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ANTIULCEROGENIC ACTIVITY OF ETHANOLIC EXTRACT OF MORINGA OLEIFERA BARK IN WISTAR ALBINO RATS

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ABSTRACT

The present study was under taken to evaluate Antiulcer activity of Ethanolic bark extract of *Moringa oleifera* against ethanol and acetic acid induced ulcer models. Ethanol is widely used ulcerogenic agent which induces acute gastric ulcers, ethanol induced ulcers which is associated with significant production of o_2 free radicals leading to lipid peroxidation with consequent damage to cell and cell membrane. *Moringa oleifera* significantly protects the gastric mucosa against the ethanol challenge, as shown by the reduced values of ulcer index. The extract had shown dose dependent significant effect when compared to ethanol control group. Acetic acid induced chronic ulcer model is another classical model for evaluation of antiulcerogenic activity. Exposure to glacial acetic acid produces deep penetrating gastric ulcers with clear vascular injury, edema, hemorrhagic streaks, epithelial thickening and necrotic lesions. The bark extract of the plant had shown pronounced dose dependent protective effect evident by reduced ulcer index. The activity of the extract was found to be significant compared to acetiacid control group in which there was enhanced ulcer index and perforations. The histopathology examination was carried out to determine effect of extracts. These were selected to reflect rate and healing of ulcers. The parameters suggested that the pre-treated rats with ethanolic extract of *Moringa oleifera* bark (200 mg/kg) or Omeprazole (20 mg/kg) *orally* significantly reduced the formation of gastric ulcer.

KEYWORDS: *Moringa oleifera*, Omeprazole, ethanol, acetic acid.

1. INTRODUCTION

For centuries people have used plants for healing ailments. Plant products as parts of foods or botanical portions and powders have been used with varying success to cure and prevent diseases throughout history[1].

Herbal medicine is the use of plants, plant parts, their water or solvent extracts, essential oils, gums, resins, exudates or other form of advanced products made from plant parts used therapeutically to provide proactive support of various physiological systems; or, in a more conventional medical sense, to treat, cure, or prevent a disease in animals or humans[2]. About 70–80% of the world populations, particularly in the developing countries, rely on non-conventional medicine in their primary healthcare as reported by the World Health Organization[3].

2. MATERIALS AND METHODS

Plant material

The *Moringa oleifera* bark is collected from the village at Warangal. Dried under shade and powdered in the laboratory. Plant material was authenticated by ayurvedic Dr. A. Sridhar he has been preserved in laboratory for future reference.

Extraction of the plant

The powered plant material was extracted using 90% ethanol, because ethanol extract is generally effective for the activity and also various chemical constituents may be present in the ethanol extract. 500 gm. of dried plant powder was taken in a round bottomed flask, the plant drugs were three times successively extracted with 1000 ml of 90% ethanol in flask by using hot maceration method. In this method the plant powder was soaked with 90% ethanol and warm at 40˚c-50˚c temperatures for 1hour. Cool it and filter it by vacuum filtration unit using watt man filter papers no-1.

Then the filtered is collected into flask and extract obtained were evaporated. Concentrated extract were dried. When the extract was completely dried take weight of evaporating dish on electronic balance and find % yield of this plant extract with reference to dried plant powder drug.

The dried plant extract was scraped by spatula and collected from evaporating dish and stored in air tight container at cool temperature. The extracts thus obtained were subjected to phytochemical analysis[4].

Determination of Phytochemicals

The qualitative phytochemical analysis were carried out according to the methods of Harborne (1973) and Trease and Evans (1989)[5].

Phyto-chemical screening of the extracts and fractions were carried out to identify the constituents, Preliminary phytochemical screening of M*oringa oleifera bark* showed the presence of alkaloids, saponins, carbohydrates, steroids, triterpenoids in ethanolic extract[6].

Solubility test

The extract was tested for solubility in water, normal saline and dimethyl sulphoxide.

Dose preparation

The ethanolic extract of *Moringa oleifera bark* was taken and dissolved in dimethyl sulphoxide which acts as a solubilizing agent? This must be done according to the concentration required.

Methodology

The experiments are done according to the CPCSEA guidelines by OESD method and approved by the Institutional Animal Ethical Committee. The test substance was dissolved in DMSO and the dose was selected at 100, 300, 500, 1000, 1500 and 2000 mg/kg body weight. The test substance pretreated with animal for 14 days by oral gavages. Albino rats were divided into groups comprising of six animals each[7].

Table 1-1: Scheme of acute toxicity in mice.[8]

Group	Treatment
Group I	Served as control and was administered vehicle only
Group II	Received 100 mg/kg bd.wt of ethanolic extract *Moringa oleifera* bark
Group III	Received 300 mg/kg bd.wt of ethanolic extract *Moringa oleifera* bark
Group IV	Received 500 mg/kg bd.wt of ethanolic extract *Moringa oleifera* bark
Group V	Received 1000 mg/kg bd.wt of ethanolic extract *Moringa oleifera* bark
Group VI	Received 2000 mg/kg bd.wt of ethanolic extract *Moringa oleifera* bark

Evalutation of antiulcerogenic activity of *Moringa oleifera* bark on normal rats

Table 1-2: Effect of ethanol induced acute gastric ulcers in rats.[9]

Group	Treatment
Group I	Control saline 1% is administered *orally*
Group II	Received ethanol 1ml/200 gms bd.wt *orally*
Group III	Received ethanol 1ml/200 gms bd.wt *orally* + test *Moringa oleifera* bark at 100 mg/kg bd.wt. *orally*

Group IV	Received ethanol 1ml/200 gms bd.wt *orally* + test *Moringa oleifera* bark at 200 mg/kg bd. wt *orally*
Group V	Received ethanol 1ml/200 gms bd.wt *orally* + standard omeprazole 20 mg/kg bd.wt *orally*

Method

Ulcer was induced by administering ethanol. All the animals were fasted for 36 hours before administration of ethanol. The standard drug (Omeprazole 20 mg/kg) and the test drug (*Moringa oleifera* 100 and 200 mg/kg, *orally*) were administered 1 hour before ethanol administration. Ethanol 90% was administered to all the animals were sacrificed to death by cervical dislocation method. Stomachs were isolated and ulcer index was determined.

Evaluation of ulcer index

UI=UN+UP×10-1

UN= average of number of ulcers per animal

US= average of severity of scores

UP= percentage of animals with ulcers

Determination of percentage protection

$$\% \text{ protective} = \frac{(\text{control ulcer index}) - (\text{test mean ulcer index})}{(\text{control mean ulcer index})} \times 100$$

Table 1-3: Effect of acetic acid induced chronic gastric ulcers in rats.

Group	Treatment
Group I	Control saline 1% is administered *orally*
Group II	Received aceticacid 0.06 ml/kg bd.wt epigastric region
Group III	Received aceticacid 0.06 ml/kg epigastric region + test *Moringa oleifera* bark at 100 mg/kg bd.wt. *orally*
Group IV	Received aceticacid 0.06 ml/kg epigastric region + test *Moringa oleifera* bark at 200 mg/kg bd. wt *orally*
Group V	Received aceticacid 0.06 ml/kg epigastric region + standard Omeprazole 20 mg/kg bd.wt *orally*

Method

Glacial acetic acid (0.06 ml/animal) was instilled into the tube and allowed to remain for 60 sec on the gastric wall. After removal of acid solution, the abdomen was closed in two layers and animals were caged and fed normally. Standard (Omeprazole 20 mg/kg bd.wt) and test (*Moringa oleifera* bark extract 100 and 200 mg/kg bd.wt) administered orally 4 h after the application acetic and continued up to 1-7 days after induction of ulcer. The animals were

then sacrificed after 18 h of the last dose of drug on 7th day of experiment to assess the ulcer size and healing. Ulcer index was calculated based upon the product of length and width (mm^2/rat) of ulcers[10].

Evaluation of ulcer index

Ulcer index=10/X

X = total mucosal area/total ulcerated area.

Ulcers scores based on intensity

0 = no ulcer.

1 = superficial mucosal erosion.

2 = deep ulcer or transmural necrosis.

3 = perforated or penetrated ulcer.

The stomach samples from groups that showed reduction in ulcer index were subsequently processed for histopathological examination three indices namely: glandular stomach, mucosal (squamous epithelial layer), submucosal layer. These were selected to reflect the rate and quality of ulcer healing.

3. RESULTS

Preliminary phytochemical analysis of *Moringa oleifera*

Phytochemical screening revealed the presence of alkaloids, glycosides, saponins, triterpenoids, steroids and carbohydrates. The results are shown in Table 4-1.

Table 1-4: Preliminary phytochemical analysis.

Phytoconstituents	Results
Alkaloids	+
Glycosides	+
Saponins	+
Triterpenoids	+
Steroids	+
Carbohydrates	+
Flavonoids	-
Phenols	-
Tannins	-

Acute toxicity studies

No behavioral changes and no mortality were observed for ethanolic extract of *Moringa oleifera* bark up to 2000 mg/kg bd.wt. After 14 days. Resulted *Moringa oleifera* bark is non-toxic and safest dose up to 2000 mg/kg.

Models

1. Effect of ethanolic extract of *Moringa oleifera* bark in ethanol induced acute gastric ulcers in rats

In control animal old 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. Ethanolic extract of *Moringa oleifera* bark has shown significant protection index of 53.8% and 62.45% with the doses of 100 and 200 mg/kg respectively in comparison to control, Omeprazole as standard drug has shown 72.44% reduction of ulcer.

Table 1-5: Antiulcerogenic activity of ethanolic extract of *Moringa oleifera* bark in ethanol induced acute gastric ulcer.

Group	Treatment	Ulcer index	% Protection
I	Ulcer control (ethanol 1 ml/200g)	40.6±7.43	--------
II	*Moringa oleifera* bark extract (100 mg/kg)	16.4±9.86*	53.8%
III	*Moringa oleifera* bark extract (200 mg/kg)	4±1.23*	62.45%
IV	Omeprazole (20 mg/kg)	2.2±1.1*	72.44%

All values are mean ± SEM, n=6, *p<0.05 when compare with control group.

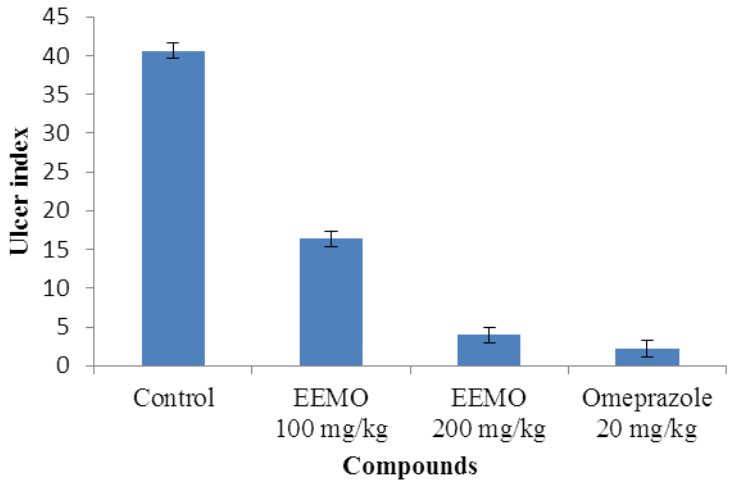

Figure 1-1: Effect of *Moringa oleifera* bark extract on ulcer index in ethanol induced acute gastric ulcer.

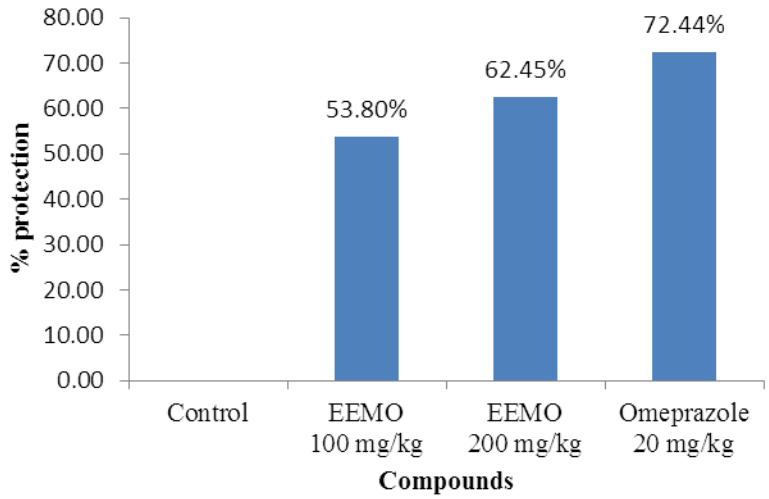

Figure 1-2: Effect of *Moringa oleifera* bark extract on % protection in ethanol induced acute gastric ulcer.

From the graph we clearly observed that ethanolic extract of *Moringa oleifera* has shown significant protective index of 53.8% and 62.45% with the doses of 100 and 200 mg/kg respectively in comparison to control, Omeprazole as standard drug has shown reduction of ulcer 72.44%.

2. Effect of ethanol extract of *Moringa oleifera* bark in aceticacid induced chronic gastric ulcers in rats

In control animal old administration of obsolute aceticacid produced superfacial erosions in the glandular portion and sub mucosal region of rat stomach which appeared as deep ulcer, perforated or penetrated ulcer. Ethanolic extract of *Moringa oleifera* has shown significant protection index of 23.96% and 60.83% with the doses of 100 and 200 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was reduction of ulcer 56.16%.

Table 1-6: Antiulcerogenic activity of ethanolic extract of *Moringa oleifera* bark in aceticacid induced chronic gastric ulcer.

Group	Treatment	Ulcer index	% Protection
I	Ulcer control aceticacid (1 ml/200g)	13.2±0.094*	--------
II	*Moringa oleifera* bark extract (100 mg/kg)	10.4±0.35*	23.96%
III	*Moringa oleifera* bark extract (200 mg/kg)	5.9±0.23*	60.83%
IV	Omeprazole (20 mg/kg)	6.47±0.032*	56.16%

All values are mean ± SEM, n=6, *p<0.05 when compare with control group.

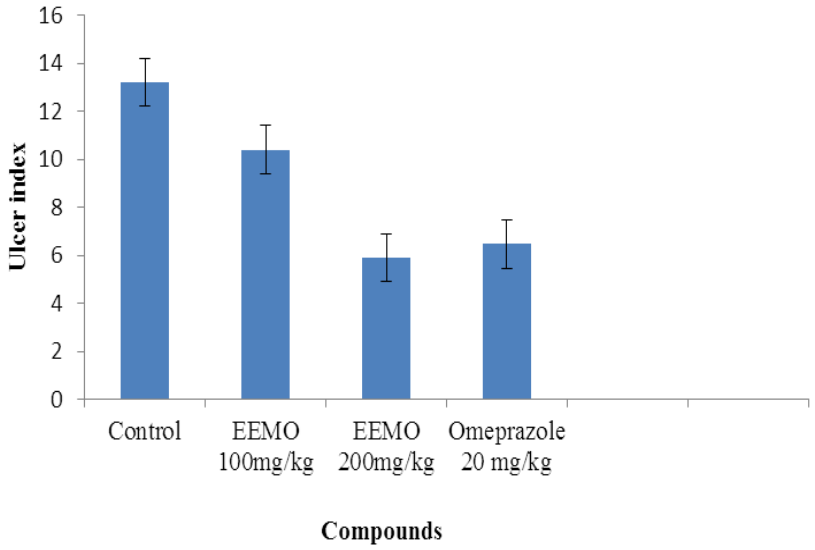

Figure 1-3: Effect of *Moringa oleifera* bark extract on ulcer index in acetic acid induced chronic gastric ulcer.

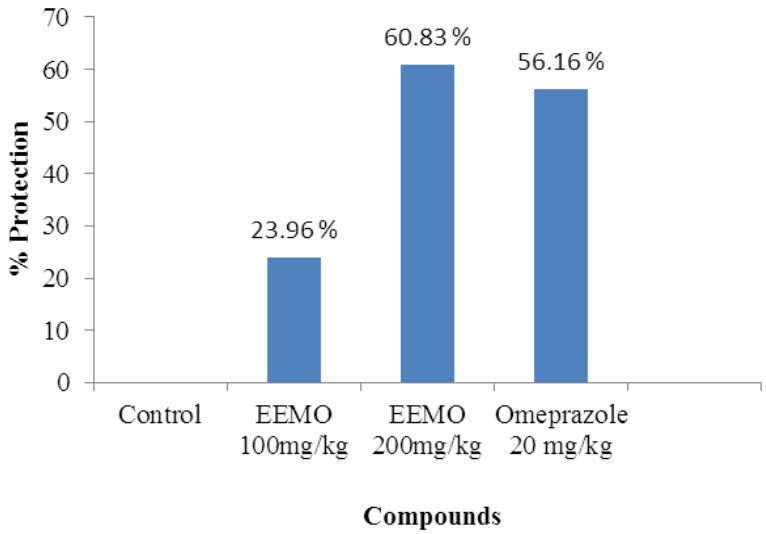

Figure 1-4: Effect of *Moringa oleifera* bark extract on % protection in acetic acid induced chronic gastric ulcer.

From the graph we clearly observed that ethanolic extract of *Moringa oleifera* has shown significant percentage protection of 23.96% and 60.83% with the doses of 100 and 200 mg/kg respectively in comparison to control, Omeprazole as standard drug has shown reduction of ulcer 56.16%.

HISTOPATHOLOGICAL STUDIES FOR ACETIC ACID INDUCED CHRONIC GASTRIC ULCERS

The histopathological examination was carried out to confirm the protective effect of the extracts on the mucosal layer of the stomach. In acetic acid control group there was marked inflammation of inflammatory cells and sub mucosal inflammatory reactions. The extract treated groups showed significant antiulcerogenic activity which was evident by moderate to negligible infiltration and sub mucosal inflammation.

The activity was dose dependent and the effect of 200mg/kg bdwt of the extract was comparable to that of standard Omeprazole 20 mg/kg bdwt. Hence the healing effect of the extract might be due to decrease acid secretion or decrease infiltration of inflammatory cells and mucosa secretion.

4. DISCUSSION: Peptic ulcer is one of the most common diseases of the GIT. It arises due to impaired mucosal resistance disturbed gastric motility and decrease gastric mucosal blood flow are also important causes of gastric ulcers.

The etiological factors of peptic ulcers by penetrating the formation of Prostaglandins hence, it can be concluded that Prostaglandins are essential for the proper formation of mucosal layer.

Application of glacial acetic acid on to the serosal surface of the stomach produces deep penetrating gastric ulcers. Ethanol induced gastric lesions formation may be due to states in gastric blood flow which contributes to the development of hemorrhagic and necrotic aspects.

Accumulation of ethanolic bark extract of *Moringa oleifera* significantly reduced the ulcers which were indicated by less haemoregic streaks and perforations. The histopathological examination determined the effect of extract on regeneration of glandular epithelium, formation of collagen, capillary density all of which are essential for healing of ulcers.

The ethanolic bark extract of *Moringa oleifera* showed dose dependent protective effect and 200mg/kg of *Moringa oleifera* dose was comparable to that of standard Omeprazole.

The phytochemical screening of the extract showed the presence of alkaloids, steroids, saponins and triterpinoids.

Devraj al, reported that alkaloids, steroids and triterpenoids present in the plant might be responsible for antiulcer activity.

Hence alkaloids, triterpinoids, saponins and steroids present in the plant extract might be responsible for the antiulcer activity of *Moringa oleifera* bark extract.

5. CONCLUSION

EEMO bark has shown significant antiulcerogenic activity against ethanol induced acute gastric ulcers and acetic acid induced chronic gastric ulcers. Its activity was comparable to that of standard Omeprozole. The histopathological studies of the stomach provided solid evidence for the antiulcerogenic activity of the extract. EEMO bark had shown significant antiulcer activity against ethanol and acetic acid induced ulcers. Its activity was comparable to that of standard Omeprazole. Further studies should be planned to isolate the responsible phytoconstituents and to develop them as potential therapeutic interventions for both ulcers and inflammation.

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