Asian Journal of Pharmaceutical and Clinical Research
Vol. 4, Issue 1, 2011 ISSN - 0974-2441

Research Article

EVALUATION OF ANTIULCER ACTIVITY OF *ZIZYPHUS OENOPLIA (L) MILL.* ROOT IN RATS

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ABSTRACT

The aim of present study is to investigate the antiulcer activity of *Zizyphus oenoplia.* In this study the powdered root of *Zizyphus oenoplia* was extracted with alcohol followed by fractionation of alcoholic extract with different solvents. The total alcoholic extract and its fractions were subjected to the screening of antiulcer activity. Results suggest that antiulcer activity of *Zizyphus oenoplia* is significant in both total alcoholic extract and its ethanol fraction at a dose of 300mg/kg, while chloroform and aqueous fraction doesn't show any significant effect at a dose of 300mg/kg. The parameters studied were Ulcer index, pH and volume of gastric juice, free acidity and total acidity; it was found that the ethanol fraction of *Zizyphus oenoplia* was more potent than total alcoholic extract.

Key words: *Zizyphus oenoplia*, antiulcer, ulcer index, flavonoids.

INTRODUCTION

Worldwide interest in natural products as preventive and therapeutic agents has led to a greater appreciation of the rich heritage of traditional systems of medicine. Traditionally peptic ulcers have been described as an imbalance between the luminal acid peptic attack versus the mucosal defense [1] The treatment of peptic ulcers with plant products used in folk medicine and the protection of induced gastric ulcer in laboratory animals using medicinal plants was reported [2] *Zizyphus oenoplia mill* belonging to the family Rhamnaceae is a thorny straggling shrub found throughout the hotter parts of India, Ceylon, Tropical Asia and Australia. Chemical investigation of this plant has shown the presence of cyclopeptide alkaloids such as Zizyphine- A, B, C, D, E, Abyssinine-B and A in stem bark of the plant. A decoction of the root bark is used to promote the healing of fresh wounds. Among the munda tribe the fruit is used as an ingredient in the preparation of stomach ache pills. [3]

MATERIALS AND METHODS

Plant material

Fresh roots of *Zizyphus oenoplia* was identified and authenticated by Dr. R.Manjunatha, Professor, and department of Botany, D.V.S College of Arts and Science, Shimoga, Karnataka. The collected roots were washed with water, dried in shade and powdered using hand grinder to make a coarse powder, sieved and packed in air tight container and stored in cool and dry place until further use.

Preparation of extract

The extraction of plant material with alcohol solvent was carried out in soxhlet extractor. 95% alcohol was used as a solvent. The extract was collected directly from round bottomed flask and solvent was evaporated using Rota flash evaporator. To detect active substance present in very small quantities in the extract a 'primary' fractionation of the total extract was carried out prior to pharmacological screening to separate polar from less-polar constituents by sequential use of solvent from high to low polarity [4]

Animal

Either sex of *Wistar albino* rats and mice weighing between 200-250 and 25-30 gm were used. Institutional Animal Ethics Committee approved the experimental protocol **ethical clearance no.NCP/IAEC/CLEAR/P.COL/06/2007-08.** Animals were maintained under standard conditions in an animal house approved by Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) [5]

Acute toxicity study

The acute toxicity study was performed according to the OPPTS (Office of prevention, pesticide and toxic substance) Up and Down procedure (Health Effect Test Guideline 2004). The different fractions were suspended using Tween 80 (0.1%) and were administered orally at the dose of 3000mg/kg. The concentration was adjusted in such a way that it did not exceed 1ml/kg b/w of the animal.

Anti-ulcerogenic activity

Pyloric ligation in rats [6]

The animals were divided in to six groups (control, standard, total alcoholic extract, ethanol fraction, chloroform fraction and aqueous fraction) of six rats in each group weighing 150-200. The pyloric ligation was carried out 30 minutes after drug administration. Under light ether anesthesia the abdomen is cut opened by a small incision below the xiphoid process; pyloric portion of the stomach is slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply.

The stomach is replaced carefully and the abdominal wall closed by interrupted sutures. The animals are deprived of both food and water during the postoperative period and are sacrificed at the end of 19 hours after operation. Stomachs are dissected out; contents are drained into tubes and centrifuged at 1000 r.p.m. for 10 min. and supernatant subjected to analysis for gastric volume, pH, and free, total acidity. The stomachs are then cut open along the greater curvature and the inner surface is examined for ulceration by giving score number. Mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated using the formula.

$$\text{Percentage of ulcer protection} = \frac{\text{UI of control} - \text{UI of test}}{\text{UI of control}} \times 100$$

Table -1: Shows ulcer severity and ulcer score

Ulcer Severity	Ulcer score
Normal rugal pattern	0
Alteration in normal rugal pattern	1
Scattered heamorrhagic lesion	2
Heamorrhage lesion and ulcer	3
Penetrating and perforation	4

Determination of total gastric output

1 ml of gastric juice was pipetted into a 100 ml conical flask, added 2 to 3 drops of Topfer's reagent and titrated with 0.01 N NaOH (which was previously standardized with 0.01 N of oxalic acid) until all traces of the red colour disappears and the colour of solution was yellowish orange. The volume of alkali added was noted. The volume corresponds to free acidity. Then 2 to 3 drops of phenolphthalein solution were added and titration was continued until a definite red tinge reappears. Again the total volume of alkali added was noted. The volume corresponds to total acidity.

Acidity was calculated by using the formula-

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \text{ meq/l/100g}$$

Healing of indomethacin induced gastric ulcer [6]

Gastric ulceration according to the method described by AL-shabanah was induced in 36 hours fasted rats by administration of ulcerogenic drug, Indomethacin. Group of 6 albino rats of either sex, 150-200 gm was fasted 36 hrs, prior to experiment, but allowed free access to water. Group of six animals each was pretreated with water or standard drug or extracts 1 hr before the administration of the indomethacin30mg/kg orally. The animals were killed after 4 hrs after ulcerogenic drugs by an overdose of anesthetic ether. The stomach were removed and opened along the greater curvature of stomach; the number of ulcer per stomach were noted and severity of the ulcer scored microscopically with the help of hand lens (10x) and scoring was done as per earlier mentioned kulkarni (1987).

RESULTS

All the fractions of alcoholic extract were found to be nontoxic when administered orally to mice in the dose 3000mg/kg b.w. and its LD_{50} was found to be safe in the same dose. Therefore $1/10^{th}$ of the LD_{50} was considered as therapeutic dose and was administered for the study of activity. In Pylorus ligation in rats the total alcoholic extract and ethanol fraction of *Zizyphus oenoplia* root significantly reduced gastric volume, free acidity, and total acidity and increased gastric pH compared to that of the control, it is evident from table No.2. Percentage of ulcer protection was found to be 37.40 and 39.8(Table No.3). Chloroform and aqueous fraction did not show any significant activity. In indomethacin induced ulcer in rats total alcoholic extract and ethanol fraction showed significantly reduction in ulcer index. The percentage of ulcer protection was 30.74 and 34.69 in total alcoholic extract and ethanol fraction comparing to control group it is evident from Table 4.

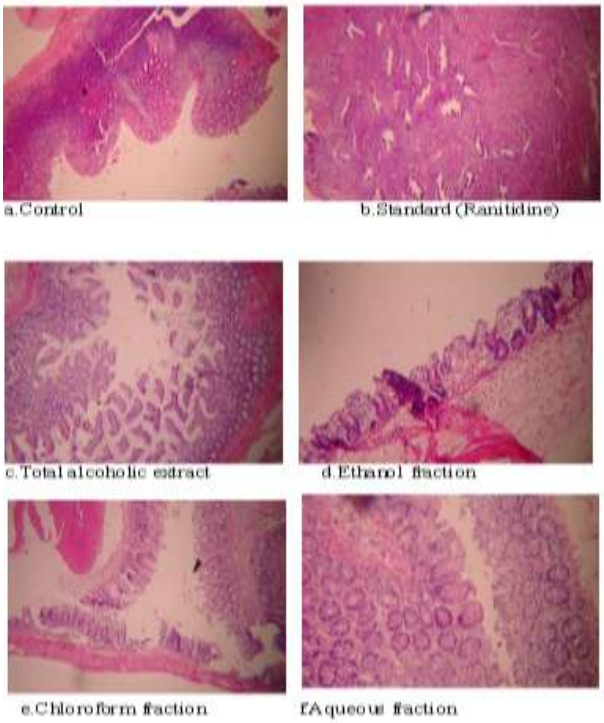

Figure -1: Shows Photograph showing Histograph of Pylorus ligation induced ulcer with treated groups in Rat model

DISCUSSION

Gastric and duodenal ulcers are illness that affects a considerable number of people in the world. Stress, smoking, ingestion of non steroidal anti-inflammatory drugs contribute to the gastric ulcer and the infection of *Helicobacter pylori*, a spiral shaped bacteria found in stomach, is also implicated in the gastric ulcer. Pyloric ligation induced ulcer are due to the auto digestion of gastric mucosa and break down of gastric mucosal barrier. Treatment with the total alcoholic extract and ethanol fraction reduced the volume of gastric secretion, free acidity, and total acidity. Decreased ulcer index and gastric contents as well as secretary parameters can be implicated with protective effects of the drug. Histopathological examination of gastric mucosal tissue of ulcer group reveals that the detectable ulcerative lesions with injury in epigastric layer and lamina propria with hemorrhagic lesions (Figure 1). Standard rat stomach section (Figure 1b) showed integrated and intact layer of gastric mucosa. Stomach from experimental control group (Figure 1a) indicated damage in the mucosal layer with moderate infiltration in the mucous membrane. In Indomethacin induced ulcer model treatment of *Zizyphus oenoplia* root, total alcoholic extract and ethanol fraction showed significantly reduction in ulcer index. The increased percentage inhibition of ulcer was observed in animals treated with total alcoholic extract and ethanol fraction when compared to animals of control group. But chloroform and aqueous fraction did not showed any significant effect. From histopathology it was found that, the rats treated with indomethacin showed loss of gland architecture with erosion, loss of the epithelial layer, evident edema and inflammation cell. The total alcoholic extract and ethanol fraction showed no ulcers in gastric mucosa, glands were regular and no inflammation was observed (Figure 2).

In both the models the total alcoholic extract and ethanol fraction inhibit the ulcer index. This may be due to increase in the prostaglandins synthesis, decrease in acid secretion and back diffusion of H^+ ion. The Indomethacin induced ulcer is mediated through tissue damage by free radicals which are generated from the conversion of hydroxyl to hydroxyl fatty acids, which are in turn generated from the degeneration of mast cells and generalized lipid peroxidation accompanying cell damage.

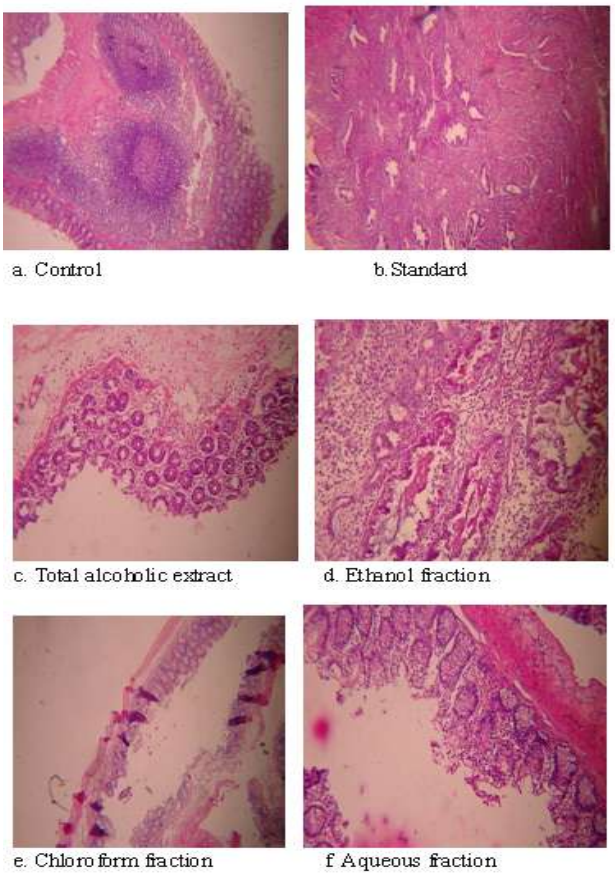

Figure -2: Shows Photograph showing Histograph of Indomethacin induced ulcer

Table -2: Shows Effect of Ranitidine, Total alcoholic extract and fractions of *Zizyphus oenoplia* on gastric volume, pH, free acidity, total acidity in pylorus ligated model

Groups	Mean vol. of Gastric juice (ml) ± SEM	Mean vol. of Acidity (mEq/L/100g)	Mean Total Acidity (mEq/L/100g)	Mean Gastric pH ±SEM
Control (0.5% CMC)	7.99±0.33	50±1.46	73.85±1.90	3.56±0.39
Standard (Ranitidine)	4.45±0.26**	33.71±1.70**	53.7±2.62**	6.34±0.29**
Total alcohol Extract	5.79±0.35**	36.07±3.10**	54.33±2.39**	5.12±0.30**
Aqueous fraction	6.65±0.51	40.83±1.95	67.5±1.5	4.31±0.32
Chloroform fraction	7.31±0.42	44.16±3.36	68.83±1.81	3.92±0.30
Ethanol fraction	6.5±0.37**	37.36±2.75**	55.00±3.40**	5.67±0.20**

Each value represents the Mean ± S.E.M. for six rats. Each value represents the Mean ± S.E.M. for six rats. ** P< 0.01,* P< 0.05compared to respective control group.

Table -3: Shows Effect of Ranitidine, total alcoholic extract and fractions of *Zizyphus oenoplia* on ulcer index and percentage of ulcer protection in pylorus ligated model

Groups	Treatment & dose (mg/ kg b.w.)	Ulcer index	% inhibition
Control (0.5% CMC)	--------	10.08±0.18	---------
Standard (Ranitidine)	30 mg/ kg	5.45±0.26**	45.93
Total alcoholic Extract	300 mg/kg	6.76±0.36**	32.93
Aqueous fraction	300 mg/kg	9.16±0.49	15.18
Chloroform fraction	300 mg/kg	9.83±0.0.82	2.48
Ethanol fraction	300 mg/kg	6.5±0.39**	35.51

Each value represents the Mean ± S.E.M. for six rats. Each value represents the Mean ± S.E.M. for six rats. ** P< 0.01,* P< 0.05compared to respective control group

Table -4: Shows Effect of Ranitidine, total alcoholic extract and fraction of *Zizyphus oenoplia* on ulcer index and percentage of ulcer protection Indomethacin induced gastric ulcer in rats.

Groups	Treatment & dose (mg/ kg b.w.)	Ulcer index	% inhibition
Control (0.5% CMC)	------	14.41±0.40	------
Standard	30 mg/ kg	7.17±0.30**	50.24 (Ranitidine)
Total alcoholic Extract	300 mg/kg	9.41±0.47**	30.74
Aqueous fraction	300 mg/kg	11.75±0.41	18.45
Chloroform fraction	300 mg/kg	12.91±0.38	10.40
Ethanol fraction	300 mg/kg	9.98±0.44**	34.69

Each value represents the Mean ± S.E.M. for six rats. Each value represents the Mean ± S.E.M. for six rats. ** P< 0.01,* P< 0.05compared to respective control group.

Observation in the current study clearly suggests that in the healing process of *Zizyphus oenoplia* extact, inhibition of acid enhances the healing of ulcers [7]. Hence we may suggest that the total ethanolic extract and ethanolic fraction of *Zizyphus oenoplia* may increase the prostaglandin synthesis.

As flavonoids have been identified in the total alcoholic extract and ethanol fraction, we believe that the antiulcer activity of this extract is probably due to the presence of flavonoids in the extract. Moreover, flavonoids have been reported to possess antiulcerogenic activity and gastric protection. Parmar and Parmar *et al*, using plant

extract of *Tephrosia purpurea*, recently demonstrated that flavonoids possess significant antiulcer activity [8]. Hence it could be conceived that *Zizyphus oenoplia* root extract exert their antiulcer activity through the flavonoids. Since flavonoids are reported to protect the mucosa by preventing the formation of lesions by various necrotic agents [9].

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