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Antidiarrheal activity of *Polypodium leucotomos*

Md. Anisuzzaman Chowdhury[1], Md. Masudur Rahman*[1], Mohammed Riaz Hasan Chowdhury[1], Mir Md. Saiful Alam[1], Md. Aziz Abdur Rahman[2]

[1]Department of Pharmacy, International Islamic University Chittagong, Chittagong-4203, Bangladesh.
[2]Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh

*Corresponding Author: mamun2001@hotmail.com

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Abstract

The rhizomes of *Polypodium leucotomos* are used in traditional medicine for the treatment of diarrhea. Thus the methanol extract of *P. leucotomos* rhizomes was investigated for its antidiarrheal property against experimental diarrhea induced by castor oil in rats. Castor oil induced diarrhea, castor oil induced enteropooling and gastrointestinal motility test were used to investigate antidiarrheal activity of the rhizome extract at different concentrations (100 and 200 mg/kg body weight). The diarrheal episode was inhibited by 19% and 41.77% at the doses of 100 and 200 mg/kg respectively. The extract significantly ($p < 0.01$) lessened the intestinal volume (1.93 ± 0.18 - 1.60 ± 0.06 ml) compared to control (2.79 ± 0.18 ml) in castor oil induced enteropooling and also decreased in intestinal transit (16.26 - 27.93%) comparable to that of standard drug loperamide (5 mg/kg). These findings demonstrate that the rhizome extract of *P. leucotomos* rhizomes have excellent antidiarrheal activity.

Keywords: Antidiarrheal; castor oil; intestinal transit; *Polypodium leucotomos*

Introduction

Polypodium leucotomos (Polypodiaceae) is a rhizomatous fern, with thick creeping rhizome 8-15 mm in diameter, densely covered in the golden-brown scales which look like fur and popularly known as Samambaia (Das, 2007). The plant historically has been used to improve health in situations such as inflammatory disorders and skin diseases, whooping cough, diarrhea, hypertension, arthritis, and pains in joints and tendons (Nilesh et al., 2001; Das, 2007). *In vitro* and *in vivo* studies have shown its potential antioxidant effects to include the scavenging of superoxide anions, hydroxyl radicals and singlet oxygen (Gonzalez & Pathak, 1996; Gomes et al., 2001; Garcia et al., 2006). It has been shown to be effective as an immunomodulator (Gonzalez et al., 2000; Reyes et al., 2006; Gonzalez et al., 2007; Philips et al., 2009), anti-inflammatory (Cuellar et al., 2003), antipsoriatic (Vasänge-Tuominen et al., 1994; Navarro-Blasco & Sempere, 1998; Capote et al., 2006), procognitive, neuroprotective (Sempere et al., 2002) and ultraviolet light protectant (Siscovick et al., 2008; Gonzalez et al., 2010; Caccialanza et al., 2011; Tanew et al., 2012). The main plant chemicals identified from *P.*

leucotomos include adenosine, alkaloids, arachidonic acid, arabinopyranosides, calagualine, ecdysone, ecdysterone, eicosapentaenoic acid, elaidic acid, juglanin, kaempferols, linoleic acids, melilotoside, oleic acid, polypodaureine, ricinoleic acid, rutin, selligueain, tannin and sulphoquinovosyldiacyl glycerols (Horvath et al., 1967; Czeczuga, 1985; Patitucci et al., 1995; Vasänge et al., 1997; Nilesh et al., 2001). From the existing information it is evident that the plant possesses many important biological activities. The literature survey revealed that there are no scientific studies carried out regarding the antidiarrheal activity, thus the experiment was designed to evaluate the antidiarrheal activity of *Polypodium leucotomos* rhizomes extract against castor oil induced diarrhea in rats.

Materials and Methods

Plant material

Polypodium leucotomos rhizomes were collected from a local area (Colonel Hat) of Chittagong district, Bangladesh and authenticated by the expert of Bangladesh Forest Research Institute, Chittagong, Bangladesh (Voucher No. EB 119).

Preparation of extract

The rhizomes were sun dried and ground. The ground rhizomes (300 g) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring then filtered through a cotton plug followed by Whitman filter paper No. 1. The solvent was evaporated under vacuum at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use.

Animals

Adult long evans rats of either sex, weighting between 85 and 90 g, were collected from International Center for Diarrheal Diseases Research, Bangladesh (ICDDRB) and housed in polypropylene cages under controlled conditions. The animals were exposed to alternative 12:12 h light and dark cycle at an ambient temperature of 25 ± 2ºC. Animals were allowed free access to drinking water and pellet diet, collected from ICDDRB Dhaka. Rats were acclimatized for 10 days in the laboratory environment prior to the study. All animal experiments were cared for in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85-23 revised 1985).

Chemicals

Loperamide hydrochloride 99.71% (Square Pharmaceuticals Ltd., Bangladesh), castor oil (WELL's Heath Care, Spain), normal saline solution, 0.9% NaCl (Popular Pharmaceuticals Ltd., Bangladesh) and charcoal meal (10% activated charcoal in 5% gum acacia) were used.

Antidiarrheal activity

Castor oil induced diarrhea

Rats were fasted for 18 h before the test with free access to water and divided into four groups of five animals each. Group I treated as control (saline 2 ml/kg body weight orally), Group II received standard drug (loperamide 5 mg/kg b. wt. i.p) and Group III-IV received methanol extract (100 and 200 mg/kg b. wt. i.p respectively). Then 1 h later, castor oil was administered orally in each rat to induce diarrhea. The rats were then housed singly in cages lined with white blotting paper. The papers were changed every hour. The total number of both dry and wet feces excreted were counted

every hour for a period of 4 h and compared with the control group. The total number of diarrheal feces of the control group was considered 100% (Awouters et al., 2008).

Castor oil induced enteropooling

Intraluminal fluid accumulation was determined by the method of Robert *et al* (1976). 18 h fasted rats were divided into four groups of four animals each. Group I treated as control (saline 2 ml/kg b. wt. orally), Group II received standard drug (loperamide 5 mg/kg b. wt. i.p) and Group III-IV received methanol extract (100 and 200 mg/kg b. wt. i.p respectively) before 1 h administration of castor oil in all rats orally to induce diarrhea. Then 1 h later, the rats were sacrificed by overdose of chloroform anesthesia, and the small intestine was ligated both at the pyloric sphincter and at the ileocecal junctions and dissected out. The small intestine was weighed. The intestinal contents were collected by milking into a graduated tube and the volume was measured. The intestines were reweighed and the differences between full and empty intestines were calculated (Qnais et al., 2007).

Gastrointestinal motility test

Rats were fasted for 18 h and divided into four groups of five animals each. Castor oil was administered orally to these animals to induce diarrhea. 1 h later Group I received saline 2 ml/kg b. wt. orally, Group II received standard drug (loperamide 5 mg/kg b. wt. i.p) and Group III-IV received methanol extract (100 and 200 mg/kg b. wt. i.p respectively). 1 h later i.p administration of treatments, all animals received 1 ml of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. Then 1 h later, the animals were sacrificed by overdose of chloroform anesthesia and the distance traveled by the charcoal meal from pylorus to caecum was measured and expressed as a percentage of the total distance of the intestine (Mascolo et al., 1994).

Statistical analysis

Experiments results were analyzed by one-way ANOVA followed by Dunnett's test using SPSS Data Editor for Windows, Version 16.0 (SPSS Inc., U.S.A.). Values are represented as mean ± SEM.

Results

Castor oil induced diarrhea

In the castor oil-induced diarrhea experiment, the rhizome extract of *P. leucotomos* produced a marked antidiarrheal effect in the rats, as shown in Table 1. At doses of 100 and 200 mg/kg, the extract produced significant ($p < 0.01$) defecation. The total number of wet feces produced upon administration of castor oil decreased (12.80 ± 1.86 at 100 mg/kg and 9.20±0.88 at 200 mg/kg) compared to the control group (15.80 ± 1.76) while loperamide decreased to 7.00 ± 1.15 at the dose of 5 mg/kg.

Table 1. Effect of methanol extract of *P. leucotomos* rhizome on castor oil induced diarrhea in rats

Group	Treatment	Total number of feces	% Inhibition of defecation	Total number of diarrheal feces	"% Inhibition of diarrhea
I	Castor oil + Saline (2 ml/kg p.o)	24.60 ± 1.20	---	15.80 ± 1.76	-
II	Castor oil + Loperamide (5 mg/kg i.p)	10.00 ± 1.15**	59.35	7.00 ± 1.15*	55.70
III	Castor oil + Extract (100 mg/kg i.p)	15.40 ± 1.45**	37.1	12.80 ± 1.86	30.82
IV	Castor oil + Extract (200 mg/kg i.p)	12.60 ± 1.45**	48.78	9.20 ± 0.88*	41.77

Values are expressed as mean ± SEM. (n = 5). * $p < 0.05$, ** $p < 0.01$ when compared with control group.

Table 2. Effect of methanol extract of *P. leucotomos* rhizome on castor oil induced enteropooling in rats.

Group	Treatment	Weight of intestinal content (g)	Volume of intestinal content (ml)	Inhibition (%)
I	Castor oil + Saline (2 ml/kg p.o)	3.18 ± 0.07	2.79 ± 0.18	--
II	Castor oil + Loperamide (5 mg/kg i.p)	1.61 ± 0.04**	1.18 ± 0.10**	57.71
III	Castor oil + Extract (100 mg/kg i.p)	2.35 ± 0.39	1.93 ± 0.18*	30.82
IV	Castor oil + Extract (200 mg/kg i.p)	1.82 ± 0.06**	1.60 ± 0.06**	42.65

Values are expressed as mean ± SEM. (n = 5). * $p < 0.05$, ** $p < 0.01$ when compared with control group.

Table 3. Effect of methanol extract of *P. leucotomos* rhizome on small intestinal transit in rats

Group	Treatment	Total length of intestine (cm)	Distance traveled by marker (cm)	Inhibition (%)
I	Castor oil + Saline (2 ml/kg p.o)	109.37 ± 1.21	100.70 ± 2.26	--
II	Castor oil + Loperamide (5 mg/kg i.p)	103.33 ± 1.32	57.33 ± 4.05**	43.07
III	Castor oil + Extract (100 mg/kg i.p)	99.31 ± 0.80	84.33 ± 3.05*	16.26
IV	Castor oil + Extract (200 mg/kg i.p)	101.30 ± 2.75	72.77 ± 3.23*	27.93

Values are expressed as mean ± SEM. (n = 5). * $p < 0.05$, ** $p < 0.01$ when compared with control group.

Castor oil induced enteropooling

Castor oil caused accumulation of water and electrolytes in intestinal loop Treatment with the *P. leucotomos* extract (100 and 200 mg/kg) produced a significant and dose-dependent reduction in intestinal weight and volume (Table 2). The intestinal volume was decreased by 30.82% and 42.65% at doses 100 and 200 mg/kg respectively. The standard drug, loperamide (5 mg/kg), also significantly inhibited ($p < 0.01$) intestinal fluid accumulation (57.71%).

Gastrointestinal motility test

The methanol extract of *P. leucotomos* was also significantly ($p < 0.05$) lessened the gastrointestinal distance (100.70 ± 2.26 cm to 72.77 ± 3.23 cm) traveled by the charcoal meal in the rat's gastrointestinal tract compared with the control group (Table 3). Loperamide (5 mg/kg) produced a marked (43.07%) decrease in the propulsion of charcoal meal through gastrointestinal tract.

Discussion

The rhizome extract of *P. leucotomos* administered at the dose of 100 and 200 mg/kg showed 37.10% and 48.78% inhibition of defecation respectively. The maximum significant ($p < 0.01$) effect is observed at the dose of 200 mg/kg comparable to reference drug loperamide (5 mg/kg). Studies on enteropooling showed that the extract reduced both the weight and volume of intraluminal contents. These effects, which are direct consequences of reduced water and electrolytes secretion into the small intestine (Shah, 2004), suggest that the extract may enhance electrolyte absorption from the intestinal lumen consistent with inhibition of hypersecretion. Hypermotility characterizes diarrhea where the secretory component is not the causative factor (Chitme et al., 2004). Pre-treatment with the extract suppressed the propulsive movement or transit of charcoal meal through the gastrointestinal tract which clearly indicates that the rhizome extract may be capable of reducing the frequency of stooling in diarrheal conditions.

Several mechanisms had been previously proposed to explain the diarrheal effect of castor oil include inhibition of intestinal Na+ K+ ATPase activity, thus reducing normal fluid absorption (Capasso et al., 1994), activation of adenylate cyclase or mucosal cAMP-mediated active secretion (Pinto et al., 1992), stimulation of prostaglandin formation and platelet activating factor (Mascolo et al., 1996).

Most recently nitric oxide has been claimed to contribute to the diarrheal effect of castor oil. However, it is well proved that castor oil produces diarrhea due to its most active component ricinoleic acid through a hypersecretory response (Racusen & Binder, 1979; Vieira et al., 2000). Above observations suggest that the extract in graded doses reduce diarrhea by inhibiting peristalsis, gastrointestinal motility and castor oil induced enteropooling. Earlier studies showed that anti-dysenteric and anti-diarrheal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, sterol and/or triterpenes (Galvez et al., 1991, 1993; Longanga et al., 2000). Hence, tannins, saponin and/or flavonoids may be responsible for the mechanism of action of *P. leucotomos* anti-diarrheal activity. *Polypodium leucotomos* has been proven effective against different physiologi-cal disorders. The results of this investigation revealed that that *P. leucotomos* rhizomes extract possess significant antidiarrheal properties. Further research is needed to fractionate the methanol extract and isolate the molecule(s) responsible for the antidiarrheal activity observed.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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