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

Ankita Misra, Sharad Srivastava, Manjoosha Srivastava

ABSTRACT
The objective of present study was to evaluate in vivo anti-diarrheal potential of Moringa oleifera leaves. Sample was analyzed for quantitative 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, flavonoid 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 (T1) and 300 mg/kg (T2) 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[15, 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, ground 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, 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 100%. The results were expressed in percentage of inhibition [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.

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

<table>
<thead>
<tr>
<th>Chemical tests</th>
<th>M. oleifera leaves (ethanol extract)</th>
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<tbody>
<tr>
<td>1) Carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>2) Protein</td>
<td>+</td>
</tr>
<tr>
<td>3) Steroid</td>
<td>+</td>
</tr>
<tr>
<td>4) Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>5) Tannin</td>
<td>+</td>
</tr>
<tr>
<td>6) Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>7) Glycosides:</td>
<td></td>
</tr>
<tr>
<td>a) Coumarin</td>
<td>-</td>
</tr>
<tr>
<td>b) Saponin</td>
<td>+</td>
</tr>
<tr>
<td>c) Cardiac</td>
<td>-</td>
</tr>
<tr>
<td>d) Anthraquinone</td>
<td>+</td>
</tr>
<tr>
<td>e) Cyanogenetic</td>
<td>+</td>
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</tbody>
</table>

+: 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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Standard</th>
<th>Test Dose 150 mg/kg</th>
<th>Test Dose 300 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>40</td>
<td>52</td>
<td>44</td>
<td>62</td>
</tr>
<tr>
<td>No of wet stools</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

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**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 secretory 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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**7. Reference**


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