group was expressed as percent inhibition (%) of diarrhea. The percent inhibition of defecation was calculated as follows:

% Inhibition of defecation=[(A-B)/A]×100

Where A indicated mean number of defecation caused by castor oil; B indicated mean number of defecation caused by drug or extract.

2.6.2. Castor oil-induced enteropooling

The castor oil-induced enteropooling was carried out according to the method of Robert *et al*[10]. Rats of either sex were divided into four groups and fasted for 18 h prior to the test. Saline water was given to Group I as control (saline 2 mL/kg body weight, orally); Group II received standard drug (loperamide 5 mg/kg body weight, orally), and the rest groups (Groups III and IV) were given methanol and petroleum ether extract (200 mg/kg body weight, orally). One hour later, all the rats were challenged with 1 mL of castor oil orally. After 1 h of castor oil received, the rats were sacrificed and the small

intestine from the pylorus to the caecum was isolated. Then the intestinal contents were weighed and volume measured by graduated tube[10,11].

2.6.3. Gastrointestinal motility test

This test was performed according to the method previously described using charcoal as a diet marker[12]. Animals of either sex were divided into four groups of five rats in each and fasted for 18 h before test. All groups received castor oil to produce diarrhea. One hour later, Group I treated as control (saline 2 mL/kg body weight, orally); Group II received standard drug (loperamide 5 mg/kg body weight, orally) and the rest two groups received two different does (200 mg/kg, body weight, orally) of methanol and petroleum ether extract. After 1 h of drug administration, all animals were received 1 mL of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour later, all animals were sacrificed, and the distance covered by the charcoal meal in the intestine from the pylorus to the caecum was measured and expressed as percentage of distance moved[13].

2.7. Statistical analysis

All analyses were carried out in triplicates. Data were presented as mean±SEM. The significance of difference between the control and treated groups was determined using two-way analysis of variance (ANOVA), followed by Student's *t*-test. *P* value of 0.05 or 0.01 was considered as significant.

3. Results

3.1. Castor oil induced diarrhea

In castor oil induced diarrhea test, both methanol and petroleum ether extracts showed considerable anti-diarrheal effect in rats. Methanol extract significantly inhibited the frequency of defecation when compared with untreated control rats ($P<0.05$). Both of the extracts decreased the total number of wet feces produced upon administration of castor oil when compared to the castor oil treated rats. The results are shown in Table 1.

3.2. Castor oil induced enteropooling

Both of the extracts showed noticeable effect in castor oil induced entropooling test in the rats (Table 2). The intestinal volume was decreased by 28.67% for methanol extract and 25.45 % for petroleum ether extract. The values were statistically significant ($P<0.05$). The standard drug, loperamide (5 mg/kg), also significantly inhibited intestinal fluid accumulation (57.71%) ($P<0.01$) and the effect of the extract was less potent in comparison to the standard drug.

3.3. Gastrointestinal motility test

The gastrointestinal distance traveled by the charcoal meal in the rats significantly ($P<0.01$) lessened by both methanol and petroleum ether extracts compared with the control group. Loperamide (5 mg/kg) produced a marked

Table 1
Effect of methanol and petroleum ether extracts of leaves of *D. pulchellum* on castor oil induced diarrhea in rats.

Groups	Treatment (*p.o.*)	Total number of feces	% Inhibition of defecation	Total number of diarrheal feces	% Inhibition of diarrhea
I	Saline (2 mL/kg)	18.18±1.92	–	11.05±1.08	–
II	Loperamide (5 mg/kg)	7.76±0.66**	57.32	5.00±0.33**	54.75
III	DPM (200 mg/kg)	12.12±0.97*	33.30	7.52±0.37*	31.95
IV	DPP (200 mg/kg)	13.25±0.92	27.12	7.92±0.51	28.33

Values are expressed as mean±SEM (n=5). *$P<0.05$, **$P<0.01$ when compared with control group. DPM: *D. pulchellum* methanol extract; DPM: *D. pulchellum* petroleum ether extract.

Table 2
Effect of methanol and petroleum ether extracts of leaves of *D. pulchellum* on castor oil induced enteropooling in rats.

Groups	Treatment (*p.o.*)	Total length of intestinal content (g)	Volume of intestinal content (mL)	Inhibition (%)
I	Saline (2 mL/kg)	3.18±0.07	2.79±0.18	–
II	Loperamide (5 mg/kg)	1.61±0.04**	1.18±0.10**	57.71
III	DPM (200 mg/kg)	2.27±0.91*	1.99±0.64*	28.67
IV	DPP (200 mg/kg)	2.48±0.20*	2.08±0.10*	25.45

Values are expressed as mean±SEM (n=5). *$P<0.05$, **$P<0.01$ when compared with control group. DPM: *D. pulchellum* methanol extract DPM: *D. pulchellum* petroleum ether extract.

Table 3
Effect of methanol and petroleum ether extracts of leaves of *D. pulchellum* on small intestinal transit in rats.

Groups	Treatment (*p.o.*)	Total length of intestine (cm)	Distance traveled by marker (cm)	Inhibition (%)
I	Saline (2 mL/kg)	109.37±1.21	100.70±2.26	–
II	Loperamide (5 mg/kg)	103.33±1.32	60.33±4.05**	41.06
III	DPM (200 mg/kg)	93.10±1.62**	70.13±0.87**	24.67
IV	DPP (200 mg/kg)	91.50±1.80**	73.63±0.52**	19.53

Values are expressed as mean±SEM (n=5). *$P<0.05$, **$P<0.01$ when compared with control group. DPM: *D. pulchellum* methanol extract DPM: *D. pulchellum* petroleum ether extract.

decrease (41.06%) in the propulsion of charcoal meal through gastrointestinal tract (Table 3).

4. Discussion

Diarrhea is usually considered a result of altered motility and fluid accumulation within the intestinal tract. The objective of diarrheal test is to determine the effect of methanol and petroleum ether extract of *D. pulchellum* on castor oil induced diarrhea. Castor oil is a triglyceride characterized by a high content of the hydroxylated unsaturated fatty acid ricinoleic acid[14]. About 90% of ricinoleate present in castor oil is mainly responsible for diarrhea production[15]. After oral ingestion of castor oil, ricinoleic acid is released by lipases in the intestinal lumen, and considerable amounts of ricinoleic acid are absorbed in the intestine[16,17]. Presence of ricinoleate in small intestine, the peristaltic activity of small intestine increases as a result of permeability of Na^+ and Cl^- changed in the intestinal mucosa[18]. Secretion of endogenous prostaglandin is also stimulated by ricinoleate[19]. Prostaglandins of the E series are considered to be good diarrheogenic agents in experimental animals as well as in human beings. The inhibitors of prostaglandins biosynthesis are therefore considered to delay castor oil-induced diarrhoea[20]. Prostaglandins are associated with changes in the bowel that stimulate diarrhea. Recent study shows that the laxative effect of ricinoleic acid present in castor oil is due to the induction of contraction of intestinal smooth muscle which is mediated by activation of EP3 receptors on intestinal smooth-muscle[21]. Many anti-diarrheal agents act by reducing the gastrointestinal motility and/or the secretions. Inhibitors of prostaglandin biosynthesis delay castor oil induced diarrhea[8]. Both the extracts of methanol and petroleum ether of *D. pulchellum* exhibit significant anti-diarrheal activity. Plant extracts containing tannin, flavonoids, alkaloids, saponins and steroids have been reported to possess anti-diarrheal activity[22,23]. The leaves of *D. pulchellum* contain several alkaloids which may be responsible for its effect. The given effects were noticeable considering with the standard drug, loperamide at 5 mg/kg. In our study, we have shown the experimental data for one dose (200 mg/kg) only, for both extracts. There are many reports on anti-diarrehal activity of plant extract using this dose level[24]. Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the intestine, reduce colon flow rates and consequently have any effect on colonic motility[25,26]. Both extracts moderately reduced intestinal transit by the decrease in the distance traveled by charcoal meal. The antimuscarinic drug and atropine decreased the propulsive movement in the charcoal meal study due to its anticholinergic effect[26]. From the result, we found the extracts suppressed the propulsion of charcoal meal by increasing the absorption of water and electrolytes.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Diarrhoeal disease is a leading cause of mortality and morbidity, especially in developing countries and is responsible for the death of millions of people each year. In developing countries, majority of people almost exclusively use traditional medicines in treating all sorts of diseases, including diarrhoea. WHO has encouraged studies for treatment and prevention of diarrhoeal diseases depending on traditional medical practices. This may reduce mortality rate in developing countries due to diarrhea. It is interesting to search for plants with anti-diarrhoeal activities that could be used against any type of diarrhoeal disease. A range of medicinal plants with anti-diarrhoeal properties have been widely used by traditional healers. *D. pulchellum* is used in cold fever, malaria and enlargement of liver and spleen, rheumatism, bone pains and swelling due to contusion or sprain. Decoction of charred roots used to reduce excessive menstrual flow. Leaves are also applied to ulcers and skin sores in hemorrhages. In Chinese medicine, this plant is used to treat rheumatic fever, infant convulsions, toothache, and also to dissolve internal blood clots, and to aid digestion. Considering the above medicinal value, investigation on anti-diarrhoeal activity is important to promote public health.

Research frontiers

The present studies are being performed to evaluate the possible anti-diarrhoeal properties of the leaf extract of *D. puchellum*. The extract was evaluated for castor oil induced diarrhoea and enterpooling as well as intestinal transit in rats.

Related reports

There are many reports on anti-diarrheal activity of plant

extract. Balaji *et al.* reported the anti-diarrheal activity of ethanol extracts of *Carum copticum* seeds in castor oil induced diarrheal rats. In this research, the authors also have carried out experimental procedures with well established methods. This is the first time report of anti-diarrheal activity of leaves extract of *D. puchellum*.

Innovations and breakthroughs

This study showed that leaves of *D. puchellum* may contain phytochemical principles which have potent anti-diarrheal activity.

Applications

Due to the anti-diarrhoeal properties of leaves extracts, it may be a good alternative for the treatment of diarrhoeal diseases after completing necessary trials.

Peer review

This is an interesting study that describes a functional characterization of *D. puchellum* leaves extracts. Study presented is scientifically sound. The results are explained in a constructive way by providing the suitable references. Overall, the methanol extract of leaves showed good anti-diarrhoeal activity than petroleum ether extract. So this will be helpful for new anti-diarrhoeal drugs exploration in the future.

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