Evaluation of Antidiarrhoeal Activity of Extract of Moringa Oleifera Pods

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ABSTRACT

The aim of the current study was to evaluate the antidiarrhoeal activity of the 70% hydroalcoholic extract of *Moringa oleifera* pods using different animal models of diarrhea in rats. Castor oil and magnesium sulfate-induced diarrhea rats were studied using loperamide as reference standard drug in rats. The onset of diarrhea, the mean number of fecal drops and mean weight of feces were determined. Prostaglandin E2 and castor oil-induced enterpooling was studied in rats by measuring the mean volume of intestinal fluid. Charcoal meal test was performed using atropine sulfate as a reference standard in rats. The mean distance passed by charcoal meal and mean percent movement of charcoal after 30 min were recorded. The results of the present study revealed that the extract of *Moringa oleifera* pods demonstrated dose-dependent antidiarrhoeal activity in all experimental animal models of diarrhea due to the presence of pharmacologically effective component(s).

Key Words: *Moringa oleifera, antidiarrhoeal, castor oil, magnesium sulfate, charcoal meal.*

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INTRODUCTION

Diarrhoeal diseases are major contributors to child and adult mortality and morbidity. It is characterized with common symptoms; the patient defecates more frequently than normal time and loose watery stools with mucus, blood pus or undigested food. Diarrhea that starts and suddenly lasts for longer than a couple of days are referred to acute diarrhea and if lasts more than two weeks is referred to chronic diarrhea [1]. Causes of diarrhea are associated with many different problems. Mainly when infectious organisms or agents come in contact with the body are more prone to produce diarrhoeal disease; for example, a virus such as rotavirus, Norwalk virus or Norovirus, Enterovirus or Hepatovirus, a bacterium such as *E. coli*, *Salmonella*, *Clostridium* or *Vibrio cholera*, a parasite that causes giardiasis or amoebiasis.

Diarrhea is primarily treated for avoiding dehydration and replacement of lost fluid which can be managed by drinking water, fruit juices and electrolyte like oral rehydration solutions. The effective antidiarrhoeals such as diphenoxylate, loperamide, diloxanide furoate and antibiotics like ofloxacin, and ornidazole are used to cure the symptoms. These drug cures diarrhea, but produce undesirable adverse effects such as intestinal obstructions, vomiting, fatigue, anxiety, constipation, dry mouth, dizziness, stomach pain, and discomfort by loperamide [2, 3].

Therefore, the search for a potent, safe and cost-effective antidiarrhoeal agent from herbal origin has become the most desirable area of research. Nature has provided us a great variety of medically valuable plants [4]. The medicinal plants have the most exceptional potential for benefitting people [5]. *Moringa oleifera* (Family: Moringaceae) has traditionally been used to improve health. Its leaves have been utilized to treat 24 medicinal conditions [6]. Hence, it can be used to market healthier products without synthetic additives [7]. It is commonly

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known as drumstick, a very popular backyard tree. Flower, tender leaves, and pods are eaten as vegetables. *Moringa* is an evergreen tree with 8-12 m in height [8]. Currently, the research on the genus *Moringa* is limited to *M. oleifera*, *M. stenopetala*, *M. concanensis*, and *M. peregrina*. It contains some amino acids, minerals, carotene, vitamins, and phenolics [9]. All parts of the plant have been used in folk medicines for the treatment of various diseases. Various parts of the title plant have been reported to possess antihypertensive, antiulcer, hepatoprotective, antitumor and anticancer, anti-inflammatory, antibacterial, antifungal, antifertility, antidiabetic, and CNS depressant activities [10]. Antidiarrhoeal activity of leaves [11] and root bark extract [12] have been already reported in the literature. However, the scientific validation of antidiarrhoeal property of pods of drumstick has not been documented in the literature so far. Hence, the present study was undertaken.

MATERIAL AND METHODS

Plant material collection

For this study, mature pods of *Moringa oleifera* were collected from the surrounding gardens of the Vijayapur, after the sample authenticated by Dr. P D Needagi, Professor and HOD of Botany, SB Arts and KCP Science College, Vijayapur, Karnataka.

Preparation of extract

Fresh mature pods were cleaned, cut into small pieces, shade dried at room temperature and powdered using a grinder. Then the powdered material was extracted with 70% hydroalcohol by Soxhlet's extraction procedure at a temperature between 60-70^{0}C. Thereafter, the extract was concentrated using rotary flash evaporator. The yield was found to be 18%. The dried extract was stored in a refrigerator below 10 ℃ for further studies.

Preliminary phytochemical screening

The preliminary phytochemical investigation of hydroalcoholic extract of *Moringa oleifera* pods (HAEMOP) was carried out for detection of different phytoconstituents. Tests for the presence of phytochemicals were performed by standard methods described by Dr. Khandelwal K. R. and Trease and Evans.

Animals used

Albino rats (Wistar strain) weighing 150-200 g of either sex and albino mice weighing 20-25 g were used in the present study. They were procured from Venkateshwar enterprises, Rajajinagar, Bangalore. The animals were acclimatized for ten days under standard laboratory condition. They were housed in polypropylene cage and maintained at 27 ±2 ℃, and relative humidity of 65±10% under 12 hr light/dark cycle. The animals were fed with rodent pellet diet and water. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC) before the initiation of the experiments. **[Reg. No. 1076/PO/Re/S/07/CPCSEA dated on 27thFeb 2017].**

Determination of acute toxicity [13, 14]

The acute toxicity (LD50) of *Moringa oleifera* pods extract was determined by fixed-dose method OECD guideline no. 423. The female albino mice weighing between 20-25g were fasted overnight prior to the experiment. 1/20th, 1/10th, and 1/5th LD50 cutoff value of the extract were selected as screening doses.

Screening of Antidiarrhoeal activity

A. Castor oil-induced diarrhea [15]

Rats weighing 150 - 200g of either sex were allocated into 5 groups of six animal each and fasted for 18h.

Group 1 - Control (Received vehicle- 5 ml/kg of 2% w/v *Acacia* suspension)
Group 2 - Standard (Loperamide 3mg/kg)
Group 3 - 100 mg/kg of HAEMOP
Group 4 - 200 mg/kg of HAEMOP
Group5 - 400 mg/kg of HAEMOP

Animals of each group received 1 ml of castor oil orally after 60 min of drug treatment. Rats were placed in transparent separate metabolic cages with pre-weighed plastic dishes placed at the base for 4 hours to monitor the onset of diarrhea, weighting fecal matter, and the number of fecal drops. The weight of the plastic dish before and after defecation was recorded. The antidiarrhoeal activity was determined by calculating percentage protection using the following formula:

$$\% \text{ Inhibition} = \frac{\text{MWFC} - \text{MWFT}}{\text{MWFC}} \times 100$$

Where MWFC = Mean weight of feces in the control group
MWFT = Mean weight of feces in the test group

B. Magnesium Sulfate-induced diarrhea [16]

Rats (150 - 200g) of either sex weighing were allocated into 5 groups of six animals in each group.

Group 1- Control (Received vehicle- 5 ml/kg of 2% w/v Acacia suspension)
Group 2- Standard (Loperamide 3mg/kg)
Group 3- 100 mg/kg of HAEMOP
Group 4- 200 mg/kg of HAEMOP
Group 5- 400 mg/kg of HAEMOP

All animals fasted for 18 hr before the above-mentioned treatment. Then after 60 min of drug treatment animals in all groups received magnesium sulfate (2 g/kg) orally. Animals were placed in transparent metabolic cages and pre-weighed plastic dishes were kept at the base and monitored for onset of diarrhea, weight fecal matter and number of fecal drops for 4 hours. The weight of the

plastic dish was noted before and after defecation and compared to control.

C. Prostaglandin E2 (PGE2) induced enterpooling [17]

Rats weighing (150 - 200g) of either sex were allocated into 6 groups of six animals in each group.

Group 1- Control (Received Vechicle-5 ml/kg of 2% w/v Acacia suspension)
Group 2- PGE2 (100 µg/kg p.o.)
Group 3- Standard (Loperamide 3mg/kg)
Group 4- 100 mg/kg of HAEMOP
Group 5- 200 mg/kg of HAEMOP
Group 6 - 400 mg/kg of HAEMOP

After 1 hr. of treatment, PGE2 was administered to each rat orally. Then each rat was sacrificed after 30 min of PGE2 administration. After that, the intestine was ligated at both pyloric and ileocaecal junction and dissected out. All intestinal contents were expelled into a graduated measuring cylinder and volume of intestinal content was measured.

D. Castor oil-induced enterpooling [18]

Rats weighing (150-200g) of either sex were allocated into 5 groups of six animals in each group.

Group 1- Control (Received vehicle- 5 ml/kg of 2% w/v Acacia suspension)
Group 2- Standard (Loperamide 3 mg/kg)
Group 3- 100 mg/kg of HAEMOP
Group 4- 200 mg/kg of HAEMOP
Group 5- 400 mg/kg of HAEMOP

The rats were fasted for 18 hr prior to above-mentioned treatment. 1 ml of castor oil was administered after 60 min of described drug treatment to all animals of the group. After 30 min of castor oil administration, all rats were sacrificed and intestine was ligated at both pyloric and ileocaecal junction and was dissected out. All intestinal contents were poured into a graduated measuring cylinder to measure the volume of intestinal content.

E. Charcoal meal test [19]

Rats weighing (150-200 g) of either sex were allocated into 5 groups of six animals in each group. Animals were fasted for 18 hr prior to drug treatment.

Group 1- Control (Received vehicle- 5 ml/kg of 2% w/v Acacia suspension)
Group 2- Standard (Atropine sulfate 5 mg/kg) i. m.
Group 3- 100 mg/kg of HAEMOP
Group 4- 200 mg/kg of HAEMOP
Group 5 - 400 mg/kg of HAEMOP

1 hr after drug treatment, 1 ml of the charcoal meal (3% charcoal suspension in 3% acacia suspension) was given by oral route. Then 30 min later all animals were sacrificed, their intestines were dissected out. It extended on a clean glass surface, the distance passed from pylorus was measured and expressed as mean % of the movement of charcoal using the following formula:

$$\% \text{ movement of charcoal} = \frac{\text{Mean distance passed by charcoal meal}}{\text{mean length of the intestine}} \times 100$$

RESULTS

Phytochemical Phytochemical Screening

The results of preliminary phytochemical screening on HAEMOP are summarized in Table -1

Table 1: Preliminary Phytochemical Screening of HAEMOP

Sr. No.	Phytochemical Constituents	Inference
1	Carbohydrate	+
2	Proteins	++
3	Alkaloids	+
4	Phenolic compounds	-
5	Tannins	++ +
6	Steroids	-
7	Flavonoids	+++
8	Saponin glycosides	-
9	Coumarin glycosides	-
10	Cardiac glycosides	+
11	Lipids	+

Determination of acute toxicity studies

In acute toxicity studies, test extract of *Moringa oleifera* plant did not produce any mortality of the animals at the dose of 2000 mg/kg. Hence, 2000 mg/kg was fixed as LD_{50} cut off value as per the fixed-dose method, OECD (Organization for Economic Corporation Development) guideline No. 423 (Annexure 2d). The screening doses selected for the antidiarrhoeal effect of test extract of title plant were:

1. 100 mg/kg – $1/20^{th}$ dose of 2000 mg/kg b.w.
2. 200 mg/kg – $1/10^{th}$ dose of 2000 mg/kg b.w.
3. 400 mg/kg – $1/5^{th}$ dose of 2000 mg/kg b.w.

Statistical analysis:

The results were expressed in mean ± SEM. All data obtained from the above study were subjected for one-way ANOVA followed by Tukey's Kremer Multiple Comparison Test by using Prism Pad 5 software. $p< 0.05$ was found statistically significant.

Evaluation of antidiarrhoeal activity

A. Castor oil-induced diarrhea

The antidiarrhoeal activity demonstrated by HAEMOP was found to be dose-dependent at different doses in castor oil-induced diarrhea model. Graded doses viz 100, 200, and 400 mg/kg produced a significant decrease in

mean fecal drops, the weight of feces, and the onset of diarrhea as compared with control. The % protection was found to be 41%, 45%, and 51% at graded doses of extract, respectively. The results are depicted in table 2.

Table 2: Effect of HAEMOP on castor oil-induced diarrhea in rats

Groups	Treatment	Dose mg/kg	The onset of diarrhea (min) ± SEM	Mean No. of fecal drops ± SEM	Mean wt of feces (g)± SEM after 4hr	% protection
1	Control	--	40.16 ±1.84	11.16±1.97	0.70±0.09	--
2	Standard (Loperamide)	3	73.13±2.1***	2.90±0.42***	0.21±0.04***	**70.00**
3	HAEMOP	100	54.18±2.86**	5.28±0.05**	0.41±0.03**	41.00
4	HAEMOP	200	61.96±2.00***	4.92±0.81**	0.38±0.03***	45.00
5	HAEMOP	400	65.90±2.12***	4.10±0.14***	0.34±0.02***	**51.00**

The values are Mean ± SEM., n=6. **p<0.01 and ***p<0.001 v/s control.

B. Magnesium sulfate ($MgSO_4$)-induced diarrhea

In $MgSO_4$-induced diarrhea model, test extract demonstrated dose-dependent antidiarrhoeal potential which was evident by monitoring significant delay in the onset of diarrhea and decrease in the number of fecal dropping and weight of stool when compared to control group. The higher dose (400 mg/kg) of test extract produced 44% protection of diarrhea which was found to be less potent than standard. The results are shown in Table 3.

Table 3: Effect of HAEMOP on $MgSO_4$-induced diarrhea in rats

Groups	Treatment	Dose mg/kg	The onset of diarrhea (min) ± SEM	Mean No of fecal drops ± SEM	Mean wt of feces (g) ± SEM after 4hr	% protection
1	Control	--	42.14±2.00	11.69±1.23	2.14±0.21	--
2	Standard (Loperamide)	3	89.96±1.98***	2.26±0.78***	0.58±0.14***	**72**
3	HAEMOP	100	51.35±1.53**	8.13±0.81^{ns}	1.41±0.04**	34
4	HAEMOP	200	57.38±1.10***	7.10±0.93**	1.29±0.02***	39
5	HAEMOP	400	66.94±1.29***	4.91±0.48***	1.19±0.02***	**44**

The values are Mean ± SEM., n=6. **p<0.01 and ***p<0.001 v/s control.

C. Prostaglandin E2 (PGE2)-induced diarrhea

There was a significant increase in intestinal fluid volume seen in PGE2 control over normal control rats. The HAEMOP significantly (p< 0.001) and dose-dependently reduced the mean volume of intestinal fluid in PGE2 induced enterpooling. In the extract-treated groups, the volume of intestinal content was found to be inhibited by 50-64% and by standard 77% in respect to that of PGE2 control rats. Results are presented in Table 4.

Table 4: Effect of HAEMOP on PGE2-induced enterpooling in rats.

Groups	Treatment	Dose mg/kg	Mean volume of intestinal fluid (ml) ± SEM	% Inhibition
1	Control	----	1.86±0.09	-----
2	PGE2	100 µg/kg	2.97±0.11@	-----
3	Standard (Loperamide)	3	0.68±0.03***	**77**
4	HAEMOP	100	1.48±0.06**	50
5	HAEMOP	200	1.21±0.03***	59
6	HAEMOP	400	1.05±0.04***	**64**

The values are Mean ± SEM., n=6. @ p<0.001 vs control ,**p<0.01 and ***p<0.001 v/s PGE2.

D. Castor oil-induced enterpooling

In castor oil-induced enterpooling model, test extract significantly inhibited volume of intestinal fluid in rats at the oral dose of 100, 200, and 400 mg/kg by 46%, 51%, and 54% respectively when compared to that of control. The standard (loperamide 3 mg/kg) also significantly suppressed intestinal fluid volume by 63%. The results are given in Table 5.

Table 5: Effect of HAEMOP on castor oil-induced enterpooling in rats

Groups	Treatment	Dose mg/kg	Mean volume of intestinal fluid (ml) ± SEM	% Inhibition
1	Control	----	2.38±0.14	-----
2	Standard (Loperamide)	3	0.86±0.08***	**63**
3	HAEMOP	100	1.28±0.07**	46
4	HAEMOP	200	1.16±0.03**	51
5	HAEMOP	400	1.09±0.04***	**54**

*The values are Mean ± SEM., n=6. **p<0.01 and ***p<0.001 v/s control.*

E. Charcoal meal test

All doses of HAEMOP and standard (atropine sulfate) demonstrated a decrease in the movement of charcoal propulsion of charcoal meal as compared to the control group of rats. The distance traveled by the charcoal meal was found to be decreased in the graded dose of test extract. Mean distance traveled by charcoal meal was found to be 20 to 17 % and the percentage inhibition of charcoal meal found to be 48-63 % respectively at 100, 200, and 400 mg/kg doses of the extract. Whereas the distance traveled by the charcoal meal and the percentage inhibition of charcoal meal observed in the standard drug-treated group was 18% and 60%, respectively. The intestinal antimotility effect at the dose of 400 mg/kg was found to be more potent when compared to atropine sulfate. The results are shown in Table 6.

Table 6: Effect of HAEMOP on gastrointestinal motility (Charcoal meal test) in rats

Groups	Treatment	Dose mg/kg	Mean length of the intestine (cm) ± SEM	Mean distance traveled by the charcoal meal (cm)± SEM	Mean %movement of charcoal	% inhibition
1	Control	--	94.98± 2.12	47.23±1.98	49	--
2	Standard (Atropine sulphate)	5	90.51± 1.98	18.67±1.17***	20	**60**
3	HAEMOP	100	89.98± 1.23	24.42±1.47***	27	48
4	HAEMOP	200	84.12± 1.91	20.23±1.32***	24	57
5	HAEMOP	400	78.53± 1.35	17.15±1.12***	21	**63**

The values are Mean ± SEM., n=6. ***p<0.001 v/s control.

DISCUSSION

In the present study, the antidiarrhoeal effect of 70% hydroalcoholic extract of *Moringa oleifera* pods was evaluated. Antidiarrhoeal potency of HAEMOP was performed using castor oil and MgSO4 induced diarrhea, PGE2 and castor oil-induced enterpooling, and charcoal meal test in rats.

Castor oil is converted to its active metabolite ricinolic acid by enzymatic hydrolysis which causes the release of prostaglandins that initiates peristaltic activity of the intestine. This mechanism leads to the alteration of water and electrolyte permeability and thus increases the volume of intestinal content by inhibiting the reabsorption of water [20]. In the present study, hydroalcoholic extract of *Moringa oleifera* pods exhibited antidiarrhoeal activity by significantly reducing the number of diarrhoeal episodes in a dose-related manner by delaying the onset of diarrhea, decreasing the number of fecal drop and weight of feces against castor oil-mediated diarrhea in rats. The extract demonstrated antidiarrhoeal activity probably by increasing the reabsorption of NaCl and water and due to antisecretory effect [21].

$MgSO_4$ has been reported to induce diarrhea by an increase in the volume of intestinal content through prevention of water reabsorption. It has been demonstrated that it provokes the release of cholecystokinin from duodenal mucosa, that increases secretion and motility of small intestine and thereby prevent water and electrolyte reabsorption [22]. In our study, the test extract exhibited a dose-dependent diminishment in fecal dropping, the onset of action and weight of stool. We postulate that the HAEMOP promisingly counteracted the increase in electrolyte secretion by means of an antielectrolyte permeability action in $MgSO_4$-challenged diarrhea.

Graded doses of HEAMOP showed significant protection against PGE2 and castor oil-induced enterpooling models (it was evidenced by monitoring the significant decrease in intestinal fluid accumulation), which might be due to inhibition of prostaglandins synthesis. Evaluation of antienterpooling effect of the extract is more relevant because the prevention of enterpooling helps in the inhibition of diarrhea [23]. In this study, loperamide used as reference drug that acts by inhibiting the peristaltic activity through an indirect effect on circular and longitudinal muscles of the intestinal wall, also by stimulating the absorption of water and electrolyte [24]. Loperamide in the current study demonstrated reduced diarrhoeal episode, the weight of fecal matter, number of fecal droppings, and volume of intestinal content in experimental models.

Gastrointestinal motility test using activated charcoal as a marker has been used for the evaluation of the antidiarrhoeal effect of test compounds for many years. Charcoal meal test was used to determine the effect of the test substance on gut motility. In gastrointestinal motility test, HAEMOP suppressed the propulsive movement of transit of charcoal meal through the GIT which demonstrates that the extract may be able to reduce the frequency of stool in diarrhoeal condition [25]. In the charcoal passage test, atropine sulfate was administered which is an antimuscarinic agent that inhibits gastrointestinal motility [26]. The mean distance traveled by the charcoal meal in standard was reduced in comparison with control due to a decrease in gastrointestinal propulsion.

Presence of tannins and flavonoids content in herbal extracts are reported to have antidiarrhoeal activity [27, 28]. The content of tannin in the plant extract form

protein tannate that decreases intestinal secretion, which might be beneficial in antidiarrhoeal effect. The qualitative phytochemical analysis of the *Moringa oleifera* pods extract reveals the presence of tannins and flavonoids and this could be the reason for exhibiting the antidiarrhoeal activity in the present study.

CONCLUSION

The results of the current study revealed that 70% hydroalcoholic extract of *Moringa oleifera* pods (HAEMOP) demonstrated dose-dependent antidiarrhoeal activity due to the presence of pharmacologically effective component(s).

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Conflict of Interest

The authors do not have any conflict of interest.

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