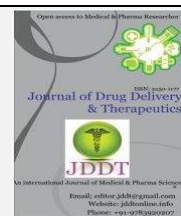




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Research Article

Evaluation of anti-ulcer activity of extract of *Moringa oleifera* Lam. using Acetic acid induced ulcer model

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ABSTRACT

Moringa oleifera Lam commonly known as Moringa, a native plant from Africa and Asia, and the most widely cultivated species in Northwestern India, is the sole genus in the family Moringaceae. It comprises 13 species from tropical and subtropical climates, ranging in size from tiny herbs to massive trees. It is grown for its nutritious pods, edible leaves and flowers and can be utilized as food, medicine, cosmetic oil or forage for livestock. Its height ranges from 5 to 10 m. Several studies have demonstrated the beneficial effects in humans. MO has been recognized as containing a great number of bioactive compounds. The most used parts of the plant are the leaves, which are rich in vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins and saponins. The present investigation was carried out to evaluate anti-ulcer activity of various extract of the plant.

Keywords: *Moringa oleifera*, Anti-ulcer activity, acetic acid induced

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INTRODUCTION

Peptic ulcers (PU) are sores or lesions in the gastrointestinal mucosa extending throughout the muscularis mucosae, typically characterized by different stages of necrosis, neutrophil infiltration, blood flow reduction, increased oxidative stress and inflammation. PU manifest as a non-fatal disease, majorly represented by periodic symptoms of epigastric pain, which are often relieved by food or alkali, besides to trigger much discomfort to patients, disrupting their daily routines and also causing mental agony. The disease is mostly categorized based on its anatomical origins, such as gastric (found along the lesser curvature of the stomach) and duodenal (occurring in the duodenal bulb—the most exposed area to gastric acid) ulcers. Studies have shown that peptic ulcer disease (PUD) occurs because of an imbalance between aggressive injurious (e.g., pepsin, HCl) and defensive mucosa-protective factors (e.g., prostaglandins, mucus and bicarbonate barrier and adequate blood flow). All ulcers of the upper gastrointestinal tract were originally thought to be caused by the aggressive action of pepsin and gastric acid on mucosa. However, the denomination “peptic ulcer” has lately pointed to *Helicobacter pylori* infection, where the chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) and

acetylsalicylic acid (ASA) are some of the disease-causing factors. Thus, based on the latest advances on this field and stress the fact that PUD is an important cause of morbidity and health care costs, the present report aims to provide a general overview on peptic ulcers, namely considering their epidemiology, main symptoms and clinical features, pathogenesis, where a particular emphasis will be given to *H. pylori* infection, pharmacological agents used in an effective management and also pointing out the latest challenges and opportunities of using plant phytochemicals as upcoming antiulcerogenic agents. Lastly, a special emphasis was given on plant products safety and security, in order to trigger the interest in deepening skills on this matter and to ensure an effective managing competence for health-related systems.¹⁻²

Drumstick tree, also known as horseradish tree and ben tree in English, is a small to medium-sized, evergreen or deciduous tree native to northern India, Pakistan and Nepal. It is developed and has turned out to be naturalized well past its local range, including all through South Asia, and in numerous nations of Southeast Asia, Central America, tropical Africa, the Caribbean, the Arabian Peninsula and tropical South America. The tree more often than not develops to 10 or 12 m in stature, with a spreading, open

crown of hanging, weak branches, padded foliage of tripinnate leaves, and thick, corky, profoundly fissured whitish bark. It is esteemed for the most part for its eatable natural products, leaves, blooms, roots, and seed oil, and is utilized widely in conventional prescription all through its local and presented ranges. It is developed and has turned out to be naturalized in different pieces of Pakistan, India, and Nepal, just as in Afghanistan, Bangladesh, Sri Lanka, Southeast Asia, West Asia, the Arabian promontory, East and West Africa, all through the West Indies and southern Florida, in Central and South America from Mexico to Peru, just as in Brazil and Paraguay. *Moringa oleifera* is a little, quickly developing evergreen or deciduous tree that generally grows up to 10 or 12 m in tallness. It has a spreading, open crown of hanging, delicate branches, fluffy foliage of tripinnate leaves, and thick, corky, whitish bark.³⁻⁵

Since the reports about the antiulcer activity of the leaves of *Moringa oleifera* sparsely documented, it was considered worthwhile to investigate the antiulcer activity of the flower and seeds extract of *Moringa oleifera* and substantiate its ethnopharmacological claim of providing relief in PUD.

METHODOLOGY

Collection of plant material and extraction procedure

The flowers and seeds of *Moringa oleifera* Lam were collected from the botanical garden of RKDF university Bhopal between 28/01/2017 to 25/09/2017. Flowers and seed were authenticated by the Head of Department of botany Dr.Zia Ul Hasan Professor of Safia College of Science Bhopal. Plant authentication no. is 346/Bot/Safia/2017 & 347/Bot/Safia/2017 on the date 14/10/2017.

Extraction of leaves and fruits⁶

The flowers and 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 material obtained was then subjected to successive extraction in batches using petroleum ether, chloroform, and acetone and methanol solvents in a Soxhlet extractor. The different extracts obtained were evaporated in rotary evaporator to get a semisolid mass.

Phytochemical estimations of the extracts

The extracts of *Moringa oleifera* Lam were subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids.⁶⁻⁷

Experimental animals

Male albino Wistar rats weighing between 200-250 gm were used. Institutional Animal Ethics Committee permitted the experimental procedure; 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).

Acute toxicity study

The intense oral poisonous quality examination was performed by the OPPTS (Office of prevention, pesticide and toxic substance) Up and Down method (Health Effect Test Guideline 2004) The different extracts were suspended using 0.5% sodium carboxy methylcellulose and were administered orally. The concentration was adjusted in such a way that it did not exceed 1ml/kg b/w of the animal.⁸

Acetic acid induced ulcer⁹⁻¹¹

The method described was followed. The creatures were fasted for 24 hours before the investigation. Under light ether anesthesia the mid-region was opened by midline entry point underneath the xiphoid procedure and the stomach was uncovered. Icy acidic corrosive (0.05ml) was added to the barrel shaped form of 6mm width set firmly over the front serosal surface of the stomach and this was permitted to stay over yonder for 60 seconds. The corrosive arrangement was evacuated by washing the shape with ordinary saline twice or thrice to keep away from harm to the encompassing tissues. The stomach was put back cautiously and the stomach divider was shut. The creatures were treated with ranitidine (50 mg/kg, p.o) and with various concentrates of blooms and seeds of *Moringa oleifera* Lam (500 mg/kg p.o) when every day for 10 days after enlistment of ulcer while the control gather got just vehicle. Rodents were relinquished on the tenth day, stomachs were expelled and they were cut open along the more noteworthy ebb and flow.

The stomach tests were examined utilizing a PC scanner and the all out mucosal zone and all out ulcerated zone were estimated utilizing open area picture preparing and examination program created at National Institute of Health, USA. The PC adaptation of the program was downloaded free from Scion (<http://www.scioncorp.com>) (Scion Image for Windows, Release Beta 4.0.2). The scale was set on 6.1 pixels/mm. The ulcer record was resolved utilizing the recipe [202]

$$\text{Ulcer index} = \frac{10}{X}$$

$$\text{where } X = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}$$

The ulcers were given scores based on their intensity as follows

0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis,

3 = perforated or penetrated ulcer.

Methodology for the evaluation of gastric lesions by using scion image

Snap on the record catch in the menu of scion picture and open the picture document from the report. Select the heart image from the device bar and follow the out line of the picture. At that point click on break down catch, next snap on set scale and set the scale as 6.1 pixels/mm. Then once more click on the study button followed by calculate which gives the area of the selected image on the info screen. The same procedure is repeated to measure the total area of the mucosal surface and ulcerated area note the area of ulcerated area and total mucosal area and calculate the ulcer index as mentioned above. The stomach samples from groups that showed reduction in ulcer index were subsequently processed for histological examination. Three lists to be specific recovered glandular epithelial width, narrow thickness and collagen content were chosen to mirror the rate and nature of ulcer recuperating [204]. Regenerated lining epithelial width was defined as the average distance from the origin of regenerated lining epithelium to the surface of the ulcer.⁹⁻¹¹

Statistical analysis

The arithmetical implication was assessed using one-way analysis of variation (ANOVA) followed by Dunnet comparison test. For comparing nonparametric ulcer scores, ANOVA followed by non-parametric Dunn posttest was used.

The standards are articulated as mean + SEM and $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The present study deals with the study of effect of the different extracts of flower and seeds of *Moringa oleifera* on gastric and duodenal ulcers. Phytochemical analysis of the flower extracts revealed that petroleum ether extract contains steroids and alkaloids, acetone extract had carbohydrates, proteins, alkaloids, tannins, flavonoids and glycosides while the methanol extract contained saponins along with all the constituents present in acetone extract.

In the acute toxicity study, no mortality was observed after treatment with the highest tested dose (5 g/kg p.o.) of all the extracts of flower and seeds. Hence, 1/10th of the tested dose (500 mg/kg p.o.) was selected for evaluation of anti-ulcer activity. The anti-ulcer effect was evaluated using the acetic acid induced ulcer. The petroleum ether, acetone, and methanolic flower extracts of *Moringa oleifera* Lam and

ranitidine showed a significant reduction in ulcer index when compared to control ($p < 0.001$). The acetone extract of the flower was most potent; it produced a 79% decrease in the ulcer index when compared to control. The seed extracts of *Moringa oleifera* Lam and the chloroform extract of the flower did not show any significant effect on ulcer index (Fig.1). None of the treatments produced any significant effect on ulcer score (Fig.2).

Sections of ulcerated area revealed that there was a significant increase in regenerated glandular epithelium width after treatment with acetone extract of the flowers ($p < 0.05$) (Fig. 3). The collagen content in the ulcerated tissue was significantly increased by petroleum ether, acetone and methanol extracts of the flowers and ranitidine, with acetone extract of the flowers showing the maximum effect (Fig. 4). No significant difference on capillary density in scar tissue was observed after treatment with different extracts of flowers or ranitidine (Table 1) (Fig. 5).

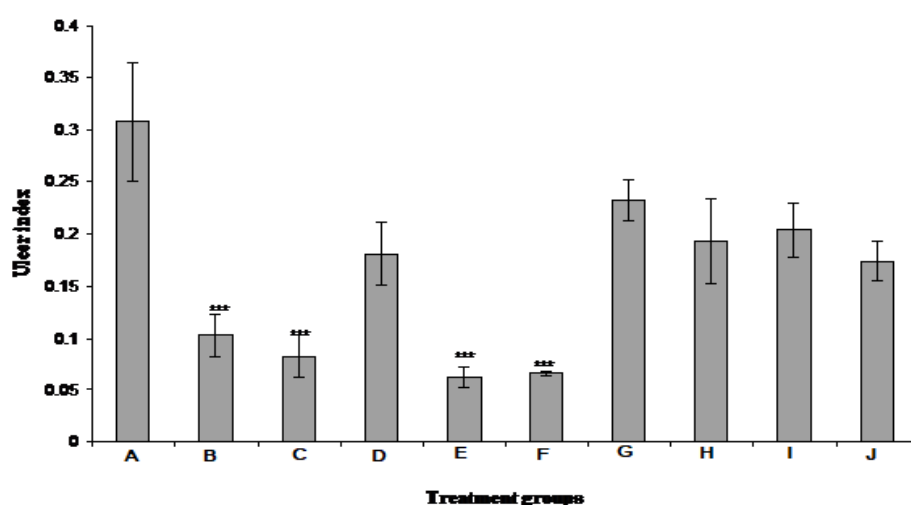


Figure 1: Effect of *Moringa oleifera* on ulcer index in acetic acid induced chronic gastric ulcer

A= Control; B= Standard (Ranitidine 50mg/kg p.o.); C= Flowers petroleum extract (500mg/kg p.o.); D= Flowers chloroform extract (500mg/kg p.o.); E= Flowers acetone extract (500mg/kg p.o.); F= Flowers methanol extract (500mg/kg p.o.); G= Seeds petroleum extract (500mg/kg p.o.); H= Seeds chloroform extract (500mg/kg p.o.); I= Seeds acetone extract (500mg/kg p.o.); J= Seeds methanol extract (500mg/kg p.o.). All values are mean ± SEM, n = 5-6. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared to control group

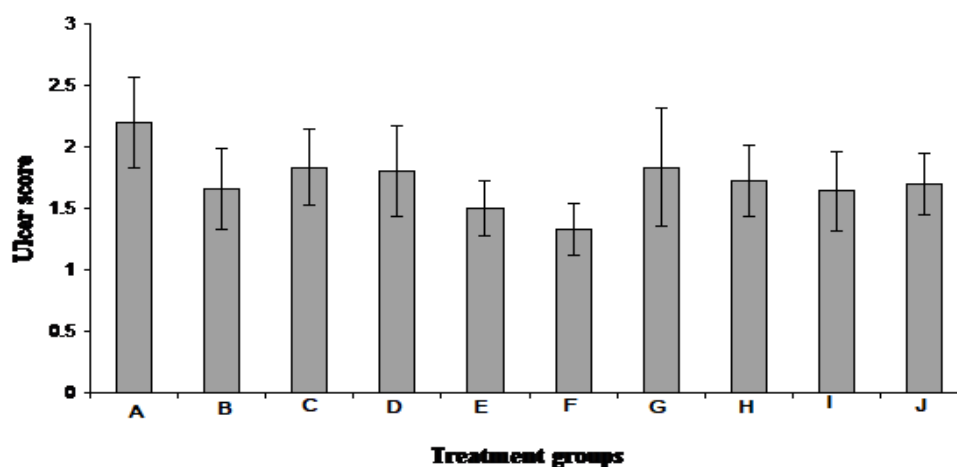


Figure 2: Effect of *Moringa oleifera* on ulcer score in acetic acid induced chronic gastric ulcer

A= Control; B= Standard (Ranitidine 50mg/kg p.o.); C= Flowers petroleum extract (500mg/kg p.o.); D= Flowers chloroform extract (500mg/kg p.o.); E= Flowers acetone extract (500mg/kg p.o.); F= Flowers methanol extract (500mg/kg p.o.); G= Seeds petroleum extract (500mg/kg p.o.); H= Seeds chloroform extract (500mg/kg p.o.); I= Seeds acetone extract (500mg/kg p.o.); J= Seeds methanol extract (500mg/kg p.o.). All values are mean ± SEM, n = 5-6. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared to control group

Table 1: Effect of *Moringa oleifera* Lam on regenerated glandular epithelium width, capillary density and volume of collagen content

Treatment	Regenerated glandular epithelium width (μm) in $19600 \mu\text{m}^2$	Capillary density (No)	Vol. of collagen content
Control	504 $\pm$ 60.39	5.4 $\pm$ 0.51	0.182 $\pm$ 0.018
Ranitidine	648 $\pm$ 35.33	5.2 $\pm$ 0.38	0.265 $\pm$ 0.019**
Petroleum Ether flower extract	564 $\pm$ 46.48	6.6 $\pm$ 0.81	0.262 $\pm$ 0.014**
Acetone flower extract	700 $\pm$ 20.94*	4.6 $\pm$ 1.89	0.291 $\pm$ 0.009***
Methanol flower extract	588 $\pm$ 24.98	4.0 $\pm$ 1.05	0.266 $\pm$ 0.012**

All values are mean $\pm$ SEM, n = 5-6. * p <0.05, ** p <0.01, *** p <0.001 when compared to control group

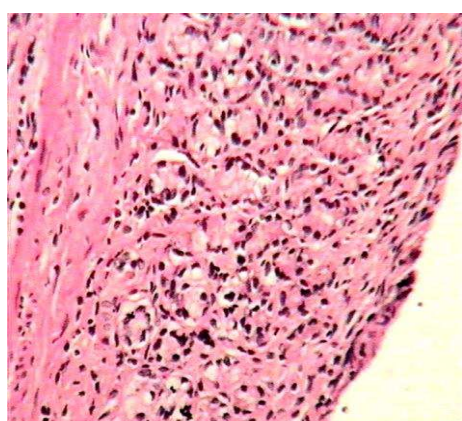
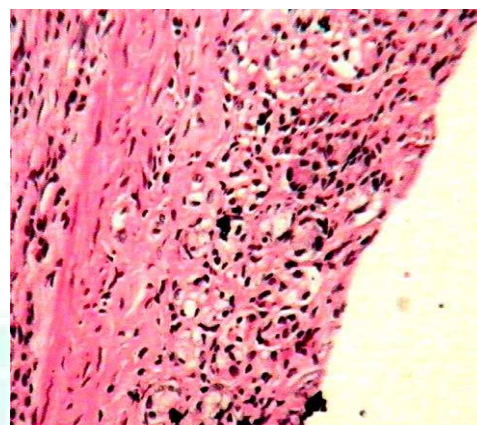
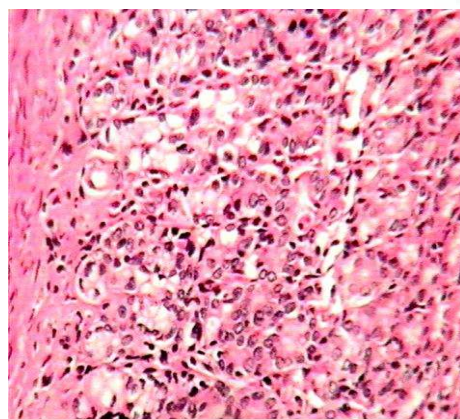
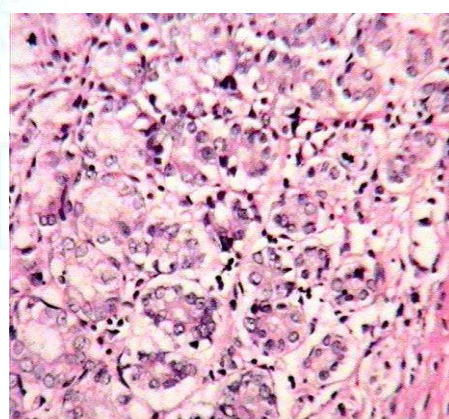
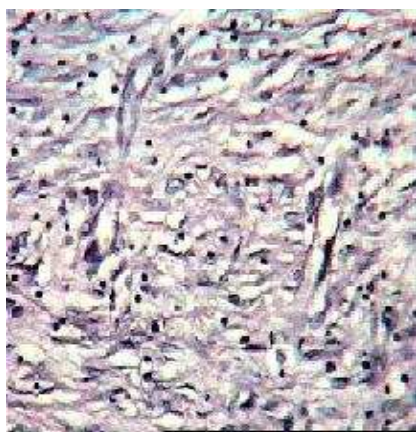
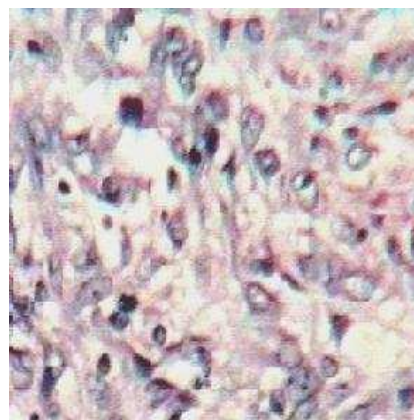
**Normal (X100)****Ulcerated Control (X100)****Ranitidine treated (50mg/kg.p.o.)****Acetone flower extract treated (500mg/kg.p.o.) (X100)**

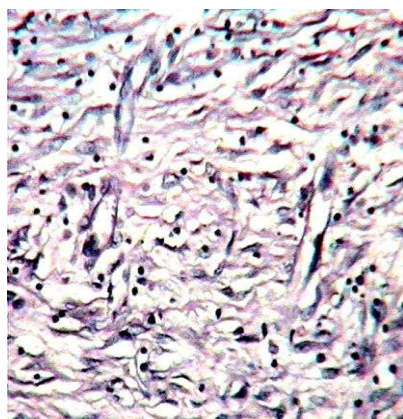
Figure 3: Sections stained with hematoxylin and eosin (H&E) displaying the regenerated glandular epithelium width in stomachs of rats treated with ranitidine and acetone flower extract of *Moringa oleifera* Lam in acetic acid induced ulcer model.



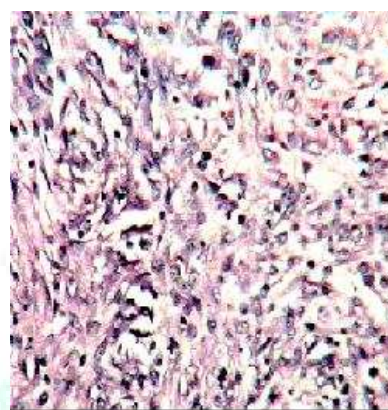
Normal (X100)



Ulcerated Control (X100)

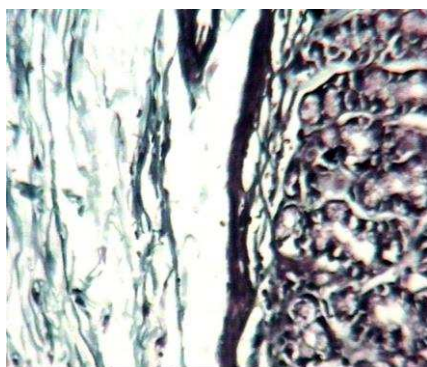


Ranitidine treated (50mg/kg.p.o.)

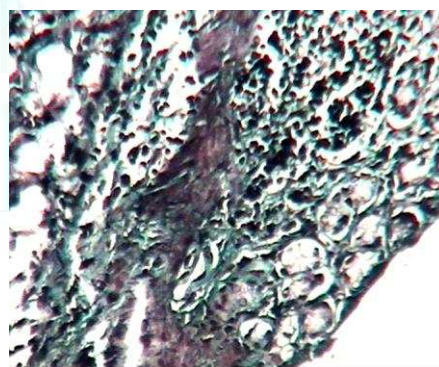


Acetone flower extract treated (500mg/kg.p.o) (X100)

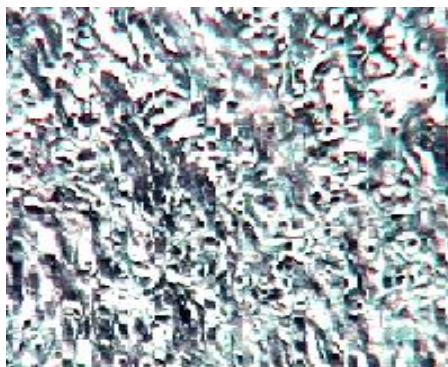
Figure 4: Sections stained with periodic acid schiff's stain displaying capillary density in stomachs of rats treated with ranitidine and acetone flower extract of *Moringa oleifera* Lam in acetic acid induced ulcer model.



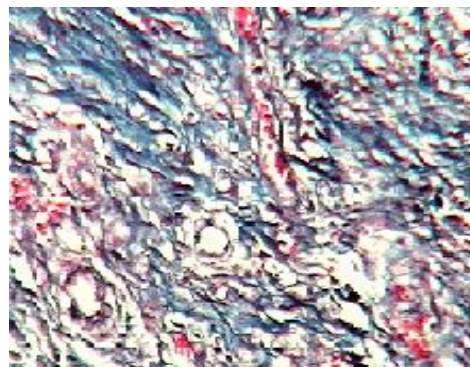
Normal (X100)



Ulcerated Control (X100)



Ranitidine treated (50mg/kg.p.o.)



Acetone flower extract treated (500mg/kg.p.o) (X100)

Figure 5: Sections stained with masson's trichome displaying collagen in stomachs of rats treated with ranitidine and acetone flower extract of *Moringa oleifera* Lam in acetic acid induced ulcer model.

CONCLUSION

The petroleum ether, acetone, and methanolic flower extracts of *Moringa oleifera* were found to be effective in healing the ulcer induced by acetic acid. The petroleum ether, acetone and methanolic extracts of *Moringa oleifera* were also found to increase the collagen content in the ulcerated tissues. The acetone flower extract significantly increased the regenerated glandular epithelium width.

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