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STUDIES OF STEM BARK OF *MORINGA OLEIFERA* (LAM.) FOR ANTIULCER ACTIVITY

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ABSTRACT

The aim of research was the studies of stem bark of *Moringa oleifera* (lam.) for antiulcer activity. The *Moringa oleifera* were obtained from the Gwalior region. It was identified and authenticated by a botanist. The powder was weighed and extracted through Soxhlet apparatus using ethanol solvent. The obtained slurry of mixture was dried under partial vacuum using a rotary evaporator or water-bath. Preliminary phytochemical screening was performed for the herbal extract. Albino rats of either sex weighing 150–200g were obtained from the animal house, Shri Ramnath Singh Mahavidhyalaya (Pharmacy) Gormi, Bhind, MP. All the rats are divided into 4 groups (n=6); group 1: rats are given only distilled water each day for 21 days; group 2: rats are given Ranitidine (20mg/kg/day, p. o.) for 21 days; group 3: rats are given ethanolic stem bark extract of *Moringa oleifera* (SBMO) (200mg/kg/day, p. o.) for 21 days; and group 4: rats are given ethanolic stem bark extract of *Moringa oleifera* (SBMO) (400mg/kg/day, p. o.) up to 21 days. The anti-ulcer effect was evaluated in pylorus ligation- induced ulcer model and parameters were performed including microscopical examination, pH of gastric acid, gastric acid volume, free & total acidity. The pH level was measured to be below the reference and comparison ranges. Decreases in pH are a well-supported treatment for ulcers, as hyperacidity is the primary determinant in their anti-ulcerogenic potential. Group 2 received ranitidine (20mg/kg), while group 4 received

ethanolic stem bark extract of *Moringa oleifera* (400m/kg) resulting in the greatest reduction in pH. In conclusion, *Moringa oleifera* has significant anti-ulcer potential at both the doses used 200mg/kg & 400mg/kg. This study suggests to isolate and develop the suitable dosage form of concerning chemical constituents present in *Moringa oleifera* for the activity.

KEYWORDS: *Moringa oleifera,* anti-ulcer, ranitidine, total acidity, ethanolic extract.

INTRODUCTION

Peptic ulcer involves acid-induced injury to the intestine that normally occurs in the stomach or upper part of the duodenum; described as having bared mucosa that extends into submucosa even. Peptic ulcer illness is estimated to affect five to ten percent of the population.[1] Poly-morphisms in the interleukin-1β gene, for example, influence mucosal interleukin 1 production, resulting in H. pylori-induced g.i.t. disorders. The incidence of gastric ulcer is four times that of intestinal ulcer.[2]

Moringa oleifera (*M. oleifera*), the "miracle tree", thrives globally in almost all tropical and subtropical regions, but it is believed to be native to Afghanistan, Bangladesh, India, and Pakistan. Nearly all parts of the tree are used for their essential nutrients. *M. oleifera* leaves have a high content of beta-carotene, minerals, calcium, and potassium.[3] Dried leaves have an oleic acid content of about 70%, which makes them suitable for making moisturizers. The bark of the tree is considered very useful in the treatment of different disorders such as ulcers, toothache, and hypertension. Roots, however, are found to have a role in the treatment of toothache, helminthiasis, and paralysis.[4] The flowers are used to treat ulcers, enlarged spleen, and to produce aphrodisiac substances. The tree is believed to have incredible properties in treating malnutrition in infants and lactating mothers.[5]

Fig. 1: Depiction of *Moringa oleifera* plant.

Taxonomy

Kingdom: Plantae

Order: Brassicales

Family: Moringaceae

Genus: *Moringa*

Species: *oleifera*

M. oleifera is widely distributed worldwide, but its indigenous origin is in India, Arabia and the East Indies. It is common in Asia, Africa, the Caribbean, Latin America, the Pacific Islands, Florida, Madagascar, Central America, Cuba, the Philippines, Ethiopia, and Nigeria.[6] The history of the plant explains that *M. oleifera* was introduced from India to Africa, Southeast Africa, and the Philippines in ancient times. It requires tropical and subtropical regions and grows at a temperature of about 25–35°C. *M. oleifera* is a deciduous type of tree typically grown in tropical and subtropical regions across the globe.[7] It grows best in indirect sunlight and without waterlogging, and the soil should be slightly acidic to alkaline. The tree begins to bear fruit at 6 to 8 months of age. Commercially, it is grown in different countries such as Africa, Mexico, Hawaii, and South America, but due to different soil conditions, the nutrient content varies from country to country.[8][9]

MATERIALS AND METHODS

Experimental requirements

Moringa oleifera stem bark, Ranitidine, distilled water, Wistar albino rats (either sex), rotatory evaporator, weighing machine and ethanol.

Collection, Identification and Authentication of plant

The *Moringa oleifera* will be obtained from the Gwalior region. It will be identified and authenticated by a botanist. They are washed making dust-free and dried at room temperature or shade after they are rendered into coarse powders and then finally into fine ones. The powder was weighed and extracted through Soxhlet apparatus using ethanol solvent. The obtained slurry of mixture is dried under partial vacuum using a rotary evaporator or water-bath. The percentage yield of the extract of the *Moringa oleifera* is calculated as below mentioned formula.[10]

$$\% \text{ Yield} = \frac{\text{Actual Yield}}{\text{Theoretical Yield}} \times 100\%$$

Phytochemical screening

The plant extracts were screened for different phytoconstituents to check their presence.

1. Detection of Alkaloids

Extracts were dissolved individually in dilute HCl and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow-colored precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hagers Reagent. Formation of yellow ppt indicates the presence of alkaloids.

2. Detection of Glycosides

Fehling's test: With distilled water dilution, Fehling's solutions A and B were heated for one minute. There were 8 drops of plant extract added to this transparent blue solution. It was then combined with 1 ml of Fehling's solution and heated for 5 minutes in a water bath. Brick red precipitation is an indication of glycoside content.

Detection of Saponins

Foam test: About 2g of the plant extract was mixed with 10ml of distilled water and shaken vigorously for a stable persistent froth. Appearance of froth indicates the presence of saponins.[11]

3. Detection of Tannins

Ferric chloride test: 0.5g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% $FeCl_3$ was added and observed for brownish green-black or a blue-black coloration.

Lead acetate test: 2ml of plant extract was combined with 2ml of distilled water. 0.01g lead acetate was added to this combined solution and shaken well. Development of white turbidity and precipitate indicates the presence of tannins.[12]

4. Detection of Flavonoids

NaOH test: A small amount of extract was treated with aqueous NaOH and HCl, and observed for the formation of yellow orange color.

H_2SO_4 test: A fraction of the extract was treated with Conc.H_2SO_4 and observed for the formation of orange color.

6. Detection of terpenoids

5 ml of the aqueous plant extract were combined with 2.0 ml of chloroform, which was then added, evaporated on the water path, and boiled with 3 ml of concentrated H2SO4. As terpenoids took shape, a grey colour emerged.

7. Detection of Steroids

2 ml of chloroform and concentrated H_2SO_4 were added with the 5 ml aqueous plant crude extract. In the lower chloroform layer red color appeared that indicated the presence of steroids.

8. Test for Reducing Sugars and Carbohydrates

Molisch's test (General test)

To 2-3ml extract of individual solvents add few drops of α-naphthol solution in alcohol, shake and add concentrate H_2SO_4 from sides of test tube. Violet ring at the junction of two liquids.

Fehling's test

It is utilised to find decreasing sugars. Make a volume of 500 mL by dissolving 34.66 grammes of copper sulphate in distilled water (solution A). 50 grammes of sodium hydroxide and 17.3 grammes of potassium sodium tartrate should be dissolved in distilled water to a volume of up to 50 millilitres (Solution B). Prior to usage, combine two solutions in an equal volume. Fehling's A and B solution in a 1 mL mixture should be boiled for one minute. Add the test solution in an equal amount. Heat in a pot of boiling water for 5-10 minutes. A first yellow and then a brick red hue was seen.

Preparation of animals

Albino rats of either sex weighing 150–200 g will be obtained from the Animal House, Shri Ramnath Singh Mahavidhyalaya (Pharmacy) Gormi, Bhind (MP). The animals are maintained in proper conditions, at room temperatures of 25 ± 1°C with 12-hour light/dark

cycle. The relative humidity is maintained at 44–56%, and are fed with standard rodent diet and water ad libitum. Animals will keep on fasting but free access to water up to 1 h before the induction of ulcers.[13]

Group design

All the rats are divided into 4 groups (n=6) as followings-

Group 1: rats are given only distilled water each day for 21 days.

Group 2: rats are given Ranitidine (20mg/kg/day, p. o.) for 21 days.

Group 3: rats are given ethanolic stem bark extract of *Moringa oleifera* (SBMO) (200mg/kg/day, p. o.) for 21 days.

Group 4: rats are given ethanolic stem bark extract of *Moringa oleifera* (SBMO) (400mg/kg/day, p. o.) up to 21 days.

Pylorus ligation-induced ulcer model

All the rats are housed in individual cages and fasted for 24 hours (water ad libitum) prior to perform pylorus ligation. Animals are monitored to avoid coprophagy. Under mild anesthesia driven by ether, a midline abdominal incision is made extending from the xiphoid process (1cm). The pyloric ligature is done with sterilized cotton thread, and care being taken that neither affects the blood supply. The abdomen wound is cleansed thoroughly with normal saline solution, dried and covered with cotton already soaked in betadine solution.

After 19 hours of pyloric ligation, the animals are sacrificed by a cervical dislocation. The pyloric segment of stomach is dissected out by clamping the lower segment of esophagus. The glandular portion of stomach is observed for ulcer index (UI). The length and width of each lesion and the sum of the area of all lesions is expressed as the ulcer area (mm2).[14]

The inhibition percentage was calculated by the following formula-

(%I) = [(UAcontrol − UAtreated) ÷ UAcontrol] X 100.

The gastric content was poured into tubes, centrifuged, and used to analysis for various biochemical tests.

- pH & volume of gastric juice
- The gastric content is titrated against 0.01N NaOH to determine the ‘free & total acidity’.[15]

Evaluation parameters[16]

1. Microscopical examination

All the groups of rodents are evaluated for deep haemorrhagic lesions of mucosal layer, submucosal oedema and leucocytes infiltration.

2. pH detection

Gastric content is taken out and kept in contaminated free petri-dish. After, the pH is easily measured by using digital pH meter. It confirms about the acidity level in the rodent.

3. Gastric Volume determination

In this test, gastric content of stomach of rat is taken out and filled into measuring cylinder to confirm the actual volume of gastric fluid. It confirms about the level of acidity developed in the rat and effect of the drug.

4. Free & total acidity

In this procedure, firstly gastric content is taken out separately. The gastric content is titrated against 0.01N NaOH to determine the 'free & total acidity'. It confirms the level of acidity and beneficial effect of drug given.

RESULTS AND DISCUSSION

Percentage yield

The percentage yield of *Moringa oleifera* stem bark was calculated as 64.28% when extracted through using ethyl alcohol.

Determination of preliminary phytochemicals

It showed that carbohydrates, tannins and steroids are found in abundance or moderate quantity. However, glycosides, phenols, and starch were found positive in extract**.** Alkaloids, saponins, and proteins were found absent. Thus, *Moringa oleifera* stem bark extract shown a rich source of numerous phytochemicals as mentioned in below table-

Table 1: Determination of Phytochemicals

S.N.	Phytochemicals	*Moringa oleifera* stem bark extract
1.	Alkaloids	_
2.	Saponins	_
3.	Carbohydrates	++
4.	Protein	_
5.	Glycosides	+

6.	Tannins	++
7.	Phenols	+
8.	Starch	+
9.	Steroids	++

Where, (+)= Positive, (++)= Moderated Positive, (-)=Negative

Evaluation of anti-ulcer activity

Ulcer index

Group 1 that was given distilled water had a UI of 1.73±0.02* and a Nil PI as there was no change ulcer. Group 2 received 20mg/kg of ranitidine, resulting in a UI of 0.41±0.06** and a percentage of inhibition of 93.02. Due to its effectiveness in preventing ulcers, it lowered the ulcer index. Ulcer index (UI) and Percentage inhibition were 0.63±0.02** and 66.02 for the group administered Ethanolic stem bark extract of *Moringa oleifera* (SBMO) (200mg/kg, p. o.). Group 4 administered the ethanolic stem bark extract of *Moringa oleifera* (SBMO) (400mg/kg, p. o.) showed the greatest anti-ulcerogenic effect, with a UI of 0.52±0.06** and a PI of 82.04.

Table 2. Ulcer Index & Percentage Inhibition role of *Moringa oleifera* in Pylorus ligation-induced ulcer model.

Treatment	**UI**	**PI**
Vehicle	1.73±0.02*	Nil
Ranitidine (20mg/kg)	0.41±0.06**	93.02
Ethanolic stem bark extract of *Moringa oleifera* (SBMO) (200mg/kg, p. o.)	0.63±0.02**	66.02
Ethanolic stem bark extract of *Moringa oleifera* (SBMO) (400mg/kg, p. o.)	0.52±0.06**	82.04

Statistical significance was indicated by an asterisk (*), and data were reported as Mean SEM; n=6.

Microscopic studies

The stomach samples were studied under a compound microscope with a 100x magnification power.

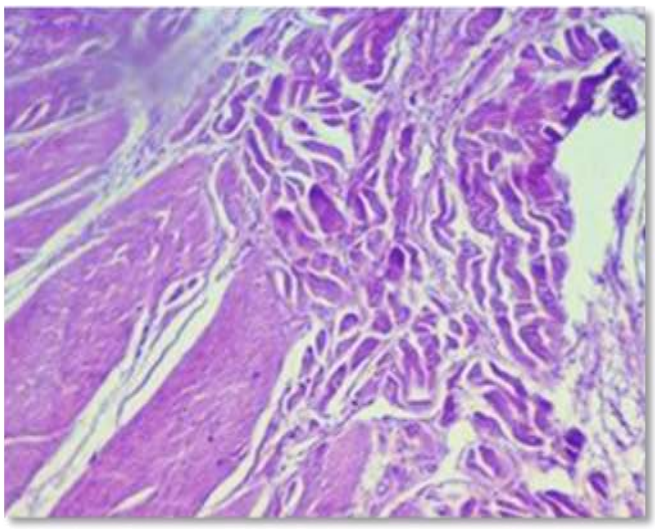

Fig. 2: Leucocytes infiltration in animal administered ethanolic stem bark extract of *Moringa oleifera* (SBMO) (200mg/kg, p. o.).

Before anything more could be done, the stomach had to be dissected and cleaned with saline solution to remove all traces of fat and gastric content. After that, it was preserved in saline solution till the high-powered compound microscope investigation was finished. The infiltration and streaking of ulcer perforations by leucocytes is seen in the next figure.

The mucosal edoema and streaks are depicted in the accompanying image. The degree of swelling in Group 4 was verified.

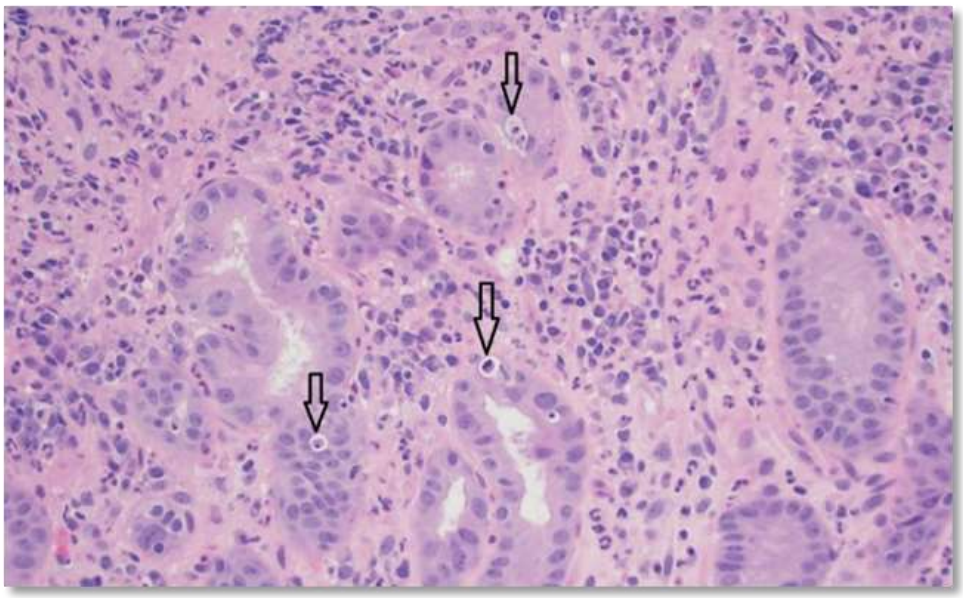

Fig. 3: Mucosal edema in animal administered ethanolic stem bark extract of *Moringa oleifera* (SBMO) (400mg/kg, p. o.).

For 15 days, every single rat received treatment on a once-daily schedule. Group 4 received ethanolic stem bark extract of *Moringa oleifera* (SBMO) ((400mg/kg, p. o.), and results from all microscopical analyses demonstrated negligible mucosal edoema and leucocyte infiltration. As a result, the observed reaction was shown to be dosage dependent. Group 3 received Ethanolic stem bark extract of *Moringa oleifera* (SBMO) (200mg/kg, p. o.), which resulted in less anti-ulcer effects. The standard group, shown in the next figure, had mild ulceration.

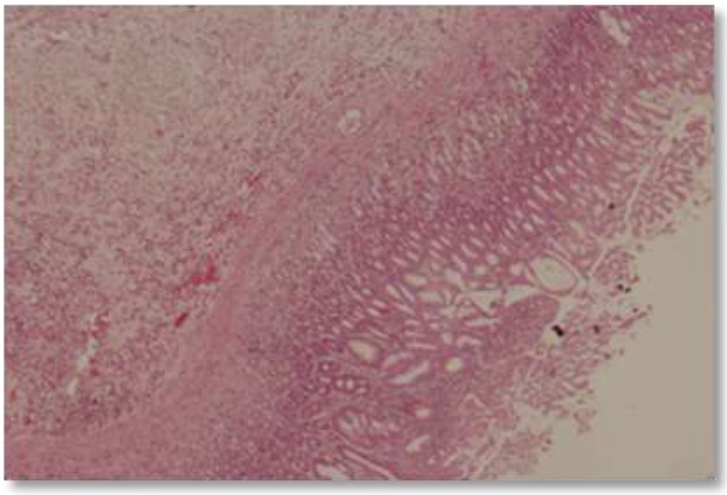

Fig. 4: Mucosal edema in animal administered Ranitidine (20mg/kg).

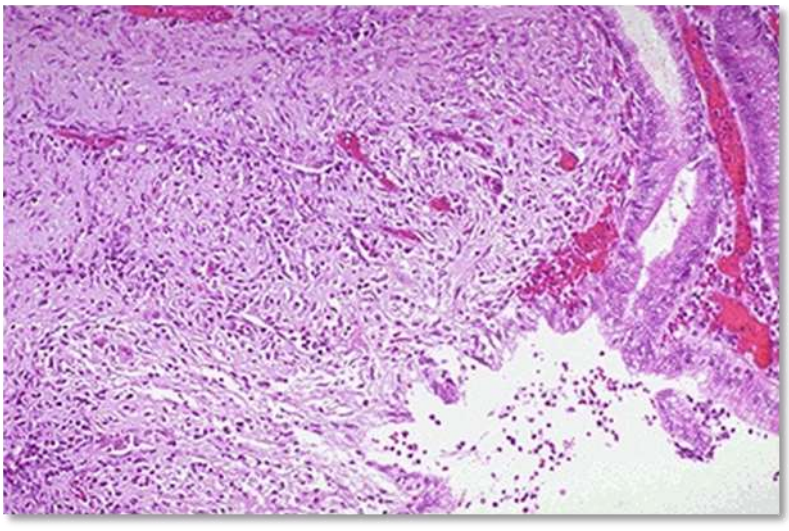

Fig. 5: Depiction of mucosal edema in control animal.

pH detection

After 21 days of once-daily dosage with distilled water, the pH of the water in Group 1 was measured as 2.36±0.10*. The optimal and raised pH in group 2 was 4.79±0.31*** after

receiving 20mg/kg of ranitidine. The pH in Group 3 was 3.27±0.22** after 21 days of continuous exposure to Ethanolic stem bark extract of *Moringa oleifera* (SBMO) (200mg/kg, p. o.), while in Group 4 it was 4.38±0.38*** after receiving Ethanolic stem bark extract of *Moringa oleifera* (SBMO) (400mg/kg, p. o.) once daily for 21 days. Thus, a greater dose of the common medicine Ranitidine (20mg/kg) was required to achieve the same pH-raising effects.

Table 3: pH range.

Treatment	pH range
Vehicle	2.36±0.10*
Ranitidine (20mg/kg)	4.79±0.31***
Ethanolic stem bark extract of *Moringa oleifera* (SBMO) (200mg/kg, p. o.)	3.27±0.22**
Ethanolic stem bark extract of *Moringa oleifera* (SBMO) (400mg/kg, p. o.)	4.38±0.38***

Statistical significance was indicated by an asterisk (*), and data were reported as Mean SEM for a no. of animals 6.

5.4.3 Volume of gastric content

The total volume of stomach contents measured after 21 days of treatment for Group 1 was 11.68±0.18* ml. The total volume of stomach contents for the group given 20 mg/kg of ranitidine was 5.42±0.26**ml. Group 3, which received ethanolic stem bark extract of *Moringa oleifera* (SBMO) (200mg/kg, p. o.) showed an increase in gastric content (ml) of 8.61±0.24** whereas Group 4, which received ethanolic stem bark extract of *Moringa oleifera* (SBMO) (400mg/kg, p. o.), showed a decrease in secreted volume of gastric content (ml) of 6.25±0.11** which is highly significant and equivalent to the standard group.

The following table demonstrates the decreased volume of gastric content (ml)-

Table 4: Volume of gastric content.

Treatment	Volume of gastric content (ml)
Vehicle	11.68±0.18*
Ranitidine (20mg/kg)	5.42±0.26**
Ethanolic stem bark extract of *Moringa oleifera* (SBMO) (200mg/kg, p. o.)	8.61±0.24**
Ethanolic stem bark extract of *Moringa oleifera* (SBMO) (400mg/kg, p. o.)	6.25±0.11**

Statistical significance was indicated by an asterisk (*), and data were reported as Mean SEM for a no. of animals 6.

5.4.4 Free acidity

The free acidity value of 36.27±1.28* in Group 1 after receiving distilled water proved fully ineffective. All of the animals in Group 2 (ranitidine 20mg/kg) had a free acidity value of 21.34±1.26** on average. The free acidity of animals given ethanolic stem bark extract of *Moringa oleifera* (SBMO) (200mg/kg, p. o.) was 29.45±1.24** whereas the free acidity of animals given the ethanolic stem bark extract of *Moringa oleifera* (SBMO) (400mg/kg, p. o.) was 24.72±1.19** putting it within striking distance of the standard medication, ranitidine.

Table 5: Free acidity.

Treatment	Free acidity (mEq/l)
Vehicle	36.27±1.28*
Ranitidine (20mg/kg)	21.34±1.26**
Ethanolic stem bark extract of *Moringa oleifera* (SBMO) (200mg/kg, p. o.)	29.45±1.24**
Ethanolic stem bark extract of *Moringa oleifera* (SBMO) (400mg/kg, p. o.)	24.72±1.19**

Statistical significance was indicated by an asterisk (*), and data were reported as Mean SEM for a no. of animals 6.

Total acidity

In group 1, which was given distilled water (20mg/kg), the total acidity was measured at 73.19±1.21**mEg/l. Total acidity was 45.14±1.37*** in group 2 after 20mg/kg Ranitidine treatment. In contrast, after 15 days of continuous dosing with ethanolic stem bark extract of *Moringa oleifera* (SBMO) (200mg/kg, p. o.), group 3's total acidity was 58.12±1.13**. Finally, total acidity was measured, and it was found to be 51.18±1.22**in group 4 after ethanolic stem bark extract of *Moringa oleifera* (SBMO) (400mg/kg, p. o.), was administered.

Table 6: Total acidity.

Treatment	Total acidity (mEq/l)
Vehicle	73.19±1.21**
Ranitidine (20mg/kg)	45.14±1.37***
Ethanolic stem bark extract of *Moringa oleifera* (SBMO) ((400mg/kg, p. o.)	58.12±1.13**
Ethanolic stem bark extract of *Moringa oleifera* (SBMO) ((400mg/kg, p. o.)	51.18±1.22**

Statistical significance was indicated by an asterisk (*), and data were reported as Mean SEM for a no. of animals 6.

The pH level was measured to be below the reference and comparison ranges. Decreases in pH are a well-supported treatment for ulcers, as hyperacidity is the primary determinant in their anti-ulcerogenic potential. Because of its acid-neutralizing properties, it can boost the release of alkaline bicarbonate ions and possibly even more. Group 2 received ranitidine (20mg/kg), while group 4 received ethanolic stem bark extract of *Moringa oleifera* (400m/kg) resulting in the greatest reduction in pH.

After being exposed to *Moringa oleifera* for 21 days straight, it was discovered that the free acidity had decreased. It could work to neutralize free acid, reducing the potentially devastating effects of free acid on the development of stomach ulcers and perforations. Perhaps this is the case because pharmacological effect is induced and accommodated by the plant extract over time. After treatment, the total acidity of the animals was also determined to be lower than before. It showed that in groups 2, 3, and 4, overall acidity decreased as well. No clear mechanism for this pharmacological effect has been identified.

CONCLUSION

In conclusion, *Moringa oleifera* has significant anti-ulcer potential at both the doses used 200mg/kg & 400mg/kg. It may prevent ulcer formation by the same action as ranitidine does. In all the parameters, it exhibited dose-dependent response in sub-siding ulcerogenic response. It selectively decreases gastric juice production and thus lowers pH of stomach. This study suggests to isolate and develop the suitable dosage form of concerning chemical constituents present in *Moringa oleifera* for the activity. Also study at molecular level to confirm its mechanism of action as anti-ulcer.

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