



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading

doi: 10.1016/S2221-1691(13)60129-X

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

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PEER REVIEW

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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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Foundation Project: Financially supported by the Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong (Grant No. 1217).

Article history:

Received 5 May 2013

Received in revised form 12 May, 2nd revised form 16 May, 3rd revised form 23 May 2013

Accepted 30 Jun 2013

Available online 28 Aug 2013

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 Health 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:

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

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

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