



Antidiarrhoeal activity of *Cucumis sativus* leaves.

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Date Received:

24th Jan 2014

Date of Accepted:

4th Feb 2014

Date Published:

11th Feb 2014

Abstract:

The present study was aimed to evaluate the antidiarrhoeal activity of crude methanol (ME) extracts of leaves of *Cucumis sativus*. The methanolic extract (ME) of the plant was studied for its antidiarrhoeal properties in experimental diarrhoea, induced by castor oil and gastrointestinal transit test in mice, at the oral doses of 250 and 500 mg/kg body weight. In antidiarrhoeal study, ME showed significant ($P < 0.001$) dose-dependent inhibitory activity against castor oil induced diarrhoea. A significant reduction ($P < 0.001$) in the gastrointestinal motility in charcoal meal test in mice was also observed, suggesting the extract might exert its antidiarrhoeal activity by antisecretory mechanism. Further investigations are, however, necessary to explore mechanism(s) of action involved in this antidiarrhoeal activities.

Keywords: Antimicrobial activity, Anti-diarrhoeal, castor oil, gastrointestinal transit, antisecretory mechanism.

Introduction

Diarrhoea is one of the main causes of high mortality rate in developing countries where over five million children under the age of five die annually from severe diarrhoeal diseases. It is a major contributor to malnutrition and also causes rapid dehydration in infant and elderly people, which could lead to death if treatment is not given (1). Although Oral Rehydration Therapy (ORT) has been very helpful in the treatment of diarrhoeal diseases, the use of traditional medicinal plants in improving the efficiency of ORT has not been much applauded (2). The world health organization (WHO) had initiated a diarrhea disease control program to study traditional medicine practices and other related aspects, together with the evaluation of health education and prevention approaches (3).

Cucumis sativus Linn. (Family: Cucurbitaceae) is an annual, rather coarse, fleshy, prostrate or climbing vine. It is widely distributed all over the world particularly in Asia, Africa and South America (4). Traditionally, this plant is used for headaches; the seeds used as cooling and diuretic, the fruit juice is

used as a nutritive and as a demulcent in anti-acne lotions; Juice of leaves used as an emetic in acute indigestion in children (5). The fruits contain erepsin enzyme, Vitamin B1 and C, ascorbic acid, proteolytic enzyme, rutin, oxidase, succinic and maleic dehydrogenases, and so on. Several investigations revealed antidiabetic (6), antiulcer (7), moisturizing (8), antioxidant and analgesic property (9) of the fruit extracts. The seed extracts were found effective on controlling the loss of body weight in diabetic rats (4) and against tapeworms (10). Cytotoxic, antifungal (11) and antibacterial activity (12) activities have been reported from leaves and stems extracts. Chemical study have demonstrated the presence of cucurbitasides B, C and ferredoxin in leaves (13,14) and α - and β -amyryn, sitosterols and cucurbitasides (15) in seeds. Identified phytoceuticals from its leaves are acylated flavone C-glycosides such as isovitexin 2''-O-(6'''-(E)-p-coumaroyl) glucoside, isovitexin 2''-O-(6'''-(E)-p-coumaroyl)glucoside-4'-O-glucoside, isovitexin 2''-O-(6'''-(E)-feruloyl) glucoside-4'-O-glucoside and

isoscoparin 2''-O-(6''-(E)-p-coumaroyl) glucoside (15). No report was found of the *C. sativus* leaves on antidiarrhoeal activity. So, an endeavor was made to assess scientifically the antidiarrhoeal effects of methanolic extract of *Cucumis sativus* leaves.

EXPERIMENTAL

Collection and Identification of the plant

The leaves of plant *Cucumis sativus* were collected from Gazipur in the month of March, 2012 and authenticated at Bangladesh National Herbarium, where a voucher specimen no-34479 has been deposited.

Preparation of the extracts

The leaves were first washed with water to remove adhering dirt and then dried at 45°C for 36 h in an electric oven, then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. The dried powdered material (1kg) was taken in a clean, flat bottomed glass container and soaked in methanol for seven days. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. The extracts were filtered and concentrated under vacuum to obtain a crude methanol extract (ME) of leaves.

Drugs and chemicals

The active drugs Loperamide, Atropine sulphate were the generous gift samples from Square Pharmaceuticals Ltd., Bangladesh. Tween-80 and DMSO was obtained from BDH Chemicals, UK. Castor oil, acacia, was purchased from CDH, India. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh. All chemicals used were of analytical reagent grade.

Animals

Swiss albino mice of either sex, weighing about 22-25 gm were used for the experiment. The mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed ICDDR formulated rodent food and water ad libitum. The set of rules followed for animal experiment were approved by the institutional animal ethical committee (17).

Statistical analysis

Oral toxicity tests

Swiss albino mice (20 - 22 g) were randomly divided into nine groups of five animals each. The animals were starved for 12 h prior to testing. Eight doses of the extract, ME (0.5-20 g/kg body weights) were administered by oral intubation to eight groups of the animals respectively. The animals in the control group received 0.2 ml distilled water. All animals were observed for 24 h and general symptoms of toxicity and mortality were recorded (18).

Antidiarrhoeal Activity

Castor oil induced diarrhoea

This study was conducted by the method described by Shoba and Thomas (19). The animals were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhea were selected for the final experiment. The animals were divided into following four groups containing five mice in each group.

Group I: Treated with vehicle (1% Tween 80 in water, 10 ml kg⁻¹ (p.o.)

Group II and Group III: Treated with 250 and 500 mg kg⁻¹ body weight (p.o.) of ME, respectively.

Group IV: Received loperamide (3mg/kg) body weight (p.o.)

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 0.5 ml castor oil to each mice, 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.

Castor Oil induced gastrointestinal motility

To test the effect of the extract on the gastrointestinal motility, the animals were randomly grouped as mentioned above only difference was Group IV was given standard drug, atropine (0.1 mg/kg). The test mice were starved for 24 h prior to the experiment but were allowed access to water. The animals were fed samples, 1 h before oral dose of castor oil (.5 ml). 1 h after castor oil administration, 0.5 ml of a 5% activated charcoal suspension in a 5% suspension of acacia powder was administered to each mice. All animals were sacrificed 30 min later, the abdomen opened and the distance moved by the plug from the pylorus to the caecum was measured and expressed as percentage of the total length of the small intestine (20).

Antidiarrhoeal activity of methanolic extract of *C.*

The data obtained in the animal experiments was subjected to statistical analysis. All values are expressed as Mean \pm S.E.M (Standard Error of Mean). The data were assessed by the analysis of variance (ANOVA) and the group means were evaluated by the post-hoc Dunnet test using SPSS program (SPSS 16.0, USA). Mean values were considered significantly different if $P < 0.01$.

RESULTS & DISCUSSION

Acute toxicity studies

Oral administration of the methanolic leaf extract of *C. sativus* produced no mortalities, even no visible signs of toxicity in the animals except for an initial huddling observed at the highest dose of 20 g/kg body weight. In addition, no toxic symptoms were observed and neither food nor water intake was found to be reduced during the period. The absence of mortality and signs of toxicity up to 5 times the maximum effective dose, with ME proves for that the plant has wide safety margin.

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Antidiarrhoeal activity in Castor oil-induced diarrhoea
 In the castor oil-induced diarrhoeal experiment in mice, the ME at the doses of 250 and 500 mg/kg, reduced the total number of faeces as well as the total number of diarrhoeic faeces in a dose dependent manner (Table 1). These results were shown to be statistically significant ($P < 0.01$).

Castor oil induced gastrointestinal transit in rats

The effect of ME on gastrointestinal transit (Table 2) revealed that 250 mg and 500mg/kg of the extract decreased the intestinal transit length by 44.39 and 57.12%, respectively. Atropine 0.1 mg/kg caused a significant ($P < 0.01$) reduction in the gastrointestinal transit by 66.51%.

Table-1: Effect of methanolic extracts of *Cucumis sativus* Leaves on castor oil induced diarrhoea in mice

Treatment	Dose(mg/Kg)	Total number of faeces in 4 h	Total number of wet faeces in 4 h	Percent inhibition of diarrhoeal faeces
Control(saline)		13 \pm 0.44	10.32 \pm 0.34	-
ME	250	9.2 \pm 0.5*	5.2 \pm 0.73*	49.61
ME	500	7.2 \pm 0.3*	4.2 \pm 0.32*	59.32
Loperamide	3	2.3 \pm 0.43*	1.2 \pm 0.33*	88.37

Results are presented as mean \pm SEM, (n=5), * $P < 0.01$ dunnet test as compared to control .

Table 2. Effect of methanolic extracts of *C. sativus* Leaves on gastrointestinal transit in mice.

Treatment	Dose(mg/Kg)	Motility (Distance moved by charcoal meal as percentage of intestinal length.)	Inhibition (%)
Control(saline)	5	67.58 \pm 2.17	-
ME	250	37.72 \pm 0.21*	44.39
ME	500	29.38 \pm 3.05*	57.12
Atropine	0.1	22.63 \pm 1.46*	66.51

Diarrhea is usually considered as a result of altered motility and fluid accumulation within the intestinal tract. Many antidiarrhoeal agents act by reducing the gastrointestinal motility and / or the secretions. Several mechanisms have been previously proposed to induce the diarrhoeal effect of castor oil (23). These include inhibition of intestinal Na⁺, K⁺-ATPase activity to diminish normal fluid absorption (24), activation of adenylate cyclase or mucosal cAMP mediated active secretion (25,26), stimulation of prostaglandin synthesis (27), platelet activating factor (28) and most recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor Oil (29). It is well documented that castor oil produces diarrhoea due to its most active component ricinoleic acid by a hypersecretory response (30). Since the methanolic extract of leaves of *C. sativus* successfully inhibited the castor oil induced diarrhoea, the extract might have exerted its antidiarrhoeal action by antisecretory mechanism. This was also evident from the reduction of total number of wet faeces in the test groups in the experiment.

The standard drug, loperamide is one of the most efficacious and widely employed antidiarrheal drug at present. Loperamide effectively antagonizes diarrheal activity induced by castor oil (31), prostaglandins (32) or cholera toxin (33). Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the intestine, reduce colon flow rates and consequently any effect on colonic motility (34, 35). The effect of extract on GI-transit was also observed to compare its Antidiarrhoeal activity to loperamide. The reduction of propulsive movement in the charcoal meal study explored the anticholinergic effect (36) of antimuscarinic drug, atropine and both the doses of ME. ME might be increased the reabsorption of water by decreasing intestinal transit of charcoal meal. Flavonoids have been ascribed the ability to inhibit contractions induced by spasmogenics (37). Phytochemical screening of ME revealed the presence of tannins, protein, saponins, steroids, alkaloids and flavonoids which have all been reported to possess activity and therefore explain its antidiarrheal action (38). There is need for study to ascertain the mechanism of action of the extract and its antimicrobial effect against diarrhea causing microorganisms.

CONCLUSION

Based on the results of the present study, we conclude that the methanolic extract of *Cucumis sativus* leaves possesses remarkable antidiarrhoeal activity in dose dependent manner. However, further studies are indispensable to examine underlying mechanisms of such pharmacological effects and to isolate the active compounds responsible for these pharmacological activities.

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