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Evaluation of Antiulcer Activity of *Moringa Oleifera* Seed Extract

Swati S. Kansara*, Manmohan Singhal

Department of Pharmacology, School of Pharmaceutical Science, Jaipur National University, Jaipur, Rajasthan, India. 302025

ABSTRACT:

Antiulcer activity of seeds of *Moringa Oleifera* was studied in rats in which gastric ulcers were induced by oral administration of ethanol and pylorus ligation. Seeds extract of *Moringa Oleifera* was administered in the dose of 150 and 200 mg/kg orally. The antiulcer activity was assessed by determining and comparing the ulcer index in the test group with that of the vehicle control group. Gastric fluid total acidity and free acidity were estimated in pylorus ligated rats. Omeprazol was used as a reference drug. The results suggested that ethanolic extract of *Moringa Oleifera* possesses significant antiulcer activity.

Keywords: Pylorus ligation, *Moringa Oleifera*, Omeprazol

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For Correspondence:

Ms. Swati S. Kansara

Department of Pharmacology, School of Pharmaceutical Science,

Jaipur National University,

Jaipur, Rajasthan, India. 302025.

Email: swati.kansara23@gmail.com

INTRODUCTION:

Moringa oleifera belonging to family Moringaceae is commonly known as "drumstick" or "horse radish tree". In Hindi it is known as Soanjana, in Guajarati- sargavo and in Sanskrit- shobhanjana. [1] It is short about 10 cm height, slender, deciduous, perennial tree widely distributed in India, Arabia & cultivated in tropical Africa, America, Shrilanka, Mexico and Malaysia. [2]

The medicinal values of the seeds and different parts of the plant have long been recognized in folklore medicine. [3] The plant has been used for the treatment of ascites and rheumatism. [4] Ayurvedic practitioners have administered the seed extract nasally in diseases like rhinitis and successfully used the dried seeds as an 'anti-allergic' agent. [1] Moringa oleifera is used in Thai tradition medicine as cardiotonic. [5]

In the West, one of the best known uses for *Moringa* is the use of powdered seeds to flocculate contaminants and purify drinking water. [6]

The seed cures eye diseases and head complaints. Oil is useful in leprous ulcers and as external application for rheumatism. The roots and seeds are prescribed for the treatment of snake bites and scorpion stings. [1]

Dried seeds are used in ophthalmic preparation, veneral affection, as anti-inflammatories, purgatives. [7]

The leaves are rubbed on the templates for head ache while the root and bark are regarded as anti-scorbutic and can be used externally as counter irritant. The juice of the leaves mixed with honey is used for the treatment of eye diseases. [4]

Peptic ulcer is a lesion in the gastric and duodenal epithelium associated with acute or chronic inflammation and it is most common gastrointestinal disorder in clinical

practice. Due to relapses and recurrences after the cessation of treatment, a search for safer potential drugs is being carried out. The use of natural drugs in gastric ulcer has been reported in many articles. [8, 9]

Report suggested that incidence of gastric ulcer in the south Asian population reveals that the occurrences is lower due to the type of food consumed by the people of this region: one of the foods that protect against ulcer is *Moringa Oleifera*. Furthermore, flower buds of *Moringa oleifera* is widely consumed in Pakistan, has been reported to possess antiulcer activity against aspirin induced gastric ulcer in rats. [10]

However, no systemic study on antiulcer activity of seeds has been reported in the literature. Therefore in the present study, we screened the seed extracts of *Moringa oleifera* for antiulcer activity.

Materials & Methods

Plant Materials

Seeds of *Moringa oleifera* Lam. (Moringaceae) were obtained from a commercial supplier in Ahmedabad and were identified as well as authenticated (HAS/ISRO/phar./JNU/04/2011) by Botanist of Department of Botany, Gujarat University, Gujarat

Preparation of Extract

The seeds of *Moringa oleifera Lam* were shade dried and reduced to coarse powder in a mechanical grinder and passed through sieve No. 40. The powdered plant material was extracted using 90% ethanol, because ethanol extract is generally effective for the activity and also various chemical constituents may be present in the ethanol extract. 500 gm of dried plant powder was taken in a round bottom flask, the plants drugs were three times successively extracted with 1000 ml of 90% ethanol in flask by using hot maceration method. In this method the plant powder was socked with 90% ethanol and warm at 40°C-50°C temperature for 1 hour. Cool it and filtered it by vacuum filtration unit using whatman filter papers no-1.

Then the filtered is collected into flask and extracts obtained were evaporated. Concentrated extract were dried .When the extract was completely dried take weight of evaporating dish on electronic balance, and find % yield of this plant extract with reference to dried plant powder drug.

The dried plant extract was scraped by spetula and collected from evaporating dish, and stored in air tied container at cool temperature.

The extracts thus obtained were subjected to phytochemical analysis.

Experimental Animals

Albino Wistar rats of either sex weighing between 180-250 gm were used. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control, and Supervision on Experiments on Animals (CPCSEA).

Dose Selection & Mode of Administration

The extracts were suspended using 0.5% sodium carboxy methyl cellulose and were administered orally. The concentration was adjusted in such a way that it did not exceed 1ml/kg b/w of the animal. All the animals fed by oral gavages with the help of feeding tube. The doses determined as low dose at rate of 150mg/kg and high dose at the rate of 200 mg/kg. Standard antiulcer drug Omeprazol used at the rate of 20mg/kg of body weight. [11, 12]

Pylorus Ligation Methods

The animals were fasted for 36 hours before pylorus ligation with water *ad libitum.*

Wistar rats of either sex were used for the experiment. They are divided in to four groups of six animals each .In this method, Group I served as disease control, 0.5 % CMC, 1ml/kg, Group IV served as positive controls received omeprazol 20mg/kg, and Group II-III received ethanolic extract of *Moringa Oleifera* seed (EEMO)150mg/kg and 200mg/kg respectively. EEMO or reference drug or control vehicle was administered 30 min prior to pyloric ligation. [13]

Under light ether anesthesia, the abdomen was opened by midline incision below the xiphoid process. The pyloric portion of the stomach was slightly lifted out and ligated, avoiding damage to its blood supply. The stomach was placed back carefully and the abdominal wall was closed with sutures. The animals were deprived of food and water during the postoperative period and the animals were sacrificed six hours after pylorus ligation by over dose of ether anesthesia.

The stomachs were isolated and the content of the stomachs were collected and centrifuged. The gastric juice collected was centrifuged for 1000 rpm for 10 minutes and the volume of gastric juice was measured and this was used for the estimation of free and total acidity.

Parameters measured: Ulcer index and % protection was measured. The volume and p^H of the gastric juice was also measured and this was used for estimation of free acidity, total acidity.

The ulcers scores were given based on their intensity as follows [14]

Normal stomach……0
Red coloration……0.5
Spot ulcer……1
Hemorrhagic streak……1.5
Ulcers……2
Perforation……3

Evaluation of Ulcer Index [15]

An ulcer index UI is calculated: (Eq. No. 1)

UI=UN+ US+ UP × 10–1……..(1)

- UN = average of number of ulcers per animal
- US = average of severity score
- UP = percentage of animals with ulcers

The % protection was calculated using the following formula (Eq.NO. 2)

$$\%\text{protective} = \frac{\text{controlmean ulcer index} - \text{Test mean ulcer index}}{\text{control mean ulcerindex}} \times 100$$

…… (2)

Determination of free and total acidity [16]

One ml of gastric juice was pipette into a 100 ml conical flask, two or three drops of Topfer's reagent were added and this was titrated with 0.1 N NaOH until all traces of red color disappeared and the color of the solution became yellowish-orange. The volume of alkali added was noted. This volume corresponds to free acidity. Two or three drops of phenolphthalein solution were added and titration was continued until a definite red tinge appeared .The total volume of alkali added was noted. The volume corresponds to total acidity. Total acidity output was expressed as Meq/L per 100 gm of body weight. Acidity was calculated using the following formula (Eq. NO. 3)

$$\text{Acidity} = \frac{volume\ of\ NaOH \times Normality\ of\ NaOH \times 100}{0.1} \frac{meq}{L} \quad \ldots\ldots(3)$$

Ethanol Induced Ulcer [9]

Wistar rats of either sex were used for the experiment. They are divided in to four groups of six animals each .In this method, Group I served as disease control, received ethanol, Group IV served as positive controls received omeprazol 20mg/kg, and Group II-III received ethanolic extract of *Moringa Oleifera* seed (EEMO)150mg/kg and 200mg/kg respectively.

The ulcer was induced by administering ethanol. All the animals were fasted for 36 hours before administration of ethanol. The standard drug (omeprazol 20mg/kg) or the seed extracts (150 & 200 mg/kg, *p.o.*) were administered one hour before ethanol administration. Ethanol (90%) was administered to all the animals at a dose of 1ml/200gm rat and after one hour all the animals were anaesthetized with ether. stomachs were isolated and ulcer index was determined as mentioned above.

Parameters measured: - Ulcer index and % protection was measured. pH of the gastric juice was also measured.

Statistical Analysis The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnet comparison test. The values are expressed as mean + SEM and $p<0.05$ was considered significant

RESULTS & DISCUSSION

Phytochemical Analysis

The seed extract contained reddish-brown residue. The yield of alcoholic extract of *Moringa oleifera* was found to be 9.7%. The results of preliminary Phytochemical screening of the ethanol extract of seeds of *M. oleifera* showed the presence of alkaloids, flavonoids, saponin, steroids, glycosides, tannins, and terpenoids.

Effect of EEMO in Pylorus Ligated Rats

Pylorus ligation induced ulcer was used to study the effect of extracts on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The original Shay rat model involves fasting of rats for 72 hours followed by ligation of pyloric end of the stomach. The ulcer index is determined 19 hours after pylorus ligation. The lesions produced by this method are located in the rumen region of the stomach.

Many authors have modified the original model. In the present study, the Shay rat model described by Kulkarni was followed. Unlike the original model, where ulcers are produced in the rumen region of the stomach, in this model, the ulcers developed as lesion in the glandular portion of the stomach. The agents that decrease gastric acid secretion and increase mucus secretion are effective in protecting the ulcers induced by this method. The ethanolic seed extract of *Moringa oleifera* and omeprazol significantly decreased the total acidity and free acidity

Ulcer index parameter was used for the evaluation of antiulcer activity since ulcer formation is directly related to factors such as reduction in gastric volume, decrease in free and total acidity. Ethanol extract of seeds of *Moringa oleifera* (EEMO) at the dose of 200 mg/kg and omeprazol (20 mg/kg) has showed significant ($p< 0.05$) reduction in the ulcer index. However, it failed to produce any significant effect on ulcer index at the dose of 150mg/kg.

It was showing protection index of 58% and 74% at the dose of 150 and 200 mg/kg respectively in comparison to control whereas Omeprazole as reference standard drug was reduction of ulcer 77%. Oral administration of EEMO in two different doses showed significant reduction in ulcer index, gastric volume, free acidity, total acidity and P^H of gastric juice as compared to the control group. However, it failed to produce significant effect statistically on free acidity at the dose of 150mg/kg. Results are shown in table 1 and figure no.1 to 5.

Table 1: Antiulcer activity of EEMO on pylorus ligated rats

Group	Treatment	Ulcer index	% Protection	pH of gastric juice	Gastric juice (ml)	Free Acidity (mEq/litre)	Total Acidity (mEq/litre)
I	Control (pyloric ligation)	12.25±0.32	-------	2.60±0.21	8.57±0.23	86.50±2.67	98.50±3.40
II	EEMO (150mg/kg)	5.103±0.46	58.34	4.03±0.31*	7.00±0.37*	74.83±3.25	81.67±4.60*
III	EEMO (200mg/kg)	3.16±0.84*	74.68	4.22±0.33*	6.83±0.51*	71.0±3.92*	80.67±3.87*
IV	Omeprazole (20mg/kg)	2.81±0.80*	77.06	4.98±0.50*	6.06±0.34*	67.67±5.29*	77.33±5.05*

All values are mean ±SEM , n = 6, * p <0.05 when compared with control group.

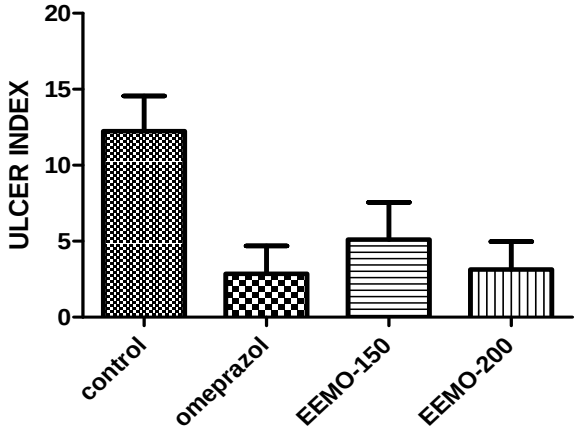

Figure 1: Effect of *Moringa Oleifera* seed extract on ulcer index

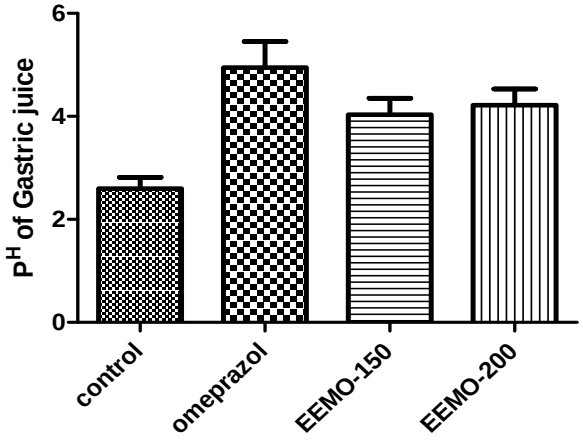

Figure 2: Effect of *Moringa Oleifera* Seed Extract on pH of gastric juice

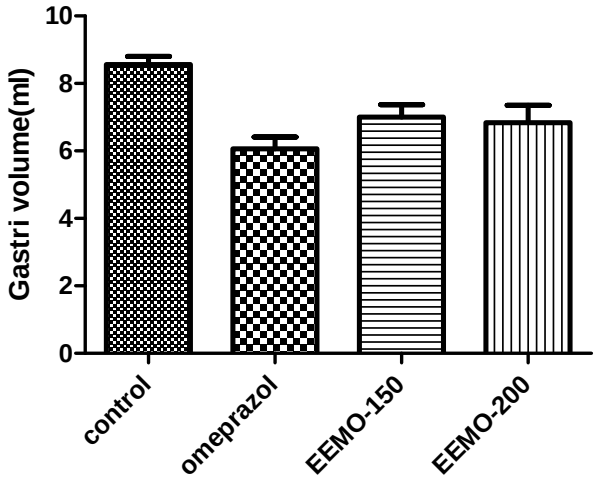

Figure 3: Effect of *Moringa Oleifera* seed extract on Gastric volume

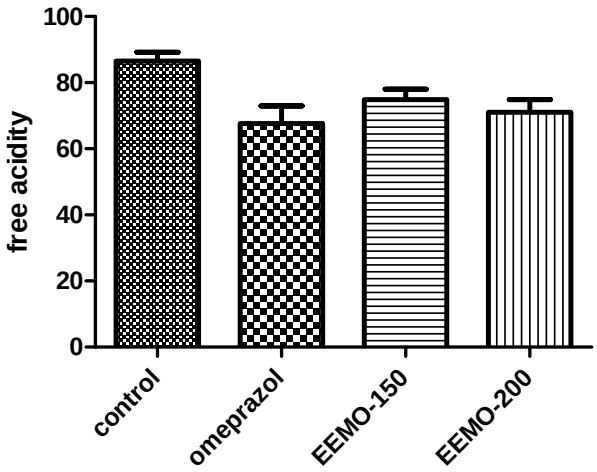

Figure 4: Effect of *Moringa Oleifera* seed extract on Free Acidity

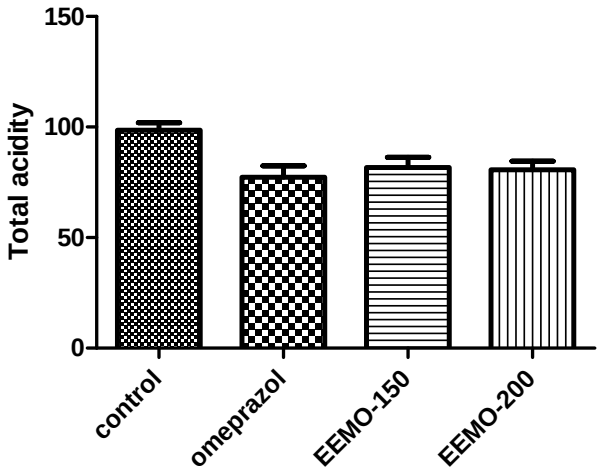

Figure 5: Effect of *Moringa Oleifera* seed extract on Total acidity

Effect of EEMO in Ethanol Induced Ulcers In Rats

In control animal, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black & dark red lesions. EEMO has shown significant protection index of 62.02% and 65.23% with the dose of 150 and 200 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was reduction of ulcer 77.44%. Results are tabulated inTable-2 and figure no. 6 & 7.

Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the hemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular

Table 2: Antiulcer activity of EEMO in ethanol induced gastric ulcer

Group	Treatment	Ulcer index	Protection %	pH of gastric juice
I	Control (pyloric ligation)	14.01±0.52	----------	3.1±0.26
II	EEMO (150mg/kg)	5.32±0.51*	62.02%	4.35±0.48*
III	EEMO (200mg/kg)	4.87±0.39*	65.23%	4.60±0.32*
IV	Omeprazol (20mg/kg)	3.16±0.82*	77.44%	4.77±0.52*

All values are mean ±SEM , n = 6, * p <0.05 when compared with control group

membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium.

The ethanolic extracts of the seed were effective in all the tested models of gastric ulcers.

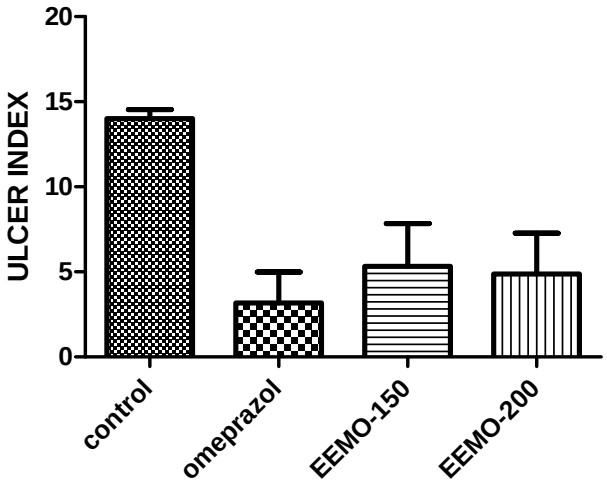

Figure 6: Effect of *Moringa Oleifera* seed extract on ulcer index in Ethanol induced ulcer

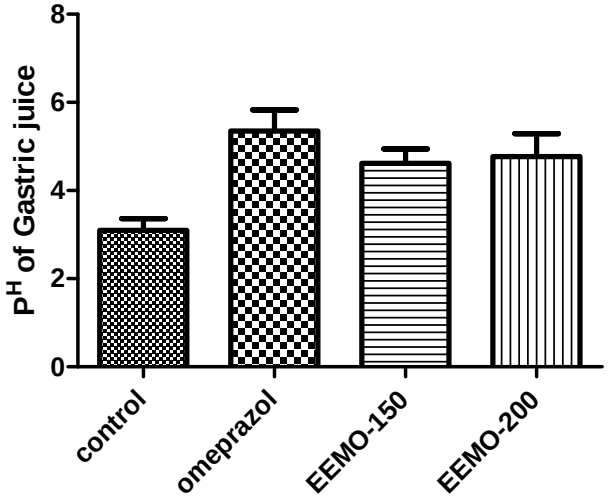

Figure 7: Effect of *Moringa Oleifera* seed extract on P^H of Gastric juice in Ethanol induced ulcer

Although a number of antiulcer drugs such as H_2 receptor antagonists, proton pump inhibitors and cyto-protectants are available for ulceration all these drugs have side effects and limitations. Complementary and alternative medicine has been used to treat PUD for hundreds of years. Herbal medicine deals with plants and plant extracts in treating diseases. These medicines are considered safer because of the natural ingredients with no side effects. [14]

Moringa oleifera contains a number of flavonoids, triterpines, steroids, alkaloids and many other chemical constituents. (12)

The flavonoids quercetin present in the seed is well known antiulcer agent. This explains more potent ulcer healing effect of ethanol extracts of the seed compared to control. The ulcer healing effect obtained with ethanolic extracts may be due to both anti-secretary and gastric cytoprotective constituents present in these extracts, as evident by a decrease in acidity in pylorus ligation and decrease in ulcer index in ethanol induced gastric ulcers. (1)

Apart from flavonoids, the seeds of the plant contain steroids such as beta sitosterol; these are known to reduce the development of gastric ulcers.

CONCLUSION

The results suggest that the seed extract of *Moringa oleifera* possesses antiulcer effect. It prevented the development of gastric ulcers induced by pylorus ligation and ethanol.

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