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Evaluation of Anti-Ulcer Activity of Whole Plant Extracts of *Ziziphus Jujuba.Mill*

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ABSTRACT

The cause of ulceration in patients is mainly due to hyper secretion of gastric juice and also due to hyper secretion of pepsin. In traditional system of medicine a number of herbal preparations have been used for the treatment of peptic ulcers. There are various medicinal plants has been used for the treatment of gastrointestinal disorders. In view of this, in present study we have to evaluate the anti-ulcer activity of *Ziziphusjujuba*. Study was carried out, by using two methods i.e., alcohol and paracetamol induced ulcers with the doses of 250 mg/kg AQEZJ and ALEZJ, 20mg/kg Omeprazole and 50 mg/kg Ranitidine through oral administration. In alcohol-induced ulcers, AQEZJ and ALEZJ were effective in reducing lesion index and increasing the gastric mucus content. It was also effective in decreasing ulcer index in paracetamol-induced ulcers. All the results obtained with ZJE were dose dependent. The results suggest that AQEZJ and ALEZJ possesses significant and dose dependent antiulcer activity. The antiulcer activity of AQEZJ and ALEZJ can be attributed to its cytoprotective and anti-secretary action.

Keywords: *Ziziphusjujuba*, anti-secretary, cytoprotective, gastric ulcer, alcohol induced ulcers, paracetamol-induced ulcers and stress induced ulcers.

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1. Introduction

Ulcer in the mucosal layer of the stomach which tends to cure with stress and is characterized by episodes of burning epigastric pain, belching and nausea, especially where the stomach is empty or after eating certain foods of which is International Journal of Medicine and Pharmaceutical Research

called gastric ulcer or also known as stress ulcer and in the duodenum is called duodenal ulcer together called as peptic ulcer.⁽¹⁾ Peptic ulcer may be acute or chronic. Acute lesions are almost always multiple and superficial. They may be

totally asymptomatic and usually heal. Chronic ulcers are true ulcers.⁽²⁾ They are deep, single, persistent and symptomatic. Peptic ulcers are caused by a combination of poorly understood factors including an excessive secretions of gastric acid, inadequate protection of mucous membrane, stress, heredity and taking of some drugs (Aspirin, Steroids, NSAIDS, Caffeine) and other ulcerogenic agents like alcohol, smoking, coffee etc. Clinically, peptic ulcer is one of the most prevalent gastrointestinal disorders commonly occurs in developed countries.⁽³⁾ Treatments are available for ulcer is generally non-specific and is usually aimed at reducing the production of gastric acid and re-enforcing gastric mucosal protection such as regular food, adequate rest and avoidance of exogenous agents.⁽⁴⁾ The drugs used in the treatment of ulcer include receptor blockers, proton pump inhibitors, drugs affecting the mucosal barrier and act on CNS. Even though a range of drugs are available for the treatment of ulcer, many of those do not fulfill all the requirements and have side effects.⁽⁵⁾ Prevention is first care better than cure to reduce gastric irritation and acid secretion with growing interest in herbal therapy. We have to find the new herbal drug which can be used for ulcer with more efficacy and least side effects.⁽⁶⁾ *Ziziphus jujuba Lam* commonly called as Indian jujube, belonging to Rhamnaceae is a small subdeciduous tree grown wild and cultivated in many parts of the India and Burma. The leaves of *Z. jujuba* are traditionally used to cure diarrhoea, syphilitic ulcers, asthma, stomatitis, gum bleeding and obesity³⁹. The leaves are reported to possess hypoglycemic⁽⁷⁾, PGI₂ inducing and permeability enhancement activity⁽⁸⁾.

Paracetamol Induced Modified Pylorus Ligated Model

The selected extractives of both plants were subjected to anti-ulcer studies using Aspirin induced model. The experimental design and dosing schedule was carried out as follows. Group I: Normal control, Group II: Ulcer control (Solvent) (10 ml/kg) + Paracetamol (200 mg/kg), Group III: Ranitidine (50mg/kg), Group IV: AQEZJ (250 mg/kg), Group V: ALEZJ (250 mg/kg). In Paracetamol induced ulcer model, one hour before pyloric ligation, aspirin at a dose of 200 mg/kg was administered orally as a suspension in 0.1% w/v CMC. The animals were orally treated with the extractives at doses of 100 and 200 mg/kg once daily for seven days and 1 hour before administration of aspirin. The standard group of animals was also treated in the same way.⁽¹⁸⁾

Evaluation of Ulcer Index :⁽¹⁹⁾

The ulcer index was calculated by counting the lesions with the aid of hand lens (10 X) and graded as follows.

0 = Normal colored stomach

0.5 = Red coloration; 1 = Spot ulcer

1.5 = Hemorrhagic streaks

2.0 = ulcers > 3 but < 5; 3.0 = ulcers > 5

Histopathological Evaluation:

The stomachs of the all groups of animals were immersed in 10% formalin to study the histopathological changes. After the standard processing the wet ulcerated tissues were embedded in paraffin and cut into thick sections. Parameters used to study histopathological changes included shedding of gastric epithelium, gastric erosions,

infiltration of neutrophils, edema and inflammation.⁽²⁰⁾ Alcohol and paracetamol induced ulcer model was carried out with the different extractives of *Ziziphus jujuba* based on the previous protocol to select the extractives with anti-ulcer activity for further evaluation on other anti-ulcer models.

Statistical analysis: The values were expressed as mean \pm SEM data was analyzed using one-way ANOVA followed by T-test. Two sets of comparison had made. i.e. Normal control Vs All treated groups. Differences between groups were considered significant at $P < 0.001$ and $P < 0.05$ levels.

2. Materials and Methods

Collection of Plant Material

The leaves of *Ziziphus jujuba. Mill* was collected from the SV University (Thirupathi) in the month of February and was identified and authenticated from Department of botany. The material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

Preparation of plant extracts

Preparation of Aqueous and Alcoholic Extract:

Fresh leaves of *Ziziphus jujuba. Mill* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of water. The contents were mixed well and then the mixture was boiled up to 80-100°C for 4-5hrs. The alcoholic leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of alcohol.⁽¹⁰⁾ The contents were mixed well and then the mixture was boiled up to 50-60°C for 4-5hrs. Further the extracts was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.⁽¹¹⁾

Experimental animals

Wister rats (150-200 g) and were procured from scarab the Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. All the animals were maintained under standard conditions, that is room temperature 26°C, relative humidity 45 - 55% and 12:12 h light - dark cycle.⁽¹²⁾ The animals were housed in large spacious hygienic cages during the course of the experimental period. Animal studies had approval of IAEC (769/2011/CPSEA).

Selection of dose for animal study: The dose considered for the experiment on rats was obtained from conversion of human dose of *Ziziphus jujuba* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats. Hence the calculated dose for the rats (considering human dose 3 and 5 g/kg) is 200mg/kg. Acute toxicity was done at dose of 2000mg/kg body weight.⁽¹³⁾

Pharmacological evaluation

Preparation of extracts:

The aqueous and alcoholic extracts of *Ziziphus jujuba* suspended in water in presence of 3% v/v Tween-80 solution. The drugs were administered at a constant volume

of 10ml/kg for each animal. All the drugs were administered orally for experimental purpose.⁽¹⁴⁾

Acute toxicity:

The acute oral toxicity of aqueous and alcoholic extracts of *Ziziphus jujuba* was determined by using wistar rats which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Rats were administered with single dose of individual extract up to 2000mg/kg and observed for its mortality during 7days and 21days study period (long term) toxicity and observed upto 7days for their mortality, behavioral and neurological profiles.⁽¹⁵⁾

Screening for Anti ulcer activity:

The aqueous and alcoholic extracts of *Ziziphus jujube* leaves were tested for antiulcer activity using various methods like Acetic acid induced, alcohol induced, paracetamol induced and pyloric ligation method.⁽¹⁶⁾

Alcohol Induced Ulcers:

Experimental design and dosing schedule was as follows. Animals were divided into four (I-V) groups. Group I - Control group received distilled water (1ml, p.o), Group II - Ulcer control, Group III - Standard group received Omeprazole for seven days (2mg/kg i.p), Group IV - Test group received aqueous extract of *Ziziphus jujube* (250mg/kg p.o) for seven days, Group V - Test group received alcoholic extract of *Ziziphus jujuba* (250mg/kg p.o) for seven days. On the final day of dosing, the animals also received extractives and the standard drug thirty minutes before administration of 1ml of ethanol. Animals were sacrificed after one hour and the contents of the gastric juice in the stomach were aspirated.⁽¹⁷⁾ Later the stomachs were removed and kept immersed in saline for 5 min. Incisions of the stomach were performed along the greater curvature and linear hemorrhagic lesions in the glandular regions were observed. A pair of dividers was used to measure the length of all the lesions in the stomachs. The length (mm) of each lesion was determined at 10 x magnification and summed up per stomach. Ulcer index was the sum of length of all lesions for each stomach.

$$\% \text{ Ulcer protection} = \frac{\text{Ulcer index in control} - \text{ulcer index in test}}{\text{Ulcer Index in Control}} \times 100$$

3. Results and Discussion

The anti-ulcer activity of ZJE was evaluated by employing alcohol, paracetamol induced gastric ulcers in rats. Alcohol and paracetamol induced ulcer models were used because they represent some of the most common causes of gastric

ulcer in humans.⁽²¹⁾ Many factors and mechanisms are implicated in the ulcer genesis and gastric mucosal damage induced by different models employed in the present study involving the increase of gastric acid output, vascular injury, depletion of gastric wall mucin, mucosal damage induced by non-steroidal anti-inflammatory drugs and free radical production.⁽²²⁾ Alcohol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid per oxidation which causes damage to cell and cell membranes. ZJE has significantly protected the gastric mucosa against alcohol challenge as shown by reduced values of lesion index as compared to solvent control group suggesting its potent cytoprotective effect. This is further substantiated by increase in gastric mucus content produced by ZJE.

Phytochemical study

The preliminary phytochemical studies revealed the presence of flavonoids in aqueous and alcoholic extracts of *Ziziphus jujuba* various flavonoids have been reported for its anti-ulcerogenic activity with good level of gastric protection. So the possible mechanism of antiulcer action of *Ziziphus jujuba* may be due to its flavonoid content. In this study we observed that *Ziziphus jujuba* provides significant anti-ulcer activity against gastric ulcers in rats.

Acute toxicity study:

Administration of the *Ziziphus jujuba* extracts in rats at doses of 250 mg/kg by oral gavage did not reveal any adverse effects or signs of toxicity. Observations twice daily for fourteen days also did not reveal any drug related observable changes or mortality. Accordingly, the acute oral LD₅₀ of the extractives was concluded to exceed 2000 mg/kg body weight, the highest dose tested in the study.

Effect on alcohol induced gastric ulcers:

Oral administration of 80% alcohol produced hemorrhagic gastric lesions in glandular portion of stomach. Pretreatment with AQEZJ and ALEZJ at the dose of 250 mg/kg and omeprazole (20 mg/kg) significantly (p<0.001) protected the gastric mucosa as shown by reduced values of lesion index (16.2 ± 0.13 and 21.11 ± 0.26 respectively) against alcohol challenge as compared to solvent control (27.48 ± 0.38).⁽²³⁾

Effect on Paracetamol induced gastric ulcers

In ZJE treated groups (250 mg/kg), the ulcer index values (0.40 ± 0.01, 0.29 respectively) were significantly reduced (p<0.001) when compared to solvent control (0.66 ± 0.01), while the ulcer index for ranitidine treated group was 0.23±0.04 (p<0.001). The %inhibition of ulcer showed by AQEZJ and ALEZJ (250mg/kg) and ranitidine was 39.4%, 56.1% and 65.2% respectively.⁽²⁴⁾

Table1: Effect of ZJ at various doses on alcohol induced gastric ulcer in rats

Treatment (n=6)	Dose mg/kg (p.o.)	% Lesion index	% Inhibition of ulcer	Mucus content (µgAlcian blue/g wet tissue)
1% CMC	-	27.48 ± 0.38	-	0.48 ± 0.02
Ulcer control	-	38.65±0.54	-	0.52±0.01
Omeprazole	20	21.11± 0.26	23.18	0.61 ± 0.02
AQEJZ	250	25.12 ± 0.35	8.58	0.55 ± 0.01
ALEZJ	250	16.2 ± 0.13	41.04***	0.90 ± 0.02

Values are mean ± S.E.M. n=number of animals in each group. Significant differences with respect to solvent control group were evaluated by Student’s t – test. (p<0.05, p<0.01 and p<0.001).

Histopathological Evaluation of Alcohol induced Ulcers:

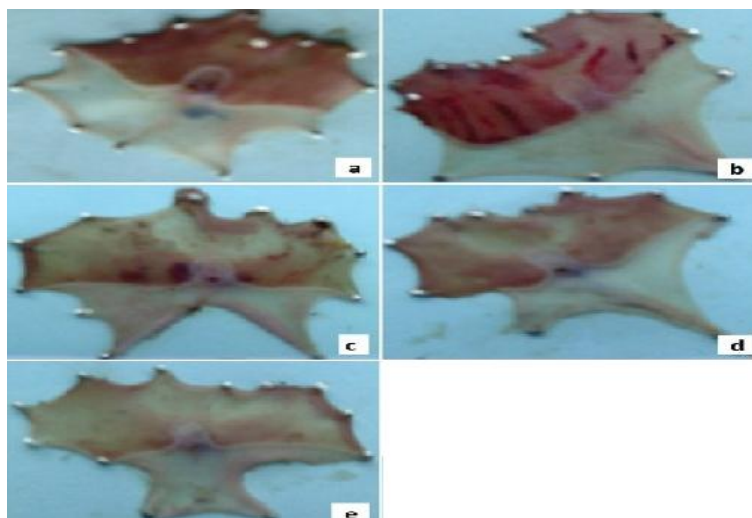


Figure 1: Effect of *Ziziphus Jujuba* on alcohol induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) *AQEZJ* (250 mg/kg) treated (d) *ALEZJ* (250 mg/kg) treated (e) Omeprazole (20 mg/kg treated).

Table 2: Effect of ZJ at various dose levels on paracetamol induced gastric ulcer in rats

Treatment(n=6)	Dose mg/kg (p.o.)	% Ulcer index	% Inhibition of ulcer
1% CMC	-	0.66 ± 0.01	-
Ulcer control	-	0.89±0.02	--
Ranitidine	50	0.23 ± 0.01	65.2
<i>AQEZJ</i>	250	0.40 ± 0.01	39.4
<i>ALEZJ</i>	250	0.29 ± 0.01	56.1***

Values are mean ± S.E.M. n=number of animals in each group; Significant differences with respect to solvent control group were evaluated by Student’s *t* - test. (a *p*<0.001).

Histopathological Evaluation of Paracetamol induced Ulcers:

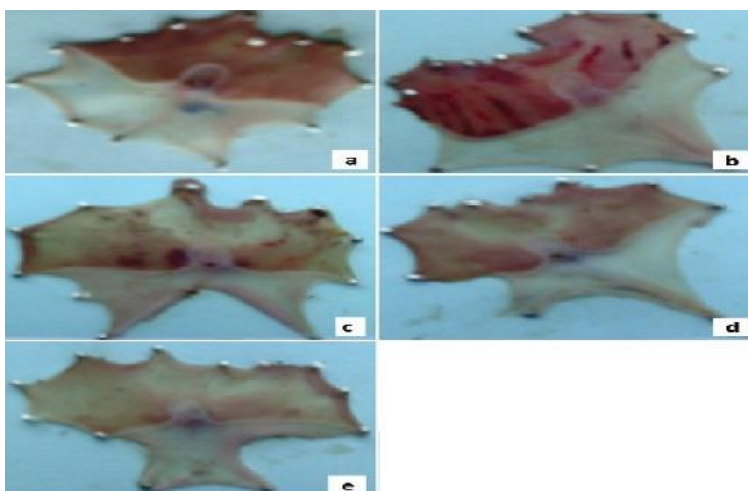


Figure 2: Effect of *Ziziphus Jujuba* on paracetamol induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) *AQEZJ* (250 mg/kg) treated (d) *ALEZJ* (250 mg/kg) treated (e) Ranitidine (50 mg/kg treated)

4. Conclusion

The anti-ulcer activity of the plant of *Ziziphusjujuba* was evaluated by employing paracetamol, alcohol and stress induced ulcer models. These models represent some of the most common causes of gastric ulcer in humans.⁽²⁴⁾ Many factors and mechanisms are implicated in the ulcero genesis and gastric mucosal damage induced by different models International Journal of Medicine and Pharmaceutical Research

employed in the present study involving, depletion of gastric wall, mucin mucosal damage induced by non steroidal anti-inflammatory drugs and free radical production.⁽²⁵⁾ Alcohol and Aqueous extract of the plant of *Ziziphusjujuba* was significantly effective in protecting gastric mucosa against paracetamol induced ulcers at all the

dose The extracts of the *Ziziphusjuzuba* has significantly protected the gastric mucosa against alcohol challenge as shown by reduced values of lesion index as compared to control group suggesting its potent cytoprotective effect.⁽²⁶⁾ It has been proposed that in pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration. The antiulcer activity of *Ziziphusjuzuba* extracts in stress induced model is evident from its significant reduction in gastric volume, ulcer index and increase in pH of gastric juice.⁽²⁷⁾ Because of animals treated with *Ziziphusjuzuba* extracts significantly inhibited the formation of ulcer in the stomach and also decreased both acid concentration, gastric volume and increased the pH values. It is suggested that *Ziziphusjuzuba* extracts can suppress gastric damage induced by aggressive factors.

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