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Original Research Article

Anti-Ulcerative Potential of *Punica granatum* L (Lythraceae) Hydroalcohol Fruit Peel Extract

Ghazaleh Moghaddam[1], Mohammad Sharifzadeh[2], Gholamreza Hassanzadeh[3], Mahnaz Khanavi[4,2], Farzaneh Dolatshahi[1], Naficeh Sadeghi[1], Mohammad Reza Oveisi[1] and Mannan Hajimahmoodi[1,5]*

[1]Department of Drug and Food Control, [2]Department of Pharmacology and Toxicology, Pharmaceutical Research Center, Faculty of Pharmacy, [3]Department of Anatomy, School of Medicine, [4]Department of Traditional Pharmacy, Faculty of Traditional Medicine, 5Department of Traditional Iranian Pharmacy Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

For correspondence: **Email: hajimah@sina.tums.ac.ir*

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Abstract

Purpose: *To evaluate the antiulcer activities of the methanol extract of pomegranate (Punica granatum L) fruit peel using 80 % ethanol-induced gastric ulcer model in rats.*
Methods: *Male Wistar rats, 175 - 220 g, were fasted for 48 h, but had free access to water. They were randomly divided into nine experimental groups. Ulcer was induced in the rats with 80 % ethanol. The control group animals received the same treatment as those in the test groups except that the extract treatment was replaced by administration of appropriate volume of the dosing vehicle. Histamine-receptor type-2 (H2) blocker, cimetidine (100 mg/kg, i.p.) was used as the reference drug. Oral pretreatment with three different extract doses (25, 50 and 100 mg/kg) was for 15 days, thereafter, ulcer index (UI) and inhibition were calculated.*
Results: *The extract, at 50 mg/kg, of black fruit peel extract produced significant ($p < 0.05$) protective effect in rats with a preventive index of 65.87 %. Other doses were significantly protective against ethanol-induced gastric ulcer in the rats. On the other hand, north white peel was not effective (50 mg/kg) showed an ulcer index of 49.52 ± 1.99. Histopathological examination of the stomach of the ulcerated animals treated with white peel (50 mg/kg) showed severe erosion of gastric mucosa, sub-mucosal edema and neutrophil infiltration.*
Conclusion: *The study shows indicates the antiulcer properties of the methanol extracts of north white peel, sour summer and black peel (25, 50, 100 mg/kg) of pomegranate. Their antiulcer activity is exerted, possibly, via its high antioxidant activity.*

Keywords: *Anti-inflammatory, Pomegranate, Punica granatum, Peel extract*

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INTRODUCTION

Peptic ulcer disease encompassing gastric and duodenal ulcer is one of the most prevalent gastrointestinal disorders. The pathophysiology of peptic ulcer diseases involves an imbalance between offensive, including acid, pepsin and H. pylori, and defensive factors such as mucin, prostaglandin, bicarbonate, nitric oxide and growth factors [1].

Gastric ulcer therapy faces a major drawback due to the unpredictable side effects of the long-term use of commercially available drugs. It is

shown that toxic oxygen radicals play an important role in the ethiopathogenesis of gastric damage [2]. In parallel to tissue damage, there is a decrease in antioxidants such as glutathione and superoxide dismutase and an increase in oxidants [2]. Hence, the search is still on to find drug possessing antioxidant and antiulcer properties, which will serve as a powerful therapeutic agent to cure gastric ulceration, and the search extends to the systematic development of natural products [3].

Currently, focus on plant research has increased all over the world and a large body of evidence has been collected to show the immense potential of medicinal plants used in various traditional systems [4]. Pomegranate (*Punica granatum* L), has been used in Iranian traditional medicine for different therapies. Today pomegranate is known as antimicrobial, antiviral and anticancer substance which has led to being the center of attention in many studies. Both pomegranate pulp and peel contain different kinds of antioxidants [5], including those which have not possibly been well characterized so far. It has been acknowledged that phenolic compounds such as flavonoids and anthocyanins are the major class of effective antioxidants in many fruits and vegetables. A plant flavonoid has been found to be effective against ulcer in experimental animals. The present study was undertaken with the aim to assess the antiulcerogenic properties of pomegranate peel methanolic extract.

EXPERIMENTAL

Plant material

Three fresh pomegranate cultivars were harvested randomly in September 2010 from different mature trees (14-year-old). The average temperature, the amount of rainfall, relative humidity, and the soil pH in growing season were 28.65 ºC, 20 mm, 26 % and 7.21, respectively. The trees were spaced 6 and 3 m between and along the rows, respectively. Trees were grown under traditional irrigation and routine cultural practices suitable for commercial fruit production. All cultivars were grown under the same geographical conditions and with the same applied agronomic practices. According to the list of Iranian pomegranate cultivars studied by Noormohammadi et al [6], North white peel (Poost-Sefid-Shirin), Sour summer (Tabestani Torsh) and Black peel (Poost Siyah Torsh) are mostly cultivated in Saveh (Markazi), and Ardakan (Yazd) respectively. Three cultivars of pomegranate, viz, North white peel, Sour summer and Black peel, were donated from Saveh Agricultural Investigation Center. The botanical identification of these peels of *Punica granatum* L (voucher no. 1399), was carried out by Prof G Amin, Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran in 2011. The voucher specimens have been deposited at the herbarium of the Kerman Faculty of Pharmacy, Kerman, Iran. Pomegranate fruits (3 nos. of each cultivar) were collected and washed three times with distilled water.

To prepare pomegranate extract, fresh fruits were peeled and 30 g of peels were weighted and extracted separately for 4 h by Soxhlet apparatus with methanol 80 %. The methanolic extracts were concentrated under control reduced pressure at 40 ^{o}C to obtain the methanolic extracts.

Chemicals

All chemicals used in the experiments were of analytical grade. . The solvent including methanol and ethanol were purchased from Merck (Darmstadt, Germany) and Sigma (St. Louis, MO) respectivelt. For laboratory experimentation, Cimetidine was obtained from Darou pakhsh Pharmaceutical Company (Tehran, Iran).

Animals

Male Wistar rats weighing 175 – 220 g (Pasteur Institute, Tehran, Iran) were used in the study. The animals were fed under normal conditions (22 °C) in 9 separate groups consisting of 5 rats. Animal experiments were performed in accordance with national guidelines for the use and care of laboratory animals and approved by the local animal care committee of Tehran University of Medical Sciences. Throughout the experiments, animals were handled according to the ethical guidelines for the care of laboratory animals as well as the rules of Tehran University of Medical Sciences (no. 88-01-33-8530).

Preparation of test samples for bioassay

The rats were deprived of food for 48 h before the experiment, but were allowed free access to drinking water (bottled tap water) up till 2 h before the experiment. Gastric ulcer in Male Wistar rats was induced by 80 % ethanol according to the method described by De Pasquale *et al* [7]. Test samples were administrated intraperitoneally (i.p.) after dissolving in saline (0.9 % NaCl). The control group animals received the same experimental

handling as those of the test groups except that the extract treatment was replaced by administration of appropriate volumes of the dosing vehicle. The histamine-receptor type-2 (H2) blocker, cimetidine (100 mg/kg, i.p.), was used as a reference compound. Oral pretreatment with all three peel extracts (25, 50 and 100 mg/kg) for 15 days protected the gastric mucosa against the damage induced by 80 % ethanol. Four hours later, the stomachs were removed and inflated with 10 ml of formalin solution and immersed in the same solution to fix the outer layer of stomach. Each stomach was then opened along the greater curvature, rinsed with tap water to remove gastric content and blood clots and examined under the dissecting microscope to assess the formation of ulcers [8]. The ulcer index [3] and inhibition percentage was calculated according to previous reported [8].

Statistical analysis

All data were analyzed by one-way ANOVA using SPSS version 16 (SPSS Inc) software. Differences among groups were obtained using the Duncan T3 test, and significance was set at $p < 0.05$. The results are expressed as mean ± SD.

RESULTS

In the current study the model of 80 % ethanol induced ulcer was used. The rat's ulcerations show significant decrease in mucosal injury by cimetidine. The body weight of the rats was a homogenous parameter. There were no significant differences between rat groups. The observations of positive control group in Figure 1 indicated that ethanol (80 %) produced severe gastric erosions and induced gastric ulcerations to the extent of 73.06 ± 4.27 (ulcer index). The data show the anti-ulcer activity of the methanol extract of the pomegranate peels in ethanol-induced gastric ulcers in rats. The results of the initial trials carried out with different extracts (25, 50 and 100 mg/kg) are presented in Table 1. Micrographs of dissected stomachs representing antiulcer effects of sour summer and in black peel extracts compared to the group induced by ethanol 80 % are presented in Figure 1.

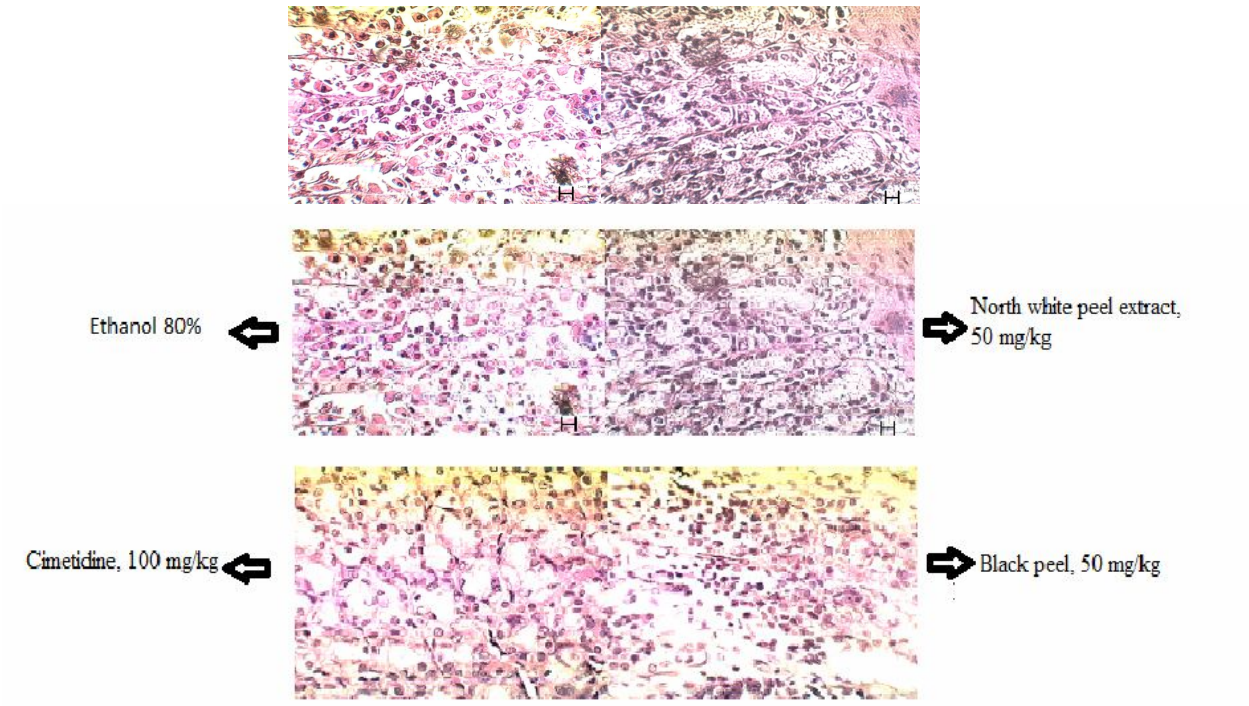

Fig 1: Micrographs of dissected stomachs showing the antiulcer effects of pomegranate peel extracts. All photomicrographs were hematoxylin and eosin-stained; ×400 magnification

Statistical analysis showed a significant difference in the ulcer indexes between the three studied cultivars in different dosages ($p < 0.05$). From the Table 1, it is evident that 50 mg/kg dosage of black peel cultivar (24.93 ± 1.82) markedly inhibited the peptic ulcer, compared with ethanol-induced gastric ulcer. There was an apparent decrease in the infiltration of polymorphonuclear leukocytes and hemorrhage after administration of black peel extracts (50 mg/kg) (Figure 1). The treatment by black peel 50 and 100 mg/kg group were protected from intraluminal bleeding with ulcer index of 24.93 ± 1.82 and 26.04 ± 1.03 ($p < 0.05$). Using other peel cultivar, methanolic extracts with the dosage of 25, 50 and 100 mg/kg decreased the level of

ulcer index significantly ($p < 0.05$) with exception of sour summer (50 mg/kg) and north white peel (50 mg/kg) which are at near ethanol induced status. In these two groups, the submucosa was edematous and there was visible infiltration of polymorphonuclear leukocytes (Figure 1). On the other hand the group pretreated by the other dosage of same cultivar extracts showed the better result with a little increase in ulcer inhibition ($p < 0.05$).

Table 1: Anti-ulcer activity of three different pomegranate peel methanol extracts in rat

Treatment (mg/kg)	Ulcer index (mean±SD)	Ulcer inhibition (%)
Control	73.06±4.2[a]	
Cimetidine, 100	12.07±0.96[j]	83.47
North White Peel, 25	39.40±1.25[f]	46.07[d]
North White Peel, 50	49.52±1.99[b]	32.22[h]
North White Peel, 100	41.51±1.84[e]	43.18[e]
Sour Summer, 25	41.45±2.06[e]	43.26[e]
Sour Summer, 50	46.64±1.23[c]	36.16[g]
Sour Summer, 100	37.94±0.78[g]	48.07[c]
Black peel, 25	43.45±1.76[d]	40.52[f]
Black peel,50	24.93±1.82[h]	65.87[a]
Black peel,100	26.04±1.03[i]	64.35[b]

Values in the same column bearing different superscripts are significantly different ($p \leq 0.05$)

DISCUSSION

The epithelium of gastrointestinal tract is continually exposed to damaging effects of noxious substances on daily basis. In Asian cultures, pomegranate is used in the traditional medicine for the treatment of a variety of ailments [8]. Based on related studies, the antioxidant activity of sour summer was higher than north white peel and black peel. The results of present study which is in the same line with previous data show that methanol peel extracts of pomegranate especially sour summer possess good potential as an antiulcer agent too [9,10].

The present study is strongly supported by the previous research whereby the antiulcerogenic effects of pomegranate peel methanol extract, was tested on rats. Oral pretreatment with peel extracts for 15 days protected the gastric mucosa against the damage induced by indomethacin. The best results were found in a dosage of 50 mg/kg in sour summer cultivar which inhibited the peptic ulcer in comparison with indomethacin induced gastric ulcer group.

Cold pressed pomegranate seed oil has anti-inflammatory properties which have been proven.. Although some papers describe the beneficial effects of pomegranate against gastro-intestinal inflammation, surprisingly selected Iranian cultivars has not been assessed against ethanol induced ulceration till now.

The stomach seems to be a location for the absorption of free ellagic acid, while ellagitannins are not absorbed. Pomegranate ellagitannins release ellagic acid in the gut, and this compound is poorly absorbed in the small intestine, conversely, ellagic acid is largely metabolized by human gut microflora in the intestinal lumen into urolithins [12]. After consumption of pomegranate, these reach relevant plasma concentrations [13]. The absorbed metabolites are conjugated with glucuronic acid and/or methylated to give ether derivatives. Ethanol-induced ulcers are the result of a direct effect of ethanol on gastric mucosa, mostly due to its capacity to induce necrosis of superficial gastric epithelial cells and erosion [14]. This study illustrated that pomegranate peel methanol extract produced a significant reduction of ulcer index in ethanol-induced gastritis in rats. The black peel extract at 50 mg/kg, inhibited ulcer index by 65.87 % and a related group was also protected from intraluminal bleeding, whereas cimetidine (100 mg/kg) showed 83.47 % inhibition. In another study, the methanol extract of the dried flowers of pomegranate (980 mg/kg) significantly reduced ulcer index by 87.5 %. In addition, a dose of 490 mg/kg and omeprazole (20 mg/kg) exhibited 65 and 49.6 % inhibition, respectively [14].

In one study, the aqueous extract of pomegranate peel showed a gastroprotective effect in rats with ethanol-induced gastric lesions [8]. The simultaneous administration of ethanol and pomegranate peel significantly decreased gastric lesions and ulcer index.

A small number of studies report that pomegranate is able to treat *H. pylori* infection. Methanol peel extract of pomegranate exhibited a remarkable anti-*H. pylori* activity, as shown by the size of inhibition zone in the disk diffusion method, which was comparable to the reference compound metronidazole (MIC 8 μg/mL) [15]. Another research revealed that administration of 70 % methanol extract of pomegranate fruit rind (250 mg/kg and 500 mg/kg) resulted in 22.37, 74.21 and 21.95, 63.41 % inhibition of aspirin- and ethanol-induced gastric ulceration, respectively. The results showed the gastroprotective activity of the extract by

antioxidant mechanism. In the test animals, in vivo antioxidant levels increased and found more or less equal to the normal amounts. In treated groups, tissue lipid peroxidation level declined [16].

Pomegranate is a deciduous tree distributed throughout the world, and no adverse effects have been reported following consumption of this fruit and its constituents; animal studies have so far not shown any toxicity at doses that are usually given [18].

CONCLUSION

The methanol extract of pomegranate peel shows protective activity against ethanol-induced gastric ulcer lesions in rats. This is significant because large quantities of pomegranate peel can easily be collected from pomegranate processing industries. Interestingly, most extracts of pomegranate peel are both anti-inflammatory and anti-ulcerogenic.

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