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Research Article

ANTIDIARRHOEAL ACTIVITY OF METHANOL EXTRACT OF THE RHIZOMES OF *CYPERUS TEGETUM* ROXB

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ABSTRACT

The present study was carried out to evaluate the antidiarrhoeal effect of methanol extract of *Cyperus tegetm* Roxb (MECT) (Family:Cypereceae) using castor oil and magnesium sulphate-induced diarrhoea models in mice. At the doses of 250, 500and 750 mg/kg b.w, the MeOH extract showed significant antidiarrhoeal activity in both models. The extract, at the dose of 250, 500 and 750 mg/kg, retarded the intestinal transit of charcoal meal in mice as compared to the control. On the basis of these findings, it can be assumed that *Cyperus tegetum* could be a potential source for novel 'lead' discovery for antidiarrhoeal drug development.

Keywords*: Cyperus tegetum* Roxb*,* antidiarrhoeal activity, Loperamide, Atropine sulphate

INTRODUCTION

Diarrhoea is characterized by increased frequency of bowel movement, wet stool and abdominal pain[1]. It is a leading cause of malnutrition and death among children in the developing countries of the world today[2]. Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and children below 5 years old especially in developing countries[3]. In recent years, there has been a great interest in herbal remedies for the treatment of number of ailments. A range of medicinal plants with anti-diarrhoeal properties is widely used by traditional healers. However, the effectiveness of many of these antidiarrhoeal traditional medicines has not been scientifically evaluated.

In present study, we carried out the antidiarrhoeal activity of the plant *Cyperus tegetum* Roxb. Belonging to the family Cyperaceae, a glabrous and robust perineal sedge found throughout India up to an altitude 1800m[4]. The plant is commonly known as mat stick, madur kathi(Bengali) and cultivated as an economic crops in Paschim Medinipur district of West Bengal and traditionally used by the tribal people for the treatment of cachexia, atrophy and snake bite[5]. Literature survey show that different cyperus plants are used by tribal people as febrifuge (reduces fever), alexiteric (anti-infective), sudorific (causes sweating), digestive, laxative, nerve tonic in cases of stress and nervous and mental disorders (including epilepsy), to treat and prevent a wide range of digestive and gastrointestinal disorders, to facilitate child birth or to induce an abortion, as a contraceptive, and for throat cancer. Activities like anticonvulsant[6], sedative[7], antimalarial[8], antidiarrhoeal[9] have been reported by several research workers on the other plants belong to Cyperaceae family, however there is no scientific report on the plant *Cyperus tegetum* Roxb of same family. Therefore the objective of the present investigation was to explore its phytoconstituents and probable pharmacological activity.

MATERIALS AND METHODS

Plant material

The plant *Cyperus tegetum* Roxb (Family:Cyperaceae) was collected from the cultivated land of Paschim Medinipur, West Bengal in the month of June-July. Botanical Survey of India, Shibpur, Howrah taxonomically identified the plant. A voucher specimen (CNH/I-I (198)/2007/Tech.II/162) has been preserved in our laboratory for further references. The rhizomes were washed, dried at room temperature under shed and then grounded in a mill to a coarse powder.

Extraction of plant materials

The powdered rhizomes were subjected to successive Soxhlet extraction using a series of solvents of increasing polarity starting from petroleum ether, chloroform, and methanol respectively. The extracts were vacuum dried and the percentage yields of the extracts were 2.1%, 3.0%, and 5.4%, respectively.

Preliminary phytochemical analysis

The phytochemical tests were performed using various reagents as described in Table1[10]. The MeOH extract was tested for the presence or absence of alkaloid, glycosides, tannins, steroids, reducing sugars, proteins and amino acids, phenolic compounds and flavonoids (Table.1).

Table 1: Result of chemical group test of the methanol extract of *Cyperus tegetum* rhizome

Phytoconstituents	Test performed/reagents used	Presence or absence
Alkaloid	Mayers test	+
	Dragendorff test	+
	Hagers test	+
Steroid	Liebermann-Burchard test	-
Flavonoid	Shinoda test	+
Tannin	Ferric chloride	+
	Lead acetate	+
Saponin	Test for stable foam	+
Glycoside	Borntrager test	-
Protein and amino acid	Ninhydrin test	+
Reducing sugar	Fehlings test	+
	Benedict test	+

(+): Present; (-): Absent

Acute toxicity study

The acute toxicity of methanol extract (MECT) of *Cyperus tegetum* Roxbwas studied on Swiss albino mice (20-25 gm) following Karber's method. The Institutional Animal Ethical Committee (CPCSEA/ORG/CH/2006/Reg.No.955) permitted the use of the animals for this purpose. After fasting condition for overnight, the animals divided into six groups (four in each group), were administered a dose of 100, 200, 400, 800, 1600 and 3000 mg/ kg b.w intraperitoneally. No animals were found died following 24h observation but sedation of the animals observed.

Animals and treatment

Swiss mice of either sex, weighing 25-30g, were used. They were housed in environmental conditions and fed with standard food for rodents and water, *ad libitum* Treatments were administered intraperitoneally in a volume of 10 ml/kg BW.

Antidiarrhoeal activity study by castor oil-induced diarrhea

The method, described by Shoba and Thomas (2001), was followed for this study with slight modification. The animals were divided into control, positive control and test groups containing five mice in each group. Control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg orally. The positive control group received loperamide at the dose of 3 mg/kg orally, test groups received the MeOH extract at the doses of 250 and 500 mg/kg and 750 mg/kg b.w intraperitonially. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. Diarrhoea was induced by oral administration of 1 ml castor oil to each mouse, 30 min after the above treatments. During an observation period of 4 h, the total number of faecal output and the number of diarrhoeic faeces excreted by the animals were recorded[11].

Table 2: Effect of MeOH extract on castor oil (1ml/mice) induced diarrhea in mice

Treatment	Dose (mg/kg bw)	Total no of faeces in 4 hrs (Mean ± SD)	Number of diarrhoeal faeces (Mean ± SD)
Control(1% tween 80, 0.1 ml/gm)	-	25.8 ± 1.92	17.6 ± 1.14
Loperamide	3	5.2 ± 0.83	2.2 ± 0.83
MECT 250	250	18.6 ±1.81	14.6 ± 1.14
MECT 500	500	14.4 ± 1.51	11.4 ± 0.89
MECT 750	750	8.4 ± 1.67	4.8 ± 1.30

Values are Mean ± SD (n=6)

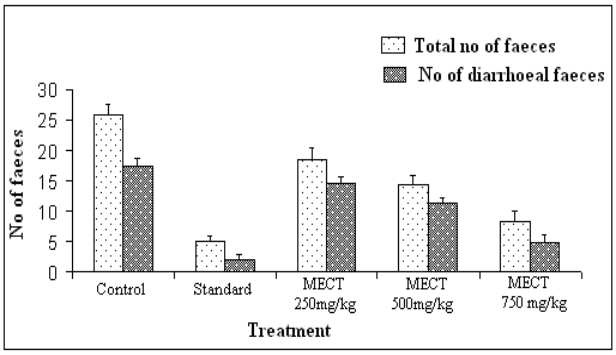

Fig. 1: Effect of MeOH extract on castor oil (1ml/mice) induced diarrhoea in mice

Antidiarrhoeal activity study by magnesium sulphate-induced diarrhoea:

A similar protocol as for castor oil-induced diarrhoea was followed. Diarrhoea was induced by oral administration of magnesium sulphate at the dose of 2 g/kg to the animals 30 min after pre-treatment with vehicle (1% Tween 80 in water, 10 ml/kg, p.o.) to the control group, loperamide (3 mg/kg) to the positive control group, the MeOH extract at the doses of 250, 500 and 750 mg/kg to the test groups. All the administrations were carried out through oral route [12]..

Table 3: Effect of MeOH extract on $MgSO_4$ (2mg/kg p.o) induced diarrhea in mice

Treatment	Dose (mg/kg bw)	Total no of faeces in 4 hrs (Mean ± SD)	Number of diarrhoeal faeces (Mean ± SD)
Control(1% tween 80, 0.1 ml/gm)	-	18.2 ± 1.30	13 ± 2.23
Loperamide	3	5.2 ± 1.48	2.4 ± 0.54
MECT 250	250	14.2 ± 2.04	9.6 ± 1.14
MECT 500	500	10.4 ± 2.60	6.6 ± 0.89
MECT 750	750	7.6 ± 0.89	3.8 ± 1.48

Values are Mean ± SD (n=6)

Small intestinal transit

The effect of the extract on small intestinal transit was studied in overnight fasted mice which were divided in different groups. Group I received 1% tween 80(0.1 ml/gm), group II received atropine (3 mg/kg, i.p.), group III, IV and V received methanol extract 250 , 500 and 750 mg/kg p.o respectively, 1 h before administration of castor oil (1ml /mice irrespective to body weight). One ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1h after castor oil treatment. The rats were sacrificed after 1h and the distance traveled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum

Table 4: Effect of MeOH extract on intestinal transit in mice

Treatment	Dose (mg/kg bw)	% Travelled by charcoal meal
Control(1% tween 80, 0.1 ml/gm)	-	75.23 ± 3.27
Atropine sulphate	0.1	35.61 ± 2.14
MECT 250	250	65.14 ± 4.32
MECT 500	500	49.36 ±1.89
MECT 750	750	35.28 ± 2.05

Values are Mean ± SD (n=6)

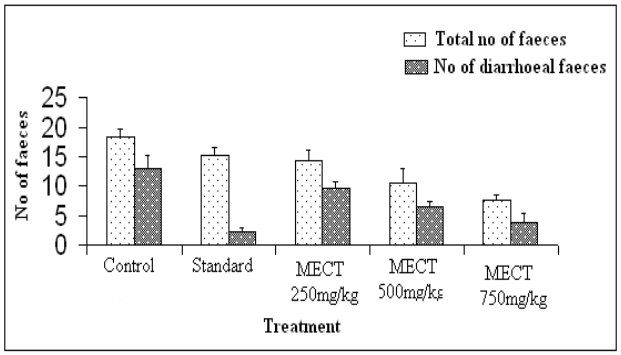

Fig. 2: Effect of MeOH extract on $MgSO_4$ (2mg/kg p.o) induced diarrhea in mice

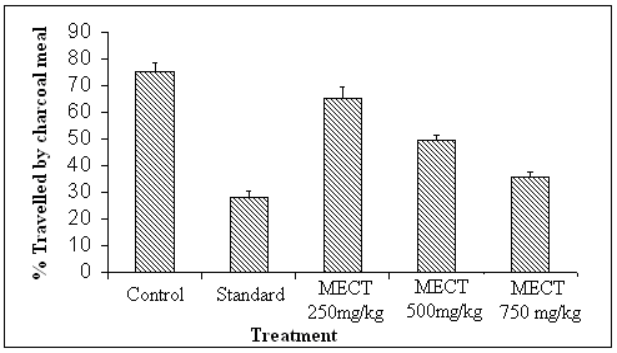

Fig. 3: Effect of MeOH extract on intestinal transit in mice

RESULTS AND DISCUSSION

Castor oil causes diarrhoea due to its active metabolite, ricinolic acid[13-14], which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin[15]. In this study, the methanol extract of *Cyperus tegetum* exhibited a significant antidiarrhoeal activity. The results were similar to that of the standard drug loperamide (3mg/kg) with regard to the severity of diarrhoea. Methanol extract significantly reduced intestinal transit as observed by the decrease in intestinal motility of charcoal meal. Phytochemical screening revealed the presence of tannins, sterol and/or triterpenes and reducing sugars. Earlier studies showed that anti-dysenteric and anti-diarrhoeal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, sterol and/or triterpenes and reducing sugars[16 - 18]. Hence, tannins, reducing sugars, sterol and/or triterpenes may be responsible for the mechanism of action of anti-diarrhoeal activity. This can be due to the fact that the extract increased the reabsorption of water by decreasing intestinal motility as observed in the decrease of intestinal transit by charcoal meal. Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the small intestine, reduce colon flow rate, and consequently any effect on colonic motility. Atropine significantly reduced intestinal transit time. This is possible due to its anticholinergic effect[19]. However, it did not inhibit castor oil induced enteropooling, thereby, suggesting that mediators other than acetylcholine are involved in castor oil-induced enteropooling. Furthermore, a decrease in intestinal transit time with atropine could also be due to reduction in gastric emptying[20]. These observations demonstrate the inhibitory effect of *C tegetum* rhizome extract on castor oil induced diarrhoea and peristaltic activity in small intestine.

CONCLUSION

The results of this investigation revealed that methanol extract contains pharmacologically active substance(s) with antidiarrhoeal properties. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecule responsible for the antidiarrhoeal activity observed.

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