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Research Article

ANTI-ULCER ACTIVITY OF *PUNICA GRANATUM* LINN. IN DIABETIC RATS

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ABSTRACT

Gastric ulcers were induced in euglycemic and diabetic rats by physical (Pylorus ligation) method. The extract of various parts (peel, rind and seed) of *Punica granatum* fruit (PGF) produced a significant reduction in the ulcer index. Administration of extract of peel, rind and seed of the PGF showed percentage of ulcer inhibition as 76.81%, 84.05% and 88.40%, respectively. The antiulcer activity was showed by changes in parameters like ulcer index, free acidity and total acidity. The antiulcer activity of the different extracts may be due to the presence of the various compound as flavonoids which are well known anti-oxidants.

Keywords: *Punica Granatum*, Punicaceae, Anti-ulcer activity

INTRODUCTION

In recent years, focus on plants research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems[1]. Peptic ulcer is a common disorder of the alimentary tract and in various countries its prevalence is estimated as 5-10% of the adult population. This disorder remains one of the most important problems, both in the practice of primary health care physicians and gastroenterologists[2]. Peptic ulcer occur due to an imbalance between offensive acid-pepsin secretion and free radical generation and defensive mucosal factors which include mucin-bicarbonate secretions, lifespan of mucosal cell proliferation, glycoproteins and antioxidant enzymes status [3].

Diabetes being a chronic disease may lead to a decrease in the mucosal defensive factors with a concomitant increase in propensity to ulceration in response to various physical (Cold restraint stress and pylorus ligation) and chemical (as aspirin, ethanol) agents. The changes induced by diabetes could be reversed by using drugs which can either promote mucosal defensive factors or correct the blood glucose level or both[4]. So we have selected *Punica granatum* linn.(Punicaceae) for the study.

P. granatum linn. is a shrub or small tree considered to be a native of Iran and Afghanistan. It is also found growing wild in the warm valleys and outer hills of the Himalayas between 900 m. and 1,800 m., and is cultivated throughout India[5].The plant is used in folklore medicine for the treatment of various disease such as ulcer, hepatic damage, snakebite, etc. The unripe fruit is a good appetizer and tonic, useful in vomiting, but causes biliousness. The ripe fruit is a tonic, astringent to bowels, aphrodisiac and is reputed to cures bliosness, fever, heart disease, sore throat, stomatitis etc. The rind of the fruit is anthelmentic and useful in diarrhoea, dysentery and ulcer[6,7,8]. Various parts of this plant have been reported to possess different biological activities as antioxidant[9,10,11,12],antidiarrhoeal activity[13,14,15], antibacterial[16,17], antifertility activity[18,19], antiulcer[20,21],wound healing activity[22], antidiabetic activity of different parts as flower[23,24], seed[25] have already been reported in the literature.

MATERIAL & METHODS

Procurement of fruits and Extraction

The peel, rind and seed of the *P. granatum* Linn. (Variety arakta) was procured from the local market of Udaipur. A specimen was identified and authenticated at the Department of Horticulture, Rajasthan College of Agriculture, Udaipur (Raj.). For extraction, the drug was soaked in solvent (50% alcohol) for 48 hrs with occasional shaking. After 48 hrs the extract was filtered using muslin cloth. The solvent was distilled to obtain the extract. The yield of the extract was found to be 44.24 % w/w.

Pharmacological studies

Animals

All studies were approved by the Institutional Animal Ethics Committee of B. N. College of Pharmacy, Udaipur (India). Healthy wistar albino rats of either sex weighing 150-180 g were maintained on standard rodent feed and water *ad libitum.* Rats were closely observed for any infection and those showing signs of infection were excluded from the study and replaced. The rats were randomly distributed into groups of 6 each for study.

Experimental methods

Induction of Diabetes

Rats were weighed and their fasting blood glucose levels were determined before inducing diabetes. Rats showing very low or high glucose levels were replaced. The Rats were injected with a single dose of Alloxan monohydrate (80 mg/kg) (S.D-Fine Chemicals Pvt Ltd, Baroda) in normal saline (freshly prepared) by I.P. route. Control rats were injected with normal saline. Fasting blood glucose was measured 3 days later to confirm the diabetic status of the rats. Rats having more than 400 mg/dl blood glucose level were taken for the study.

Estimation of blood glucose level

The blood samples were collected by tail puncture and the blood glucose level was estimated with the glucometer (Accuchek sensor).

Anti ulcer study

For the study of antiulcer activity in diabetic rats, the diabetic rats were divided into 6 groups. Group I (normal control) and groups II (diabetic control) were administered vehicle, groups III (positive control) were administered standard drug (ranitidine, 50 mg/kg, orally, group IV, V & VI (diabetic experimental) were administered peel, rind & seed extract in dose of 100, 500 & 500 mg/kg, orally, respectively.

Pylorus- ligation – induced ulcers

Drugs were administered for period of 5 days in diabetic rats. On day 6th, 1 hr, after administration of the test drug, the rats (fasted for 18 hrs) were anaesthetized using anaesthetic ether, the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen closed in layers. The animals were deprived of water during the postoperative period and were sacrificed by cervical dislocation after 4 hrs. Stomach was dissected out and the contents collected for estimation of biochemical parameters and ulcer index [4].

Ulcer index was calculated as –

$$\text{Ulcer index} = \text{ulcerated area} / \text{total mucosal area} \times 10$$

Biochemical estimation

The volume of the gastric content (in ml) was measured and then centrifuged for 10 min. at 3000 rpm.

Free acidity

One ml of supernatant fluid was pipetted out and 9 ml of distilled water was added to it. Free acid was determined by titration of this solution with 0.01 NaOH using Topfer's reagent as indicator (till development of orange color). Free acidity was calculated as-

Free acidity = Volume of NaOH X Normality X 100/0.1

Total acidity

Total acidity was determined by titration with 0.01 NaOH using Phenolphthalein reagent as indicator (till development of pink colour). The calculating formula was the same as for free acidity. Free acidity & Total acidity were expressed in m Eq /litre /100 gm[26].

Statistical analysis

Statistical analysis was done by student's t-test. The difference were considered to be significance when $p < 0.05$.

RESULTS

Antiulcer activity

This experiment was carried out to study the effect of orally administered peel, rind and seed extract on gastric ulcer induced by pylorus ligation in diabetic rats. Peel (100 mg/kg) (group IV), Rind (500 mg/kg) (group V) and Seed (500mg/kg) (group VI) decreased the mucosal injury significantly at 6th day (last day) and showed antiulcer effect. Ranitidine (50 mg/kg) group (group III) also showed significantly decreased mucosal injury. (Table-1)

Biochemical estimation

Free acidity & total acidity

This experiment was carried out to study the effect of orally administered peel, rind and seed extract on free acid and total acid production in pylorus ligation in diabetic animals. Peel (100 mg/kg) (group IV), Rind (500 mg/kg) (group V) and Seed (500mg/kg) (group VI) decreased free and total acidity significantly at 6th day (last day). Ranitidine (50 mg/kg) (group III) also showed significantly decreased in free acidity only. The both acidity was compared to the diabetic control (group II). (Table- 2)

Table 1: Anti-ulcer activity of *P. granatum* linn. extracts in pylorus ligated diabetic rats

Group	Ulcer index	Percentage ulcer inhibition	Gastric content (ml/100 gm)
Normal control	0.21 ± 0.06	-	2.10±0.24
Diabetic control	0.69 ± 0.14	-	2.35±0.38
Positive control	0.30 ± 0.09*	56.52	2.63±0.35
Diabetic Experimental (Peel)	0.16 ±.0.06*	76.81	2.42±0.47
Diabetic Experimental (Rind)	0.11 ± 0.05**	84.05	2.08±0.40
Diabetic Experimental (Seed)	0.08 ± 0.02**	88.40	1.92±0.28

Values are mean ± SEM, * P<0.05, **P<0.01 were considered statistically significant when compared to diabetic control group.

Table 2: Effect of *P. granatum* linn. extracts on free acidity and total acidity in pylorus ligated diabetic rats

Group	Free acidity (mEq/lit./100 gm)	Total acidity (mEq/lit./ 100 gm)
Normal control	11.0±1.91	37.0±5.56
Diabetic control	10.67±1.49	37.17±5.03
Positive control	6.33±0.98*	25.17±2.94
Diabetic experimental(Peel)	3.75±0.47**	17.0±1.95*
Diabetic experimental (Rind)	3.5±0.61**	14.67±1.97**
Diabetic experimental (Seed)	3.2±0.37**	14.2±1.49**

Values are mean ± SEM, * P<0.05, **P<0.01 were considered statistically significant when compared to diabetic control group.

DISCUSSION

In the present study different parts of the fruit have shown significant antiulcer activity as evidenced by changes in parameters like ulcer index, free acidity and total acidity in pylorus ligation in alloxan monohydrate induced diabetes in rats. The increased susceptibility to ulceration could be due to back diffusion of hydrogen ions in the stomach of diabetic rats which played an important role in the formation of acute hemorrhagic ulcers. Mucus is secreted by the mucus neck cells and coats the gastric mucosa, thereby preventing physical damage and back diffusion of hydrogen ions[4].

The ulcer protective effects of *Punica granatum* exract may be due to its actions on gastric mucosal defensive factors like an increase in dissolved mucus and decrease in mucosal cell exfoliation[27].

P.granatum is reported to contain alkaloids, such as pelletierine, pseudopelletierine[6] and ellagic acid, ellagitannins (including punicalagins), punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonols and flavones[28]. Flavonoids are beneficial for prevention of cardiovascular, inflammatory and other diseases. It has been suggested that free radical scavenging and antioxidant activities play an important role in prevention of free radical related- diseases, including aging and ulcer[20]. Flavonoids, by virtue of their high chemical reactivity with membrane phospholipids, have been reported to affect enzymes altering endogenous phospholipids metabolism leading to either stimulation or inhibition of PGs synthesis, leukotrienes(LTs) and platelets activating factor (PAF). It is well known that the products of arachidonate metabolism play an important role in gastro-intestinal mucosal damage; cyclooxygenase products like PGE and PGI_2 protect the gastric mucosa against damage, while LTC_4, a5-lipoxygenase product and PAF, mediate damage and induce gastric and colonic ulceration[27].

Hence, in the present study it can be concluded that the ulcer protective activity of *P. granatum may* be due to the presence of these compounds. Further phytochemical investigation and isolation of active constituents offers the promise of yielding compounds which may be useful as antiulcer drugs.

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REFERENCES

1. Dandagi PM, Patil MB, Mastiholimath VS, Gadad AP, Kulkarni AR. Antiulcer activity of extracts of *Calotropis gigantia* root extracts in pylorus ligated rat models. Int J Pharmacol Biol Sci 2008; 2(1), 127.
2. Patil VP, Viswanathswamy, AHM, Thippeswamy AHM, Kamblekar YJ, Hallikeri, CS, Hatapakki, BC. Gastroprotective and antiulcer properties of clozapine in pylorus ligated rats. Int J Pharmacol Biol Sci 2008; 2(1), 121.
3. Tripathi KD. Essentials of Medical Pharmacology. New Delhi: Jaypee Brothers Medical Publishers Ltd.; 2002.
4. Joshi MC, Dorababu M, Prabha T, Kumar MM, Goel, RK, Effect of *Pterocarpus marsupium* on NIDDM- induced rat gastric ulceration and mucosal offensive and defensive factors. Indian Journal of Pharmacology 2004; 36(5), 296.
5. Satyavati GV, Gupta AK, Tandon N. Medicinal plants of India, New delhi: Indian Council of Medical Research; 1987.
6. Kirtikar KR & Basu B D, *Punica granatum,* in *Indian medicinal plants,* Sri Satguru Publications; 2000, p.1084-87.
7. Nadkarni AK, Indian material medica, Bombay Popular Prakashan; 1991.
8. Sharma PC, Yelne MB, Dennis TJ. Database on medicinal plants used in ayurveda, New Delhi: Central council for Research in Ayurveda and Siddha; 2001.
9. Kaur G, Jabbar Z, Athar M, Alam MS. *Punica granatum* (Pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. Food Chemistry and Toxicology 2006; 44(7), 984-93.
10. Ricci D, Giam Peri L, Bucchini A, Fraterne D. Antioxidant activity of *Punica granatum* fruits. Fitoterapia 2006; 77(4), 310-12.
11. Noda Y, Kaneyuki T, Mori A, Packer L. Antioxidant activities of pomegranate fruit extract and its anthocyanidins, delphinidin, cyanidin and pelargonidin. Journal of Agricultural and Food Chemistry 2002; 50(17), 4791-95.
12. Negi PS, Jayaprakasha GK. Antioxidant and antibacterial activites of *Punica granatum* peel extracts. Journal of Food Science 2003; 68(4), 1473-77.
13. Venkatrao N, Korths MD. Satyanarayana S, Hemamalini R, Santakumar SM. Antidiarrhoeal and anti-inflammatory activity of fruit rind extract of *Punica granatum* Indian Drugs 2007; 44(12), 909.
14. Das AK, Mandal SC, Banerjee SK, Sinha S, Das J, Saha BP, Pal M. Studies on antidiarrhoeal activity of *Punica granatum* seed extract in rats. J Etanopharmacol 1999; 68(1-3), 205-08.
15. Bandawane DP, Javekar AR, Judhavs B, Dhole SN. Antidiarrhoeal activity of pomegranate fruit rind a comparative study, Indian Drugs 2006; 43(21), 102.
16. Methabe MC, Nikolova RV, Lall N, Nyazema NZ. Antibacterial activity of medicinal plants used for the treatment of diarrhoea in limpopo province, South Africa. J Etanopharmacol 2006; 105 (1-2), 286-93.
17. Aqil F, Khan SA, Owais M, Ahmed I. Effect of certain bioactive plant extract on clinical isolates of beta lactamase producing methicillin resistence *Staphylococcus aureus*. Journal of Microbiology 2005; 45(2), 106-14.
18. Mori-Okamoto J, Otawar-Hamamoto Y, Yamato H, Yoshimura H. Pomegranate extracts improves a depressive state and bone properties in menopausal syndrome model ovriectomized mice. Journal of Ethanopharmacology 2004; 92(1), 93-101.
19. Prakash AO. Potentialities of some indigenous plants for antifertility activity. International Journal of Crude Drug Research 1986;24 (1), 19-24.
20. Ajaikumar KB, Asheef M, Babu BH, Padikkala J. The inhibition of gastric mucosal injury by *Punica granatum* L. (pomegranate) methanolic extract. J Etanopharmacol 2000; 96, 171.
21. Gharzouli K, Khennouf S, Amira S, Gharzouli A. Effects of aqueous extracts from *Querces ilex* L. root bark, *Punica granatam* L. fruit peel and *Artemisia herba-alba* Asso leaves on ethanol induced gastric damage in rats. Phytotherapy Research 1999; 13 (1), 75-6.
22. Murthy KN, Reddy VK, Veigas JM, Murthy UD. Study on wound healing activity of *Punica granatum* peel. J Med food 200; 7 (2), 256-9.
23. Jafri MA, Aslam M, Kalin J, Surendr, S. Effect of *Punica granatum* Linn. (Flowers) on blood glucose level in normal and alloxan induced diabetic rats. J Etanopharmacol 2000; 70(3), 309-314.
24. Huang TW, Gang P, Bhavani P, Kota GQ, Li J Y. Antidiabetic action of *Punica granatum* flower extract: Activation of PPAR-gamma and identification an active component. Toxicology and Applied pharmacology 2005; 207(2), 160-69.
25. Das AK, Mandal SC, Banerjee SK, Sinha S, Saha BP, Pal M. Studies on the hypoglycaemic activity of *Punica granatum* seed in streptozotocin induced diabetic rats. Phytotherapy Research 2001. 15(7), 628-29.
26. Goyal RK. Practicals in Pharmacology. Ahmadabad: BS Shah Prakashan; 2003-04.
27. Dorababu M, Prabha T, Priyambada S, Agarwal VK, Aryya NC, Goel R K. Effect of *Bacopa monniera* and *Azadirachta indica* on gastric ulceration and healing in experimental NIDDM rats. Indian J Exp Biol 2004; 42, 389.
28. Julie Jurenka, M T, Therapeutic application of Pomegranate (*Punica granatum* L): A review. Alt Med Rev 2008; 13 (2), 128.