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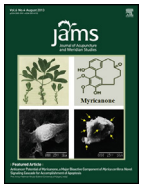

RESEARCH ARTICLE

Assessment of the Antiulcer Potential of *Moringa oleifera* Root-Bark Extract in Rats

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Abstract
In the present study, an ethanolic root-bark extract of *Moringa oleifera* (MO) was examined for its antiulcer potential in albino Wistar rats using two experimental models: ethanol-induced and pylorus ligation-induced gastric ulceration. The extract was orally administered at three different doses (150, 350, and 500 mg/kg) for 15 consecutive days. The antiulcer effects in rats treated with different doses of the extract and omeprazole (30 mg/kg, p.o.) were determined and compared statistically with the antiulcer effects in the control rats treated with saline (NaCl, 0.9%). The MO at doses of 350 and 500 mg/kg decreased the ulcer index significantly as compared to the control group ($p < 0.01$). The percentage protections against gastric ulcers were 82.58%, 85.13%, and 86.15% for MO doses of 150, 350, and 500 mg/kg, respectively, in the pylorus-ligated ulcer model and 55.75%, 59.33%, and 78.51%, respectively, in the ethanol-induced ulcer model. The MO significantly reduced the free acidity, total acidity, and ulcer index ($p < 0.01$) and increased the pH of gastric content compared with the control group. This study suggests that MO possesses valuable antiulcer, antisecretory, and cytoprotective activity. Thus, an ethanolic root-bark extract of *Moringa oleifera* can be used as source for an antiulcer drug.

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1. Introduction

Peptic ulcer disease is the most common gastrointestinal tract disorder and includes gastric and duodenal ulcers, which are usually acidic and, thus, extremely painful [1]. The pathophysiology of peptic ulcer disease involves an imbalance between offensive (acid, pepsin, and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors) [2]. Several factors—such as improper digestion, metabolism, and elimination of food, and mental and physical stress—enhance the development of peptic ulcers. A number of drugs are available for the treatment of peptic ulcers, but the clinical evaluation of these drugs indicates high incidences of side effects and drug interactions. These negative effects are the rationale for the development of new antiulcer drugs and the search for novel molecules in plants, such as *Ocimum sanctum*, *Azadirachta indica*, *Asparagus racemosus*, *Musa sapientum*, *Centella asiatica*, *Bacopa monnieri*, and *Bidens pilosa*, that could offer better protection and decreased relapse [3]. In the present study, the antiulcer potential of an ethanolic extract of *Moringa oleifera* root bark (MO) was studied using ethanol- and pylorus ligation-induced gastric lesions in experimental rats.

M. oleifera Lamm (Family: Moringaceae), also known as the drumstick tree and horseradish tree, is indigenous to Northwest India, Pakistan, Bangladesh, and Afghanistan [4]. This tree is the most widely cultivated species of the genus *Moringa*. Leaves of *M. oleifera* are highly nutritious, being a significant source of β-carotene, vitamin C, protein, iron, and potassium [5]. Almost all parts of this plant—root, leaves, fruit, bark, seeds, and flowers—are reported to have important medicinal values as cardiac and circulatory stimulants and to have antitumor, antipyretic, anti-inflammatory, antihypertensive, diuretic, cholesterol-lowering, antidiabetic, antioxidant, antispasmodic, antibacterial, and antifungal activities [6—11]. Moreover, the ethanolic extract of leaves of *M. oleifera* is reported to have antiulcer activity [12]. In view of the above, this study was performed to investigate the antiulcer potential of an ethanolic root-bark extract of *M. oleifera* by using ethanol-induced gastric ulcers and pylorus ligation-induced ulcers.

2. Material and methods

2.1. Animals

Male albino rats of the Wistar strain weighing about 200—250 g were procured from the Indian Institute of Chemical Biology, Kolkata, India. The rats were housed in solid-bottomed polypropylene cages and kept under standard husbandry conditions. The rats were fed with standard diet and water *ad libitum*. The experiments were designed and conducted in accordance with the ethical norms approved by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) and the Institutional Animal Ethics Committee of the SLT (Sulochna Lakhanlal Trivedi) Institute of Pharmaceutical Sciences, Guru Ghasidas Central University, Bilaspur (994/a/GO/06/CPCSEA, IACE/Pharmacy/2012/42).

2.2. Materials

The root barks of *M. oleifera* were collected from the local area of Chhattisgarh, India. The plant was taxonomically identified and authenticated by Dr. H.B. Singh (head of the Raw Materials and Herbarium Museum, National Institute of Science Communication and Information Resources, New Delhi, India). The specimens were deposited in the Pharmacology Department of the SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Central University, Bilaspur (C.G.), India. The powdered plant material was packed in soxhlet extractor and heated under reflux using 70% ethanol as a solvent. The percentage yield of the ethanolic extract from the root bark of *M. oleifera* was found to be 11.8%. The extract was filtered, concentrated under reduced pressure, dried, and stored in a tightly closed container at 4 °C for future use. The following chemicals and reagents were procured: ethanol (Jiangsu Huaxi International, China), diethyl ether (Fischer Scientific, Mumbai, India), and omeprazole (Cipla Ltd, India). All chemicals used were of analytical grade.

2.3. Phytochemical analysis

The MO was subjected to the following test for the phytochemical screening methods [13,14]: carbohydrates were identified by using Molisch's test (Molish's reagent, Organo Biotech Laboratories Pvt. Ltd., New Delhi, India), alkaloids by using Mayer's test (Mayer's reagent, Oxford Laboratory, Maharashtra, India), and Hager's test (Hager's reagent, Alpha Chemika, Mumbai, India), phenols by using a ferric chloride test (Ferric chloride, Alpha Chemika, Mumbai, India), tannins by using a gelatin test (Gelatin, Triveni Chemicals, Vapi, India) and a ferric chloride test, flavonoids by using an alkaline reagent test (Sodium hydroxide, Alpha Chemica, Mumbai, India), proteins and amino acids by using a xanthoproteic test (Nitric acid, Vats International, Delhi, India) and ninhydrin test (Ninhydrin reagent, Shinton Chemicals Pvt. Ltd., Indore, India), cardiac glycosides by using Legal's test (Sodium nitropruside, Brisben Chemicals, Mumbai, India), and saponins by using a foam test.

The acute oral toxicity study was performed as per the OECD (Organisation for Economic Co-operation and Development)-425 guidelines. The extract was suspended using Tween-80 (0.1%) and was administered orally at doses in the range of 50—2000 mg/kg. The concentration was adjusted in such a way that it did not exceed 1 mL/kg body weight of the rats. LD_{50} was calculated, and three different doses of MO (150, 350, and 500 mg/kg) were selected for the evaluation of the antiulcer activity.

2.4. Evaluation of the antiulcer activity using the pylorus ligation-induced ulcer model

Thirty male Wistar rats were divided into five different groups with six rats in each group ($n = 6$) [1,15]. Pylorus ligation was performed in all groups of rats for the induction of gastric ulcers, which was followed by the respective treatments. Group 1 (control) received normal saline (0.9%), Group 2 was treated with a standard drug (omeprazole, 30 mg/kg), and Groups 3, 4, and 5 were treated with

MO at doses of 150, 350, and 500 mg/kg, respectively. All treatments were given orally to the pylorus-ligated rats.

Male Wistar rats were deprived of food for 18 hours before pyloric ligation, but had free access to water. Under light ether anesthesia, the abdomen was opened by using a small incision below the xiphoid process. The pyloric position of the stomach was slightly lifted and ligated to avoid traction into the pylorus or damage to its blood supply. The stomachs were removed, and the contents were drained into tubes and centrifuged at 1000 rpm for 10 minutes. Supernatants were subjected to analysis for gastric volume, pH, free acidity, and total acidity. The stomachs were then cut along the greater curvature, the inner surface was examined for ulceration, and the ulcer index (UI) was calculated [16—18].

2.5. Evaluation of the antiulcer activity using the ethanol-induced ulcer model

Intragastric application of absolute ethanol is a reproducible method to produce gastric lesions in experimental animals [1,15]. The animals were divided into different groups similar to the groups used for the pylorus-ligated ulcer model. All treatments were given orally to the rats. The rats were deprived of food for 18 hours before the experiment, but had free access to water. Rats received the treatments 30 minutes before the administration of 1 mL of absolute ethanol. One hour after the administration of ethanol, the rats were sacrificed by overdosing with diethyl ether. The stomachs were excised and gently rinsed with saline (0.9%), inflated with 1% formalin solution (10 mL), and immersed in the same solution to fix the outer layer of the stomach. After 10 min, each stomach was then opened along the greater curvature and examined under a dissecting microscope to assess the formation of ulcers [19].

2.6. Measurement of the UI

The opened stomach was examined for ulceration and was given a scoring number [18]. Ulcer scores were estimated by viewing the ulcers with a magnifying glass and are as follows: 0, no ulcer; 1, superficial mucosal erosion; 2, deep ulcer; 3, perforated or penetrated ulcer. The UI was determined by using a measuring scale to measure the ulcerated area. The ulcerated area and the total mucosal area were calculated, and the UI was determined as

$$UI = 10/X,$$

where X is the ratio of the total mucosal area to the ulcerated area. The percentage protection was calculated using the formula

$$\%\,\text{ulcer protection} = \{(\text{UI of control} - \text{UI of test}) \times 100\}/\text{UI of control}$$

2.7. Biochemical parameters used to investigate gastric juice

Gastric juice was collected from pylorus-ligated rats [20]. The volume and the pH of the gastric juice were measured. For the determinations of the free and the total acidities in the gastric juice, 1 mL of gastric juice was pipetted into a 100-mL conical flask, and two to three drops of Topfer's reagent were added and titrated with 0.01N NaOH until all traces of red color had disappeared and the solution had become yellowish orange.

2.8. Statistical analysis

All the values are expressed as mean ± SEM ($n = 6$). The data of all groups were analyzed using a one-way analysis of variance (ANOVA), followed by the Dunnett's t-test. The criterion for statistical significance was $p < 0.05$.

3. Results

Preliminary phytochemical studies revealed the presence of alkaloids, carbohydrates, proteins, tannins, phenols, saponins, triterpinoids, and steroids in MO. The MO was found to be nontoxic when administered orally to the albino rats at doses in the range of 50—2000 mg/kg, and the LD_{50} was found to be safe at the highest dose.

The rats treated with MO had significantly ($p < 0.01$) reduced ulcer indices compared to the saline-treated pylorus-ligated rats (Fig. 1). The percentages of ulcer protection at doses of 150, 350, and 500 mg/kg were 82.58%, 85.13%, and 86.15%, respectively. Compared to the control group, the MO-treated group showed an increased gastric pH and reduced free acidity and total acidity (Table 1). For rats with ulceration induced using absolute ethanol (1 mL/kg), the use of MO significantly ($p < 0.01$) reduced the UI (Fig. 2), and the percentages of ulcer protection at doses of 150, 350, and 500 mg/kg were 55.75%, 59.33%, and 78.51%, respectively (Table 2).

4. Discussion

Gastric hyperacidity and ulcers are very common nowadays, causing tremendous human suffering [21]. Although many products are available for the treatment of gastric ulcers (e.g., antacids and antihistaminics), most of those drugs produce several adverse reactions, such as arrhythmias, impotence, gynecomastia, and hematopoeitic changes [22]. The extracts of many herbal plants have been shown to produce promising results for the treatment of gastric ulcers with fewer or negligible side effects [23]. Almost all parts of the *M. oleifera* plant are reported to have medicinal values for the treatment of various ailments, such as gastrointestinal disorders, tumors, diabetes, hypertension, renal disorders, bacterial and fungal diseases, and fever [6—11]. Furthermore, an ethanolic leaf extract of *M. oleifera* is reported to have potential for the treatment of gastric lesions [12, 24]. In view of this, the present study was performed to investigate the antiulcer potential of the ethanolic root-bark extract of *M. oleifera* by using two experimental models in experimental rats: ethanol-induced gastric lesions and pylorus ligation-induced gastric ulcers.

An acute oral toxicity study of the MO did not produce any sign of mortality or toxicity in the rats during the experiment. The LD_{50} was found to be safe at the doses

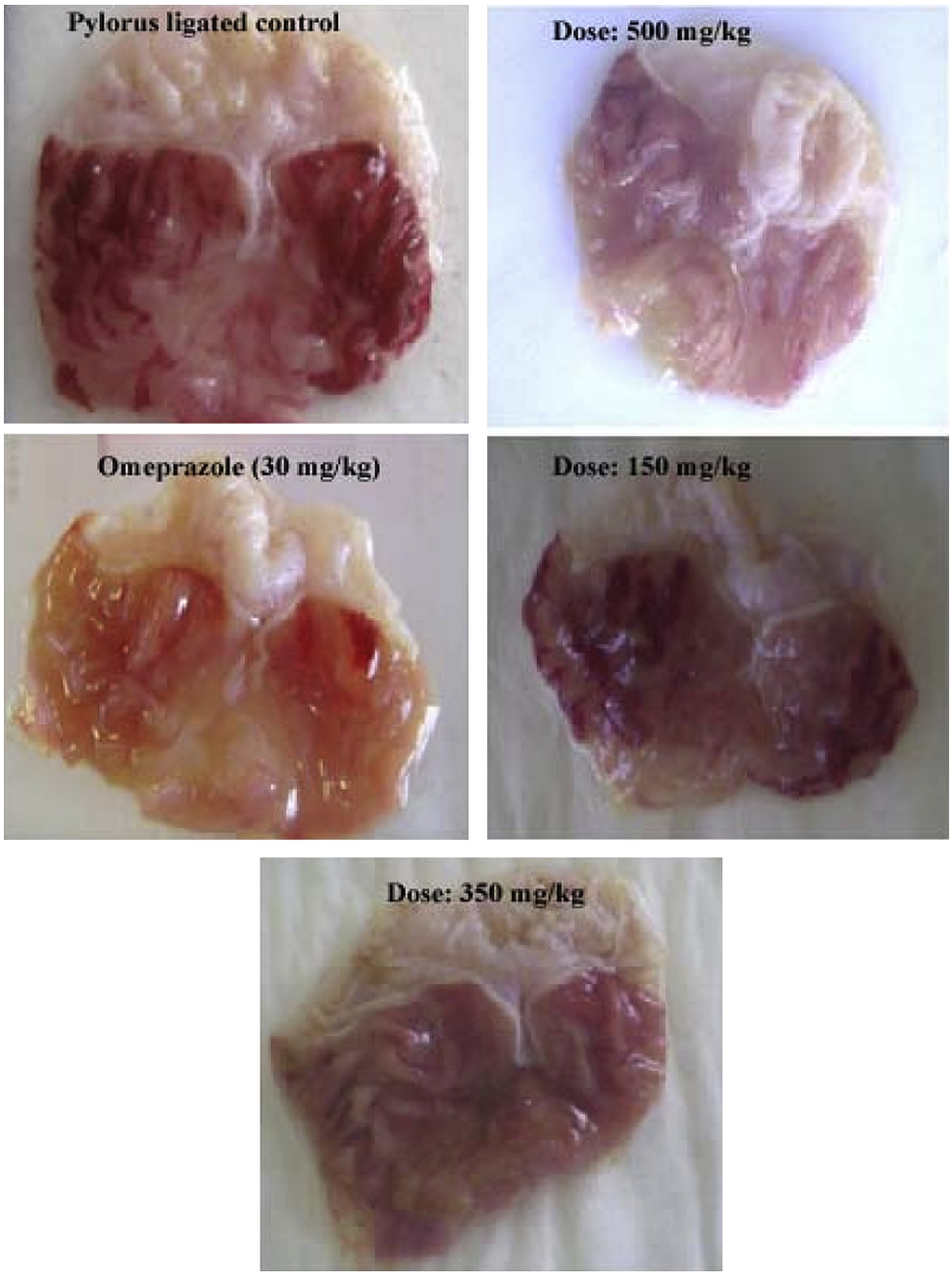

Figure 1 Antiulcer effect of different doses of *Moringa oleifera* (MO) and omeprazole (30 mg/kg) on pylorus ligation-induced gastric ulceration in rats.

Table 1 Antiulcer effect of omeprazole and *Moringa oleifera* (MO) on pylorus ligation-induced ulcers in rats.

Groups	Ulcer index (UI)	% Protection	Free acidity	Total acidity	pH
Control (saline water)	4.91 ± 0.11	—	56.00 ± 2.70	85.00 ± 2.51	1.25 ± 0.02
Omeprazole (30 mg/kg)	0.42 ± 0.05*	91.44%	2.00 ± 0.40*	16.20 ± 1.02*	2.74 ± 0.09*
MO (150 mg/kg)	0.85 ± 0.11†	82.58%	16.40 ± 1.34†	39.80 ± 1.62†	1.79 ± 0.04†
MO (350 mg/kg)	0.73 ± 0.03*	85.13%	12.20 ± 1.12*	32.80 ± 1.51*	1.91 ± 0.04*
MO (500 mg/kg)	0.68 ± 0.01*	86.15%	6.40 ± 0.96*	23.00 ± 0.72*	2.21 ± 0.07*

Values are expressed as mean ± SEM ($n = 6$).
*$p < 0.01$ and †$p < 0.05$ as compared to control (saline-treated) group (one-way ANOVA followed by Dunnett's test). Percentage is calculated as compared to control group.

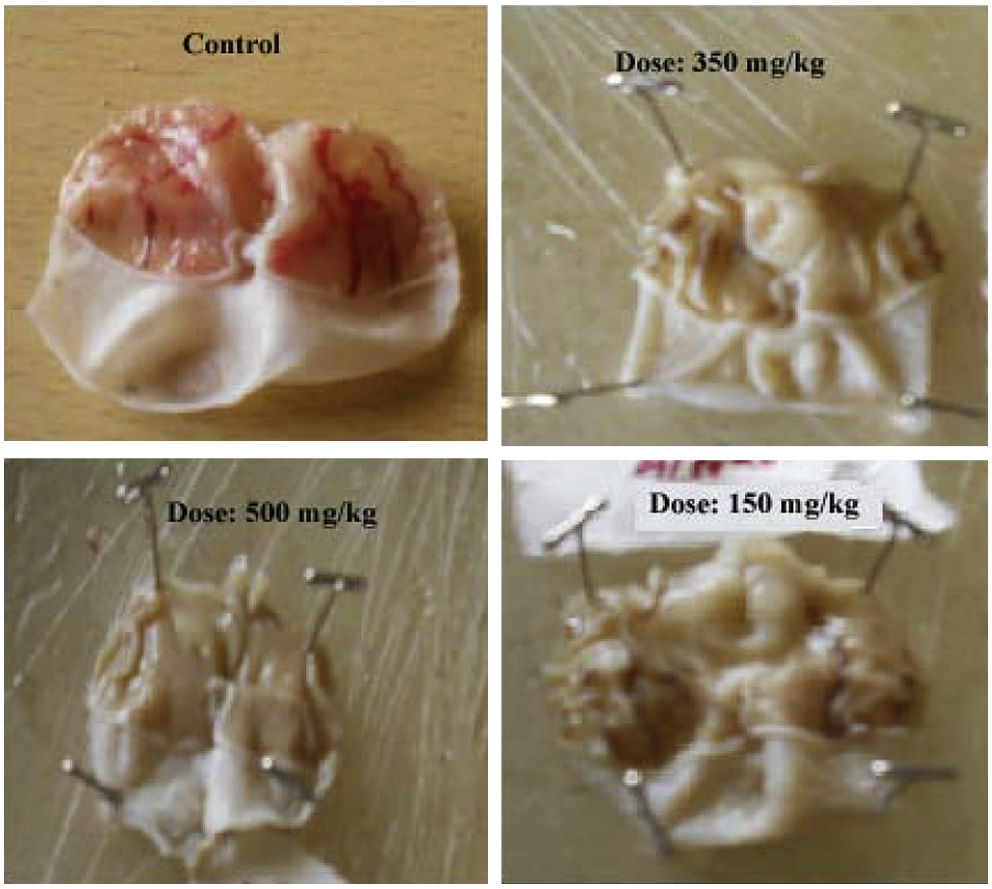

Figure 2 Antiulcer effect of different doses of *Moringa oleifera* (MO) on ethanol-induced gastric ulceration in rats.

used in this study. A dose—response antiulcer study was made using 150, 350, and 500 mg/kg of MO for both anti-ulcer screening models. The pylorus ligation-induced ulcer model is a simple, reproducible, and highly predictable model for the screening of antiulcer drugs. It neither utilizes exogenous ulcerogens nor is induced by exogenous interfering factors. Ulcers are believed to develop because of an excess of acid and pepsin for a given degree of mucosal defense [25,26]. Pylorus ligation-induced ulcers may be attributable to autodigestion of gastric juice, decreased mucosal blood flow, and breakdown of the mucosal barrier [27]. In our study, pylorus ligation produced a marked increase in gastric lesions and damaged the stomach mucosal layer in the experimental rats. The stomachs of rats in the control group (saline treated) showed higher inductions of gastric ulcers due to increased levels of gastric juice in the rat's stomachs; the values of the pH for the gastric contents were decreased. Group 2 (omeprazole treated, 30 mg/kg) showed significant ($p < 0.01$) protection (91.44 %) against gastric ulceration caused by pylorus ligation compared to the ulcers produced in the control group. The MO treatment (Groups 3—5) at different doses (350 and 500 mg/kg) also showed significant ($p < 0.01$) decreases in the UI as compared to the control group, but the effect of the dose of 150 mg/kg was found to be less significant ($p < 0.05$) (Fig. 1).

Table 2 Antiulcer effect of omeprazole and *Moringa oleifera* (MO) on alcohol-induced ulcers in rats.

Groups	Ulcer index (UI)	% Protection
Control (saline, 0.9%)	3.92 ± 0.11	—
Omeprazole (30 mg/kg)	0.44 ± 0.03*	88.47%
MO (150 mg/kg)	1.73 ± 0.14†	55.75%
MO (350 mg/kg)	1.59 ± 0.15*	59.33%
MO (500 mg/kg)	0.84 ± 0.16*	78.51%

Values are expressed as mean ± SEM ($n = 6$).
*$p < 0.01$ and †$p < 0.05$ as compared to control group (one-way ANOVA followed by Dunnett's test). Percentage is calculated as compared to control group.

The gastric content of the incised stomachs were analyzed for the gastric pH, free acidity, and total acidity. The free acidity and the total acidity were significantly decreased ($p < 0.01$), and the pH value of the gastric content was significantly increased ($p < 0.01$) by oral administration of omeprazole (30 mg/kg) and MO (350 and 500 mg/kg) as compared to pylorus-ligated control group, but administration of MO at 150 mg/kg showed a less significant result ($p < 0.05$). These findings indicate that the MO is effective in reducing gastric ulcers produced by hyperacidity in the stomach. Even the low dose (150 mg/kg) has shown sufficient cytoprotective action against pylorus ligation-induced gastric ulcers and mucosal damage. The agents that decrease gastric acid secretion or

increase mucus secretion are effective in protecting against ulcers induced by using these methods. The MO reduced in gastric acid secretion, proving its antisecretory effect [24].

The absolute ethanol method of inducing gastric lesions is a rapid and convenient way of screening herbal extracts for antiulcer activity and cytoprotection in macroscopically and microscopically visible lesions [28]. The incidence of ethanol-induced ulcers is predominant in the glandular part of the stomach and has been reported to stimulate the formation of leukotriens C4, mast-cell secretory products, and reactive oxygen species, resulting in damage to the gastric mucosal layers [29,30]. The formation of gastric mucosal lesions following ethanol administration involves several mechanisms, such as a reduction in gastric blood flow, solubilization of mucus constituents, induction of oxidative stress, an increase in xanthine oxidase activity, and increase in malondialdehyde levels, and a decrease in total glutathione content [31,32]. Ethanol has also been reported to cause gastric mucosal lesions through vasoconstriction, the release of vasoactive substances such as histamine, and the production of free radicals that cause a discontinuity in the mucosal cell membrane. The high production of free radicals is another factor associated with gastric injury and is due to increased lipid peroxidation and damage to the gastric surface. Accumulation of activated neutrophils in the gastric mucosa may be a source of this free radical generation [31]. Free radical scavengers protect the gastric mucosa against alcohol-induced gastric ulceration [32].

In our experiment, the oral administration of absolute ethanol (1 mL) to the rats produced gastric ulcers and mucosal layer damage in the stomachs of the rats. The UI and the mucosal layer damage were significantly decreased in the omeprazole (30 mg/kg)-treated group as compared to the control group ($p < 0.01$). The MO at different doses decreased the UI significantly ($p < 0.01$), but the group that was administered a dose of 150 mg/kg showed a less significant result than the control group ($p < 0.05$). The results indicated that all selected doses showed reductions in the UI, but the dose of 500 mg/kg showed the highest protection against gastric lesions. The mechanisms involved in the reduction of ulcers caused by administration of ethanol may be regeneration of the glandular epithelium, formation of collagen, increased capillary density, increased pH, and increased free-radical scavenging action [24].

The qualitative phytochemical analysis of the MO showed the presence of alkaloids, carbohydrates, proteins, saponins, tannins, triterpinoids, and steroids. Moringine and moringinine are the alkaloids found in the root-bark extract that are useful for treating ulcers. Pterygospermin is a powerful antibiotic found in *M. oleifera* and is effective against a broad range of bacteria, (e.g., *H. pylori*). Saponins are widely distributed in plants and are a particular form of glycoside. Plant materials with antiulcer activity often contain triterpenoids and saponins in considerable amounts. The protective activities of these active saponins are probably due to the activation of mucous membrane protective factors [33]. Tannins are used in medicine primarily because of their astringent properties, which are attributable to the fact that they react with the proteins in the layers of tissue with which they come into contact. Tannins are known to "tan" the outermost layer of the mucosa and to render it less permeable and more resistant to chemical and mechanical injury or irritation [34].

5. Conclusion

On the basis of the present study, we conclude that MO has the potential to cure gastric ulcers and gastric mucosal lesions. It also decreases the acidity and increases the pH of gastric juice. These findings indicate that MO possesses antiulcer and antisecretory activity and, hence, can be used as a source for antiulcer drugs in future. Further experimental work is ongoing to isolate and identify the active principles present in the extract that are responsible for the antiulcer activity.

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