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Methanolic extract of *Pycreus polystachyos* possesses potent antidiarrheal activity that varies in male and female mice

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Abstract
Pycreus polystachyos is a well-known weed grown typically in ditches, grasslands, rice fields, etc. The whole plant is traditionally used against diarrhea. The present study was aimed to examine antidiarrheal of whole plant of *Pycreus polystachyos* in male and female mice model. Antidiarrheal activity was evaluated using two tests such as castor oil induced diarrhea and castor oil induced enteropooling at three extract concentrations- 100, 200 and 400 mg/kg.bw. Total flavonoids and tannins contents were also measured. The plant extract showed potent effect in prevention of castor oil induced diarrhea. Mice of both sexes showed greater effect than standard drug loperamide at 400 mg/kg.bw; the effect was substantially higher in male mice than female producing 64.77% and 38.04% in male and female respectively. Loperamide produced 35.28% and 33.70% inhibition in male and female mice respectively. In enteropooling test, both percent reduction in weight and volume of intestinal content were increased as doses of extract increased. Potent inhibition in intestinal fluid accumulation was evident from percent reduction in mean fluid volume. Male and female mice showed fluid reductions by 45.87% and 33.29% respectively while loperamide reduced it by 44.73% and 25.32%. Total flavonoids and tannins contents were found 13.44 mg Quercetin equivalent/g and 118.5 mg Gallic Acid equivalent/g respectively. The experiment endorses the folkloric use of *P. polystachyos* in diarrhea.

Keywords: *Pycreus polystachyos,* castor oil, diarrhea, enteropooling, mice

1. Introduction
Pycreus polystachyos (Rottb.) (Family: Cyperaceae) is a widely distributed weed found with almost cosmopolitan presence in tropics, subtropics and warm temperate countries [1]. The plant is typically grown in ditches, grasslands, rice fields, riverbanks, etc. Despite its unwelcomed presence as weed in rice fields, the plant is used in the treatment of diarrhea in folk medicine [2].

Diarrhea, a disease characterized by passage of loose or liquid stools, claims around 5,25,000 death of children under five year from 1.7 billion annual reported cases [3]. The pathophysiological processes involved in diarrhea include abnormal intestinal motility and imbalance in electrolyte secretion and absorption. Frequent watery defecation leads to increased loss of water and electrolytes from body resulting dehydration that can become fatal if remains untreated. Replenishment of water and electrolyte with oral rehydration therapy is the cornerstone in patients with acute illness and save thousands of lives particularly in developing countries. Antimotility and antisecretory drugs are the mainstay in persistent and severe diarrheal cases. Opioids and their derivatives such as loperamide, diphenoxylate, difenoxin, etc. are widely used in the treatment of diarrhea; these drugs interact with opioid μ- and δ-receptors resulting reduced intestinal motility and secretion respectively. Antimicrobial therapy is also chosen to reduce severity and duration of infectious diarrhea. The aim of the study was, therefore, to investigate the putative antidiarrheal activity of *P. polystachyos* in mice model.

2. Material and Methods
2.1 Materials and reagents
Standard drug- loperamide was a commercial product of Square Pharmaceuticals Limited, Bangladesh. Methanol of analytical grade was purchased from Merck, Germany. Whole plant of *Pycreus polystachyos* was collected from Noakhali, Bangladesh (22° 86’ N and 91° 09’ E). The plant name was checked online (theplantlist.org) and authenticated by Bangladesh National Herbarium (Voucher No.: DACB-48124).

2.2 *P. polystachyos* extraction
Methanolic extract of whole plant of *P. polystachyos* was prepared according to previously published method [4]. Total phenolics and tannins content were also determined [5].

2.3 Grouping of animals and doses of plant extract
Both male and female Swiss albino mice (20-25 g) were obtained from International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh. Prior approval was taken regarding use of experimental animals from concerning committee. The animals were housed in polycarbonate cages with stainless steel grid as lid (40 cm x 30 cm x 17 cm) under controlled environment (12/12 h dark/light cycle, 23-25 °C temperature, 50-55% relative humidity). Formulated pellet and water were given ad libitum during entire study period. Mice were acclimatized with laboratory environment for one week before commencement of experiments. In each experiment, 30 male and 30 female mice were used. Each sex was then divided into five groups- control group (water, 10 ml/kg.bw), standard group (loperamide, 3 mg/kg.bw) and three groups having three different doses of plant extract- 100, 200 and 400 mg/kg.bw.

2.4 Castor oil induced diarrhea
In this experiment, diarrhoea was induced by administering castor oil (0.2 mL/animal) 30 min prior to sample or standard treatment [6]. Immediately after administering castor oil, each animal was kept in an individual cage with the floor lined with blotting paper and observed for 4 h. The following parameters were recorded: onset of defecation and consistency of feces categorized as normal, semi-solid and liquid. Evacuation index (EI) value was calculated according to the formula: EI = solid feces*1 + semi-solid feces*2 + liquid feces*3. From EI values, percentage inhibition of diarrhea was calculated as (EI of control - EI of sample) x 100/ (EI of control).

2.5 Castor oil-induced enteropooling
In enteropooling experiment, the animals were treated in the same fashion as conducted for castor oil-induced diarrhea [7]. 1 h later, 0.5 ml of castor oil was administered per mouse in all groups. The mice were sacrificed 1 h after the administration of castor oil. Small intestine of each mouse from the pylorus to the caecum was isolated and weighed. Then, the intestinal content of all individual animals was collected by milking into a graduated tube and the volume was measured. Weight of intestine of each mouse was reweighed after collection of intestinal content. Then, percent reductions in the weight and volume of intestinal content, relative to control group, were calculated using the following formulae:

$$\%\ \text{reduction in weight of intestinal content (g)} = \frac{\text{weight in control group} - \text{weight in sample group}}{\text{weight in control group}}\ x\ 100$$

$$\%\ \text{reduction in volume of intestinal content (mL)} = \frac{\text{volume in control group} - \text{volume in sample group}}{\text{volume in control group}}\ x\ 100$$

3. Result and Discussion
This study was designed and conducted to evaluate putative antidiarrheal effect of *P. polystachyos* using two antidiarrheal activity test models in male and female mice. Methanolic extract of the plant showed dose dependent antidiarrheal effect in both castor oil induced diarrhea and enteropooling tests. In diarrhea test, lower evacuation index of a test sample than control indicates higher antidiarrheal effect that consequently converts into higher percentage inhibition. Here, evacuation indices were decreased from 10.33 to 5.17 as the dose of extract increased from 100 to 400 mg/kg.bw in male mice; females showed similar trend but varies in intensity such as 16.67 at 100 mg/kg.bw and 9.5 at 400 mg/kg.bw (Table 1). Both male and female model at 400 mg/kg.bw inhibited diarrhea at greater extent than loperamide. Moreover, noticeably higher effect was seen in male mice than in female at 400 mg/kg.bw showing 64.77% and 38.04% respectively while loperamide showed 35.28% and 33.70% in male and female. Analogous findings were observed in castor oil induced enteropooling test. Here, reduced mean weight and volume of intestinal content were observed in all doses of plant extract as compared with control. Percent reduction in weight and volume of intestinal content was increased as the dose increased. In male mice, loperamide showed weight reduction by 38.89% while extract at 400 mg/kg.bw decreased it by 20.04% (Figure 1). In case of female mice, percent reductions were 49.2% and 33.53% for loperamide and highest extract dose respectively. This model also revealed potent inhibitory effect on intestinal fluid accumulation as evident from percent reduction in volume of intestinal content. In male mice, both loperamide and extract at 400 mg/kg.bw produced equal effect (~45%) while the same extract dose produced noticeable greater effect (33.29%) than loperamide (25.32%) in female mice.

Table 1: Effect of methanolic leaf extract of *P. polystachyos* on castor oil-induced diarrhea in mice.

	Group	Onset of defecation	Evacuation classification			Evacuation index	% inhibition
			Solid	Semi-solid	Liquid		
Male	Control	74.5±22.5	4.83±0.95	1.67±0.33	2.17±0.48	14.67	
	Loperamide	88.2±17.5	3.33±0.33	2.83±0.48	0.17±0.17**	9.5	35.28
	PP100	62.8±5.72	2.67±1.02	3.33±0.56*	0.33±0.21	10.33	29.55
	PP200	98.0±1.65	0.50±0.22***	1.67±0.42	2.00±0.52**	9.83	32.95
	PP400	107±21.4	3.00±0.45	0.33±0.21	0.50±0.22**	5.17	64.77
Female	Control	80.2±16.2	3.00±0.82	2.67±0.56	2.33±0.42	15.33	
	Loperamide	85.5±21.0	2.83±0.79	2.67±0.62	0.67±0.21	10.17	33.70
	PP100	86.2±5.42	2.83±0.79	2.17±0.31	3.17±0.79	16.67	(-)8.70
	PP200	91.8±7.56	3.83±0.70	2.33±0.33	0.67±0.33	10.5	31.52

	PP400	92.0±6.33	2.83±0.31	1.33±0.21	1.33±0.33	9.50	38.04

PP stands for *P. polystachyos* while 100, 200 and 400 indicates dose of plant extract at mg/kg.bw. Negative value in female mice for PP100 might possibly be an inadvertent mistake in data collection. Data were presented as Mean ± SEM. One-way ANOVA with Dunnett's multiple comparisons test was performed to determine statistical significance of the test results; here, **P*<0.05, ***P*<0.01 and ****P*<0.001 vs control.

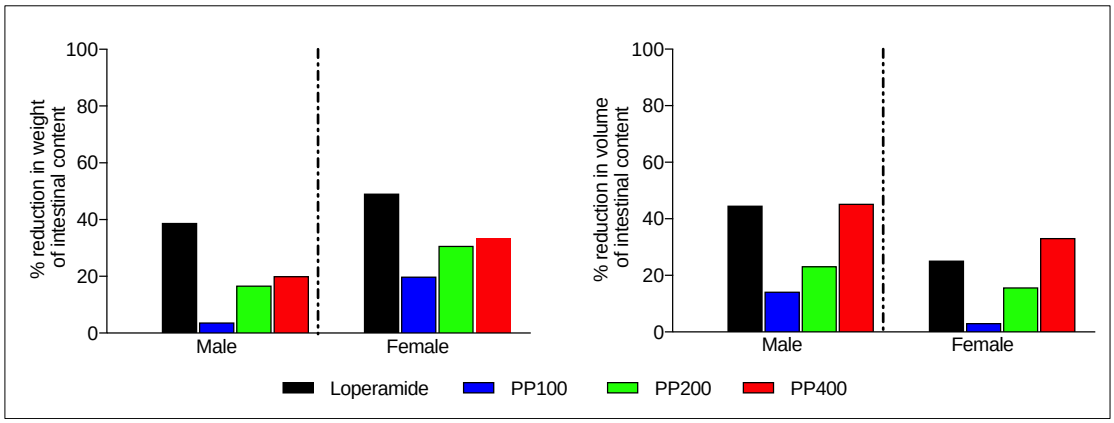

Fig 1: Percent reduction in weight (left panel) and volume (right panel) of intestinal content by plant extract in castor oil induced enteropooling test. 'PP' stands for *P. polystachyos* while 100, 200 and 400 indicates dose of plant extract at mg/kg.bw.

Ricinoleic acid, the metabolic product of castor oil in gut, irritates gastrointestinal mucosa resulting release of several mediators such as prostaglandins, nitric oxide, cAMP, tachykinins, etc. that subsequently increases GI motility and electrolyte secretion and decreases electrolyte and fluid absorption [8]. As extract of *P. polystachyos* inhibited castor oil induced diarrhea, it can be suggested that synthesis of these mediators can be impeded by the extract providing greater transit time and reabsorption of fluid and electrolyte. Remarkable inhibition of intestinal fluid accumulation also suggests antisecretory effect of the methanolic extract. Though, the exact mechanism of antidiarrheal effect of the extract could not be established from our study, a good number of researches reported plant secondary metabolites particularly flavonoids and tannins possess antidiarrheal properties. Quantitative determination of phytochemicals in *P. polystachyos* showed high amount of tannins (118.5 mg GAE/g) and flavonoids (13.44 mg QE/g). Several works reported inhibition of prostaglandin and other mediators by tannins and flavonoids [9-11]. Tannins also have antispasmodic and gastroprotective effect [12]. Various plant extracts rich in tannins and flavonoids showed potent antidiarrheal effects [13-15]. Thus, it could be speculated that high contents of tannins and flavonoids in our plant extract contributed to its potent antidiarrheal effect in mice model.

4. Conclusion

The experimental evidence in male and female mice model confirms that *P. polystachyous* possesses antidiarrheal activity and endorses the usefulness of the plant for the treatment of diarrhea.

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Conflict of Interest: The authors declare no conflict of interest.

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