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Aqueous methanolic extract of *Punica granatum* protect against alcohol induced gastric ulcer in rats

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ABSTRACT

The study was designed to evaluate the anti-ulcer activity of *P.granatum* in rats. Different extracts viz Aqueous methanol extract (AM), chloroform soluble fraction (CSF), chloroform insoluble fraction (CIF), water soluble fraction (WSF), and water insoluble fraction (WIF) in a dose of 980 mg/kg. Omeprazole 20 mg/kg was used as standard. Ulcer was induced by administering absolute alcohol (96% v/v) in a dose of 5 ml/kg. Aq. mehanolic extract extract exhibit significant antiulcer activity, no other extract showed significant antiulcer activity. Present investigation suggest that Aq. Methanolic ext. of *P.granatum* possess potent antiulcer activity.

KEYWORDS

Gulnar farsi;
Punica granatum;
Anti ulcer;
Aq. methanolic ext.

INTRODUCTION

Gulnar farsi (Abortive flowers of *Punica granatum linn*) has been frequently prescribed in the treatment of gastrointestinal disorders since ancient times. In standard text of Unani medicine Gulnar Farsi has been reported to be effective in the treatment of gastric ulcer, abrasion and stomatitis. It is tonic for liver, heart and stomach Gulnar Farsi has also been mentioned as a constituent of compound formulation like Qurs-e-Gulnar, Qurse-e-Zaibetis, Qurse-e-Kehruba, Majoon-e-Kalan, Sufoof Asl-us-soos[1].

Peptic ulcer is one of the major gastrointestinal disorders that occurs due to an imbalance between offensive and defensive factors. Major offensive factors include acid, pepsin and *Helicobacter pylori* infection and defensive factors mainly involve mucus-bicarbonate secretion and prostaglandins. Consequently, reduction of gastric acid production as well as re-inforcement of gastric mucosal protection has been the major approaches for therapy of peptic ulcer disease[2].

Gastrointestinal disorders have been attributed to various causes viz. stress, hormones, synthetic drugs, alcohol, smoking and ingestion of certain foods[3].

The involvement of the pathogenic organism was later confirmed with the discovery of *H.pylori* in the 1980[4]. An imbalance between mucus bicarbonate secretion and prostaglandins was also reported to be involved in the ulcer pathogenesis[2].

To regain the balance, different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production, stabilizing the surface epithelial cells or interfering with the PG_s syn-

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thesis[5].

Flowers of Gulnar farsi (Punica granatum) have been reported to contain pelargonidine,3,5, diglucoside, delphenidine, diglycoside, Malvidine pentos glycoside, Isoquercetrine[6].

Literature survey revealed that the *Punica granatum* has been reported to possess anti-fertility- activity, antibacterial activity, antihelmentic activity[7], antioxidant activity[8], hypoglycemic activity[9], molluscidal activity[10], antidiarrhoeal activity[11], and gastropro tective activity[12].

There was no scientific report available on the traditional claim (useful in peptic ulcer disorders) of the flowers of the plant. Therefore, we investigated the antiulcerogenic effects of aqueous methanolic extract on gastric lesions induced model by alcohol in rats to determine the antiulcer properties of Gulnar farsi (*Punica granatum*).

MATERIAL AND METHODS

Drugs and chemicals

Ethanol (S.D. Fine Chemicals Ltd, Bombay), Chloroform (S.D. Fine Chemicals Ltd, Bombay), Omeprazole (Dr Reddy's labs Ltd, Hyderabad).

Plant material

The dried flowers of Gulnar farsi *(Punica granatum*) were procured from the Khari Bawli, Delhi and authenticated at NISCARE, New Delhi.

Preparation of fraction

200gm-dried flowers of Gulnar farsi (*Punica Granatum*) were taken in a Soxhlet's apparatus. The drug was extracted with 80% methanol for 12 hours. 81.61gm (40.8%of the drug) extract was obtained. This total aqueous methanol extract (AM) was taken in a separating funnel and shaken with 100 ml chloroform thrice. The chloroform fractions were added together. 1.77gm (0.88% of the drug) chloroform soluble fraction (CSF) was obtained after drying on water bath. The chloroform insoluble fraction (CIF) 75.84 gm (37.92% of the drug) was separated out. Chloroform insoluble fraction (CIF) was taken in a 250 ml of conical flask and well-shaken with 150 ml of distilled water and then filtered. Water-soluble fraction (WSF) was dried and weighed to obtained 47.65gm (23.82% of the drug) and water insoluble fraction (WIF) 15.36gm (7.68% of the drug) was separated out. The different fractions were marked for pharmacological study.

Experimental animals

Albino rats of wistar strain weighing in between 180-260 gm, used in the study, were obtained from Central Animal House, Jamia Hamdard. The animals were kept under standard laboratory conditions and fed diet supplied by Amrut Lab Animal-Feed, Pehladpur, New Delhi. Water was allowed *ad libitum*.

Treatment schedule

Animals were divided into eight groups, of six animals each. Animals were fasted for 24 hours with water *ad libitium*. Saline 1ml/kg (Group I, II), omeprazole 20 mg/kg as a standard drugs[13]. (Group III), test drugs like aqueous methanol extract (AM), chloroform soluble fraction (CSF), chloroform insoluble fraction (CIF), water soluble fraction (WSF), and water insoluble fraction (WIF) at the dose of 980mg/kg, (Corresponding to 7 gm dried flowers), suspended in the vehicle (10%, V/V tween 80 in distal water, were administered orally in a volume of 10ml/kg (Group IV, V, VI, VII, VIII) at zero hours. After 30 minutes of oral administration, ulceration was induced by absolute ethanol 5 ml/kg (96%; v/v) except group I.

Determination of gastric ulcer

The animals were sacrificed 1 hour later by an overdose of anesthesia ether vapors and the stomachs were removed along the greater curvature and sum of length of lesions was evaluated for ulcer index as given below.

Erosions	Score
1mm or less	1
1mm to 2 mm	2
More than 2 mm	3

The over all score was divided by a factor of 10, which was designated as the ulcer index[14].

Statistical analysis

All the values have been expressed as mean ± SEM. one way ANOVA followed by Dunnett's multiple comparison test using Graph Pad PRISM software. P value <0.05 was considered significant.

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TABLE 1: Effect of different fraction of Gulnar Farsi on alcohol induced gastric ulcer in rats

Group	Treatment	Dose (Mg/kg b.w.)	Ulcer index (mm) (Mean±S.E.)	Inhibition (%)
I	Control	N.Saline	0	
II	Toxic control	980	3.1±0.13	
III	Omeprazole	20	1.41±0.083**	49.64
IV	AM	980	0.35±0.084**	87.50
V	CSF	980	2.08±0.241^{ns}	33.11
VI	CIF	980	2.68±0.238^{ns}	13.82
VII	WSF	980	2.63±0.296^{ns}	15.43
VIII	WIF	980	2.91±0.153^{ns}	6.43

n = 6, **P<0.01

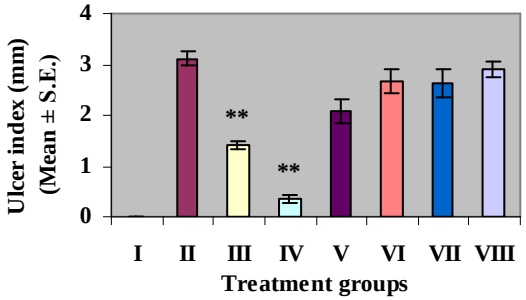

Figure 1: Bar graph showing protection of alcohol induced gastric ulcer in Rats using different fraction of Gulnar Farsi

RESULTS AND DISCUSSION

The pretreatment with aqueous methanolic extract (AM) of *Punica granatum* showed reduction in the severity of ulcer lesions. AM given in a dose of 980mg/kg (Group IV) showed ulcer index of 0.35±0.08 (P<0.01) with a significant inhibition of ulceration which was 87.5% as compared to 3.11±0.13 in toxic control (Group II). CSF Group V 2.08±0.241 with a non significant inhibition of 33.11%, CIF Group VI 2.68±0.238 with a non significant inhibition of 13.82%, WSF Group VII 2.63±0.296 with a non significant inhibition of 15.43% and WIF Group VIII at the dose of 980 mg/kg 2.91±0.153 with a non significant inhibition of 6.43%. Omeprazole given in the dose of 20 mg/kg (Group III), exhibited ulcer index of 1.41±0.08 (p<0.01) with the inhibition of 49.64%. The values obtained are given in TABLE 1 and the protection against ulcer can be seen in figure 1.

Alcohol induced ulcers are due to direct nercotizing effect of ethanonol on gastric mucosa[15]. Ethanol causes necrosis of superficial epithelial cells on gastric mucosa and erosion[16]. In the present study, it was observed that AM extract significantly reduced the ulcer index highlighting its cytoprotective effect of Aqueous methanolic extract of Gulnar farsi. Results obtained with experimental models of ethanol induced acute ulcer in rats showed 87.5% protection when 980mg/kg aqueous methanolic extract (AM) of Gulnar farsi was administered. Whereas, the inhibition of omeprazole 20 mg/kg was 49.64%.

In the present study, AM showed prevention of gastric lesion in the experimental models. We observed that AM 980 mg/kg and omeprazole 20 mg/kg showed significant inhibition of ulceration 80.32% and 69.55%. Thus AM reduced the gastric acid secretion in rats.

On the basis of above data, it may be concluded that Gulnar farsi has antiulcer potential. It may be further concluded that Gulnar farsi showed antiulcer activity by various mechanisms including cytoprotection and antisecretory activity. It is also documented that herbal drugs augment the defensives factors, and are reliable and safe. Hence Gulnar farsi may be considered for use alone or in a combination with other antiulcer drugs.

The present study in this study, we observed that aqueous methanolic extract of Gulnar farsi exhibited significant antiulcer activity against alcohol induced models of gastric ulcer in rats.

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