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Antidiarrhoeal activity of leaf extract of *Moringa Oleifera* in experimentally induced diarrhoea in rats

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Abstract

To evalauate the antidiarrhoeal activity of the hydroalcoholic extract of moringa oleifera leaves. The hydroalchoholic extract was evaluated using rodent animal models of diarrhoea like the castor oil and magnesium sulfate induced gastrointestinal motility, in a model of enteropooling induced by the administration of castor oil and PGE2, Charcoal meal test. Acute toxicity and phytochemical constituents were also been evaluated using standardized methods. The results of the present study indicates that the hydroalcoholic extract of moringa oleifera leaves was effective in inducing a significant protection against experimentally induced diarrhoea by castor oil and magnesium sulfate, as evidenced by a decrease in the number of frequency, weight of stools after 4 hours with respect to control. The extract also prevented the enteropooling induced by castor oil and PGE2 at all the doses tested. Acute toxicity studies indicated that the extract is safe till 2500 mg/kg. The antidiarrhoeal activity though, not ascribed to any particular phytochemical present, general tests performed indicated the presence of flavonoids, tannis which were reported to produce antidiarrhoeal activity. These results showed that Moringa oleifera leaves possess anti-diarrheal properties mediated through inhibition of hyper secretion and gastrointestinal motility that substantiate its use in the treatment of diarrhea in traditional medicines or folklore use.

Keywords: enteropooling, hyper secretion, Moringa oleifera.

Introduction

Diarrhoea is characterized as rapid movement of faecal matter through intestine resulting in poor absorption of water, nutritive elements and electrolytes producing abnormal frequent evacuation of watery stools. According to world health organization, it is the one of the most common cause of morbidity and mortality in many developing countries effecting mainly the infants and children's [1]. It is often caused by enterotoxins which are produced by bacteria such as Escherichia coli, Salmonella typhi, Salmonella typhimurium, Clostridium difficile, Clostridium freundii, Aeromonas hydrophila, Campylobacter jejuni and Vibrio cholerae, to name a few [2]. These bacterial are commonly infested either by means of polluted water or consumption of contaminated food and by physical contact like handshake etc.

These bacteria cause the influx of water and ions to the intestinal lumen and thus increase the intestinal motility, thereby causing watery stools. Such secretory diarrhoea is treated by the administration of oral rehydration salts in children or adults to reduce the loss of essential electrolytes and maintain the body fluids osmolality [2]. Alternatively, many opioid drugs like Diphenoxylate, Loperamide, Diloxanide furoate for protozoal infections induced diarrhea

and dysentry, racecadotril, muscarinic receptor blockers like atropine sulfate etc; are available in the market for treating diarrhoea. But all of the existing drugs suffer from adverse effects like the induction of bronchospasm, vomiting by racecadotril; intestinal obstruction and constipation by loperamide [3]. For this reason, present days there has been great interest in herbal remedies for the treatment of such ailments. Although several medicinal plants have gained importance for the treatment of diarrhoea, many remain to be evaluated scientifically.

Moringa oleifera (family: Moringaceae) is one among such plants cultivated for different purposes such as medicine, vegetable, as spice for cooking and as a cosmetic. All the parts of the tree has been used in folk medicine for the treatment of various diseases such as urinary tract infections, HIV-aids, external sores and ulcers, diabetes, cancer, gastritis etc [4]. The plant has been reported to possess anti-inflammatory [5], antioxidant [6], anti-ulcer [7], anti-cancer [8], anti-hyperlipidaemic [9], anti-diabetic [10], anti-asthmatic [11], hepatoprotective [12] and anti-hypertensive properties [13]. However, literature review failed to offer any scientific validation on the antidiarrhoeal activity of Moringa oleifera leaves. In view of this, the present study was undertaken to justify the traditional use of leaves of Moringa oleifera as an antidiarrhoeal.

Materials and methods:

Plant material:

Fresh leaves of Moringa oleifera were collected in and around Davangere, Karnataka. Voucher herbarium specimens have been authenticated by Prof. K. Prabhu, department of Pharmacognosy, S.C.S. College of pharmacy, Harapanahalli and deposited at the Museum of SCS College of pharmacy.

Chemicals:

Ketamine (Themis medicare, Uttarakhand); Gum acacia, castor oil, Charcoal meal, Magnesium sulfate (SD Fine chemicals, Mumbai); Lopermaide, the standard drug (Micro labs, bangalore), Ethanol (Merck, Mumbai) and PGE2 (Astra zeneca, Bangalore).

Preparation of crude extracts and characterization:

The shade dried plant material was powdered coarsely and loaded onto a soxhlet evaporator, extracted with circulating 70% hydroalcohol at 80^0C. Later, for concentrating, the obtained extract was flash evaporated at 50^0C and used for the study. Concentration of the extract produced 15.25 gm (22.6% W/W) yield. Preliminary phytochemical screening of the obtained extract was performed in accordance to the tests described elsewhere by Kokate and K.R. Khandalwal[14]. The extract was found to posse's alkaloids, carbohydrates, flavonoids, and tannins. Tests for glycosides and steroids revealed their absence in the obtained hydroalcoholic extract.

Dose selection and treatment: Hydroalcoholic extract prepared thus was administered to mice for LD50 determinations using the fixed dose method in accordance to OECD guidelines [15]. Healthy adult female Swiss albino mice weighing between 25 to 35 g were used for the study. After oral administration of extracts various parameters like body temperature, CNS activity, micturation, defecation etc. were observed for 24 h. Accordingly 100, 250 and 500 mg/kg were selected for treatment during the study.

Animals: Albino mice (25-30 g) and Wistar rats (200-250 g) were used in the present study. All the animals were housed in polypropylene cages and maintained in a standardized animal house under standard conditions of temperature (24 ± 2ºC), relative humidity, and 12 hr light/dark cycle. Animals were fed with standardized chow and water ad libitum. All the tests were performed according to OECD guidelines [15] and institutional animal ethics committee approved the study protocol.

Five groups of animals were used for the study. First group always served as vehicle or control group in all the cases. The second group of animals received the standard drug, lopermaide.

All the other three groups of animals received the hydroalcoholic extract in suspension using acacia gum as the suspending agent, at the doses mentioned above. For prostaglandin (PGE2) induced enteropooling model, six groups of animals were used. One group served as negative control and the other one as a positive control.

Castor oil induced diarrhoea [16]:
Rats of either sex (150-250gm) were fasted for 18 h. They were divided into four groups (n=6). The first group of animals, which served as control was administered with aqueous 2% acacia suspension. The second group received standard drug, loperamide (3 mg/kg) orally as suspension. The extract was administered orally at 100, 250 and 500 mg/kg dose to third, fourth and fifth group respectively. After 60 min of drug treatment, the animals of each group received 1ml of castor oil orally and the watery faecal material and frequency of defecation was noted up to 4 h in the transparent metabolic cages with pre weighed plastic dishes placed at the base. Weight of plastic dish before and after defecation was noted and compared to control.

Magnesium sulphate induced diarrhoea [17]**:**
Animals and treatment was similar to castor oil induced diarrhoea model. After 60 min of drug treatment, the animals in each group received magnesium sulphate (2 g/kg) orally. Again, the faecal material and the frequency of defecation were noted up to 4 hr in the transparent metabolic cages with pre weighed plastic dishes placed at the base. Weight of plastic dish before and after defecation was noted and compared to control.

Castor oil induced enteropooling [18]:
Treatment remained the same as mentioned previously. 60 min after drug administration, 1 mL of castor oil was administered to all animals including the control or vehicle treated group. After 30 min, following administration of castor oil, all rats were sacrificed by overdose of ketamine, whole length of the intestine from pylorus to caecum was dissected out, its content collected in measuring cylinder and volume measured.

Prostaglandin E2 (PGE 2) induced enteropooling [19]:
Rats of either sex were used in the study and divided into six groups of six in each group. The first group, which served as negative control was administered with vehicle (2% acacia suspension) and1 ml of 5%v/v ethanol and normal saline by oral route. The second group, which served as positive control, was administered with PGE 2 (100 µg/kg p.o.) only. The extract was administered orally at 100, 250 and 500 mg/kg to third, fourth and fifth group respectively. Immediately after extract administration, PGE2 was administered. After 30 min following administration of PGE 2 each rat was sacrificed by administering excessive dose of ketamine and the whole length of the intestine from pylorus to caecum was dissected out, its content collected in measuring cylinder and volume measured.

Effect on Charcoal meal test [20]:
Albino Wistar rats were treated as described earlier except that atropine sulfate was used as a standard drug and administered at a dose of 5 mg/kg i.m. 1 mL of Castor oil was administered orally to rats and left for one hour after which drug treatment was done. One hour later after drug treatments, each of these animals were given 1mL of charcoal meal (3% charcoal suspension in 5% suspension of acacia) by oral route. All animals were sacrificed after 30 min; the stomach and small intestine were removed and extended on a clean glass surface. The distance moved by the charcoal meal from the pylorus was measured and then expressed as a percentage of the distance from the pylorus to the caecum.

Statistical analysis:

Values are expressed as mean ± S.E.M of n =6 animals. Mean values were evaluated by Analysis of variance and Dunnet`s posttest. Values with $p < 0.05$ were considered significant.

Table 1: Effect of hydroalcoholic extract of Moringa oleifera (HMO) on Castor Oil Induced Diarrhoea in Rats.

Groups	Treatment	Dose Mg/kg	Mean frequency of diarrhoea ± SEM	Mean wt of fecal drops ± SEM	Mean wt of faeces ± SEM after 4hr	% protection
I	Control	1 mL	3.6±0.021	10.6±0.24	1.16±0.031***	0.00
II	Loperamide	3	0.4±0.2***	1.8±0.37***	0.154±0.033***	86.72
III	HMO	100	2.2±0.2*	6.6±0.5***	0.6±0.07***	48.27
IV	HMO	250	1.6±0.4**	6±0.44***	0.54±0.05***	53.44
V	HMO	500	1.2±0.2**	5±0.044***	0.3±0.044***	74.13

HMO, Hydroalcoholic extract of Moringa oleifera; g,gram;
The values are mean ± SEM., n=6. *p<0.05, **p<0.01 and ***p<0.001 vs control.

Table 2: Effect of hydroalcoholic extract of Moringa oleifera (HMO) on Magnesium sulfate induced Diarrhoea in Rats.

Groups	Treatment	Dose mg/kg	Mean frequency of diarrhoea ± SEM	Mean wt of fecal drops (g) ± SEM	Mean wt of faeces (g) ± SEM after 4hr	% protection
I	Control	1 mL	4.2±0.48	11.4±1.778	1.4±0.12	0.00
II	Loperamide	3	0.6±0.24***	1.6±0.67***	0.12±0.05***	91.42
III	HMO	100	1.6±0.24***	3.0±0.83***	0.28±0.80***	80.00
IV	HMO	250	1.2±0.5***	2.6±0.92***	0.26±0.81***	81.42
V	HMO	500	1.0±0.31***	2.0±0.54***	0.14±0.05***	90.00

HMO, Hydroalcoholic extract of Moringa oleifera; g,gram;
The values are mean ± SEM., n=6. *p<0.05, **p<0.01 and ***p<0.001 vs control.

Results:

In a model of castor oil and magnesium sulfate induced diarrhoea, among all the extracts being effective, the higher dose produced a significant decrease in mean frequency, mean number of faecal drops and means weight of faeces with respective to control as shown in Table 1 and 2.

Both, Castor oil and PGE2 failed to produce enteropooling in animals at all doses of the extract administered as shown in Tables 3 and 4. The protective effect of the extract indicates, the anti enteropooling effect of Moringa oleifera. The low dose of the extract produced more inhibition of enteropooling than the other doses tested in PGE2 model of diarrhoea, the reason for which could not be explained.

Table 3: Effect of hydroalcoholic extract of Moringa oleifera (HMO) on castor oil induced Enteropooling in Rats.

Groups	**Treatment**	**Dose mg/kg**	**Mean volume of intestinal fluid (ml) ± SEM**	**% protection**
I	Control	1 mL	2.82±0.058	0.00
II	Loperamide	3	0.34±0.050***	87.94
III	HMO	100	1.54±0.050***	46.09
IV	HMO	250	0.7±0.070***	75.17
V	HMO	500	1.4±0.083***	50.35

mL, Millilitres; The values are mean ± SEM., n=6. ***p<0.001 vs control.

Table 4: Effect of hydroalcoholic extract of Moringa oleifera (HMO) on PGE2 induced Enteropooling in Rats.

Groups	Treatment	Dose mg/kg	Mean volume of intestinal fluid (ml) ± SEM	% Protection
I	-ve control	1 mL	1.35±0.067	--
II	+ve control	PGE2 100µg	2.58±0.10	87.94
III	Loperamide	3	0.49±0.08***	81.00
IV	HMO	100	0.7±0.079***	72.86
V	HMO	250	1.1±0.103***	57.36
V1	HMO	500	1.85±0.055***	28.29

mL, Millilitres; µg, microgram; -ve, negative; +ve, positive; The values are mean ± SEM., n=6. ***p<0.001 vs control.

Table 5: Effect of hydroalcoholic extract of Moringa oleifera (HMO) on charcoal meal induced hyperperistalsis.

Groups	Treatment	Dose mg/kg	Mean length of intestine (cm)± SEM	Mean distance travelled by charcoal meal (cm) ± SEM	Mean % movement of charcoal ± SEM after 30min	% Inhibition
I	Control	1mL	47.20±1.02	40.40±3.20	85.59±2.27	14.26±2.24
II	Atropine sulfate	5	44.80±1.7	22.83±2.75***	51.04±0.54***	48.95±0.54
III	HMO	100	43.33±1.30	26.50±1.52***	61.01±3.35***	38.99±3.35
IV	HMO	250	46.3±1.514	25.50±1.04***	55.12±2.66***	44.87±2.66
V	HMO	500	46.2±1.490	27.10±1.38***	58.24±2.25***	41.86±2.25

cm, centimeter; the values are mean ± SEM., n=6. **p<0.01 and ***p<0.001 vs control.

In a model of charcoal meal induced hyperperistalsis, at all the doses tested, a decrease in the movement of charcoal from pylorus to caecum was observed as given in Table 5.

Discussion:

The results of the present study could suggest Moringa oleifera mechanism of action in inhibiting diarrhea involves anti propulsive and antisecretory effects. Hydroalcoholic extract of Moringa oleifera leaves showed significant inhibitory activity against castor oil and magnesium sulfate induced diarrhea, PGE2- and castor oil induced enteropooling, a significant reduction in gastrointestinal motility during charcoal meal test in rats. The extract also posse's non-specific spasmolytic activities as it inhibited the motility in charcoal meal test.

Castor oil or its triglyceride is hydrolyzed by lipases in the small bowel to glycerol and ricinoleic acid, which acts primarily in the small intestine to stimulate secretion of fluid and electrolytes and speed up the intestinal transit. Further, it is supported by the release of prostaglandins, which results from the inflammation and irritation effect of the ricinoleic acid [21]. These prostaglandins then cause an increase of intestinal motility and secretions into the lumen of the intestine [22]. The antidiarrhoeal activity of the extract against experimentally induced diarrhoea by castor oil may be attributed to an anti-electrolyte permeability action. The significant reduction in frequency of defecation, number of faecal droppings and mean weight of stool demonstrates the efficacy of moringa oleifera leaves as an effective antidiarrhoeal agent. Magnesium sulfate similarly causes an increase in the electrolyte secretion by creating an osmotic imbalance [23]. The extract sufficiently counteracted the increase in electrolyte secretion by means of an anti-electrolyte permeability action.

All the doses of tested, the extract showed a significant protection against PGE2 and castor oil

induced enteropooling, which might be due to the inhibition of synthesis of prostaglandins. Anti-enteropooling effect of the extract is more relevant because the prevention of enteropooling helps in the inhibition of diarrhea, especially by PGE2 induced diarrhea as it is involved in the onset of diarrhoea in intestinal mucosal cells. Although intraluminally administered PGE2 is known to induce duodenal and jejunal secretion of water and of electrolytes such as CL and Na, fluid content is the principal determinant of stool volume and consistency [24]. Net stool fluid content reflects a balance between luminal input (ingestion and secretion of water and electrolytes) and output (absorption) along gastrointestinal tract.

Tannic acid present in many of the plant extracts are shown to form a complex with the luminal proteins which then precipitate and form a coat over the intestinal line and reduce secretion in a model of charcoal induced hyper peristalsis [25]. The extract possessed anti spasmodic effect and anti enteropooling effect by which the extract produced relief in diarrhoea.

The specific constituent(s) responsible for the anti-diarrheal properties of leaves of this plant are yet to be identified. None of the several phytochemical constituents identified and isolated from the leaves has been reported to possess anti-diarrheal properties. Although phytochemical analysis of the extract in this study revealed the presence of alkaloids, carbohydrates, flavonoids, and terpenes, our current experimental data is insufficient to directly ascribe the anti-diarrheal activity to any of them. Elsewhere, the anti-diarrheal properties of some medicinal plants have been attributed to their phytochemical constituents like tannins and some flavonoids [25], which have been shown to possess anti-diarrheal activity attributable to their ability to inhibit intestinal motility and hydro-electrolytic secretion.

To conclude, the results of the present study indicate that hydroalcoholic extract of leaves of Moringa oleifera posse's significant antidiarrhoeal properties, thus supports the traditional use of Moringa oleifera leaves in treatment of diarrhoea.

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