Gastro-protective and therapeutic effect of Punica granatum against stomach ulcer caused by Helicobacter Pylori


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ABSTRACT

Gastric ulcer is a chronic condition that occurs when the mucosa of the stomach is broken. There is a physiological equilibrium between aggressive factors and mucosal defense. The purpose of this research was to compare the prevention level and efficiency of herbal medicinal plants (Punica granatum) to the omeprazole drug. Many groups were prepared from Albino male rats, the first control group (inoculate with H. pylori and fed with standard pellet), the second group, rats inoculated by H. pylori and prevented with Punica granatum aqueous extracts (PGAE) in two dosages (250mg/kg, 500mg/kg), and last group inoculated by H. pylori and prevented with standard drug omeprazole at the dose (20mg/kg). The results showed that the Ulcer Inhibition % of Punica granatum with a high dose of 500mg/kg and a low dose of 250mg/kg was 84.60±5.48 and 42.87±7.14, respectively. While in the omeprazole treatment group, Ulcer Inhibition % was 24.50±6.35. This Ulcer Inhibition % in the Punica granatum treatment groups was significant compared to the omeprazole treatment group and the control group (P=0.0001). PGAE displayed a significant lessening in stomach index and infectious cell proliferation with much cell damage. Although the result of the current study improves, a high dosage of aqueous extracts of plants has more effectiveness than a low dosage of aqueous extracts plants.

Materials and Methods

Extraction of peel part of Punica granatum

Fresh Pomegranate fruit was collected from a market

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in Erbil city. Mainly the yellow peel is separated from the pomegranate fruit collected in August 2020 and is used in the study. The samples were air-dried indoors to protect them from direct sunlight. The method described by (12,13) was used for the aqueous extract preparation of the finely powdered samples. Hot distilled water (85°C) was added and left overnight to prepare a high dose (25g/250ml), and a low dose (25g/500ml), then the extract was filtered by filter paper Whatman No.1. Finally, the filtered extract, were stored inside the sterile cup at refrigerator until used.

Omeprazole

Omeprazole is the class of medicines that have been used to treat disorders including peptic ulcers as proton pump inhibitors. Omeprazole inhibits the acid-forming enzymes in the stomach wall. The output of the stomach acid is reduced by blocking these enzymes and thus the stomach can be healed. Omeprazole was the reference anti-ulcer treatment in this sample and was extracted in Erbil city. The medication was supplied by mouth to rats at 20mg/kg body weight suspended in distilled water (5ml/kg) (14).

Animal used in the experiment

Forty-eight male albino rats were used for the trials. Rats body weight ranged from 160±20 gram 7–9-week-old and the rats were randomly allocated to four groups, each group of twelve rats were purchased from Zakho University and controlled in well-ventilated cages under sanitary conditions. The rats were provided with a regular diet for one week in the animal house of Salahaddin University's College of Education.

Induction of gastric ulcer by H. Pylori

Rats were given the Helicobacter pylori bacterium, which was obtained from biopsy samples of clinical patients, and this generated gastritis in the rats. The rats were held in reserve in the animal house of the Salahaddin University-College Erbil's of Education for a week and provided a basic meal. In this study, the rats were fasted in the morning and afternoon first inoculated with plant extracts then immediately inoculated with 1ml H. pylori suspension (5×10⁸-5×10¹⁰ CFU/ml) by gavage twice daily at an interval of 4h for three consecutive days, then left on normal feeding for 15 days to see the effect of the plants extracts with H. pylori activity (15), before inoculating animals with plant extract and H. pylori the animals do the antigen test via stool to ensure that did not infect with H. pylori before. In last period of prevention, anesthetized of the rats done by intramuscular injection of mixed xylazine-ketamine (1:9) as a single dose in the same syringe, then the Stomach was removed of each rat from each group, then dissects the stomach.

Evaluating large gastric lesions

Ulcers in the gastric mucosa appeared as extended bands of hemorrhagic lesions parallel to the long stomach axis. As a result, the damage was investigated in each stomach mucosa sample. The overall surrounding place of all lesions for each stomach was added to the size of the ulcer zone (UA) whereby the sum of tiny squares (×4×1.8 = UA mm²) as described earlier by a prior with minimal modification (16). The interference proportion (I%) was computed using the following equation with minor modifications. (I%) = [(UA control - UA treated) ÷ UA control] × 100% (17).

Acute toxicity test of pomegranate extract

Male Wistar rats were used to determine the acute toxicity in vivo. The research was initially carried out to determine the safety of the doses that were installed. Three separate dosages of 100, 1000, and 2000 mg/kg were administered orally through gastric gavage to three distinct groups of rats (n=3) in order to determine the lethal dose (LD50) that results in the death of 50% of the experimental rats in a category. This was carried out in accordance with the Organization for Economic Cooperation and Development's ethics for chemical testing (18). To track any weight changes, male Wistar rats were weighed before, during, and after the treatment. After dosing at least once in the first 30 minutes and on occasion over the following 24 hours, rats were also watched to look for any potential changes in their behavior. For 14 days, special attention was given daily throughout the first four hours. The skin, eyes, breathing, seizures, and overall health of the rats were followed for any signs of poisoning (19).

Acid content of gastric juice measurement and evaluation (pH)

The pH level of the stomach was individually recorded for evaluation of the pH level of all groups of rats to know the effect and correlation of plant extract with pH level.

Microbiological analysis

Microbiological analysis was directly carried out on the day of sample collection, Stool antigen test was done on all rat groups and was analyzed by strip H. pylori antigen test to see if the rats were infected with Helicobacter pylori or not.

Histopathology investigation

After the necropsy, stomach samples were taken and preserved in 10% formalin for 48 hours. The tissue samples were cut into 0.5 cm thick slices and put in plastic cassettes to be dehydrated and cleared before being embedded in paraffin. Subsequently, the tissue samples were cut into 4 μm sections. The tissue layers used to be cautiously put in a water bath and mounted on glass slides using a hot plate. Sections of stomach tissue were deparaffinized twice with xylene for two minutes each, rehydrated three times with ethanol diluted differently (100%, 90%, and 70%) for two minutes each, and stained with hematoxylin and eosin (H&E) stain. At magnifications of 40x, 100x, 200x, and 400x, tissue sections were seen and studied under a light microscope.

Statistical method

To investigate the effect of an aqueous extract of Punica granatum and antibacterial omeprazole in gastric ulcer production and ulcer inhibition, the data were expressed as mean ± S.E.M. (number = 6 mice/group). One-way ANOVA followed by Tukey's post-mortem multiple comparison tests was performed to measure the significance of differences between groups. The significance level was determined to be less than 0.05%.
Results

Effect of PGAE and omeprazole

Animals of the second and third groups protected by PGAE showed a significant reduction in the ulcer area. The results showed that in the Punica granatum treatment group with a dose above 500 mg/kg, Ulcer Area (mm2) was 125.50±1.24 and Ulcer Inhibition % was 84.60. In the Punica granatum treatment group with a dose of 250 mg/kg Ulcer Area (mm2) was 465.60±8.54 and Ulcer Inhibition % 42.87. In the omeprazole treatment group, Ulcer Area (mm2) was 615.25±11.68 and Ulcer Inhibition was 24.50. While in the control group Ulcer Area (mm2) was 815.00±24.78 and Ulcer Inhibition % 14.76. And this Ulcer Inhibition % in the Punica granatum treatment groups was significant compared to the omeprazole treatment group and the control group (P=0.0001).

The ulcer area was dramatically reduced in a dose-dependent manner when compared to the control group. The largest reduction proportion of ulcer area development was (84.60%) in the rats prevented with a high dosage (500mg/kg) of PGAE. However, ulcer area was greatly decreased or suppressed from (815.00mm2 control group rats) to (125.50 mm2) as shown in Table 1.

The antulcer activity of the drug omeprazole in a model of H. pylori-induced gastric lesion. The results demonstrated that rats' stomachs protected with omeprazole and given H. pylori solution reduced areas of gastric ulcer formation at a rate (24.50%) of ulcer area inhibition in comparison to the gastric ulcer control group.

Evaluation of pH level of gastric content between Punica granatum and antibacterial omeprazole

When PAGE was used to prevent ulcers in experimental animals, it was observed that this treatment method was associated with a decrease in gastric acidity. Examination of stomach acidity after treatment showed that the pH of stomach acid in the P. granatum 500 mg/kg treatment group was 6.3 and in P. granatum 250 mg/kg treatment group it was 5.

Stomach pH was 5.5 in the omeprazole treatment group and 3.5 in the control group, which shows the positive effects of P. granatum treatment in reducing stomach acidity (P=0.001) (Figure 1).

Evaluation of stomach color change in rats between Punica granatum and antibacterial omeprazole

The color of prevented rat stomachs with PGAE significantly changed compared with the ulcer control group, white bright color was found in the stomach of the rats with PGAE, and a white to pink color was found in each of the stomachs, otherwise, the control groups stomach was totally dark red, while the color of prevented rat stomach with omeprazole are changed comparing with the ulcer control group, red bright color was found in the stomach of the rats with omeprazole that showed in Figure 2.

Histological examinations

Figure 3 shows the histological analysis and macroscopic appearance of the stomachs of the several rat groups that were evaluated. The macroscopic inspection of the opened stomachs of normal control rats revealed normal morphological features (20), while the ulcer control group displayed hemorrhagic lesions and specks of coagulated blood. Additionally, the PGAE pre-treatment group's sto-

Table 1. Anti-ulcer effects of P. granatum and omeprazole treatments in male albino rats.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Prevention (5ml/kg) dose</th>
<th>Ulcer Area (mm²) Mean ± SD</th>
<th>Ulcer Inhibition %</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ulcer control group</td>
<td>815.00 ± 24.78</td>
<td>14.76</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>High dose</td>
<td>125.50 ± 1.24</td>
<td>84.60</td>
<td>0.0001</td>
</tr>
<tr>
<td>3</td>
<td>Low dose</td>
<td>465.60 ± 8.54</td>
<td>42.87</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Omeprazole</td>
<td>615.25 ± 11.68</td>
<td>24.50</td>
<td></td>
</tr>
</tbody>
</table>

P-Value based on One-way ANOVA followed by Tukey's posthoc.
machs were equivalent to those of a normal control group with few mucosal lesions. The histopathology structure of the gastric layers was discovered by histologically analyzing the stomachs of healthy control rats (mucosa, submucosa, and serosa) (21). Rats without ulcers had focal gastric mucosal necrosis associated with inflammatory cell infiltration, submucosal edema, and submucosal inflammatory cell infiltration in their stomachs. The PGAE group’s negligible damages demonstrated the ulcer formation’s suppression (22). While other analyzed sections from the pomegranate peel treatment group exhibited no histological alterations, some of them showed minor submucosal edema (23). The ulcer scores significantly decreased after receiving PGAE treatment (p = 0.0013). (400x).

Discussion

The present findings agree with the results of their findings show that done (24). Gastric ulcer was prevented with PGAE, producing significant healing in an induced gastric ulcer model (25). The current investigation concluded that components of the PGAE are effective for gastric ulcer prevention, as claimed by traditional medicine practitioners (26). Numerous psychological and physical stress cause stomach ulcers in both humans and laboratory animals. PGAE acts as a protection against gastric ulcers (27).

Current findings suggest that the anti-ulcer effect is associated with increased secretion of adherent mucus and increased pH of gastric contents (28), which can inhibit the production of oxygen-derived free radicals and keep the contents of MDA in a normal state. Our result was agreeing with the result finding in (25) PGAE prevents ulcers by increasing mucus secretion and pH level in pyloric ligation rats (29). Also shows the anti-ulcer activity of AMP in experimentally induced gastric ulcers. H. pylori formed widely visible black hemorrhagic injuries on the stomach mucosa (30), moreover plant extraction of pomegranate peel had an effect (84.60%) inhibition formation of stomach ulcers compared with the control group in rats gastro-protective with the high dose plant extract of PGAE (500mg/kg) and (42.87%) inhibition formation of the stomach ulcers in rats with low dose *Punica granatum* extract (250mg/kg), however, another study result (31) were agrees with our study result finding that PGAE plays a major role on inhibition formation of stomach ulcers gastro-protective in rat animal model (32).

The active chemicals found in pomegranate are assumed to be responsible for the fruit’s ability to boost local gastric defense mechanisms, re-epithelialization, and regeneration of the glandular architecture of the stomach mucosa as evidenced in histological sections (33). Prostaglandins, eNOS-mediated NO generation, tissue development factors, mucosal endogenous antioxidant status, chelating oxidative agents, and anti-angiogenic factors are all enhanced by pomegranate polyphenols (34). By promoting the precipitation of microproteins at the ulcerative site, constructing a barrier against irritants across the stomach mucosa, and inhibiting gastric secretions at the damage site, tannins—another beneficial component of PGAE—also helped with ulcer healing (35). It was expected that pomegranate rind extract generated in methanol would have a better antioxidant capacity (27).

In addition to the juice, pomegranate extracts from the peels, blossoms, and seeds also exhibit a substantial anti-inflammatory effect in the gut. Some inferences can be made from all the studies included in the current evaluation (36). Also, it appears that the pure substances found in pomegranate fruits function in various ways (37).

In a study, the ability of pomegranate (*Punica granatum* Linn) flower diethyl ether extracts to cure wounds in diabetic male Wistar rats was examined (38). Healthy wounded rats were given basic ointment as treatment. Others received basic ointment base treatment (39). In groups, rats with injuries were given nitrofurazone, a common medicine, while others were given a simple base ointment containing a 1:1 combination of the two extracts (40). Histopathological examinations revealed that all-natural extract-treated rats had significantly lower wound areas than the control and nitrofurazone groups (41). Among the natural extract groups, pomegranate flower extract produced the best results, with lesions completely healed by day 18 (42). In vitro, PGAE displayed significant activity against *H. pylori* with a MIC of 0.156 mg/mL and significant urease inhibitory activity with an IC50 of 6 mg/mL, in contrast to the confirmed strain. PGAE and metronidazole together have a synergistic impact on *H. pylori* with a MIC of fewer than 0.5. Rats given PPEE orally for 9 days experienced a considerable decrease in *H. pylori* gastritis, and even at quite large dosages, PGAE exhibited no symptoms of acute in vivo toxicity. PGAE can therefore be utilized as a possible substitute or additional therapy to reduce *H. pylori* contamination that is accompanied by clinical symptoms.

There are many reports (43-) about the extracts of medicinal plants and their biological and therapeutic effects, and the effects of one of them on pomegranate were discussed in this research.

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Interest conflict

The authors declare that they have no conflict of interest.

Author’s contribution

AJM and ZZA: Experimental design and achievement of data; AMH, RKF and ZBT: Analysis and writing; MMH and SM: Revising and reviewing

Financial interest statement

Partial financial support was received from Cihan University-Erbil.

Ethics statement

(A) This research was approved by the Ethics Committee for Animal Experimentation, Faculty of Science, Cihan University-Erbil and Department of Biology, College of Education, Salahaddin University, Erbil (No.140, D,11.4.2021) Rats recognized the principles outlined in the United States National Academy of Sciences Guide for Care and Use of Research Laboratory Animals, which was issued by the National Institutes of Health (OECD, 2002).
References


