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Evaluation of anti diarrheal potential of *Moringa oleifera* (Lam.) leaves

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ABSTRACT
The objective of present study was to evaluate in vivo anti-diarrheal potential of *Moringa oleifera* leaves. Sample was analyzed for quantative estimation of phytochemicals and anti-diarrheal activity of ethanol extract, at 150 and 300 mg/kg body weight (b.w) was investigated using castor oil induced model. Study reveals that protein was maximum in leaves (23.35%) followed by fiber, starch, oil, sugar, alkaloid, tannin, flavanoid and phenolic. At dosages of 150 and 300 mg/kg b.w extract showed significant ($p<0.01$) activity, as compared to control. In course of observation for 4 hr, oral dose of 300mg/kg b.w showed remarkable dose dependent delay in onset of copious diarrhoea, decrease in the frequency of purging, weight of wet stools, weight of total stool and percentage protection. Study, thus signifies the potential clinical effect of extract in disease and needs to be extended further for activity guided extraction/isolation of the active chemical moiety.

Keywords: Castor oil, Diarrhea, Loperamide, *Moringa oleifera*

1. Introduction
Moringa oleifera Lam. (Moringaceae) is a small- to medium-sized tree, abundantly found in almost all over the plains of India. It is mentioned as "Shigon" in the "Shushruta Samhita", supporting the evidence that cultivation of this tree in India was dates back to thousands of years. Several parts of the specie were used in tribal/traditional medicine for the diseases like sores, dysentery, pneumonia, cancer, etc. *Moringa* contains various phytochemicals, some of which are of high interest because of their medicinal values; in particular this plant is rich in a fairly unique group of glycoside compounds called as glucosinolates and isothiocyanates.
In developing countries, a majority of people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases including diarrhea, which is very common and recurring disease in community [1-3]. Diarrhea is a major health problem especially for children under the age of 5 and up to 17% of infected children die off with this disease. Worldwide distribution of diarrhea accounts for more than 5-8 million deaths each year in infants and children below 5 years especially in developing countries [4]. According to W.H.O. estimates, about 7.1 million deaths were caused by diarrhea [5]. It is therefore important to identify and evaluate available natural alternatives to currently used anti diarrheal drugs, which are not always free from adverse effects[6]. Literature suggested that some parts of this species had potential as an anti diarrheal agent. A study on anti diarrheal activity of *Moringa* roots had reported earlier [1]. In other experiments anti diarrheal activity in methanol, aqueous and hydroalcoholic extract of *M. oleifera* leaves were also evaluated [7, 8]. Some studies were also carried out on antibacterial activity of various parts of species [9-11], which includes assay on coliform bacteria (major causative microorganism for diarrhea), also [12]. In similar series of action an attempt has been made to analyze the various phytochemicals present in the leaves and to evaluate the activity potential in ethanol extract of *M. oleifera* leaves through castor oil induced model in experimental animals.

2. Materials and Methods
2.1 Drugs
Doses of the extract (Test) was selected as 150 mg/kg (T_1) and 300 mg/kg (T_2) b.w on the basis of toxicity studies, which was observed at maximum dose of 5000 mg/kg b.w. Loperamide hydrochloride is used as standard drug.

0.5% Carboxy Methyl Cellulose (CMC) in distilled water is used as vehicle to make suitable dilutions of standard and extract[1, 13, 14]. All the chemicals and reagents (AR grade) are purchased from Sigma-Aldrich.

2.2 Plant material
Fresh leaves of *Moringa oleifera* were collected from Lucknow cantonment, authenticated and deposited in institute's herbarium [LWG 97845]. Sample was shade dried, grinded and sieved (40 mesh) to get uniformly coarse powder.

2.3 Phytochemical characterization
Quantification of various phytochemicals viz. Sugar & Starch[15], Oil[15], Tannin[16], Phenolic & Flavonoid[17], Fiber[18], Total protein[19] and Crude alkaloid content[20] were determined in *M. oleifera* leaves.

2.4 Preparation of extract and preliminary phytochemical screening
Powdered sample (500 g) was initially macerated for 7 hr with petroleum ether (40-60 °C) to remove the fatty component and then subjected to alcohol (absolute ethanol, 99.99%) as a solvent for 7 days (Temp. 27±2 °C) with intermittent shaking. Crude extract was filtered and dried under reduced pressure at 40 °C.
Qualitative estimation of carbohydrates, proteins, alkaloids flavanoids, tannins, saponins, steroids, anthraquinones and cyanogenic glycosides were performed as per standard procedures [21, 22] in ethanol extract of leaves.

2.5 Experimental animals
Animals (Sprague-Dawley rats) of 125-175 g were selected and randomly divided into six groups (n=6) for screening. Two groups for test doses (150 and 300 mg/kg) of ethanol extract, while one each for standard drug and control respectively. Animals were placed in cages, fed with standard diet and water (Temp 27±2 °C). Before treatment animals were fasted overnight of food but not water [23]. Conditions were maintained as per animal ethical committee guidelines.

2.6 Castor oil-induced model
The animals were divided into four groups of 6 animals each. The group 1 served as the control and received 0.5% CMC suspended in distilled water. The next three groups received castor oil (Paras Chemicals) in the dose of 1 ml per animal p.o. [24]. Half an hour after castor oil administration, group 2 and 3 receive extract at dose of 150 and 300 mg/kg body weight, p.o. and the group 4 receive Loperamide (3 mg/kg; p.o.) respectively. Following their administration, the animals were placed separately in acrylic cages with filter paper, which was changed every hour. The severity of diarrhoea was assessed each hour till 4 h. The total number of faeces (dry and wet stool) and diarrhoeal faeces (wet stool) excreted in record time were scored and compared with control group. The total score of diarrhoeal faeces of control group was considered that of 100%. The results were expressed in percentage of inhibition [23, 25, 26].

2.7 Statistical analysis: Results obtained from the study were expressed as mean ± SEM. The data were analyzed using one-way ANOVA followed by Dunnett's test. Results were considered significant when $P<0.01$.

3. Results
The study supports the presence of various phytochemicals in *M. oleifera* leaves as shown in figure 1 and there quantification reveals that protein content was found to be highest (23.35%) followed by fiber, starch, oil, sugar, alkaloid, tannin, flavonoids and phenolic i.e. 23.35, 16.94, 9.86, 9.21, 4.81, 1.8, 1.78, 1.46, 0.65 % respectively. This depicts the nutritional importance and socio-economic use of leaves apart from edible fruits, more in rural and under-nourished part of the world. *M. oleifera* leaves when treated with ethanol, yielded 9.75% of extract on weight basis. Qualitative estimation of phytochemicals in ethanol extract of leaves as summarized in table 1 shows the presence of carbohydrate, protein, steroid, flavonoids, tannin, alkaloid and glycosides.

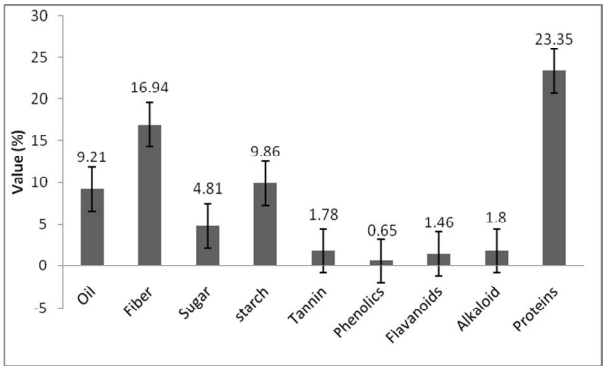

Fig 1: Quantitative estimation of phytochemicals presents in *M. oleifera* leaves.

Table 1: Preliminary phytochemical screening of *Moringa* leaves (alcoholic) extract.

Chemical tests	*M. oleifera* leaves (ethanol extract)
1) Carbohydrate	+
2) Protein	+
3) Steroid	+
4) Flavonoids	+
5) Tannin	+
6)Alkaloids	+
7) Glycosides:	
a)Coumarin	-
b)Saponin	+
c)Cardiac	-
d)Anthraquinone	+
e)Cynogenetic	+

+: Present, - : Absent

In vivo screening as in table 2 showed that ethanol extract of *M. oleifera* leaves produced a statistically significant ($p < 0.01$) anti diarrheal activity. The onset of diarrhea is delayed, after administration of doses i.e. 52 and 62 min for 150 and 300 mg/kg b.w respectively and is significant to that of control, which shows diarrheal symptom only after 40 min. In the course of observation for 4 hrs after castor oil administration, all the animals in tested groups which previously produces copious diarrhoea, shows decrease in frequency of purging (reduction in no. of wet stool and total no. of stools), weight of wet stools and weight of total stool. Mean no of wet stool in control, standard and test doses were shown in Figure 2 and are observed that frequency of wet stool decreases as dose increases. In selected doses of 150 and 300 mg/kg b.w percentage inhibition was found to be 37.50 and 43.75% respectively as compared to control (100%) and standard Loperamide, having 81.25% inhibition respectively as represented in figure 3).

Table 2: Effect of *Moringa* leaves (ethanol extract) in castor oil induced diarrhea model for control, standard & test groups

Groups	Dose (mg/kg)	Onset of Diarrhea (min)	Mean number of total stools	Weight of wet stools (gm)	Weight of total stools (gm)
Control	--	After 40	9.5±0.35	0.290	0.320
Standard	3	After 67	2±0.70	0.070	0.112
Test groups	150 (T_1)	After 52*	7±0.70	0.095	0.130
	300 (T_2)	After 62*	6.5±0.35	0.135	0.195

Values are expressed as mean ± S.E.M (n=6). *P<0.01 when compared with control.

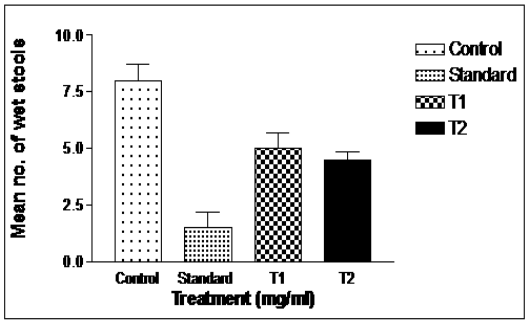

Fig 2: Graphical representation of mean no. of wet stools for all groups. (Test doses were compared with control at P<0.01).

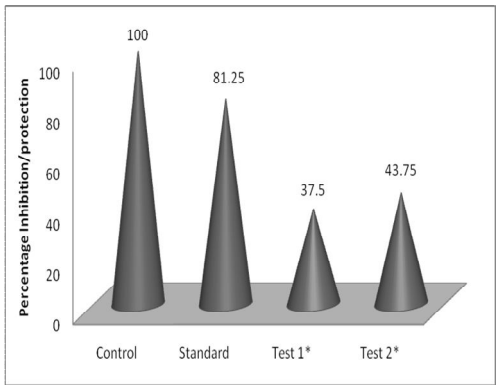

Fig 3: Percentage inhibition of diarrhoea in control, standard and test doses. (*P<0.01, when compared with control).

4. Discussion

The result of present study would suggest that ethanolic extract of *M. oleifera* leaves exhibit significant action against castor oil induced diarrhea. Castor oil causes diarrhoea due to its active metabolite, ricinoleic acid [27, 28], which stimulate peristaltic activity in small intestine, leading to changes in the electrolytic permeability of the intestinal mucosa and thus increases the volume of intestinal content by preventing the reabsorption of water. The liberation of ricinoleic acid also results in irritation and inflammation of intestinal mucosa leading to release of prostaglandins and autocoids [13, 29]. Loperamide at present is one of the most efficacious and widely employed anti diarrheal agents and effectively antagonizes the action of castor oil due to its antimotility and antisecretary property.

Since the alcoholic extract successfully inhibits the castor oil induced diarrhoea, the action might be via anti-secretary mechanism. The significant reduction in frequency of defecation, number of wet stool, mean stool count, weight of wet stool and weight of total stool signifies the efficacy of ethanolic extract of moringa leaves as an effective anti diarrheal agent. Traditionally heelers and common man uses alcoholic decoction of various herbs to cure the disease and this is in fact the reason to analyze the anti diarrheal activity in ethanol extract. In addition to this it is well reported that several group/classes of phytochemicals have anti dysenteric and antidiarrhoeal property i.e. tannins, alkaloids, saponins, flavanoids, sterols/triterpenoids, reducing sugars and anthraquinone glycosides, especially known for their laxative effect. Thus the presence of tannin, alkaloid, flavonoids, sugar and anthraquinone may be the underlying cause of mechanism. Beside this the potentiating action may also be due to denatured proteins, which form protein tannates, these complexes of tannin make the intestinal mucosa more resistant and therefore reduces the secretion [30]. Thus the overall possible mechanism of action may be due to anti secretary mechanism.

5. Conclusion

Present investigation revealed that alcoholic extract of *M. oleifera* contains pharmacologically active phyto molecule(s) with potential anti diarrhoeal properties and can be used as non-specific anti diarrhoeal agent. Since the extract contains a range of compounds, the observed activity may be due to single chemical moiety and/or group of therapeutically active components like protein, flavonoids, tannin etc. which may adds to underlying cause of the action. Hence, further extensive and elaborated studies are needed to extract and isolate the bioactive compound (s) for better understanding of such actions in more scientific manner.

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