

Research Article

Anti-Ulcer Activity of Fruit of *Zizyphus Nummularia* In Pyloric Ligation Method In Rats

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ABSTRACT

The aim is to evaluate the anti-ulcer activity of methanol extract of fruit of *Zizyphus nummularia*, Anti-ulcer activity of the three doses (100, 200 & 400 mg/kg) of extract was studied in rats by using pylorus ligated ulcer model and it was subjected to preliminary phytochemical studies for the identification of phytoconstituents. Omeprazole was used as the standard drug for comparison. The animals were sacrificed after 8 hrs after the ligation. Stomach was dissected out and contents were drained into tubes and were centrifuged at 3000 rpm for 10 min and volume was noted. The PH of gastric juice was recorded using a pH meter. The contents were subjected for analysis of free and total acidity. The numbers of ulcers per stomach was noted and severity of ulcers scored. The expected result is to get an anti-ulcer activity of the methanol extract of fruit of *Zizyphus nummularia* should owing to the presence of one or more phytoconstituents, which may reduce the acidity of the gastric juice and also prevents the mucosal damage and ulcer formation. The extract 200 & 400 mg /kg showed significant decrease ulcer index, gastric juice, acidity and rising pH compared to 100 mg/kg which is non significant to ulcer control. Extract 400mg/kg expected to showed comparable anti ulcer-activity as that of standard Omeprazole.

Keywords: Ulcer, Fruit, *Zizyphus nummularia*, pyloric ligation, Omeprazole.

INTRODUCTION

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly for the population of non industrialized countries¹. Several factors are implicated in the pathogenesis of gastric ulcer including increased acid-pepsin secretion, impaired bicarbonate neutralization, impaired mucus secretion and precipitate lesions on the mucosal layer^{2,3}. In recent years, a powerful association between peptic ulcers and infection of *Helicobacter pylori* has been adopted. At least 70-90% of patients with gastric ulcers and 80-95% with duodenal

ulcers are infected by *H.pylori* and eradication of this Microorganism seems to be curative for the disease⁴. There is a balance between the aggressive (i.e. acid, pepsin, active oxidants, *H. pylori*) and the mucosal protective (i.e. mucus, bicarbonate, prostaglandin's) factors in stomach. Thus, drug therapy of peptic ulcer has been commonly targeted at either counteracting the aggressive factors or stimulating defensive one⁵. Despite the progress in conventional chemistry and pharmacology in producing highly effective drugs, some of them are expensive and have different adverse effects^{6,7}. However, screening plants for active drugs

is still important and might provide a useful source of new anti-ulcer compounds for developing pharmaceutical drugs or alternatively as simple dietary adjuncts to existing therapies⁸.

The plant *Zizyphus nummularia* belonging to family *Rhamnaceae* and commonly known as Aja-priya in Sanskrit, Jhar Beri in Hindi, Korgodi in Tamil and Nelaregu in Telugu^{9,10}. The plant used for anthelmintic, blood purification and digestion¹¹. As per the literature review plant having traditional claim of anti-ulcer activity, this is not scientifically documented. In the present study, we reported anti-ulcer activity methanol extract of fruit of *Zizyphus nummularia* in pyloric ligation method in rat model.

MATERIAL AND METHODS

Identification, Authentication and collection of plant materials

The fruit of *Zizyphus nummularia* is collected from Chittoor district, Madanapalle region, Andhra Pradesh. The plant was identified and authenticated by Dr.K.Madhava chetty, Assistant Professor, Department of Botany of S.V.University, Tirupathi.

Preparation of extracts

The collected fruit of *Zizyphus nummularia* were washed fussy and dried under shade for 15 days. The dried fruit were prepared into a boorish powder using dry grinder. The powder was conceded through sieve no. 40 and stored in an air tense vase at 25°C, used for further study. Powdered plant material (250 gm) was extracted with Soxhlet apparatus using the solvents in methanol. The extracts were concentrated by condense the solvent in a rotary vacuum evaporator and vanish to dryness. The yield was calculated (9.56%) with reference to the dried plant material.¹²

Preliminary Phytochemical studies¹³

The extracts of fruit of *Zizyphus nummularia* was subjected to qualitative analysis for various phytoconstituents.

Experimental animals

The albino rats & mice, weighing 150-200 & 25-30g were used in the present study. All the rats were kept at room temperature (22°C) in the animal house. All the animals were housed and treated as per the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food and were acclimatized to laboratory conditions. All the experimental procedures were reviewed and approved by Institutional Animal Ethics Committee and in

accordance with the recommendations for the proper care and use of laboratory animals.

Acute toxicity Studies¹⁴

The acute toxicity study was carried out with extracts of *Zizyphus nummularia* as per OECD 423 Guidelines. Swiss albino mice with weight ranging (25-30g) were in use for the experiment. The animals were fasted for the night, provided with water after which extract of *Zizyphus nummularia* was administered orally at a dose level of 5 mg/kg, p.o. If mortality was observed in animals, then the dose administered was assigned as a toxic dose. If death was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If death was not observed, the method was repeated for further upper doses such as 50, 300 and 2000 mg/kg body weight. Dose volume was administered 0.1 ml / 100 gm body weight to the animal by oral route. After giving the dose the toxic signs were observed within 3-4 hours. Onset of toxicity and signs of toxicity like changes in skin and fur, eyes, and mucous membrane and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behaviour pattern, signs of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma was also to be noted, if any, was observed for 14 days. It was observed that the methanol extracts of fruit of *Zizyphus nummularia* were not lethal to the mice even at the 2000 mg/kg doses. Hence LD50 of extracts found to be 2000 mg/kg. Therefore, 1/10th (200mg/kg) of this dose consider as ED50 and 100, 200 and 400 mg/kg doses selected for further study.

Anti-ulcer activity by pyloric ligation method

Experimental design

Albino rats weighed about 150-180g were divided into six groups of six mice each.

Group I - Received 1% Methyl cellulose (1.0ml/kg p.o) as normal control.

Group II - Received 1% Methyl cellulose (1.0ml/kg p.o) as negative control.

Group III - Received Omeprazole as standard (20mg/kg).

Group IV - Received Methanolic extract of *Zizyphus nummularia* (100mg/kg, p.o).

Group V - Received Methanolic extract of *Zizyphus nummularia* (200mg/kg, p.o).

Group VI - Received Methanolic extract of *Zizyphus nummularia* (400mg/kg, p.o).

Pylorus ligation model

The standard and test samples were administered to respective group of animals

through oral treatment were carried out 30 min before pyloric ligation, respectively. After 12 hours of fasting, ulcer induction was undertaken according to Shay et al.¹⁵. The rats of all groups except Group I, was quickly and mildly anaesthetized with chloroform and the abdomen was cut open through a midline incision. The pylorus was secured and ligated with silk sutures, after which the wound was closed and the animals were allowed to recover from anesthesia. After ligation of the pylorus, drinking water was withheld and the gastric examinations were under taken 8 hours after pylorus ligation.

Measurement of gastric juice volume, and pH

The stomach was carefully excised keeping esophagus closed and opened along greater curvature and luminal contents were removed. The gastric contents were collected in a test tube and centrifuged. The gastric juice thus collected was centrifuged at 3000 rpm for 10 min. The volume of supernatant was measured and expressed as ml/100g body weight. The free acidity, total acidity and pH were measured from the supernatant liquid.

Ulcer index (UI)

The mucosa was flushed with saline and stomach was pinned on frog board. The lesion in glandular portion was examined under a 10x

magnifying glass and length was measured using a divider and scale and gastric ulcer was scored by following method. Ulcer index of each animal was calculated by adding the values and their mean values were determined.¹⁶

0 – Normal colored stomach, 0.5 – Red coloration, 1 – Spot ulceration, 1.5 – Haemorrhagic streak, 2– ulcers and 3– Perforations. Percentage inhibition was calculated using the following formula.

$$\frac{\text{Negative control} - \text{test}}{\text{Negative control}} \times 100$$

Statistical Analysis

Results of the above experiments were expressed as Mean \pm SEM, and the difference between means was analyzed by one way analysis of variance (ANOVA) using graph pad prism 5 software followed by Dunnett's test, with $p < 0.05$, $p < 0.01$, & $p < 0.001$ being considered as statistical significant

RESULTS

Phytochemical screening

The preliminary phytochemical investigation on methanol extracts of fruit of *Ziziphus nummularia* showed the presence of alkaloids, saponins, flavonoids, terpenoids, tannins, gums and phytosteroids.

Ulcer index on pyloric ligation rat

Table 1: Effect of *Ziziphus nummularia* on Ulcer Index in pyloric ligation induced gastric ulcer

All values are expressed as mean \pm S.E.M.; (n=6) animals in each group. * $p < 0.05$, ** $P < 0.01$ when Omeprazole and extract treated groups were compared with ulcer control group

| Group | Ulcer index (UI) | Percentage inhibition (%) |
|----------------------|-------------------|---------------------------|
| Normal Control | 00.00 \pm 0.00 | 100 |
| Ulcer Control | 11.7 \pm 2.13 | 0 |
| Omeprazole (20mg/kg) | 4.54 \pm 0.83** | 61.19 |
| MEZN (100mg/kg) | 8.87 \pm 1.61* | 24.18 |
| MEZN (200mg/kg) | 5.45 \pm 1.07** | 53.41 |
| MEZN (400 mg/kg) | 4.37 \pm 1.63** | 62.64 |



Group. I: Normal control



Group. II: Ulcer control



Group. III: Standard



Group. IV: MEZN 100mg/kg



Group. V: MEZN 200mg/kg



Group. VI: MEZN 400mg/kg

Table 2: Effect of *Ziziphus nummularia* on Gastric acidity, secretion & pH in pyloric ligation method

| Group | Gastric volume (ml/100g) | pH of gastric juice | Total acidity meq/L | Free acidity meq/L |
|----------------------|--------------------------|---------------------------|--------------------------|-------------------------|
| Normal Control | 1.20 ± 0.08 | 2.46 ± 0.06 | 23.27±1.52 | 18.39±0.9 |
| Ulcer control | 5.74 ± 0.71 | 1.05 ± 0.39 | 53.61±0.98 | 35.68±0.7 |
| Omeprazole (20mg/kg) | 1.42±0.22** | 2.98±0.68** | 24.48±1.86** | 17.54±0.3** |
| MEZN (100mg/kg) | 3.99 ± 0.45* | 1.09 ± 0.61 ^{ns} | 49.72±2.01 ^{ns} | 33.49±0.5 ^{ns} |
| MEZN (200mg/kg) | 2.84 ± 0.30** | 2.16 ± 0.23* | 30.55±1.82** | 24.26±1.4** |
| MEZN (400mg/kg) | 1.47 ± 0.35** | 2.80 ± 0.54** | 27.49±3.7** | 18.57±0.8** |

All values are expressed as mean ± S.E.M.; (n=6) animals in each group. *p<0.05, **P<0.01 & ^{ns} - non significant when Omeprazole and Extract treated groups were compared with ulcer control group.

RESULT AND DISCUSSION

The present study was undertaken to determine the antiulcer activity of the Methanolic extract from the fruits of *Ziziphus nummularia*. Earlier literatures shows that the phytoconstituents like flavonoids, tannins, terpenoids, and saponins have been reported in several anti-ulcer as possible gastroprotective agents. Flavonoids, tannins and triterpenes are among the cytoprotective active materials for which antiulcerogenic efficacy has been extensively confirmed.¹⁷

It is suggested that these compounds will be able to stimulate mucus, bicarbonate and prostaglandin secretion, and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen.¹⁸ Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants.¹⁹ Table 1 show the severity of the Gastric ulceration in pylorus ligation method as assessed is based on the ulcer index. The ulcer index in negative control group was significantly increase compared to normal control. The methanol extract at 400 mg/kg exhibited significant decrease in ulcer index, which is almost comparable to that of Omeprazole and proved its protection effect against ulcer formation due to pylorus ligation. The ulcer curative ratio of methanol extract at 400mg/kg was almost comparable to that of standard Omeprazole. The extract at dose of 100 mg/kg exhibited a less inhibition percentage than the 200 & 400mg/kg doses respectively.

Table 2 show in negative control group gastric juice was 5.74 ml it shows significant variation when compared to normal control. Omeprazole a standard drug decreased the mean gastric volume, which is statistically significant. Methanol extract at 400mg/kg decreased the significant gastric juice volume compared to negative control. All the dose of extract showed the decreased in gastric juice

volume on comparison to negative control group. But extract 400mg/kg showed equipotent effects as that of Omeprazole in reducing the gastric juice volume and also showed elevation in pH indicating their capacity to reduce the acidity of the gastric juice. The pH value of methanol extract at 400mg/kg is almost equipotent as that of Omeprazole than the other doses.

The present study, Methanol extract of *Ziziphus nummularia*, showed the presence flavonoids and their glycosides, tannins, triterpenoids and saponins. These phytoconstituents present in the extract could be the possible agents involved in the prevention of gastric lesions induced by pyloric ligation method.

CONCLUSION

From the above results, it can be inferred that, the methanol extracts showed marked anti ulcer activity in pylorus ligation induced gastric ulcer model in a dose dependent manner, but overall methanol extract of *Ziziphus nummularia* at 400 mg/kg reduced the gastric volume, free acidity, total acidity and ulcer index thus showing the anti-secretory mechanism involved in the extract for their anti-ulcerogenic activity. So, methanol extract at 400 mg/kg exhibited significant anti ulcer activity and almost equipotent effect as that of Omeprazole and these results offer pharmacological evidence and support on the traditional use of *Ziziphus nummularia* fruit as an Anti ulcer agent. In future, this work has been extended by including more ulcer models to confirm the anti ulcer potency of for meaningful and tangible conclusion.

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