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EVALUATION OF ANTIULCER ACTIVITY OF *MORINGA OLEIFERA* PODS EXTRACT

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ABSTRACT

The aim of current study is to evaluate antiulcer activity of the 70% hydroalcoholic extract of *Moringa oleifera* pods (HAEMOP) using different animal models of ulcer in rats. Pylorus ligation method was studied using Ranitidine (150mg/Kg) in rats as reference standard. The ulcer index, pH of gastric juice, volume of gastric juice was determined. Ethanol and aspirin induced ulcer was studied and the ulcer index was measured. In antiulcer studies, *Moringa oleifera* pods extract showed reduction in ulcer score each experimental model sucessessfully. In pylorus ligation ulcer model it also reduced gastric volume and increased pH of gastric juice. The results have shown that the *Moringa oleifera* pods extract contain some active ingredients with the potential of being antiulcer agents.

KEYWORDS: *Moringa oleifera*, Pylorus Ligation Ulcer, Ethanol, Aspirin, Ulcer Index.

INTRODUCTION

Ulcer is most common gastrointestinal disorder characterized with break in lining of stomach, initial part of small intestine or infrequently the lower oesophagus. The ulcer mainly associated with symptoms like burning abdominal pain which extends from novel to chest, loss of apetite, nausea, blood and dark stools, unexplained weight loss, indigestions and vomiting.

Ulcers in stomach called gastric ulcer and ulcer formed in duodenum call duodenal ulcer, togetherly it called as peptic ulcer.[1]

These are examined under endoscopy (gastroscopy) and upper gastrointestinal series. The curative treatment for peptic ulcers involves H_2 antihistaminic, proton pump inhibitor, ulcer protectants and anti *H. pylori*therapy.[2]

The conventional antiulcer drugs used in management of peptic ulcer may cause undesirable side effect or drug interaction in body upon their prolonged use.

In traditional medicine, various herbal preparations are used to cure the gastrointestinal disease with adeal aim to relieve symptoms and delay its recurrence.

To the date, no drugs meets all these goals therapy. Therefore the search for potent, safe and economically effective antidiarrhoeal and antiulcer agents from herbal origin has become most desirable area of research.

Moringa oleifera Lam belongs to family Moringaceae is medium sized tree as valuable food source. It is also known as drumstick tree. It is fast growing tree with soft trunk, white corcky and gummy bark bearing branches. It is rich in some of amino acids, minerals, vitamins, tannins, sterols, saponin and alkaloids.[3]

The different parts of *Moringa oleifera* tree have been studied for numerous pharmacological actions like antifungal, antimicrobial, antifertilty, CNS depressant, anti- inflammatory, diuretic and regulating hypothyroidism.[4,5]

The literature survey reveals no scientific validation of antiulcer activities of *Moringa oleifera* pods till date. Hence, the present investigation aimed to evaluate the antidiarrhoeal and antiulcer activies of *Moringa oleifera* pods extract in various experimental animal models.

MATERIAL AND METHODS

1. Plant material collection

For this study, mature pods of *Moringa oleifera* were collected from the surrounding gardens of the Vijayapur, after it was authenticated by Dr. P. D. Needagi, HOD Botony and professor, K. C. P. Science, Arts and Commerece College, vijayapur, Kanataka.

2. Preparation of extract

Fresh mature pods were cleaned, cut in to small pieces, shade dried at room temperature and powedered using grinder. Then the powdered material was be extracted with 70% hydroalcoholic by Soxhlet extraction procedure at temperature between 60-70^{0}C. Thereafter,

the extract was concentrated using rotary flash evaporator. The yield was found to be 18gm. The dried extract was stored in refrigerator below 10^0C for further studies.

3. Preliminary phytochemical screening

The preliminary phytochemical investigation of HAEMOP was carried out for detection of different phytoconstituents. Tests for presence of phytochemicals were performed by standard methods described by Dr. Khandelwal K. R. and Trease and Evans.

4. Animals used

Albino rats (Wistar strain) weighing 150-200 g of either sex and albino mice weighing 20-25g of either sex were used in the present study. They were procured from Venkateshwar enterprises, Rajajinagar, Bangalore. The animals were acclimatized for ten days under standard laboratory condition. They were housed in polypropylene cage and maintained at 27^0C ± 2^0C, relative humidity 65 ± 10% under 12 hr light/dark cycle. The animals were fed with rodent pellet diet and water. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC) before initiation of the experiments. **[Reg. No. 1076/PO/Re/S/07/CPCSEA dated on 27th Feb 2017].**

DETERMINATION OF ACUTE TOXICITY[6,7]

The acute toxicity (LD50) of extracts of *Moringa oleifera* pods was determined by fixed dose method (OECD guide line no. 423) of CPCSEA. The female albino mice weighing between 20-25g were fasted for over night prior to experiment 1/20th, 1/10th, 1/5th LD50 cutoff value of the extract were selected as screening doses.

SCREENING OF ANTIULCER ACTIVITY

A. Pylorus ligation method[8]

Rats weighing (150-200g) of either sex were allocated into 5 groups of six animal in each group. Animals were fasted for 18hr prior to drug treatment but had free access to water.

Group 1- Control (Received vehicle- Normal saline 5ml/kg)
Group 2- Standard (Ranitidine 150 mg/kg) orally
Group 3- 100mg/kg of HAEMOP
Group 4- 200mg/kg of HAEMOP
Group 5- 400mg/kg of HAEMOP

Pylorus ligation was carried out in all groups of rats for the induction of gastric ulcers and followed by the respective treatments orally. After 6 hrs of ligation all animals were sacrificed, the abdomen was opened by using a small incision. The stomachs were dissected out and contents were drained into tubes and centrifuged for 10 minutes at 1000 rpm. Supernatants were subjected to investigation of gastric volume and pH of gastric juice. The stomachs were then cut along the greater curvature and examined for ulceration and the ulcer index (UI) was calculated.

B. Ethanol induced ulcer

Rats weighing (150-200g) of either sexwere allocated into 5 groups of six animal in each group. Animals were fasted for 18hr prior to drug treatment but had free access to water.

Group 1- Control (Received vehicle- Normal saline 5ml/kg)
Group 2- Standard (Ranitidine 150 mg/kg) orally
Group 3- 100mg/kg of HAEMOP
Group 4- 200mg/kg of HAEMOP
Group 5- 400mg/kg of HAEMOP

Animals were given test extract or standard drug. 1 hr later 1ml/200g of 99.80% alcohol was given orally to every animal. After 1 hr of treatment animals were sacrificed and stomach was incised along the greater curvature and ulceration was scored. The number of ulcers and the ulceration area were measured. Ulcer index was calculated using following formula.

UI = UN + US + UP x 10-1

Where,
UI = Ulcer Index
UN = Average of number of ulcer per animal US = Average of severity score
UP = Percentage of animal with ulcer

C. Aspirin induced ulcer[9]

Rats weighing (150-200g) of either sex were allocated into 5 groups of six animal in each group. Animals were fasted for 18hr prior to drug treatment but had free access to water.

Group 1- Control (Received vehicle- Normal Saline 5ml/kg)
Group 2- Standard (Ranitidine 150 mg/kg) orally
Group 3- 100mg/kg of HAEMOP
Group 4- 200mg/kg of HAEMOP
Group 5-400mg/kg of HAEMOP

1hr after extract and standard drug treatment aspirin was administered orally to all groups of rat on the day of experiment in the form of an aqueous water suspension (200 mg/kg, per oral). Animals of group were sacrificed after 4 hrs of aspirin administration. The stomach was incised along with greater curvature and further examined for ulcer index using following formula-

UI = UN + US + UP x 10-1

Where,
UI = Ulcer Index
UN = Average of number of ulcer per animal US = Average of severity score
UP = Percentage of animal with ulcer

STATICAL ANALYSIS

The results were expressed in mean □SEM. All data obtained from the above study were subjected for One way ANOVA followed by Tukey's Kremer Multiple Comparison Test by using Prism Pad 5 software. The $p< 0.05$ was found statistically significant.

RESULT

1. Phytochemical Phytochemical Screening

The results of preliminary phytochemical screening on HAEMOP are summarized in following Table no-1.

Table no-1: Preliminary Phytochemical Screening of HAEMOP.

Sr. No.	Phytochemical Constituents	Inference
1	Carbohydrate	+
2	Proteins	++
3	Alkaloids	+
4	Phenolic compounds	-
5	Tannins	++ +
6	Steroids	-
7	Flavonoids	+++
8	Saponin glycosides	-
9	Coumarin glycosides	-
10	Cardiac glycosides	+
11	Lipids	+

2. Determination of acute toxicity studies

In acute toxicity studies, test extract of *Moringa oleifera* plant did not produce any mortality of the animals at dose of 2000mg/kg. Hence, 2000mg/kg was fixed as LD50 cut off value as per fixed dose method, OECD (Organization for Economic Corporation Development) guideline No. 423 (Annexure 2d) of CPCSEA. The screening doses selected for antiulcer activities of test extract of title plant were:

i. 100mg/kg – 1/20th dose of 2000mg/kg b.w.
ii. 200mg/kg – 1/10th dose of 2000mg/kg b.w.
iii. 400 mg/kg – 1/5th dose of 2000mg/kg b.w.

3. EVALUATION OF ANTIULCER ACTIVITY

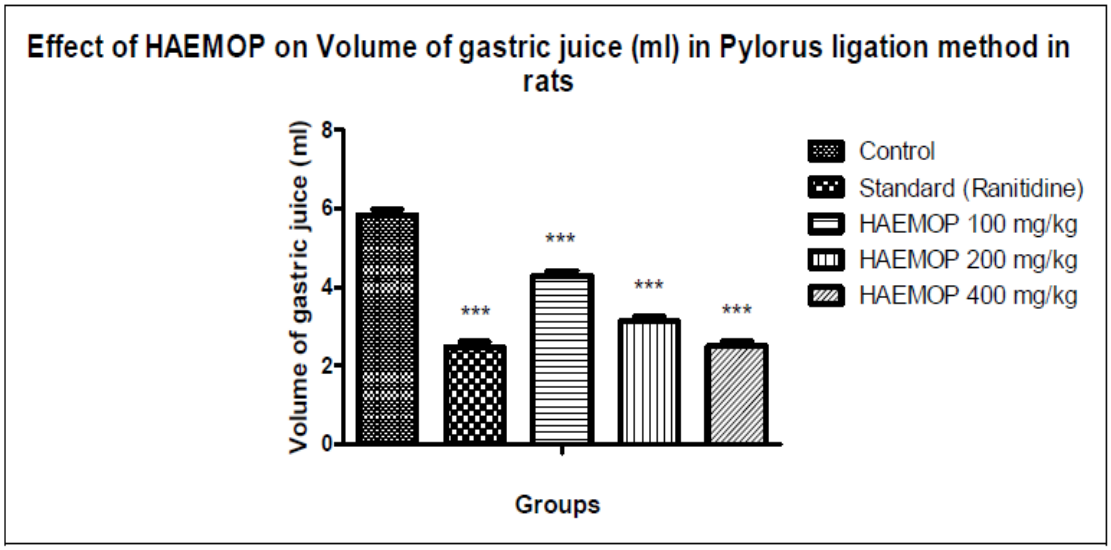
A. Pylorus ligation method

There was significant increase in volume of gastric juice and ulcer index of the stomach and increase pH of gastric juice seen in control, untreated pylorus ligated rats. The dose dependent antiscretory and antiulcer effect of HAEMOP has been observed in pylorus ligation model which was evident by significant decrease in volume of gastric juice and ulcer score and increase in pH of gastric juice. The percentage protection at doses of 100, 200 and 400 mg/kg was found to be 32%, 45% and 53% respectively. The result of the test extract found to be lesser potent than ranitidine (71%). The results emphasizes in Table- 1.

Table No. 1: Effect of HAEMOP on Pylorus ligation method in rats.

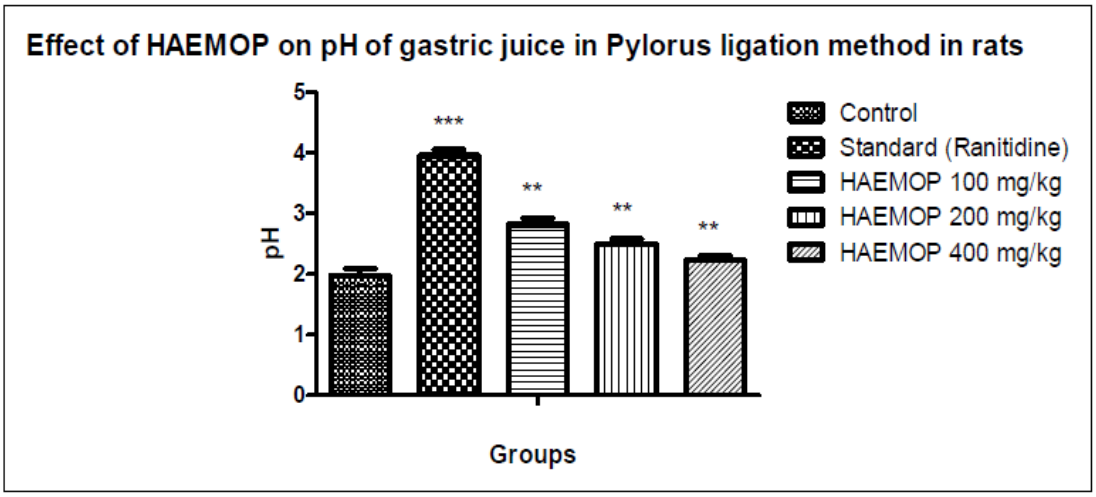
Groups	Treatment	Dose mg/kg	Volume of gastric juice in ml	pH	Ulcer index	% Inhibition
1	Control	--	5.84 ± 0.14	1.98 ± 0.11	3.86 ± 0.20	---
2	Standard (Ranitidine)	150	2.48 ± 0.13***	3.96 ± 0.10***	1.10 ± 0.13***	**71**
3	HAEMOP	100	4.30 ± 0.11***	2.82 ± 0.11**	2.60 ± 0.12***	32
4	HAEMOP	200	3.14 ± 0.10***	2.50 ± 0.09**	2.10 ± 0.11***	45
5	HAEMOP	400	2.51 ± 0.12***	2.23 ± 0.08**	1.78 ± 0.10***	**53**

The values are Mean □ SEM., n=6. **p<0.01 and ***p<0.001 vs control



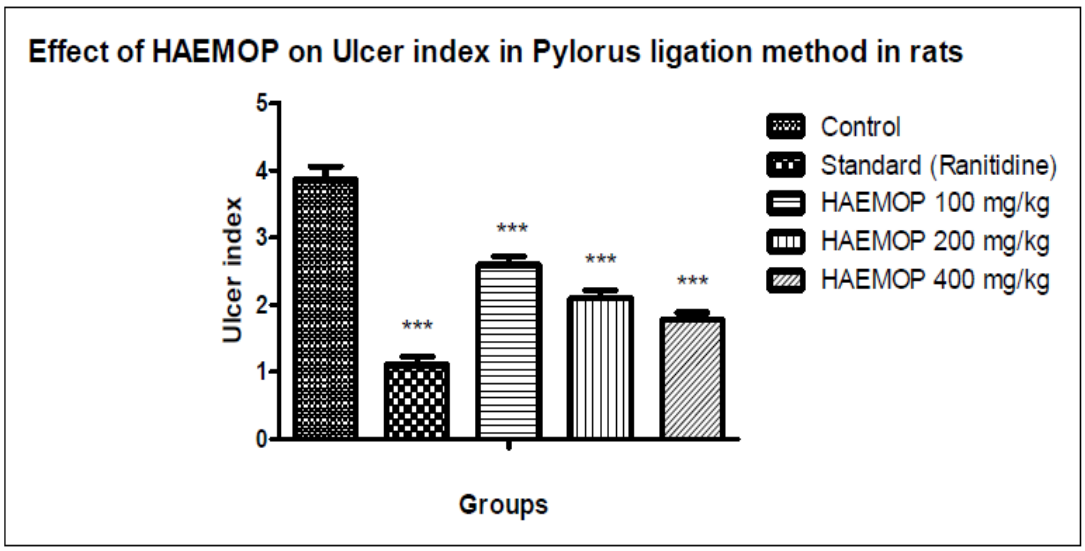
The values are Mean ± SEM., n=6. ***p<0.001 vs control.

Fig. 1: Effect of HAEMOP on volume of gastric juice (ml) in pylorus ligation method in rats.



The values are Mean ± SEM., n=6. **p<0.01 and ***p<0.001 vs control.

Fig. 2: Effect of HAEMOP on pH of gastric juice in pylorus ligation method in rats.



The values are Mean ± SEM., n=6. ***p<0.001 vs control

Fig. 3: Effect of HAEMOP on ulcer index in pylorus ligation method in rats.

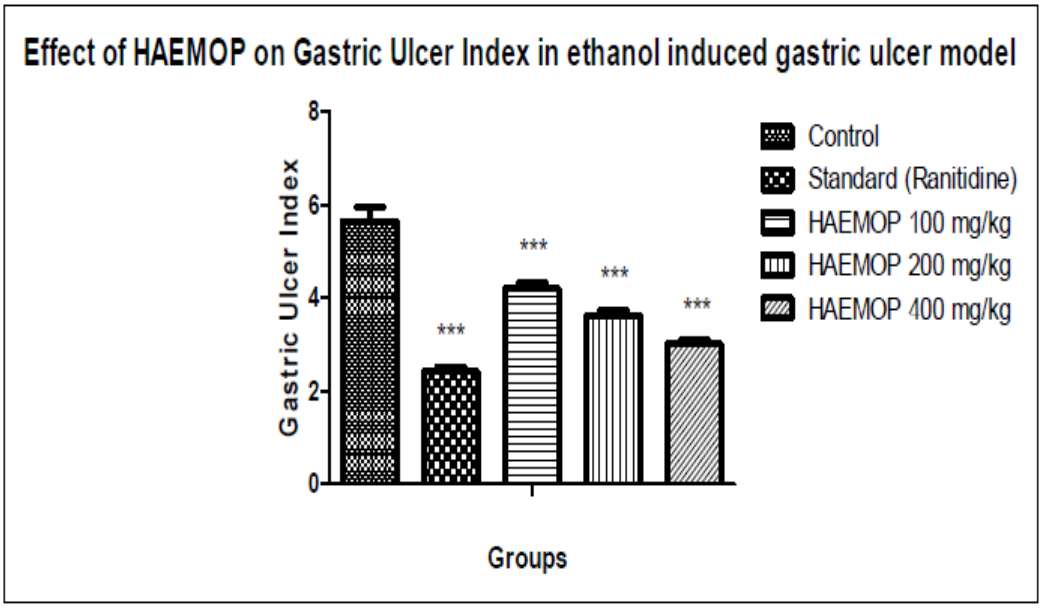
B. Ethanol induced ulcer

In ethanol induced ulcer model, HAEMOP has exhibited significant decrease in ulcerogenic effect as compared to control group. The % inhibition in extract treated group of animal at doses of 100, 200 and 400 mg/kg was found to be 25%, 35% and 46% respectivelyto that of standard (Ranitidine 57%). Results are shown in Table- 2.

Table No. 2: Effect of HAEMOP on ethanol induced gastric ulcer in rats.

Groups	Treatment	Dose mg/kg	Gastric Ulcer Index	% Inhibition
1	Control	--	5.65 ± 0.30	---
2	Standard (Ranitidine)	150	2.42 ± 0.11***	**57**
3	HAEMOP	100	4.20 ± 0.13***	25
4	HAEMOP	200	3.63 ± 0.10***	35
5	HAEMOP	400	3.01 ± 0.09***	**46**

The values are Mean ± SEM., n=6. ***p<0.001 vs control.



The values are Mean ± SEM., n=6. ***p<0.001 vs control.

Fig. 4: Effect of HAEMOP on gastric ulcer index on ethanol induced gastric ulcer model.

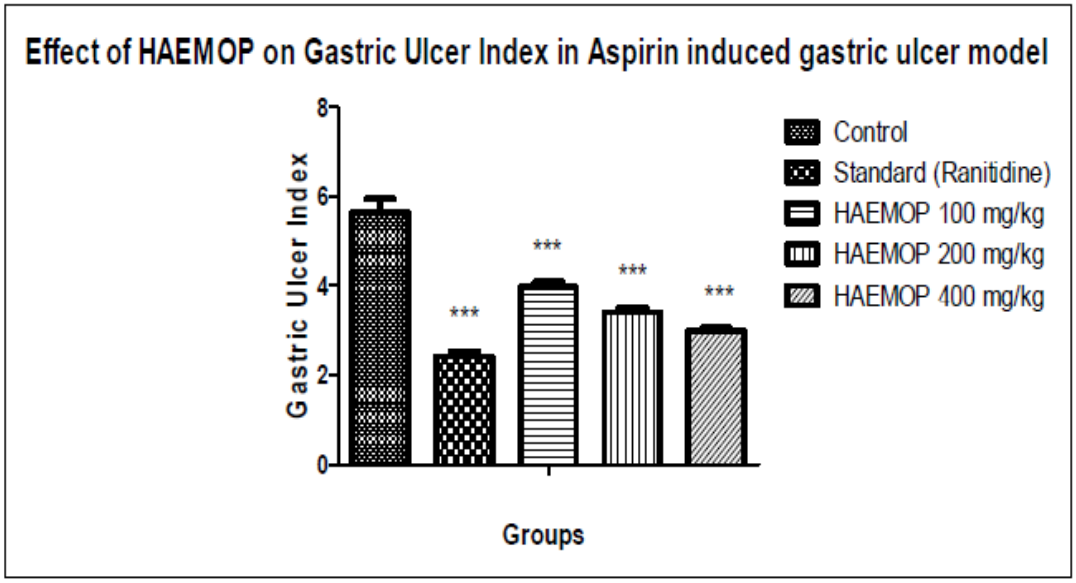
C. Aspirin induced ulcer

The result depicted in table 3 reveal the dose dependent antiulcerogenic effect of HAEMOP against aspirin induced ulcer model. At higher dose, test extract exhibited reduction of ulcer index by 3.00 ± 0.07 to that of control 5.65 ± 0.30. Also at doses of 100 and 200 mg/kg showed decrease in ulcer index. The percentage inhibition of ulcer score was found to be 29% to 46% at graded doses of test extract respectively and by standard 57%.

Table No. 3: Effect of HAEMOP on Aspirin induced gastric ulcer in rats.

Groups	Treatment	Dose mg/kg	Gastric Ulcer Index	% Inhibition
1	Control	--	5.65 ± 0.30	---
2	Standard (Ranitidine)	150	2.42 ± 0.11***	**57**
3	HAEMOP	100	3.98 ± 0.11***	29
4	HAEMOP	200	3.41 ± 0.09***	39
5	HAEMOP	400	3.00 ± 0.07***	**46**

The values are Mean ± SEM., n=6. ***p<0.001 vs control



The values are Mean ± SEM., n=6. ***p<0.001 vs control.

Fig. 5: Effect of HAEMOP on gastric ulcer index in aspirin induced gastric model.

DISCUSSION

In present study, the evaluation antiulcer effect of 70% hydroalcoholic extract of *Moringa oleifera* pods was performed. The antiulcer effect of HAEMOP was checked by pylorus ligation method, ethanol and aspirin induced ulcer models in wistar rats.

In pylorus ligation model, ligation for 6 Hrs resulted in accumulation of gastric secreation resulting in increased gastric acid volume. The accumulated acid may be responsible for gastric ulceration.[5] The HAEMOP at graded doses significantly reduced the volume of gastric juice and ulcer index and also increased the pH of gastric juice in dose dependent fashion.

The ethanol induced ulcer model has been used for the screening of antiulcer property of HAEMOP. Ethanol causes an inflammatory response consequently destroying the stomach protecting layers and provoking haemorrhages ulceration of stomach in experimental animals.[10] The results obtained from ethanol mediated ulcer model showed that hydroalcoholic extract exerted 25-46% of protection of the gastric mucosa which was evidenced by observing significant decrease in ulcer scores in treated groups over control.

Aspirin is NSAID which induces ulcer by inhibiting prostaglandins synthesis in stomach by blocking the cyclooxygenase enzyme. NSAIDs also cause an inflammatory response increasing the reactive oxygen species in gastric mucosa.[11] In present study, HAEMO in all tested doses caused significant reduction in the ulcer index by 29- 46% indicating its possible involvement in prostaglandin pathway.

Ranitidine, reference standard drug exhibited significant (at least $p<0.01$) reduction in ulcer score and volume of gastric juice and increases in pH of gastric juice. Similarly in ethanol and aspirin induced ulcer models also ranitidine significantly ($p< 0.001$) reduced the ulcer index. Ranitidine decreases gastric acid and increases mucus secreation by antisecretory mechanism via inhibition of gastric secreation and pepsin actity.[12]

The flavonoids present in the plant extract claimed to exhibit antiulcer effect. The ulcer healng effect produced by extract may be due to both antisecretary and gastric cytoprotective constituents present in these extract.[13] Plant extracts containing tannins are used in medicine for treating ulcer primarily because of their astringent properties.[14] It precipitates proteins in outer most layer of the mucosa and to render it less permeable and more resistant to chemical and mechanical injury or irritation.

The qualitative phytochemical analysis of the *Moringa oleifera* pod extract reveals presence of tannins and flavonoids and this could be the reason for exhibiting the antiulcer activity in present study.

CONCLUSION

The results of the current study conclude that 70% hydroalcoholic extract of *Moringa oleifera* pods (HAEMOP) demonstrated dose dependent antiulcer activity due to presence of pharmacologically effective component(s).

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