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Anti-ulcer properties of *Ziziphus jujuba* Lam leaves extract in rats

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Abstract

Objective: To evaluate the antiulcer activity of *Ziziphus jujuba* leaves extract (ZJE) at various doses using different experimentally induced gastric ulcer models in rats. Methods: Gastric ulcers were induced in rats by Pylorus ligation, 80% ethanol (1ml/rat) and aspirin (200mg/kg). In pylorus ligation induced ulcer model the parameters studied were gastric volume, free acidity, total acidity and ulcer index. Lesion index and gastric mucus content were determined in ethanol induced ulcer model and in aspirin induced ulcer model the ulcer index was determined. Results: In pylorus ligation model, ZJE pre-treatment caused significant reduction in gastric volume, free acidity, total acidity and ulcer index as compared to control group. In ethanol-induced ulcers, ZJE was effective in reducing lesion index and increasing the gastric mucus content. It was also effective in decreasing ulcer index in aspirin-induced ulcers. All the results obtained with ZJE were dose dependent. Conclusions: The results suggest that ZJE possesses significant and dose dependent antiulcer activity. The antiulcer activity of ZJE can be attributed to its cytoprotective and antisecretory action.

Key words: *Ziziphus jujuba*, antisecretory, cytoprotective, gastric ulcer.

1. Introduction

Peptic ulcer is a benign lesion of gastric or duodenal mucosa, occurs due to an imbalance between the aggressive (acid, pepsin and *H.pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors [1]. To regain the balance, different therapeutic agents including plant extracts are used to inhibit gastric acid secretion or to boost the mucosal defensive mechanism. Today large section of world's population relies on traditional remedies to treat plethora of diseases due to their low costs, easy access and reduced side effects [2]. *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

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ulcers, asthma, stomatitis, gum bleeding and obesity [3]. The leaves are reported to possess hypoglycaemic [4], PGI2 inducing [5] and permeability enhancement activity [6].

However, no study has been carried out to reveal its Anti-ulcer property. Hence the present study was undertaken to evaluate Anti-ulcer effect of ZJE at 200, 400 and 600mg/kg doses on pylorus ligation, ethanol and aspirin induced acute gastric ulcer models in rats.

2. Materials and methods

2.1 Animals

Inbred Wistar rats (150-200g) of either sex were used. Animals were housed under standard conditions of temperature (23±1°C), 12 h light/dark cycle and fed with standard chow diet and water *ad libitum*. Before performing the experiment, ethical clearance was obtained from Institutional Animal Ethics Committee.

2.2 Preparation of extract

The fresh leaves of *Z.jujuba* were collected from damp fields near Belgaum in the month of August 2002 and identified by Prof. S.B.Sasalatti, Head, Department of Botany, RL Science institute, Belgaum, where a voucher specimen is deposited. The leaves were shade dried, powdered and extraction was carried out by percolation using 70% ethanol (500ml for 100g) at room temperature for 24 h. After filtration the dark green solution obtained was evaporated at 50°C under reduced pressure till a viscous mass was obtained. The yield (6% v/v) was stored at 2-4°C. For animal testing, the extract was prepared fresh as an aqueous suspension using 1% CMC solution.

2.3 Anti-ulcer studies

2.3.1 Pylorus ligation induced gastric ulcers

Thirty rats of either sex were randomly divided into five groups and fasted for 48 h with free access to water. Pyloric ligation was performed under light ether anaesthesia to each animal [7].

Animals were given 1% CMC solution or ZJE 200, 400 and 600mg/kg or ranitidine 50mg/kg orally immediately after pylorus ligation. Animals were sacrificed 4 h later. The stomach was carefully removed and the gastric contents were collected. The gastric juice was centrifuged at 3000rpm for 30 min and the volume of gastric juice was measured. Free and total acidities in the supernatant were determined by titration with 0.1N NaOH and expressed as mEq/L/100g [8]. The stomach was cut open along the greater curvature and pinned on a soft board for evaluating gastric ulcers and ulcer index was calculated [9].

The percentage inhibition of ulcer was calculated as mean ulcer index of control-mean ulcer index of test/mean ulcer index of control x100.

2.3.2 Ethanol induced gastric ulcers

Thirty rats of either sex were randomly divided into five groups and fasted for 24 h with free access to water. Animals were given 1% CMC solution or ZJE 200, 400 and 600mg/kg or omeprazole (20mg/kg). 1 h later, 1ml of 80% ethanol was administered p.o. to each animal [10].

Animals were sacrificed 1h after ethanol administration, stomachs were isolated and cut open along the greater curvature and pinned on a soft board. The length of each gastric lesion was measured and the lesion index was expressed as sum of the length of all the lesions in mm [11]. The percentage inhibition was calculated as mean lesion index of control-mean lesion index of test/mean lesion index of control x100. The glandular portion of the stomach was carefully separated from the luminal part and the mucus content was estimated and expressed as Alcian blue μg/g wet glandular tissue [12].

2.3.4 Aspirin induced gastric ulcers

Thirty rats of either sex were randomly divided into five groups and fasted for 24 h with free access to water. Animals were given 1% CMC solution or ZJE 200, 400 and 600 mg/kg or ranitidine 50 mg/kg orally. After 1 h, 200mg/kg of aspirin was orally given to each rat [13]. Animals were sacrificed 4 h later, stomachs were isolated and ulcer index and %inhibition of ulcer was determined as explained in pylorus ligation model.

2.4 Statistical analysis

The values are expressed as mean ± SEM (n=6). Data analysis was carried out by using unpaired Student's *t* - test. Discrepancies with $p<0.05$ were considered to be statistically significant.

3. Results

3.1 Effect on pylorus ligation induced gastric ulcers

The ZJE 400 and 600 mg/kg treated animals showed ulcer index values of 0.40 ± 0.03 and 0.33 ± 0.02 respectively, which were statistically significant ($p<0.001$), while the animals which received ZJE 200 mg/kg had modest ulcer index value 0.68 ± 0.04 ($p<0.02$) when compared to the solvent control group (0.86 ± 0.02). The ZJE treated groups at the dose of 200 ($p<0.02$), 400 ($p<0.001$) and 600 mg/kg ($p<0.001$) showed significant reduced values of free and total acidity when compared to solvent control group.

There was also significant reduction in gastric volume for all the ZJE treated groups at the dose level of 200, 400 and 600 mg/kg, 8.31±0.21ml ($p<0.01$), 5.66±0.12ml and 4.46±0.10ml ($p<0.001$) respectively when compared to solvent control group 9.25±0.34ml. The %inhibition of ulcer displayed by ZJE (200, 400 and 600 mg/kg) and ranitidine treated group of animals was 20.93%, 53.5%, 61.6% and 58.1% respectively. All the results obtained with ZJE at the dose of 400 and 600 mg/kg were comparable with that of standard group (Ranitidine 50 mg/kg, p.o.). (Refer Table 1)

3.2 Effect on ethanol induced gastric ulcers

Oral administration of 80% ethanol produced haemorrhagic gastric lesions in glandular portion of stomach. Pretreatment with ZJE at the dose of 400 and 600 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, 12.68 ± 0.24 and 21.11 ± 0.26 respectively) against ethanol challenge as compared to solvent control (27.48 ± 0.38).

Table 1.
Effect of ZJE at various doses on pylorus ligation induced gastric ulcers in rats.

Treatment (n=6)	Dose mg/kg (p.o.)	Gastric volume (ml/100g)	Free acidity (mEq/L/100g)	Total acidity (mEq/L/100g)	Ulcer index	% Inhibition of ulcer
1% CMC	-	9.25 ± 0.34	19.6 ± 0.24	36.49 ± 0.28	0.86 ± 0.02	-
Ranitidine	50	5.68 ± 0.23^c	7.76 ± 0.29^c	21.79 ± 0.33^c	0.36 ± 0.01^c	58.1
ZJE	200	8.31 ± 0.21^b	17.92 ± 0.48^a	32.24 ± 0.69^a	0.68 ± 0.04^a	20.93
ZJE	400	5.66 ± 0.12^c	11.01± 0.09^c	24.23 ± 0.28^c	0.40 ± 0.03^c	53.5
ZJE	600	4.46 ± 0.1^c	7.9 ± 0.19^c	20.46 ± 0.21^c	0.33 ± 0.02^c	61.6

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.
($^a p<0.02$, $^b p<0.01$ and $^c p<0.001$).

Table 2.
Effect of ZJE at various doses on ethanol induced gastric ulcer in rats.

Treatment (n=6)	Dose mg/kg (p.o.)	Lesion index	% Inhibition of ulcer	Mucus content (µg Alcian blue/g wet tissue)
1% CMC	-	27.48 ± 0.38	-	0.48 ± 0.02
Omeprazole	20	21.11± 0.26^{c}	23.18	0.61 ± 0.02^{c}
ZJE	200	25.12 ± 0.35^{b}	8.58	0.55 ± 0.01^{a}
ZJE	400	16.2 ± 0.13^{c}	41.04	0.90 ± 0.02^{c}
ZJE	600	12.68 ± 0.24^{c}	53.85	1.08 ± 0.03^{c}

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. ($^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$)

The low dose of ZJE (200 mg/kg) showed a little significant ($p<0.01$) effect on lesion index (25.12 ± 0.35) as compared to solvent control group. The % of protection however was less for omeprazole and ZJE 200mg/kg treated rats i.e. 23.18% and 8.58% respectively, while ZJE at the dose of 400 and 600 mg/kg offered modest protection 41.04% and 53.85% respectively against ethanol damage.

The animals, which received 400 and 600 mg/kg, p.o. of ZJE showed highly significant increased values of mean mucus content (0.90 ± 0.02 and 1.08 ± 0.03; $p<0.001$) when compared to solvent control group 0.48 ± 0.02, though 200 mg/kg of ZJE also showed moderate but significant increase in mean mucus amount (0.55 ± 0.01; $p<0.05$). The results were comparable with that of standard (omeprazole 20mg/kg) whose mean mucus content was 0.61 ± 0.02 ($p<0.001$). (Refer Table 2)

3.3 Effect on aspirin induced gastric ulcers

In ZJE treated groups (200, 400 and 600 mg/kg), the ulcer index values (0.40 ± 0.01, 0.29 ± 0.02 and 0.20 ± 0.02 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 ZJE (200, 400 and 600 mg/kg) and ranitidine was 39.4%, 56.1%, 69.7% and 65.2% respectively. (Refer Table 3)

4. Discussion

The anti-ulcer activity of ZJE was evaluated by employing pylorus ligation/ethanol/aspirin induced gastric ulcers in rats. Ethanol and aspirin induced ulcer models were used because they represent some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis 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 [14].

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 [15]. The Anti-ulcer activity of ZJE in pylorus ligation model is evident from its significant reduction in gastric volume, free acidity, total acidity and

Table 3.
Effect of ZJE at various dose levels on aspirin induced gastric ulcer in rats.

Treatment (n=6)	Dose mg/kg (p.o.)	Ulcer index	% Inhibition of ulcer
1% CMC	-	0.66 ± 0.01	-
Ranitidine	50	0.23 ± 0.01[a]	65.2
ZJE	200	0.40 ± 0.01[a]	39.4
ZJE	400	0.29 ± 0.01[a]	56.1
ZJE	600	0.20 ± 0.02[a]	69.7

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).

ulcer index. Because ZJE treated animals significantly inhibited the formation of pylorus ligated ulcer in the stomach and also decreased both acid concentration and gastric volume, it is suggested that ZJE can suppress gastric damage induced by aggressive factors.

Ethanol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation which causes damage to cell and cell membranes [16]. ZJE has significantly protected the gastric mucosa against ethanol 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.

NSAID's like aspirin, indomethacin cause gastric mucosal damage by decreasing prostaglandin levels through inhibition of PG synthesis [17]. ZJE was significantly effective in protecting gastric mucosa against aspirin induced ulcers at all the dose level studied.

Hence ZJE affords effective protection to gastric mucosa against various insults by increasing gastric mucus content and decreasing the acid volume, free and total acidity in rats. The effects in all the 3 models studied were dose dependent.

In conclusion, to the best of our knowledge for the first time, we have demonstrated that ZJE has gastroprotective activity against experimentally induced ulcers in rats. The mechanism of gastroprotective action can be attributed to its antisecretory and cytoprotective property. However further experiments are required to establish and elaborate the molecular mechanism(s) of its Anti-ulcer activity.

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