Prophylactic Anti-Ulcer Effect of Punica Granatum (Pomegranate) Peel and Seed Extract in Murine Peptic Ulcer Model

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Abstract

Objective: To assess the prophylactic antiulcer effect of methanolic extracts of Punica granatum (Pomegranate) peel and seed and its comparison to pantoprazole in diclofenac induced murine model of peptic ulcer.

Methods: Eighty-one male rats were segregated into 9 groups having 9 rats each. Control groups; G-1 (healthy control) and G-2 (disease control) were given only distilled water orally. Pantoprazole and Punica granatum peel (PGPE) and seed (PGSE) extracts were given orally once daily to the treatment groups (3-9) for 15 days as follow: G-3: pantoprazole 60mg/kg/d, G-4: PGPE 100mg/kg/d, G-5 PGSE 500mg/kg/d, G-6: PGPE 50mg/kg/d + PGSE 250mg/kg/d, G-7: Pantoprazole 30mg/kg/d + PGPE 50mg/kg/d, G-8: Pantoprazole 30mg/kg/d + PGSE 250mg/kg/d, G-9: Pantoprazole 30mg/kg/d + PGPE 50mg/kg/d + PGSE 250mg/kg/d. Groups 2-9 were then given 100mg/kg diclofenac orally on day 17 for induction of toxicity. Gastric parameters (gastric juice volume & pH, ulcer index) were assessed.

Results: All treatment groups demonstrated significant gastroprotective activity against diclofenac. However, group 9 (pantoprazole 30mg/kg/d + PGPE 50mg/kg/d + PGSE 250mg/kg/d) exhibited greater antiulcer activity than pantoprazole as well, shown by improvement in gastric parameters.

Conclusion: The study revealed that Punica granatum peel and seeds, generally considered worthless could be used for prevention of peptic ulcer.

Keywords: Peptic ulcer disease, Punica granatum, Proton pump inhibitor, Diclofenac

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Introduction

Peptic ulcer disease (PUD) is a common, chronic multifactorial disease with periods of activation and remission represented by discontinuity in the mucosal lining of upper gastrointestinal tract (GIT).[1] It affects 4% people globally between the ages of 30-60 years resulting in loss of productivity and increase in financial burden.[2] Its debilitating plethora of symptoms and complications range from anorexia, nausea, vomiting, dyspepsia, severe nocturnal epigastric pain to upper GI hemorrhage, perforation, and even gastric outlet obstruction.[3,4] PUD related morbidity and mortality resulted in 267,500 deaths in 2015.[2]

Peptic ulceration is characterized by decrease in gastric mucosal defensive factors and enhancement of offensive factors damaging the mucosa and is a result of various physical and psychological stressors with H.pylori infection and NSAIDs being the leading causes.[5] Diclofenac is frequently used as over the counter drug for pain, fever, inflammation and is one of the most commonly prescribed NSAID for a variety of musculoskeletal

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and rheumatological disorders, but has numerous adverse effects. NSAID-induced peptic ulcer is one of the most serious adverse effects due to any drug therapy and is mediated by inhibiting cyclooxygenase enzymes and subsequently prostaglandin synthesis and their protective effects.[6] According to international research approximately 16,500 arthritis patients receiving NSAIDs die from its gastrointestinal toxicity annually.[7]

Therapy of PUD is targeted at either counteracting aggressive factors or stimulating the mucosal defensive factors. Treatment options include; antacids; anti-secretory drugs like H2 antagonists and proton pump inhibitors (PPIs); mucosal protective agents; antibiotics for H. pylori eradication.[8] Proton pump inhibitors like Pantoprazole, irreversibly blocking the H+/K+ ATPase in the gastric parietal cells, provide superior acid suppression, symptom relief and healing rates and are thus recommended as initial therapy for most of the patients but have adverse effects and drug interactions.[9] Thus, novel, non-toxic, anti-ulcer preparation preferably of plant origin as an alternative for managing peptic ulcer is the demand of time. Several plants like cabbage leaves, ginger, bitter gourd, grapes, gooseberry, sweet potato, and pomegranate have been prescribed by healers to prevent and cure peptic ulcer because of their anti-secretory and gastroprotective properties but most of them have not been studied scientifically yet to confirm their pharmacological activity and safety profile.[10]

Punica granatum (PG) known as Pomegranate belonging to Punicaceae family, is an ancient, mystical fruit mentioned in various mythologies and religious writings as a sacred fruit and has been used for the treatment of a variety of diseases in traditional medicine. It is grown and utilized almost throughout the world. All parts of PG; roots, bark, leaves, flowers, peel, seeds have medicinal value and have been used since millennia across different cultures and civilizations.[11] Therefore, the current study was conducted to analyze the potential of PG peel and seeds in the prevention of peptic ulcer.

Materials and Methods

This experimental animal study was conducted in Post graduate medical institute (PGMI) Lahore, after approval from Institutional Review Board (IRB) of Federal post graduate medical institute (FPGMI) and was completed in 17 days. 81 healthy male albino rats weighing 130-170 grams were purchased from University of Veterinary and Animal Sciences (UVAS), Lahore, were kept in the animal house of PGMI in polypropylene cages under standard housing and lighting conditions and were given standard laboratory diet and water ad libitum. They were randomly assigned to 9 groups (G-1 to G-9) having 9 rats each by lottery method. Before starting the experiment, the rats were acclimatized to the new environment for one week. Pantoprazole tablets (40mg) and Diclofenac tablets (50mg) were bought from Servaid Pharmacy, Lahore, Fresh PG fruits were purchased from local fruit market of Lahore. The identification, verification and extract preparation were done in Applied Chemistry and Research Centre at Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Lahore. Fruits were washed thrice with distilled water and peeled manually. 1 kilo peel and seeds were shade-dried and finely ground in the grinder. Separate extraction was carried out for the peel and seeds with 80% methanol in a Soxhlet apparatus for 4hrs, and further concentration was performed at 40°C under controlled reduced pressure using a rotary vacuum evaporator. The extracts were collected in capped bottles and stored at 4°C till further use.[12]

After acclimatization the animals in the healthy (G-1) and disease control groups (G-2) were given only 0.5ml distilled water orally daily for 15 days and were left untreated. The remaining seven groups were given prophylactic oral treatment once daily for 15 days as per group designation detailed below.

Group 3: (standard group) Pantoprazole 60mg/kg/d
Group 4: PG peel extract (PGPE) 100mg/kg/d
Group 5: PG seed extract (PGSE) 500mg/kg/d
Group 6: PGPE + PGSE (50+250mg/kg/d)
Group 7: Pantoprazole + PGPE (30+50mg/kg/d)
Group 8: Pantoprazole + PGSE (30+250mg/kg/d)
Group 9: Pantoprazole + PGPE + PGSE (30+50+250 mg/ kg/d)

Animals of groups 2-9 were then fasted for 24 hrs (day 16) and given Diclofenac 100mg/kg orally in a single dose on the 17th day.[13] The animals were surgically sacrificed 4hrs later to obtain stomach for evaluation of gastric parameters; gastric juice volume, pH and ulcer index. The upper and lower ends of stomach were tied by threads and stomach was isolated. A small cut was given at the lower end of the stomach and contents were collected in glass tubes. The collected gastric juice was centrifuged at 1000rpm for 10 min.[14] Total volume was directly read from marking of centrifuge tubes and pH was determined using digital pH meter. The stomach was then dissected along its greater curvature, fixed on a board with the help of thumb pins (Fig 1). Ulcer was noticeable as hemorrhagic streaks or linear / punctate discontinuity in the mucosal lining. Ulcers were scored

by examining the stomach macroscopically with hand lens based on grading on a 0–5 scale as presented in the following table.[15]

The ulcer index (U.I) was determined for each group

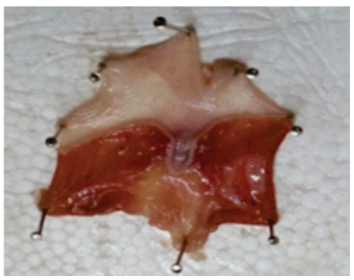
Score	Remarks
0	No lesion
1	Mucosal edema and petechiae
2	One to five small lesions (1-2mm)
3	More than five small lesions or one intermediate lesion(3-4mm)
4	Two or more intermediate lesions or one gross lesion (>4mm)
5	Perforated ulcers

using the following equation[15]:

Ulcer index (UI) = Total ulcer score / no. of animals ulcerated.

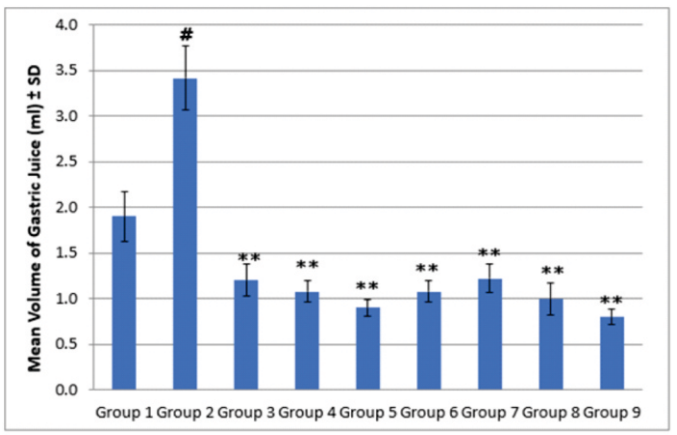
Data was analyzed using SPSS version 23.0 and was described by Mean + SD for each group. Comparison among groups was made by using one-way ANOVA test and pair wise comparison was done by Tukey's test. p-value < 0.05 was considered statistically significant and < 0.01 highly significant.

Fig 1: *Macroscopic examination of stomach*



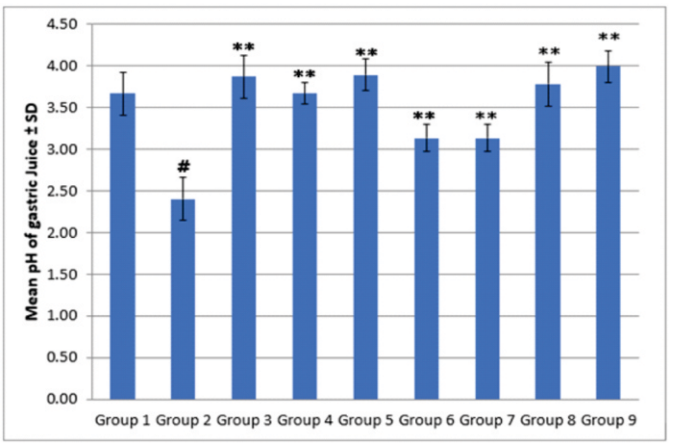
Results

*** (Highly significant values compared to group 2) # (significance of group 2 in comparison to group 1)*



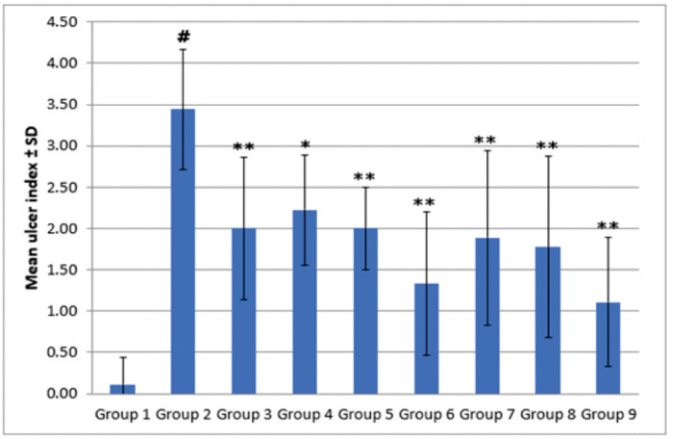
Graph 1: *Graphical representation of total volume of gastric juice (ml) of rats given Pantoprazole, PGPE and PGSE as per group designation (n=9)*

*** (Highly significant values compared to group 2) # (significance of group 2 in comparison to group 1)*



Graph 2: *Graphical representation of pH of Gastric Juice of rats given Pantoprazole, PGPE and PGSE as per group designation (n=9)*

** (Significant values) ** (Highly significant values) # (significance of group 2 in comparison to group 1)*



Graph 3: *Graphical representation of Ulcer Index of rats given Pantoprazole, PGPE and PGSE as per group designation (n=9)*

Discussion

PUD is a widespread gastrointestinal disorder characterized by breach in the mucosal lining of upper GIT, secondary to imbalance between the gastric mucosal defensive factors and offensive factors mostly caused by H. pylori infection and NSAIDs.[1] Several drugs have been available for the prevention and treatment of peptic ulcer but none is free of adverse effects.[10] Prevention and complete remission of PUD with minimal or no toxic effects is the main health concern nowadays.

Phytotherapy is one of the most promising alternative therapies for the treatment of peptic ulcer due to its minimal side effects, cost effectiveness, accessibility and wide range of antioxidant and cytoprotective properties.[16] Pomegranate fruit, peels and seeds have shown anti-ulcer effects in murine models due to their strong gastro-protective and antioxidant activity attributed to presence of phenolic compounds, flavonoids, tannins and anthocyanins.[17] Keeping this in mind, the current research project was designed to investigate the prophylactic antiulcer effect of PG peel and seed extracts (PGPE, PGSE) individually and in combination in comparison to Pantoprazole, a standard antiulcer drug (PPI) in the murine diclofenac induced gastric ulcer model. This specific model was chosen because diclofenac an NSAID, has a pronounced history of acute gastric ulceration secondary to cyclooxygenase pathway inhibition suppressing prostaglandin synthesis.[18] Moreover, no previous research existed to compare the amelioration of these parameters with PGPE and PGSE in comparison to the standard drug pantoprazole against diclofenac. Results of the research project are discussed as follows in detail: Group 1 (healthy control) showed a mean gastric juice volume, pH and ulcer index of 1.90 ± 0.27 ml, 3.67±0.26 and 0.11±0.33 respectively (Graph 1, 2 and 3). Group 2 (disease control) treated with diclofenac caused the highest increase in volume (44%) and maximum reduction in pH(34%) of 3.41±0.35 ml and 2.40± 0.26 respectively as compared to the healthy control group. It also had the highest ulcer index of 3.44±0.73 amongst all the groups which was 97% higher as compared to group 1, indicating its ulcerogenic potential which is due to inhibition of prostaglandins, increasing lipid peroxidation, production of reactive oxygen species and decreasing gastric mucosal blood flow.[7]

All the treatment groups (3-9) showed varying percentage of statistically significant (p-value=0.000) reduction in gastric juice volume (65%–77%), significant increment in pH of gastric juice (30% - 66%) and significant reduction in ulcer index (35% - 68%) as compared to the disease group. PG peel and seeds possess a variety of phytochemicals and their anti-ulcer and gastroprotective effects might be due to free radical scavenging (ellagic acid), inhibiting lipid peroxidation (tannins and ellagic acid), increasing mucus and bicarbonate secretion (flavonoids), promoting NO generation (proanthocyanidin) and increasing mucosal blood supply and strong antioxidant activity (punicalagin, flavonoids). Flavonoids are cytoprotective constituents that have been proven to stimulate prostaglandin, mucus and bicarbonate secretion and possess antioxidant activity as well.[19] Furthermore, tannins present abundantly in PG peel precipitate proteins at the site of ulcer, and form a protective covering that prevents further mucosal damage by toxins, a mechanism similar to sucralfate.[20]

Group 5 (PGSE 500mg/kg/d) showed 74% reduction in volume and 62% increase in pH of gastric juice as compared to disease group, and the results were even better than the standard group (Pantoprazole 60mg/kg/d). These results were in line with a previous study by Gautam et al in which PGSE showed maximum reduction in gastric volume as compared to PGPE and standard drug (Ranitidine 50mg/kg) in diabetic rats.[21] These effects prove strong antisecretory activity of PGSE. The best results were seen in Group 9 (Pantoprazole 30mg/kg/d+PGPE 50mg/kg/d+PGSE 250mg/kg/d) which showed 77% reduction in volume, 66% increase in pH of gastric juice and 68% reduction in ulcer index as compared to disease group. The results were even better than the standard and healthy control groups. This combination showed "synergistic activity" of pantoprazole with PG peel and seed extracts due to combination of the above-mentioned mechanisms of phytochemicals present in peel and seeds and additionally the anti-secretory and cytoprotective activity of pantoprazole by inhibiting H+/K+ ATPase and reducing mucosal oxidative damage respectively.[22] No previous study could be found to support or refute this synergistic effect.

Conclusion

The current study demonstrated that Punica granatum peel and seed extracts administered prophylactically, individually and in combination showed significant anti-ulcer potential in the acute diclofenac model comparable to the standard drug, pantoprazole; evidenced by significant reduction in gastric juice volume, ulcer index and increase in pH of gastric juice as compared to disease group. Therefore, it can be inferred that the peels and seeds of Punica granatum, a commonly used delicious fruit are an invaluable yet wasted resource which may provide new options for prevention of peptic ulcer disease.

Conflict of Interest *None*

Funding Source *None*

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Authors Contribution

MIP: Conceptualization of Project
SSA: Data Collection
FAK: Literature Search
SMNZ: Statistical Analysis
SSA: Drafting, Revision
TZ: Writing of Manuscript