

Antiulcer activity of aqueous and ethanolic extracts of *Ocimum sanctum* leaves in albino rats

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ABSTRACT

Background: Peptic ulcer is one of the most common gastrointestinal disorders and a major cause of morbidity. The incidence and prevalence of peptic ulcer has been increasing worldwide. Persisting peptic ulcer leads to complications like gastrointestinal bleeding, gastric perforation and pyloric obstruction. The complications further increase the morbidity and mortality. The objectives of this study were to evaluate the antiulcer activity of aqueous and ethanolic extracts of *Ocimum sanctum* leaves in albino rats.

Methods: 48 apparently healthy wistar albino rats of either sex weighing about 150-200 grams were divided randomly into 12 groups (n=4). All the drugs were given as pre-treatment, per oral daily for 3 days. Test drugs, aqueous leaf extract (100 mg/kg, 200 mg/kg) and ethanolic leaf extract (50 mg/kg, 100 mg/kg) in 1% carboxymethylcellulose (CMC) were administered. Pyloric ligation and cold restrain stress were used as experimental models in the study. Parameters like free acid, gastric volume and ulcer index were observed.

Results: All the test drugs significantly reduced the free acid, gastric volume and ulcer index in pyloric ligation model. 100 mg/kg of aqueous leaf extract failed to decrease the ulcer index in cold restrain stress, but other doses of test drugs reduced the ulcer index.

Conclusions: Both the doses of aqueous and ethanolic extracts of *Ocimum sanctum* leaves exhibited significant antiulcer activity in pyloric ligation model, whereas only 200 mg/kg of aqueous leaf extract was effective in cold restrain stress model.

Keywords: Pyloric ligation, Cold restrains stress, CMC, Ranitidine, *Ocimum sanctum* leaves extracts

INTRODUCTION

Peptic ulcer disease (PUD) is one of the most prevalent gastrointestinal disorders affecting all age groups worldwide. An ulcer is defined as “disruption of the mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation”.¹ Various environmental factors like

culture, religion and ethnicity have an important bearing on the occurrence, pathogenesis and treatment of peptic ulcer disease in different populations. The prevalence of peptic ulcer in particular is more in low socio-economic groups and more common in the developing countries.

A random population based study in a general adult population, found a point prevalence of 4% for PUD and

that the persons with ulcer frequently show atypical symptoms. In India, PUD is common and the Indian Pharmaceutical Industries share 6.2 billion rupees and occupy 4.3% of the market share in consuming antacids and other antiulcer drugs.² The estimated deaths due to peptic ulcer disease in India are about 15,000 every year. The prevalence of peptic ulcer is more in southern than in northern India. Peptic ulcer disease involves both gastric and duodenal ulcers and can occur in any portion of the gastrointestinal tract but most common in the first part of duodenum and lesser curvature of the stomach. The clinical presentation of peptic ulcer is variable. Most common symptom of peptic ulcer is abdominal discomfort. Other symptoms include epigastric burning, poor appetite, weight loss, anemia, bloating, nausea and vomiting. In patients with advanced stage, the symptoms are sharp, persistent and sudden accompanied by stomach pain, melena and hematemesis. Some patients are asymptomatic until life threatening complications like haemorrhage, perforation and stricture develop.³ Duodenal ulcers are more common than gastric ulcers but sometimes symptoms are indistinguishable.

Peptic ulcer is a multi-factorial disease with imbalance of offensive and defensive factors. The etiopathogenesis of peptic ulcer has changed from Schwartz's dictum "No acid-no ulcer" to "no mucosal damage-no ulcer" and recently to "no helicobacter pylori-no ulcer". Peptic ulcer can occur even with regurgitation of bile due to incompetent pyloric sphincter or mucosal disruption due to alcohol. Ayurveda has been practiced in India for centuries. The attention and interest in the use of traditional medicine has increased globally. According to WHO traditional medicine strategy 2002-2005 document, the use of ayurveda and medicinal plants helps to meet the primary health care needs of 65% of Indians. WHO and ICMR are encouraging wide spread researches on herbal drugs.

Although different treatment modalities are available for the prevention and treatment of peptic ulcer, side effects and high recurrence rates demand search for potent and less toxic anti-ulcer drugs. Plant extracts are the most attractive source since long time and a large number of plants have been shown to have promising anti-ulcer effects. Further the plant extracts have advantages like safety, economic, effective and easy availability. *Ocimum sanctum* popularly known as 'Tulasi' in Hindi and 'Holybasil' in English is an herbaceous plant. Various extracts of different parts of the plant have been used for medicinal purposes.

In ayurveda, Tulasi has been well documented for its therapeutic potentials like anti asthmatic, anti-diabetic, anti-oxidant effect.⁴ The antiulcer effect of various extracts of leaves and essential oil of *Ocimum sanctum* has been documented. Aqueous and alcoholic leaf extracts have been reported to have antiulcer activity.⁵⁻⁷ With these backgrounds, the present study was

undertaken to study the antiulcer effect of aqueous and ethanolic extracts of *Ocimum sanctum* leaves.

METHODS

The study was conducted at research laboratory of department of pharmacology, NRI Medical College and General Hospital, Chinakakani, Andhra Pradesh, India.

48 Wistar albino rats were divided into 12 groups using computer generated random numbers. All the animals were maintained in a well-ventilated animal house with 12 hour light and dark cycle.

Ranitidine powder was obtained from GlaxoSmithline pharmaceuticals. Aqueous and alcoholic extracts of *Ocimum sanctum* leaves were obtained from Laila Pharmaceuticals, Vijayawada.

Table 1: Plan of study.

| Group | Treatment | Method |
|-------|-------------------------------------------------------------|----------------------|
| 1 | 0.5 ml 1% CMC | pyloric ligation |
| 2 | Ranitidine 10 mg/kg | pyloric ligation |
| 3 | Aqueous extract of <i>Ocimum sanctum</i> leaves 100 mg/kg | pyloric ligation |
| 4 | Ethanolic extract of <i>Ocimum sanctum</i> leaves 50 mg/kg | pyloric ligation |
| 5 | Aqueous extract of <i>Ocimum sanctum</i> leaves 200 mg/kg | pyloric ligation |
| 6 | Ethanolic extract of <i>Ocimum sanctum</i> leaves 100 mg/kg | pyloric ligation |
| 7 | 0.5 ml 1% CMC | cold restrain stress |
| 8 | Ranitidine 10 mg/kg | cold restrain stress |
| 9 | Aqueous extract of <i>Ocimum sanctum</i> leaves 100 mg/kg | cold restrain stress |
| 10 | Ethanolic extract of <i>Ocimum sanctum</i> leaves 50 mg/kg | cold restrain stress |
| 11 | Aqueous extract of <i>Ocimum sanctum</i> leaves 200 mg/kg | cold restrain stress |
| 12 | Ethanolic extract of <i>Ocimum sanctum</i> leaves 100 mg/kg | cold restrain stress |

Pyloric ligation model of this study was 24 wistar albino rats of group 1-6 were used. Test and standard drugs were administered to respective groups orally once daily for 3 days.⁸⁻¹⁰ On day 2, the animals were fasted overnight. On

day 3, 1 hour after drug administration, abdomen was opened under light ether anaesthesia by a midline incision; pyloric end of the stomach was identified and ligated with silk sutures. Care was taken not to damage the blood supply. Stomach was carefully placed inside abdominal cavity and the abdominal wall was closed with interrupted sutures. The animal was placed in a cage. There was no access to food and water for a period of 6 hours.¹¹⁻¹³ At the end of 6th hours, the animals were sacrificed by cervical dislocation, abdomen was opened, stomach was clamped on both ends and isolated. The gastric contents were collected, centrifuged and parameters like volume of gastric contents, free acidity were estimated. The stomach was cut along the greater curvature, the edges of the stomach fixed to a myoboard and ulcer scoring was done with the help of a 10X magnifying lens.

Parameters

Volume of gastric content

Gastric contents collected and the volume was measured and noted.

*Estimation of free acid*¹⁴⁻¹⁶

The gastric contents were collected in test tubes and centrifuged at 1000 rpm for about 10 minutes. 1ml of supernatant, diluted with 9 ml distilled water was titrated with N/10 NaOH with 1 drop of Topfer's reagent as indicator. Titration was done till the pink colour changed to yellow. This volume of N/10 NaOH was noted, represents free acidity. Free acidity represents the amount of gastric HCl present in the gastric content whereas Total acidity is a combination of HCl and weak acids like protein, acid phosphate and organic acids like lactic acid which are produced by digestion and fermentation in the stomach. To express the value as mEq/Lt, the titre value is multiplied by 10.

Estimation of ulcer index

The animals were sacrificed by cervical dislocation, abdominal wall was opened, stomach was dissected out carefully, opened along the greater curvature, the edges fixed to the myoboard and with the help of magnifying lens, ulcer scoring was done for individual ulcers and ulcer index was calculated for the group.

Ulcer scoring was done as per the following score^{17,18}

- 0-Normal mucosa
- 1- 0.5- red colorations/petechial haemorrhage
- 2- 1- spot ulcers
- 3- 1.5- hemorrhagic streaks
- 4- 2- ulcers between 3 mm-5 mm,

- 5- 2.5- ulcers >5 mm.

Ulcer index was calculated using formula^{19,20}

$$UI = UN + US + UP \times 10^{-1}$$

UN - Average number of ulcers per animal,

US - Average of severity scores,

UP - Percentage of animals with ulcers in that group.

Cold-restrain stress model^{21,22}

24 wistar albino rats of group 7-12 groups were used. Test and standard drugs were administered to respective groups orally, once daily for 3 days 8-10. On day 2, the animals were fasted overnight. On day 3, 1 hour after drug administration, the animals were completely restrained in wire mesh and placed in the refrigerator at 4°C, for about 4 hours.²³ The temperature 40C was constantly monitored by digital thermometer. At the end of 4 hours, the animals were sacrificed by cervical dislocation. Abdomen was opened up by midline incision. Pyloric and cardiac ends of the stomach were identified and ligated. The stomach was cut opened along the greater curvature, the contents were collected and the edges of the stomach fixed to the myoboard. Ulcer scoring was done and ulcer index calculated as above.¹⁶⁻¹⁸

Statistical analysis

Continuous data were analysed by one way analysis of variance (ANOVA) followed by Scheffe multiple range test to find out the significantly different groups. Kruskal-Wallis test was used for discrete data. The level of significance was 5% ($p \leq 0.05$ was taken as significant).

RESULTS

Free acid

Standard drug ranitidine and both the test drugs significantly reduced free acid than the CMC treated control group (group 1) ($p < 0.05$).

Gastric volume

Standard and the test drugs significantly reduced the gastric volume in comparison to control group ($p < 0.05$).

Ulcer index (pyloric ligation model)

Standard and the test drugs significantly reduced ulcer index in pyloric ligation model compared to group 1 ($p < 0.05$). Reduction in ulcer index was more with Aqueous extract 200 mg/kg.

Table 2: Effect of drugs on parameters in pyloric ligation method.

| Group no. | Drug | Free acid (Mean+SEM) | Gastric volume (Mean+SEM) | Ulcer index (mean rank) |
|-----------|--------|----------------------|---------------------------|-------------------------|
| 1 | CMC | 24.0±1.581 | 9.33±0.364 | 22.50 |
| 2 | Ran 10 | 9.80±0.854* | 2.85±0.155* | 13.50* |
| 3 | AE 100 | 5.50±0.645* | 3.93±1.109* | 17.50* |
| 4 | EE 50 | 7.50±0.645* | 5.80±0.227* | 10.75* |
| 5 | AE 200 | 6.00±0.408* | 3.50±0.438* | 3.25* |
| 6 | EE 100 | 6.50±1.041* | 3.78±1.133* | 7.63* |

CMC= carboxymethylcellulose; Ran 10= Rantidine 10 mg/kg, AE 100 = Aqueous extract of *Ocimum sanctum* leaves 100 mg/kg, AE 200 = Aqueous extract of *Ocimum sanctum* leaves 200 mg/kg; EE 50 = Ethanolic extract of *Ocimum sanctum* leaves 50 mg/kg, EE 100 = Ethanolic extract of *Ocimum sanctum* leaves 100 mg/kg. * => p < 0.05 as compared to control.

Table 3: Effect of drugs on ulcer index in cold restrains stress method.

| Group no. | Drug | Ulcer index (mean rank) |
|-----------|--------|-------------------------|
| 7 | CMC | 22.50 |
| 8 | Ran 10 | 2.50* |
| 9 | AE 100 | 18.50 |
| 10 | EE 50 | 14.50* |
| 11 | AE 200 | 10.50* |
| 12 | EE 100 | 6.50* |

CMC= carboxymethylcellulose; Ran 10= Rantidine 10 mg/kg, AE 100 = Aqueous extract of *Ocimum sanctum* leaves 100mg/kg, AE 200 = Aqueous extract of *Ocimum sanctum* leaves 200 mg/kg; EE 50 = Ethanolic extract of *Ocimum sanctum* leaves 50 mg/kg, EE 100 = Ethanolic extract of *Ocimum sanctum* leaves 100 mg/kg. * => p < 0.05 as compared to control.

Ulcer index (cold restrain model)

Ethanolic leaf extract 50 mg/kg, 100 mg/kg and aqueous leaf extract 200 mg/kg significantly reduced ulcer index in cold restrain model compared to CMC treated control group. Rantidine 10 mg/kg, exhibited more reduction in ulcer index. Aqueous leaf extract 100 mg/kg failed to produce reduction in ulcer index in cold restrain stress model.

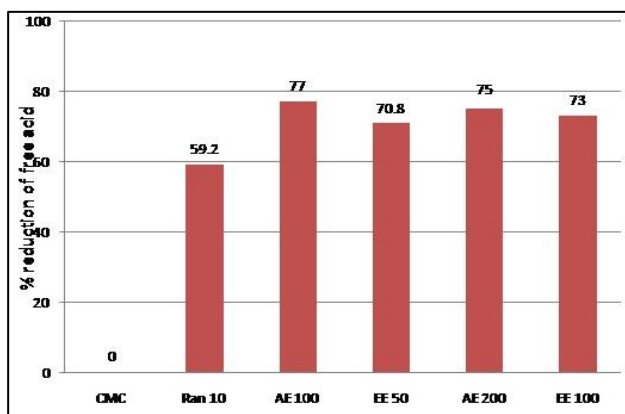


Figure 1: Effect of drugs on free acid in pyloric ligation method.

It was observed that ranitidine reduced free acid by 59.2%, Aqueous leaf extracts 100 mg/kg and 200 mg/kg reduced the free acid level by 77% and 75% respectively and ethanolic leaf extracts 50 mg/kg and 100 mg/kg reduced the free acid level by 70.8% and 73% respectively. The test drugs viz. aqueous leaf extracts in both the doses and ethanolic leaf extracts in both the doses; exhibited more reduction in free acid compared to ranitidine in pyloric ligation model.

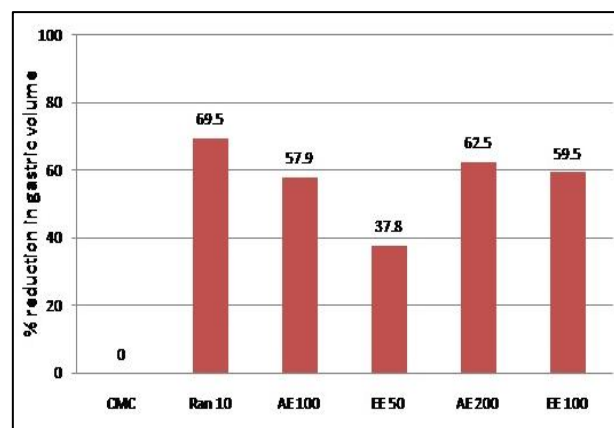


Figure 2: Effect of drugs on gastric volume in pyloric ligation model.

The gastric volume increased after pyloric ligation. Ranitidine reduced the gastric volume by 69.5%, aqueous leaf extracts 100 mg/kg and 200 mg/kg by 57.9% and 62.5% respectively, ethanolic leaf extracts 50 mg/kg and 100 mg/kg, reduced by 37.8% and 59.5% respectively. All doses of test drugs studied exhibited identical effect to that of ranitidine.

Ulcer index is a measure of severity of gastric ulceration. Ranitidine reduced the ulcer index by 32.9%. Aqueous leaf extracts 100 mg/kg and 200 mg/kg, reduced ulcer index by 21.7% and 46.7% respectively. Ethanolic leaf extracts 50 mg/kg and 100 mg/kg, reduced ulcer index by 33.3% and 40.4% respectively. 100 mg/kg of ethanolic leaf extract and 200 mg/kg aqueous leaf extract exhibited more reduction of the ulcer index than ranitidine. 50

mg/kg of ethanolic leaf extract produced effect identical to that of ranitidine.

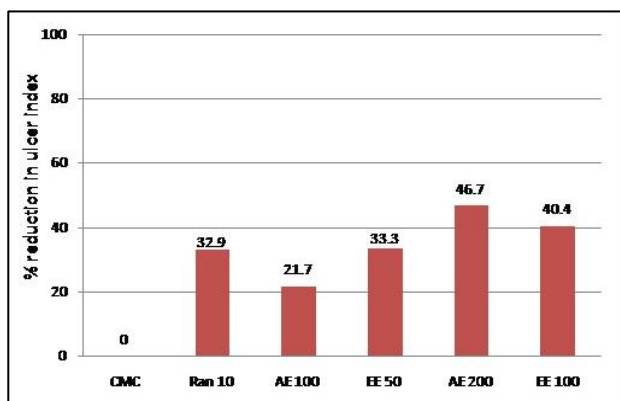


Figure 3: Effect of drugs on ulcer index in pyloric ligation model.

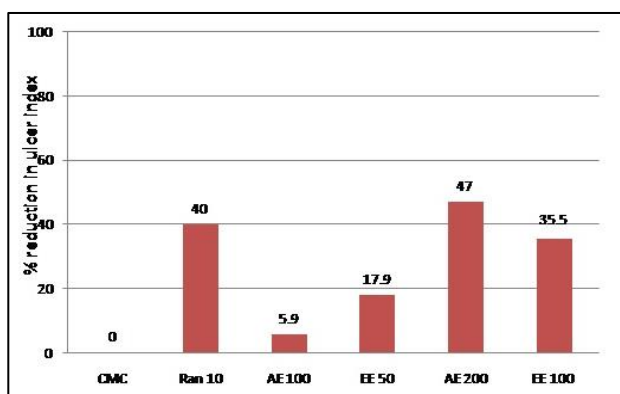


Figure 4: Effect of drugs on ulcer index in cold stress restrain model.

Ranitidine reduced the ulcer index by 40%. Aqueous leaf extract 200 mg/kg, ethanolic leaf extracts 50 mg/kg and 100 mg/kg, reduced the ulcer index by 47%, 35.5% and 17.9% respectively. Aqueous leaf extract 100mg/kg failed to reduce ulcer index in cold restrain stress model. Aqueous leaf extract 200 mg/kg exhibited better protection than ranitidine.

DISCUSSION

Ranitidine is a potent and selective H₂ receptor antagonist.²⁴ It can be administered by both oral and parenteral routes. Studies have shown that ranitidine 10 mg/kg was effective in reducing the gastric acid secretion by 90-95%.²⁵ So it has been used as a standard drug. Pyloric ligation for 6 hours resulted in accumulation of gastric secretion resulting in increased volume and titratable acid. The accumulated acid may be responsible for gastric ulceration. Gastric hydrochloric acid (HCl) accounts for the free acidity of gastric juice. The role of hydrochloric acid in the pathogenesis of gastric ulcer is well established. The volume of gastric secretion is also an important factor in the formation of ulcer. In pyloric

ligation model, acid accumulates leading to ulcer formation.

The test drugs viz. aqueous leaf extracts in both the doses and ethanolic leaf extracts in both the doses; significantly reduced free acid and gastric volume in pyloric ligation model. Decrease in free acid may be responsible for antiulcer effect similar to that of ranitidine.

Pyloric ligation induces ulcers by auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier. Ulcer index is a measure of severity of gastric ulceration. Acid output is closely associated with the occurrence of stress-induced lesions. Standard and test drugs in all doses studied reduced the ulcer index. These results are similar to the observations of Ghangale et al and Harshal et al.^{5,7}

Ranitidine reduced the ulcer index by 40% in cold restrain stress. Although aqueous leaf extract 100 mg/kg failed to reduce ulcer index in cold restrain stress model, aqueous leaf extract 200 mg/kg, ethanolic leaf extracts 50 mg/kg and 100 mg/kg produced significant reduction in ulcer index similar to that of Ranitidine. The observations are similar to that of Bharat et al.⁶

CONCLUSION

Aqueous leaf extracts (100 mg/kg and 200 mg/kg) and ethanolic leaf extracts (50 mg/kg and 100 mg/kg) exhibited better protection than ranitidine in reducing free acid in pyloric ligation model. All doses of test drugs studied exhibited identical effect to that of ranitidine in reducing free acid and gastric volume. Ethanolic leaf extract 100 and 200 mg/kg aqueous leaf extract exhibited better protection compared to ranitidine in reducing the ulcer index in pyloric ligation model. 50 mg/kg of ethanolic leaf extract produced effect identical to ranitidine. Aqueous leaf extract 200 mg/kg exhibited better protection compared to ranitidine in reducing the ulcer index but aqueous leaf extract 100 mg/kg was ineffective in cold restrain stress model.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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