



Research Article

Antidiarrheal Activity of Methanolic Extract of *Moringa oleifera* Lam Roots in Experimental Animal Models

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ABSTRACT

Among medicinal plants, *Moringa oleifera* Lam. has been recommended for several disorders in folk medicine. Indian Materia Medica describes the use of roots of *Moringa oleifera* Lam in the treatment of a number of ailments, including asthma, gout, lumbago, rheumatism, enlarged spleen or liver, etc. Nevertheless, no pharmacological studies of *Moringa oleifera* Lam root have thus far evaluated for its anti diarrheal activity.

Thus the purpose of the present study is to evaluate scientifically the effect of hydroalcoholic (50:50) extract of root of *Moringa oleifera* Lam against castor oil induced diarrhea models in rats. The parameters used for the evaluations are the decrease in severity and frequency in diarrheas caused due to castor oil, further to understand the probable mechanism of its anti-diarrhoeal activity, its effect was evaluated on intestinal transit, castor oil induced intestinal fluid accumulation (enteropooling) and electrolyte concentration in the small intestinal fluid. The methanolic root extract of *Moringa oleifera* Lam 200 (p<0.01) and 400 mg/kg (p<0.001) produced a significant reduction in the severity and frequency of diarrhea, intestinal fluid accumulation, the volume of intestinal content and intestinal transit compared to normal saline control group, dose dependently more than atropine (3mg/kg i.p). This signifies the usefulness of this model and the clinical effect of the extract.

Moringa oleifera Lam root extract may be useful in a wide range of diarrheal states due to both disorders of transit e.g. functional diarrhoeas, radiation diarrhoea or due to abnormal secretory mechanisms like in cholera or E.coli entero-toxin induced diarrhoea. Further studies are necessary for chemical characterization of the active principles and more extensive biological evaluations.

Keywords: *Moringa oleifera*, intestinal fluid accumulation, volume of intestinal content, intestinal transit, radiation diarrhoea.

INTRODUCTION

Moringa oleifera Lam. or the horseradish tree, (Moringaceae) is a small- to medium-sized tree, found almost all over the plains of India. Although the name "Shigon" for *M. oleifera* is mentioned in the "Shushruta Sanhita" of India, which was written in the beginning of the first century AD, there is evidence that the cultivation of this tree in India dates back many thousands of years [1]. Various parts of this plant were used in tribal medicine for the diseases like sores, dysentery, pneumonia, cancer etc [2]

Moringa oleifera Lam contains several phytochemicals, some of which are of high interest because of their medicinal value, in particular this plant family is rich in a fairly unique group of glycoside compounds called glucosinolates and isothiocyanates.

Recent studies demonstrate that isothiocyanates have antitumor activity in cancers of the lung, breast, skin, esophagus, and pancreas [3, 4]. Small proteins/peptides were isolated from the leaves of *Moringa oleifera* possessing antifungal and antibacterial activity [5]. The methanolic extract of the root (ME) contains moringine and moringinine which are reported to possess analgesic and anticonvulsive activity [6].

In developing countries, a majority of people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases including diarrhoea. Diarrhoea is a major health problem especially for children under the age of 5 and up to 17% of children admitted in the paediatrics ward die of diarrhoea. 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 [7]. According to W.H.O. estimates for 1998, about 7.1 million deaths were caused by diarrhea [8]. The incidence of diarrhoeal diseases still remains high despite the efforts of many governments and international organisations to curb it. It is therefore important to identify and evaluate available natural drugs as alternatives to currently used anti-diarrhoeal drugs, which are not always free from adverse effects [9]. A range of medicinal plants with anti-diarrhoeal properties is widely used by traditional healers. However, the effectiveness of many of these anti-diarrhoeal traditional medicines has not been scientifically evaluated.

Hence, the study was undertaken to evaluate the ethanolic root extract of *Moringa oleifera* Lam for its

antidiarrheal activity in castor oil induced diarrhoea models.

EXPERIMENTAL METHODS

Experimental animals

Wistar rats (200-250 g) of either sex bred in Central Animal House facility of the Institute were used. The animals were housed under standard conditions, maintained on a 12 h light/dark cycle and had free access to food and water up to the time of experimentation. The animals were acclimatized to the laboratory environment 1 h before the experiments. Animals were randomly distributed into groups of 6 animals each. Each animal was used only once. All experiments were conducted during the light period (08.00-16.00 h). All the protocols were approved by the Institutional Animal Ethical Committee (SDPC/IAEC-RP-087) and conducted according to the Indian National Science Academy Guidelines (INSA) for the use and care of experimental animals.

Plant material and preparation of extract

The roots of *Moringa oleifera* Lam were collected from Surat (Gujarat). Their authenticity was confirmed by a Taxonomist, Department of Bioscience, Veer Narmada south Gujarat university, Surat, Gujarat. A specimen of plant is kept in the herbarium of our institute (Voucher No. SDPC/PA/MOR-08). The roots were completely dried in the sunlight and powdered. Root powder was extracted exhaustively with 50% methanol by maceration for 2 days at room temperature with frequent shaking. Crude (hydroalcoholic) extract was filtered and dried under reduced pressure at 40°C (Yield - 9.3% w/w of dried plant material). Aqueous solution of dried extract (MO) in suitable dilution in distilled water is administered in test animals.

Preliminary phytochemical screening

The presence of carbohydrates, proteins, alkaloids, flavanoids, glycosides, saponins, tannins and essential oils were tested using standard procedures [10].

Drugs

Atropine sulphate (inj.) served as a standard drug for castor oil induced diarrhea, enteropooling and small intestine transit time. The dose of the methanolic extract of *Moringa oleifera* Lam was selected randomly as 100,200 and 400 mg/kg body weight of the animal used.

Toxicity studies

LD₅₀ and lowest published toxic dose (TDLo) of root extract of *Moringa oleifera* Lam are 500 mg/kg

and 184 mg/kg, respectively, when used intraperitoneally in rodents (mice) [11].

Castor oil-induced diarrhoea

Rats were divided into five groups of six animals each, diarrhoea was induced by administering 1ml of castor oil orally to rats. Group 1 served as control (2ml/kg, i.p.saline), group 2 received atropine (3mg/kg, i.p.) served as standard and group 3, 4, and 5 received the methanolic extract of *Moringa oleifera* (100,200 and 400 mg/kg, i.p.), 1 h before castor oil administration. The numbers of both wet and dry diarrheal droppings were counted every hour for a period of 4 h mean of the positive control group consisted of animals given an intraperitoneal injection of saline (2ml/kg,ip)[12].

Castor oil-induced enteropooling

Overnight fasted rats were divided in to five groups of six animals each. Group 1 received normal saline orally (2ml/kg.) served as a control, group 2 received atropine (3mg/kg, i.p.) and groups 3, 4 and 5 received the methanolic extract of *Moringa oleifera* (100,200 and 400 mg/kg) intraperitoneally respectively 1h before the oral administration of castor oil. Two hours later the rats were sacrificed, the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by milking in to a graduated tube and their volume was measured. The intestine was reweighed and the difference between full and empty intestines was calculated [13].

Small intestinal transit

Rats were fasted for 18 h and divided into six groups of six animals each, Group 1 received 2ml of castor oil orally with saline 2 ml/kg intraperitoneally, group 2 received atropine (3mg/kg, i.p.), group 3,4, and 5 received 100,200 and 400mg /kg intraperitoneally of the methanolic plant extract respectively, 1 h before the administration of castor oil. One ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 h after castor oil treatment. The rats were sacrificed after 1h and the distance travelled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum [14].

Statistical analysis

The data was expressed as mean \pm S.E.M. (standard error of the mean). student's t-test was used for the evaluation of data and $p < 0.05$ accepted as significant.

RESULTS

The results of the present study showed that the methanolic root extract of *moringa olifera* Lam produced a statistically significant reduction in the sever-

ity and frequency of diarrhoea produced by castor oil in a dose dependent manner 100mg/kg (47.67%), 200 mg/kg (56.25%) and 400mg/kg of the extract had inhibited defecation up to 61.74%(p<0.001 compared to normal saline control group), while atropine (3 mg/kg i.p) is 33.38%only.

It is also noted that the extract (400mg/kg)significantly inhibited castor oil induced intestinal fluid

accumulation and the volume of intestinal content up to 82.51% (p<0.001 compared to normal saline control group), dose dependently more than atropine (24.54%).Additionally the extract in the same dose had significantly reduced the castor oil induced intestinal transit up to 37.29% (p<0.001 compared to normal saline control group), while the atropine is 40.56%.

Table 1: Effect of methanolic extract of *Moringa olifera* Lam root on castor oil induced diarrhoea in rats.

Treatment/ Dose (mg/kg)	Mean defecation in 4 Hr.	% inhibition of defecation
Castor oil (1ml p.o) +Control (saline2ml/kg i.p)	21.33 ± 2.7039	-----
C.O+Atropine(3mg/kg i.p)	14.21± 0.9098	33.38 **
M.O(100mg/kg i.p) +Castor oil (1ml p.o)	11.16 ± 0.8027	47.67 **
M.O(200mg/kg i.p) + Castor oil (1ml p.o)	9.33 ± 1.8236	56.25 ***
M.O(400mg/kg i.p) + Castor oil (1ml p.o)	08.16 ± 1.3017	61.74 ***

Values are expressed as mean ± SEM from the experiments. *P<0.05, **P<0.01, ***P<0.001 when compared with CO+ saline treated group.

Table 2: Effect of methanolic extract of *Moringa olifera* Lam root on castor oil induced enteropooling in rats.

Treatment/ Dose (mg/kg)	Weight intestinal content	% inhibition in wt of intestinal content
Castor oil (1ml p.o) +Control (saline2ml/kg i.p)	7.09 ± 0.1227	-----
Castor Oil + Atropine(3mg/kg i.p)	5.35 ± 0.2054	24.54 *
M.O(100mg/kg i.p) +Castor oil (1ml p.o)	3.23 ± 0.1163	54.44 **
M.O(200mg/kg i.p) + Castor oil (1ml p.o)	1.98 ± 0.0497	72.07***
M.O(400mg/kg i.p) + Castor oil (1ml p.o)	1.24 ± 0.1172	82.51 ***

Values are expressed as mean ± SEM from the experiments *P<0.05, *P<0.01, **P<0.001 when compared with CO+ saline treated group.

Table 3: Effect of methanolic extract of *Moringa olifera* Lam root on castor oil induced small intestinal transit in rats.

Treatment/ Dose (mg/kg)	Total length of intestine	Distance traveled by marker	% of intestinal transit
Castor oil (1ml p.o) +Control (saline2ml/kg i.p)	112.65 ± 2.86	96.5 ± 3.01	85.66 ± 1.056
CastorOil+Atropine(3mg/kgi.p)	105.21 ± 2.82	42.68 ± 2.76	40.56 ± 1.146**
M.O(100mg/kg i.p) +Castor oil (1ml p.o)	99.51 ± 4.82	51.52 ± 4.36	51.77 ± 2.623*
M.O(200mg/kg i.p) + Castor oil (1ml p.o)	106.33 ± 3.04	44.23 ± 1.74	41.59 ± 0.963 **
M.O(400mg/kg i.p) + Castor oil (1ml p.o)	103.69 ± 3.04	38.58 ± 2.761	37.29 ± 2.626***

Values are expressed as mean ± SEM from the experiments *P<0.05, *P<0.01, **P<0.001 when compared with CO+ saline treated group.

DISCUSSION

The use of castor oil induced diarrhoea model in our study, is well justified because the autocoids and prostaglandins are involved these have been implicated in the causation of diarrhoeas in man [15,16]. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion [17].

In this study, atropine produced a significant reduction in the number of stools and increased intestinal transit time possibly due to its anti-cholinergic effect [18]. However, it did not inhibit castor oil induced enteropooling and gain in weight of intestinal content suggesting thereby that mediators other than acetylcholine are involved in castor oil induced enteropooling. An increase in intestinal transit time with atropine could also result due to reduction in gastric emptying [19].

Castor oil is also reported to induce diarrhoea by increasing the volume of intestinal content by prevention of the reabsorption of Water. The liberation of ricinoleic acid results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [17]. Thereby prevents the reabsorption of sodium chloride and water [20]. Probably extract increased the reabsorption of sodium chloride and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal suggesting its sympatholytic activity of *Moringa olifera Lam* [21].

The antidiarrheal activity of the extract may also be due to the presence of denature proteins forming protein tannates, which make the intestinal mucosa more resistant and reduce secretion [22].

The secretory diarrhoea is associated with an activation of Cl⁻ channels, causing Cl⁻ efflux from the cell, the efflux of Cl⁻ results in massive secretion of water into the intestinal lumen and profuse watery diarrhea [23]. The extract may inhibit the secretion of the water into the lumen by reverting this mechanism.

Anti-dysentric and antidiarrheal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars [24]. The preliminary phytochemical analysis of the root extract of *Moringa olifera Lam* revealed the presence of sugars, alkaloids (moringine and moringinine), phenols, flavanoides, isothiocyanates, thiocarabamate glycosides, tannins [25,26,27]. These constituent may mediated the antidiarrhoeal activity of the root extract of *Moringa olifera Lam*. The over all possible mechanism may be due to, inhibition of release of autocoids and prostaglandins thus inhibiting the motility and secretion induced by castor oil or alteration of the activity of Na⁺K⁺ATPase or activation of chloride channels by

the root extract of the *Moringa olifera Lam* which is still to be understood.

Castor oil induced is a suitable model of diarrhea in rats, since it allows the observation of measurable changes in the number of stools, enteropooling and intestinal transit. The extract resulted in a marked reduction in the number of diarrhoea stools and the reduction in the weight and volume of the intestinal contents, as well as a modest reduction in intestinal transit. This signifies the usefulness of this model and the clinical effect of the extract.

CONCLUSION

In conclusion, the remarkable antidiarrhoeal effect of *Moringa olifera Lam* root extract against castor oil induced diarrhea model attest to a wide range of utility in secretory and functional diarrhoeas [28]. Whatever, may be the mechanism of action, *Moringa oleifera Lam* root extract may be useful in a wide range of diarrheal states due to both disorders of transit e.g. functional diarrhoeas, radiation diarrhea or due to abnormal secretory mechanisms like in cholera or *E.coli* entero-toxin induced diarrhea. Further, in our laboratories studies are in progress to completely understand the mechanism of anti-diarrheal action of *Moringa olifera Lam* root extract.

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