

Antiulcerogenic Activity of *Moringa Oleifera* Root Extract Against Ethanol-Induced Gastric Ulcer in Rats

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ABSTRACT

The present study was carried out to evaluate the anti-ulcerogenic activity of ethanol extract of *Moringa oleifera* root against ethanol-induced gastric mucosal injury in rats. Five groups of adult *Sprague Dawley* rats were orally pre-treated respectively with carboxymethyl cellulose (CMC) solution (ulcer control group), Omeprazole 20 mg/kg (reference group), and 100, 200 and 400 mg/kg *M. oleifera* root extract in distilled water (experimental groups), one hour before oral administration of absolute ethanol to generate gastric mucosal injury. Rats were sacrificed and the ulcer areas of the gastric walls were determined. Grossly, the ulcer control group exhibited severe mucosal injury, whereas pre-treatment with *M.oleifera* root extract exhibited significant protection of gastric mucosal injury. These results strongly document the beneficial cytoprotective effects of plant extract against ethanol-induced gastric ulcer in rats.

Keywords: *Moringa oleifera*, cytoprotection, gastric ulcer.

INTRODUCTION

Peptic ulcer is one of the most common diseases affecting mankind. It is now well recognized that in peptic ulcer disease, there is shift in balance between mucosal damaging mechanisms to mucosal protecting mechanisms. Pathogenesis of gastric ulcers is mainly attributed to impaired mucosal resistance. Disturbed gastric motility and reduced gastric mucosal blood flow are also important pathogenic elements in gastric ulcer malignancy. The etiological factors of this disorder include stress, smoking, nutritional deficiencies, infections, frequent and indiscriminate use of nonsteroidal anti-inflammatory drugs (NSAIDs)¹. For more than a century, peptic ulcer disease has been a major cause of morbidity and mortality. The well known pathophysiology of peptic ulcer has been centralized on an imbalance between aggressive (gastric acid secretion) and protective factors (gastric mucosal integrity) in the stomach and infection with *Helicobacter pylori*². The modern drugs used in the treatment of gastric ulcers is based on the inhibition of gastric acid secretion by histamine

receptors blockers, proton pump inhibitors or enhancing the mucosal production like prostaglandin analogues or certain antimicrobials like metronidazole, amoxicillin etc.³ However, some studies have revealed that reactive oxygen species and lipid peroxidation are implicated in the pathogenesis of ethanol-induced gastric lesions and they damage many biological molecules such as prostaglandins⁴⁻⁶. Therefore, treatment with antioxidants and free radical scavengers can be effective in gastric ulcers.

Moringa oleifera Lam (Moringaceae), native to the western and sub-Himalayan region, India, Pakistan, Asia minor, Africa and Arabia^{7,8} is now distributed in the Philippines, Cambodia, Central, North and South America and the Caribbean Islands⁹. *M. oleifera* is a tropical tree whose numerous economic applications and facility of propagation are arousing growing international interest. The *Moringa* tree is cultivated and used as a vegetable (leaves, green pods, flowers, roasted seeds), for spice (mainly roots), for cooking and cosmetic oil (seeds) and

as a medicinal plant (all plant organs)¹⁰. *Moringa oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, β – carotene, amino acids and various phenolics¹¹. The *Moringa* plant provides a rich and rare combination of zeatin, quercetin, kaempferol and many other phytochemicals. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumour¹², antipyretic, antiepileptic, antiinflammatory, antiulcer¹³. Recently, widespread efforts have been launched to identify novel anti-ulcer drugs from natural resources. A number of models are available in which to test substances for their anti-ulcer effects. Here, we report on the effect of an ethanolic extract from *Moringa oleifera* roots on gastric lesion induced in different animal models employing necrotizing or stressor agents.

MATERIALS AND METHODS

Collection of plant materials

The plant roots of *Moringa oleifera* were collected in June 2011 from different localities of Mangalore district, Karnataka state. The plant materials authenticated by botanist Dr. Noeline J.Pinto, Department of botany, ST Agnes college (Autonomous), Mangalore. The roots were cut, washed with distilled water and dried in shade, pulverized by mechanical grinder to get coarse powder and stored in an airtight container.

Preparation of Plants Extracts

The powdered material obtained was then subjected to successive extraction by Hot Percolation Method using ethanol solvent in a Soxhlet extractor. The different extracts obtained were evaporated at 45°C to get a semisolid mass. The extracts thus obtained were subjected to phytochemical analysis. The percentage yield of Alcoholic extract was found to be

45.50%w/w and the ethanolic extract was used for further studies¹⁴.

Preliminary phytochemical screening

The phytochemical examination of the *M.oleifera* extract was performed by the standard methods¹⁵.

Acute toxicity test LD50

Adult male and female *Sprague Dawley* rats (6 - 8 weeks old) were weighed between 150 - 180 g. The animals were given standard rat pellets and tap water *ad libitum*. The acute toxic study was used to determine a safe dose for the root extract. Eighteen rats (9 males and 9 females) were assigned equally each into 3 groups labelled as vehicle (CMC, 0.25% w/v, 5 ml/kg); 2 and 5 g/kg of *M.oleifera* root extract preparation, respectively. The animals were fasted overnight (water but not food) prior to dosing. Food was withheld for a further 3 to 4 h after dosing. The animals were observed for 30 min and 2, 4, 8, 24 and 48 h after the administration for the onset of clinical or toxicological symptoms. Mortality, if any was observed over a period of 2 weeks. The acute toxicity LD50 was calculated as the geometric mean of the dose that resulted in 100% lethality and that which caused no lethality at all.

Experimental Animals

Wistar albino rats of either sex weighing around 150-250g were taken from inbred colony animals, which were housed in polypropylene cages under standard laboratory conditions (light period 7.00 A.M. to 7.00 P.M., 21±2 °C, relative humidity 55%). The animals were given standard rat pellets and tap water *ad libitum*, but they were deprived of food 36 h before the experiments. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. All procedures involving laboratory animal use were in accordance to the Institute Animal Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Animal treatment

The rats were fasted for 36 h before the experiment¹⁶, but were allowed free access drinking water up till 2 h before the experiment. Gastric ulcer in *Sprague Dawley* was induced by orogastric incubation of absolute ethanol (5 ml/kg) according to the method described previously by Pasquale et al. (1995) with slight modification. Ulcer control group was orally administered with vehicle (carboxymethyl cellulose, CMC, 0.25% w/v, 5ml/kg). The reference group received oral doses of 20 mg/kg omeprazole in CMC (5 ml/kg) as positive controls. Experimental groups were orally administered with 100, 200 and 400 mg/kg of ethanol extract of *M.oleifera* root in distilled water (5 ml/kg), respectively. One hour after this pre-treatment; all groups of rats were gavaged with absolute ethanol (5 ml/kg) in order to induce gastric ulcers¹⁷. The rats were euthanized by cervical dislocation 60 min later¹⁸ under an over dose of diethyl ether anaesthesia and their stomachs were immediately excised.

Gross gastric lesions evaluation

Assessment of gastric lesions was also carried out by gross pathological examination of the stomach mucosa. One hour after ethanol administration, the animals were euthanized by cervical dislocation. The stomachs were excised, cut along the greater curvature, and gently rinsed under ice cold PBS. Massive gastric haemorrhage was observed in the rats' stomachs 1 h after oral administration of absolute ethanol. The stomachs were stretched on a thermocol board with mucosal surface up then to be examined in a standard position for macroscopic examination and the scoring of ulcer was performed with the help of a magnifying hand lens.

Scoring of ulcer will be made as follows¹⁹⁻²¹

- Normal stomach..... (0)
- Red coloration..... (0.5)
- Spot ulcer..... (1)
- Hemorrhagic streak... (1.5)
- Ulcers..... (2)

Perforation..... (3)

Ulcer index was calculated using the formula:

$$UI = UN + US + UP \times 10^{-1}$$

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

The percentage of ulcer protection was determined as follows:-

%protective

$$= \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}}$$

Statistical analysis

The values are represented as mean \pm S.E.M, and statistical significance between treated and control groups was analyzed using of One way ANOVA, followed by Dunnett's test where $P < 0.01$ was considered statistically significant.

RESULTS**Phytochemical screening**

The results of preliminary phytochemical screening of the ethanolic extract of *M.oleifera* root revealed that presence of flavonoids, cinnamates, terpenoids, anthocyanins, Phenols and absence of fixed oils and steroids.

Acute toxicity study

An Acute toxicity study was carried out in which the animals were treated with the root extract at a dose of 2 and 5 g/kg of *M.oleifera* root extracts and were kept under observation for 14 days. All the animals remain alive and did not manifest any significant visible signs of toxicity at these doses. There were no abnormal signs, behavioural changes, body weight changes, or macroscopic finding at any time during the observation period.

Ethanol-induced gastric ulcer

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. *M.oleifera* has shown significant protection

index of 13.93%, 22.95% and 59.83% with the dose of 100, 200 and 400 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was reduction of ulcer 55.16%.

DISCUSSION

Administration of absolute ethanol into the gastric lumen induced gross lesions in the glandular part of the stomach²². It was also apparent that ethanol caused gastric damage, which was confirmed by significant increase in the number of haemorrhage and gastric erosions²³. In our study, the ulcerogenesis, pronounced destructive changes associated with haemorrhage in the stomach were observed in the ethanol-induced group. Pre-treatment of rats with a single oral dose of *M.oleifera* could partly reduce ulcer area and promote healing of gastric lesions induced by acute intake of ethanol. The present results demonstrate that the ethanolic extract of *M.oleifera* root protect the rat gastric mucosa against hemorrhagic lesions produced by absolute ethanol. Absolute ethanol method of inducing gastric lesions is rapid and convenient way of screening plant extracts for anti-ulcer potency and cytoprotection in macroscopically and microscopically visible lesions. Disturbances in gastric secretion, damage to gastric mucosa, alterations in permeability, gastric mucus depletion and free-radical production are reported to be the pathogenic effects of ethanol²⁴. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspect of tissue injury²⁵. It is of interest to note that administration of antioxidants inhibit ethanol-induced gastric injury in the rats²⁶. It could be conceived that ethanolic extract of *M.oleifera* root exert their anti-ulcer activity through the flavonoids, since flavonoids are reported to protect the mucosa by preventing the formation of lesions by various necrotic agents²⁷. We

can suggest that it may be possible to use plant root extract as remedy to prevent ulcers. However, further investigations are required to elucidate their exact mechanism of action of anti-ulcer activity. Grossly, the results of the current study showed that pre-treated rats with ethanolic extract of *M.oleifera* root or cimetidine significantly reduced the formation of gastric ulcer induced by absolute ethanol compared to animals pre-treated with PBS and administered absolute ethanol.

CONCLUSION

From the above results, it can be inferred that, the ethanolic, extracts showed marked anti ulcer activity in ethanol induced gastric ulcer model in a dose dependent manner. Ethanolic extract of *Moringa oleifera* at 400 mg/kg reduced the ulcer index thus showing the anti-secretory mechanism involved in the extracts for their anti-ulcerogenic activity. So, ethonolic extract at 400 mg/kg exhibited significant anti ulcer activity and almost equipotent effect as that of Omeprazole and these results offer pharmacological evidence and support on the folkloric use of *Moringa oleifera* roots as an Anti ulcer agent. In future, this work has been extended by including more ulcer models to confirm the anti ulcer potency of for meaningful and tangible conclusion. Toxicological studies can also be carried out to know about the toxic non-toxic nature of the drug. Isolation and characterization of the Phyto-constituents responsible for pharmacological activity can also be attempted in future.

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Table I: Effect of *Moringa oleifera* root extracts on ulcer index and percentage protection in ethanol induced ulcer model

Values are expressed as mean \pm S.E.M., (n=6).
 *P<0.01 as compared with ethanol.



Fig. 1: Ulcer Control (Ethanol-5ml/kg)



Fig. 2: Reference group (Omeprazol-20mg/kg)



Fig. 3: Moringa oleifera extract(100 mg/kg)



Fig. 4: Moringa oleifera extract (200 mg/kg)



Fig. 5: Moringa oleifera extract (400 mg/kg)

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Group	Treatment	Ulcer index	% Protection
1.	Ulcer Control (CMC-5 ml/kg)	12.2 \pm 0.095
2.	Omeprazole (20 mg/kg)	5.47 \pm 0.047*	55.16 %
3.	<i>M.oleifera</i> extract (100 mg/kg)	10.5 \pm 0.033*	13.93 %
4.	<i>M.oleifera</i> extract (200 mg/kg)	9.4 \pm 0.036*	22.95 %
5.	<i>M.oleifera</i> extract (400 mg/kg)	4.9 \pm 0.24*	59.83 %

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