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ANTIDIARRHEAL ACTIVITY OF LEAVES EXTRACT OF *MICROCOS PANICULATA* LINN IN MICE

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ABSTRACT

The experiment of methanolic extract of *Microcos paniculata* leaves showed significant ($p<0.001$) inhibitor activity against castor oil induced diarrhea and castor oil induced enteropooling in mice at dose of 400 mg/kg body weight. There was also significant ($p<0.01$) reduction in gastrointestinal motility in the charcoal meal test. Loperamide (5 mg/kg b. wt) was used as positive control. These results revealed that the leaves extract possess pharmacological activity against diarrhea and may possibly explain the use of the plant in traditional medicine.

Keywords: Antidiarrheal activity, castor oil induced diarrhea, enteropooling method, gastrointestinal motility, *Microcos paniculata*

INTRODUCTION

Diarrhea is the condition of having three or more loose or liquid bowel movements per day. Diarrheal disease is the second leading cause of death in children under five years old, and is responsible for killing 1.5 million children every year [1].

In the developing countries, diarrheal disease was the sixth leading cause of death in the year of 2007, causing 5.2% of deaths overall [2]. In Bangladesh, one third of the total child death burden is due to diarrhea [3]. Use of traditional medicines to combat the episodes of diarrhea has been emphasized by WHO in its Diarrhea Control Programme [4].

Microcos paniculata (Linn.) is a small tree with palmately nerved, sparsely piles leaves, calve flower buds and suburbanite edible fruits belongs to the family Tiliaceae (Patka in Bengali, Shiral in Hindi and Microcos in English) that is native to Asia.

Boiled leaves along with turmeric and shell of snail are taken for the treatment of jaundice. Traditionally it is used to improve digestion and is also used for other health problems including colds, hepatitis, diarrhea, heat stroke and dyspepsia [5, 6] but have no scientific evidence. *M. paniculata* has been added in Chinese herbal tea. A new alkaloid, N-Methyl-6 beta-(deca-1',3',5'-trienyl)-3 beta-methoxy-2 beta-methylpiperidine, was isolated from the stem bark of *M. paniculata*, showed good insecticidal activity against Aedes aegypti second instar larvae [7].

Another two new piperidine alkaloids, microcosamines A (1) and B (2), were isolated from leaves, showed significant larvicidal activity against *Culex quinquefasciatus* [8]. A new triterpene named methyl 3beta-O-p hydroxy-Ecinnamoyloxy- 2 alpha, 23-dihydroxyolean-12-en-28-oate (1), epicatechin (2), 3-trans-feruloyl maslinic acid (3), maslinic acid (4) and sucrose (5) were identified from the stem bark. Among them, compound 2 displayed significant free-radical-scavenging activity [9].

Analgesic and cytotoxic activity of leaves extract were also experimentally reported[10]. From the existing information it is evident that the plant may possess some important biological activities, thus the experiment was designed to evaluate the antidiarrheal activity of extracts of *Microcos paniculata* leaves against experimentally induced diarrhea in mice.

MATERIALS AND METHODS

Plant material: Microcos paniculata leaves were collected from the local forest of Chittagong district, Bangladesh in the month of July-August and authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, and Bangladesh.

Preparation of extract: The leaves were dried for a period of 2 weeks under shade and ground. The ground leaves (250 gm) 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 number 1. The solvent was evaporated under reduced pressure at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use.

Experimental Animals:
Swiss Albino mice weighing 25-30 gm of both sexes 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 hours light and dark cycle at an ambient temperature of 26±2ºC. Animals were allowed free access to drinking water and pellet diet, collected from ICDDRB Dhaka. Mice were acclimatized for 7 days in the laboratory environment prior to the study.

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

Castor oil-induced diarrhea:
The experiment employed the method described by Awouters *et al* [11]. Mice were fasted for 18 h before the test with free access to water and divided into five groups of five animals each. Group I treated as control (saline 2 ml/kg body weight intraperitoneally), Group II received standard drug (loperamide 5 mg/kg b. wt. ip) and Group III-IV received methanolic extract (200 and 400 mg/kg b. wt. ip). Then 1 h later, castor oil was administered orally to these animals to induce diarrhea. The mice's 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%.

Castor oil induced enteropooling:
Intraluminal fluid accumulation was determined by the method of Robert *et al* [12]. 18 h fasted mice were divided into five groups of five animals each. Group I served as control (saline 2 ml/kg body weight intraperitoneally), Group II received standard drug (loperamide 5 mg/kg b. wt. ip) and Group III-IV received methanolic extract (200 and 400 mg/kg b. wt. ip). Then 1 h later, castor oil was administered orally to these animals to induce diarrhea. Two hours later, the mice 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.

Gastrointestinal motility test:
This experiment was carried out by the method described by Mascolo *et al* [13]. Mice were fasted for 18 h and divided into five groups of five animals each. Castor oil was administered orally to these animals to induce diarrhea. One hour later Group I received saline 2 ml/kg body weight intraperitoneally, Group II received standard drug (loperamide 5 mg/kg b. wt. ip) and Group III-IV received methanolic extract (200 and 400 mg/kg b. wt. ip). One hour after ip administration of treatments, animals received 1 ml of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour 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.

Statistical analysis:
Experiments results were analyzed by one-way ANOVA followed by Dunnett's *t*-test using SPSS Data Editor for Windows, Version 11.5.0 (SPSS Inc., U.S.A.).

RESULTS

Castor oil induced diarrhea:
Diarrhea was apparent 1 hour after administration of castor oil in all the animals of control group for the next 4 h. Diarrheal episodes predominantly reduced by the ip injection of loperamide 5 mg/kg (60.53%). The antidirrhoeal effect was not potent as loperamide

in the dose of 200 mg/kg, but in the dose of 400 mg/kg, the extract exhibited significant ($p<0.001$) reduction of diarrhea over 4 h [Table 1].

Castor oil induced enteropooling:
Castor oil caused accumulation of water and electrolytes in intestinal loop. Treatment with the *M. paniculata* extract (200 and 400 mg/kg) produced a significant ($p<0.001$) and dose-dependent reduction in intestinal weight and volume [Table 2].

Gastrointestinal motility test:
The methanolic extract of M. paniculata was also significantly ($p<0.05$) reduced the gastrointestinal distance traveled by the charcoal meal in animals at 400 mg/kg dose, compared with the control group [Table 3]. Loperamide (5 mg/kg) produced a marked ($p<0.001$) decrease the propulsion of charcoal meal through gastrointestinal tract.

DISCUSSION

Microcos paniculata leaves extract showed activity against diarrhea by inhibiting intestinal motility, intraluminal fluid accumulation and significantly reducing the frequency of defecation. The methanol extract was administered at the dose of 200 and 400 mg/kg showed 36.85% and 50.04% reduction of diarrhea respectively. Significant ($p<0.001$) reduction (50.04%) in diarrheal episodes with maximum effect at 400 mg/kg dose level was found compared to loperamide (60.53%).

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 [14], activation of adenylate cyclase or mucosal cAMP-mediated active secretion [15], stimulation of prostaglandin formation and platelet activating factor [16]. Most recently nitric oxide has been claimed to contribute to the diarrheal effect of castor oil [17]. However, it is well proved that castor oil produces diarrhea due to its most active component ricinoleic acid through a hypersecretory response [18, 19]. Therefore, it can be assumed that the antidiarrheal action of the extract was mediated by an antisecretory mechanism and also by reducing gastrointestinal motility.

CONCLUSION

The results of this experiment demonstrated that *M. paniculata* leaves extract possess pharmacologically active ingredient (s) with antidiarrheal properties. The active constituent(s) responsible for antidiarrheal activity remain to be identified; further studies are required to understand the pharmacological action of its antidiarrheal activity.

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Table 1: Effect of methanolic extract of leaves of *M. paniculata* on castor oil induced diarrhea in mice

Group	Treatment	Total number of feces	% Inhibition of defecation	Total number of diarrheal feces	% Inhibition of diarrhea
I	Castor oil + Saline (2 ml/kg ip)	15.66 ± 0.88	---	12.67 ± 1.20	---
II	Castor oil + Loperamide (5 mg/kg ip)	7.76 ± 1.15*	50.44	5.0 ± 0.58**	60.53
III	Castor oil + Extract (200 mg/kg ip)	11.33 ± 1.21*	27.65	8.0 ± 0.82	36.85
IV	Castor oil + Extract (400 mg/kg ip)	9.67 ± 0.85*	38.25	6.33 ± 0.88**	50.04

Values are expressed as mean ± S.E.M. (n=5). *p <0.01, **p <0.001 when compared with control group.

Table 2: Effect of methanolic extract of leaves of *M. paniculata* on castor oil induced enteropooling in mice

Group	Treatment	Volume of intestinal content (ml)	Weight of intestinal content (g)	% Inhibition of intestinal content
I	Castor oil + Saline (2 ml/kg ip)	0.67 ± 0.04	1.76 ± 0.03	---
II	Castor oil + Loperamide (5 mg/kg ip)	0.48 ± 0.04	1.18 ± 0.04*	32.95
III	Castor oil + Extract (200 mg/kg ip)	0.57 ± 0.12	1.31 ± 0.05**	25.56
IV	Castor oil + Extract (400 mg/kg ip)	0.45 ± 0.02	1.06 ± 0.07**	39.77

Values are expressed as mean ± S.E.M. (n=5). *p <0.01, **p <0.001 when compared with control group.

Table 3: Effect of methanolic extract of leaves of *M. paniculata* on charcoal induced gut transit changes in mice

Group	Treatment	Total length of intestine (cm)	Distance traveled by marker (cm)	% of inhibition
I	Castor oil + Saline (2 ml/kg ip)	59.60 ± 0.83	54.43 ± 0.87	---
II	Castor oil + Loperamide (5 mg/kg ip)	60.60 ± 1.13	32.37 ± 2.15**	40.52
III	Castor oil + Extract (200 mg/kg ip)	58.30 ± 0.58	43.36 ± 1.27	20.34
IV	Castor oil + Extract (400 mg/kg ip)	55.13 ± 2.27	38.38± 2.78*	29.49

Values are expressed as mean ± S.E.M. (n=5). *p <0.01, **p <0.001 when compared with control group.

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