



Evaluation of Antidiarrhoeal Potential of *Bryophyllum Pinnatum* in Experimental Animals

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ABSTRACT

The present study was undertaken to evaluate the antidiarrhoeal potential of *Bryophyllum pinnatum* against several experimental models of diarrhoea in Albino Wistar rats. We studied the effects of aqueous extract of the leaves of *Bryophyllum pinnatum* in the castor oil induced diarrhoea model. Weight and volume of intestinal content induced by castor oil were studied by the enteropooling method. The gastrointestinal transit rate was expressed as the percentage of the longest distance traversed by charcoal divided by the total length of the small intestine. Loperamide (5 mg/kg), diphenoxylate (5 mg/kg) and atropine (3 mg/kg) were used as standard drugs. Extract was used in 100, 200 and 300 mg/kg doses. At all the three doses the aqueous extract showed significant antidiarrhoeal activity against castor oil induced diarrhoea and castor oil induced enteropooling in rats. There was also significant reduction in gastrointestinal motility in the small intestinal transit test. Results obtained in this study substantiate the antidiarrheal effect of the aqueous extract of *Bryophyllum pinnatum* leaves and its use by traditional practitioners in the treatment of diarrhoea. Further studies are needed to completely understand its mechanism of anti-diarrhoeal action.

1. INTRODUCTION

Diarrhoea can be regarded as a global menace which can culminate in mortality and morbidity of the incumbent due to loss of fluids and electrolytes from the body [1]. Diarrheal disease ranks second only to lower respiratory infection as the most common infectious cause of death worldwide. Among children <5 years old, diarrheal disease is a particularly important cause of death. Every year nearly 2 million children in this age group die of diarrheal disease [2].

It is defined as an increase in the frequency, fluidity or volume of bowel movements and characterized by increased frequency of bowel sound and movement, wet stools, and abdominal pain. In clinical terms, it is used to describe increased liquidity of stools, usually associated with increased stool weight and frequency [3]. The plausible implicating factors inducing diarrhoea can range from infective to immunological and nutritive factors [1].

In order to combat the problems of diarrhoea globally, the World Health Organization in its Diarrhoeal Disease

Control programme has given a special emphasis on the use of traditional folklore medicines in the control and management of diarrhoea [4]. It is therefore important to identify and evaluate available natural drugs as alternatives to current antidiarrheal drugs, which are not always free from adverse effects. Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of diarrhoea.

Bryophyllum pinnatum (Crassulaceae) is a widely used medicinal plant in traditional system with a wide range of biological activities [5]. It is classified as a weed, and its common names include: "African never die", "Resurrection plant", "Love plant", "life plant", "air plant", Zakham-e-hyat, parnabija etc. It is a perennial herb growing widely and used in folklore medicine in tropical Africa, tropical America, India, China, Australia and southern part of Nigeria. The plant grows all over India in hot and moist areas, especially in Bengal. The plant

flowers in November-March and fruits in April [6]. Flavonoids, polyphenols, and triterpenoids have been identified from the leaves of *B. pinnatum*. Quercetin-3- α -L-rhu- β -D-xyl; a flavonoid B; ryophyllin B, a novel potent cytotoxic bufadienolide and Malic acid were isolated from the leaves of *B. pinnatum* [7]. The leaves and bark is bitter tonic, astringent to bowels, analgesic, carminative, and are useful in diarrhoea and vomiting. Antimicrobial, antifungal, anti ulcer, anti-inflammatory and analgesic activities of leaf extract were reported. Juice of the fresh leaves is used very effectively for treatment of jaundice in folk medicine of Bundelkhand region of India [8]. A water extract of *Bryophyllum* leaves administered topically and internally has been shown to prevent and treat Leishmaniasis [9]. The plant is used traditionally for the treatment of earache, in ophthalmic preparations, sprains & in dysmenorrhoea [10]. Methanol extract of the leaves have histamine receptor (H1) antagonism in the ileum, peripheral vasculature and bronchial muscle [11].

Though *B. pinnatum* is used in diarrhoea in traditional practice [12], but no scientific study has been reported on its Antidiarrhoeal activity. Therefore this study was undertaken with the objective of studying the antidiarrhoeal potential of aqueous extract of *Bryophyllum pinnatum* (AEBP) leaves.

METHODS

Experimental Animals:

Albino Wistar rats of either sex, weighing between 150-200 gm, were procured from animal house of Department of Pharmacology, Gauhati Medical College. The animals were housed at 25 \pm 2 °C with 12:12 h light and dark cycle and allowed to standard rat pellets and water ad libitum. They were acclimatized to laboratory condition for 1 week before the study. The study was approved by the Institutional Animal Ethics Committee of Gauhati Medical College & Hospital (No.MCI 32/2012/4). CPCSEA guidelines were adhered during the experiment.

Plant Materials:

The leaves of *Bryophyllum pinnatum* were collected from in and around Guwahati and were identified by Dr Triguna Ranjan Sharma, Lecturer of department of Botany, Swadeshi Academy, Assam. The leaves were dried & pulverized in a mechanical grinder to obtain fine powder. The powdered leaves (200 gm) were successively extracted by cold maceration process, which was further evaporated to dryness to obtain the aqueous extract. The yield was 28.41% (w/w).

Drugs and Chemicals:

Drugs and chemicals needed for the present study were Castor oil, Loperamide, Diphenoxylate, Atropine sulphate and Charcoal black.

Acute Toxicity Study:

LD₅₀ of aqueous extract of *Bryophyllum pinnatum* is reported to be 1.8 gm/kg body weight after intraperitoneal administration in rats [13].

2. Methodology:

Castor oil induced diarrhoea:

A total of 30 animals were divided into 5 groups containing 6 in each. They were fasted for 24 hrs before the test with free access to water. After 1 hour of administration of drugs, diarrhoea was induced by administering 1 ml of castor oil orally. The animals were placed in individual cages over clean filter paper. Total number of both wet and dry diarrheal droppings was counted every hour for a period of 4 hours. The mean of the stools passed by the treated groups were compared with that of the control group [14].

Group 1 – Control, received normal saline 2 ml/kg i.p

Group 2 – Standard, received loperamide 5 mg/kg i.p

Group 3 – AEBP 100 mg/kg i.p

Group 4 – AEBP 200 mg/kg i.p

Group 5 - AEBP 300 mg/kg i.p

Castor oil induced Enteropooling:

Intraluminal fluid accumulation was determined by castor oil induced enteropooling. Five groups of 6 rats in each were taken and fasted for 24 hrs before the test with free access to water. After 1 hour of administration of drugs, diarrhoea was induced by administering 2 ml of castor oil orally. Two hours later, the rats were sacrificed; small intestine was ligated at both the pyloric sphincter and the ileocaecal junction and dissected. The small intestine was weighed and its contents were collected by milking into a graduated tube allowing the volume to be measured; the intestine was reweighed and the difference between full and empty intestines was calculated [15].

Group 1- Control, received normal saline 2 ml/kg p.o.

Group 2- Standard, received diphenoxylate 5 mg/kg p.o.

Group 3- AEBP 100 mg/kg i.p

Group 4- AEBP 200 mg/kg i.p

Group 5- AEBP 300 mg/kg i.p

Small Intestinal Transit:

Rats were divided into 5 groups of 6 in each, and were fasted for 18 hours before the test. Castor oil was given 1 hour after administration of drugs. One ml of 10% activated charcoal suspended in 5% gum acacia was administered orally 1hour after castor oil treatment. The rats were sacrificed after 1 hour of charcoal administration. The small intestine was carefully removed and the distance travelled by charcoal meal from the pylorus was measured, this length was expressed as percentage of the total length of the intestine from the pylorus to caecum [16].

Group 1- Control, received normal saline 2 ml/kg p.o.

Group 2- Standard, received atropine 3 mg/kg i.p

Group 3- AEBP 100 mg/kg i.p

Group 4- AEBP 200 mg/kg i.p

Group 5- AEBP 300 mg/kg i.p

Statistical Analysis:

The mean \pm S.E.M. (standard error of mean) values were calculated for each group. Significant differences between the groups were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's t tests. $P < 0.05$ was considered to be statistically significant. All analyses were performed using the SPSS 16.

3. RESULTS

Aqueous extract of *B. Pinnatum* leaves reduced the total number of faeces in a dose dependant manner in castor oil induced diarrhoea. At 100, 200 and 300 mg/kg extract, a significant ($P < 0.05$) reduction in diarrhoea was observed representing 31.60%, 48.39% and 64.50 % inhibition respectively. Loperamide 5 mg/kg inhibited the castor oil induced diarrhoea by 78.05%. (Table 1).

Group	Treatment	Mean defecation in 4 hours	% Inhibition of Defecation
1	Castor oil + Normal Saline 2 ml/kg i.p	25.83 \pm 0.31	-----
2	Castor oil + Loperamide 5 mg/kg i.p	5.67 \pm 0.21*	78.05
3	Castor oil + AEBP 100 mg/kg i.p	17.67 \pm 0.33*	31.60
4	Castor oil + AEBP 200 mg/kg i.p	13.33 \pm 0.42*	48.39
5	Castor oil + AEBP 300 mg/kg i.p	9.17 \pm 0.31*	64.50

Results are expressed as mean \pm S.E.M (n=6). $P < 0.05$ *Statistical significance ($P < 0.05$) on comparison with control group

Table 1: Effect of aqueous extract of *B. Pinnatum* leaves on castor oil induced diarrhoea in albino rat

Similarly, the extract at all the three doses significantly ($P < 0.05$) reduced the extent of castor oil induced enteropooling in test animals. AEBP 100, 200 & 300 mg/kg significantly ($P < 0.05$) inhibited fluid accumulation at the levels of 33%, 41.5% and 48.7% respectively. Diphenoxylate 5 mg/kg showed the highest inhibition of 60%. (Table 2).

In gastrointestinal motility test, AEBP significantly retarded ($P < 0.05$) the intestinal transit of charcoal meal compared to the control group. 100, 200 & 300 mg/kg of the extract decreased the intestinal transit length by 85.83%, 78.33% & 72.83%, respectively. Atropine 3 mg/kg caused a highest reduction in the gastrointestinal transit by 64.17%. (Table 3).

Conflict of Interest: None Declared

Group	Treatment	Volume of intestinal content (ml)	% Inhibition
1	Castor oil + Normal Saline 2 ml/kg p.o	2.65 \pm 0.09	-----
2	Castor oil + Diphenoxylate 5 mg/kg p.o	1.06 \pm 0.01*	60
3	Castor oil + AEBP 100 mg/kg i.p	1.77 \pm 0.02*	33
4	Castor oil + AEBP 200 mg/kg i.p	1.55 \pm 0.01*	41.5
5	Castor oil + AEBP 300 mg/kg i.p	1.36 \pm 0.02*	48.7

Results are expressed as mean \pm S.E.M (n=6). $P < 0.05$ *Statistical significance ($P < 0.05$) on comparison with control group

Table 2: Effect of aqueous extract of *B. Pinnatum* leaves on castor oil induced enteropooling in albino rat

Group	Treatment	% of Intestinal transit
1	Castor oil + Normal Saline 2 ml/kg p.o	89.67 \pm 0.42
2	Castor oil + Atropine 3 mg/kg i.p	64.17 \pm 0.31*
3	Castor oil + AEBP 100 mg/kg i.p	85.83 \pm 0.31*
4	Castor oil + AEBP 200 mg/kg i.p	78.33 \pm 0.42*
5	Castor oil + AEBP 300 mg/kg i.p	72.83 \pm 0.31*

Results are expressed as mean \pm S.E.M (n=6). $P < 0.05$ *Statistical significance ($P < 0.05$) on comparison with control group

Table 3: Effect of aqueous extract of *B. Pinnatum* leaves on castor oil induced small intestinal transit in albino rat

4. DISCUSSION

Several mechanisms have been supposed to be involved in the diarrhoeal effect of castor oil. Castor oil produces diarrhoeal effect due to its active metabolite ricinoleic acid, activation of adenylate cyclase, stimulation of prostaglandin E & F formation, and recently nitric oxide was found to contribute to the diarrhoeal effect of castor oil [17]. Since the aqueous extract of leaves of *Bryophyllum pinnatum* successfully inhibited the castor oil-induced diarrhoea, the extract might have exerted its antidiarrhoeal action by any one of the above mechanism.

Studies on enteropooling showed that *Bryophyllum pinnatum* reduced the volume of intraluminal contents. Castor oil caused accumulation of water and electrolytes in intestinal loop. The involvement of muscarinic receptor effect was confirmed by increased production of both gastric secretion and intraluminal fluid accumulation induced by castor oil [15]. The significant inhibition of castor oil-induced enteropooling by AEBP suggests that extract might have exerted its antidiarrhoeal action by antisecretory mechanism.

Atropine increased intestinal transit time possibly due to its anti-cholinergic effect [18]. The pre-treatment with

AEBP suppressed the propulsive movement of charcoal meal through the gastrointestinal tract [16]. The anti-diarrhoeal effect of the extract 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 [3].

Antidiarrhoeal properties of medicinal plants are found to be due to tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenoids [14]. Flavonoids, saponins & triterpenoids have been identified from aqueous extract of leaves of *B. pinnatum* [7, 19]. These constituents may mediate the antidiarrhoeal property of *B. pinnatum*.

From this study, we can conclude that the aqueous leaf extract of *Bryophyllum pinnatum* possesses significant antidiarrhoeal activity, thus justifying its widespread use by the local population for this purpose. There is need for further studies to isolate and characterize the anti-diarrheal constituents of this plant & to ascertain the mechanism of action.

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