https://doi.org/10.15218/zjms.2019.038

Antibacterial and anti-ulcerogenic effects of *Punicagranatum* peel extract against ethanol-induced acute gastric lesion in rats

Received: 20/5/2018 Accepted: 3/9/2018

Sheila M. Nuraddin[1] Zahra A. Amin[1]* Sargul H. Sofi[1] Shokhan Osman[1]

Abstract

Background and objectives: *Punicagranatum* (pomegranate) is a fruit-bearing deciduous shrub or small tree from the family Lythraceae. It has been cultivated since ancient times throughout the Mediterranean region. Different parts of it have been used for research, such as fruits, peels, and juice. This study aimed to investigate the antibacterial and anti-ulcer effect of *Punicagranatum* peel extract and to screen the expression of transforming growth factor β1 (TGF β1) in rat's blood serum after stomach ulcer was induced by ethanol.

Methods: Twenty four rats were divided randomly into 4 groups; GroupI(ulcer positive group) rats were (experimental) orally administered 5 ml/kg sterilized distilled water (the vehicle). Group II (ulcer negative group) rats were orally administered 5 ml/kg of 20 mg/kg esomeprazole. Groups III and groups IV were orally administered 5 ml/kg of 250 and 500 mg/kg of *Punicagranatum* plant extract, respectively.

Results: Data showed an antibacterial activity of *Punicagranatum* peel extract against gram positive and gram negative bacteria and the best antibiotic in which both bacteria were sensitive to was norfloxacin(10 µg). Treatment with *Punicagranatum* peel extract and esomeprazole had protective effects on stomach gastric mucosa, which indicate an anti-ulcer effect confirmed by the high levels of TGFβ1 in serum.

Conclusion: We conclude that *Punicagranatum* peel extract exhibit antibacterial effect and possess a protective role against ethanol induced gastric ulcer in rats.

Keywords: *Punicagranatum*; TGF β1; Anti-ulcer; Esomeprazole.

Introduction

The potential therapeutic properties of indigenous plants have been studied worldwide and were used either for treatment or prevention of various diseases. *Punicagranatum* (pomegranate), a plant from family Lythraceae, has been reported to enclose many medical properties like chemopreventive,[1] antioxidant,[2] antifungal,[3] anti-inflammatory,[4] antibacterial,[5] and wound healing.[6] It also has been reported to have a preventive role against obesity.[7] Phytochemical screening of *Punicagranatum* extract found to contain steroids, triterpenoids, saponins, glycosides, flavonoids, alkaloids, carbohydrate tannins, and vitamin C.[8] The red color of juice can be pointed to anthocyanins such as cyanidin, delphinidin and pelargonidin glycosides.[9,10] The peel contains three times more polyphenols compared to the pulp such as catechins, condensed tannins[11] gallocatechins and prodelphinidins.[12] This study aimed to study the antibacterial and anti-ulcerogenic activities of *Punicagranatum* peel extract on ethanol induced gastric lesions on experimental rats.

Methods

This experimental study was conducted from 25th March to 25th May 2018. The antiulcer part of the study was done in the animal house unit, College of Medicine, while the extraction and the antibacterial

[1] Department of Pharmacognosy, College of Pharmacy, Hawler Medical University, Erbil, Iraq.
* Correspondence: zahraaalnajaar@yahoo.com

https://doi.org/10.15218/zjms.2019.038

work were done at the laboratories of Pharmacognosy and Microbiology, College of Pharmacognosy. The peel of *Punicagranatum* was dried in the oven at (40-50 ^{o}C) for one week. The dried peels were powdered using a mixer grinder. Subjected to ultrasonic bath, extractor with 99% ethanol for 3 hours at 40 °C. The mixture was evaporated to dryness in a rotary evaporator and stored in the refrigerator until use. The experimental model of this study was following the model of Mahmood et al.[13] with slight modifications. Gastric ulcer was induced by orogastric intubation with absolute ethanol (5 ml/kg) to adult albino rats weighing 200-240 gm that were obtained from the animal house, College of Medicine, Hawler Medical University. The rats fasted for 48 hours prior to the experiment but permitted free access drinking water until two hours prior to the experiment. They were divided into four groups, each of six rats. Group I (ulcer positive group) rats were (experimental orally administered 5 ml/kg sterilized distilled water (the vehicle). Group II (ulcer negative group) rats were orally administered 5 ml/kg of 20 mg/kg esomeprazole. Groups III and groups IV were orally administered 5 ml/kg of 250 and 500 mg/kg of *Punicagranatum* plant extract, respectively. After one hour, all rat groups were administered with 5 ml/kg absolute ethanol (gastric ulcer inducer). One hour later, the rats were sacrificed, and blood was collected. Subsequently, serum was separated, and TGFß1 was measured using (Al-Shkairate Establishment for Medical Supply, Jordan) kit following the instruction manual. The stomachs were removed immediately, and ulcer measurements were observed then immersed in 10% buffered formalin solution. The ulcer measurements were done by opening the stomach along the greater curvature. Samples of gastric contents were analyzed for hydrogen ion concentration by pH-meter titration with 0.1 N NaOH solutions using digital pH meter. The gastric mucosa of each stomach was softly scraped using a glass slide, and the mucus obtained was weighed using a precision electronic balance. Ulcers have been seen as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach were reported. The length (mm) and width (mm) of the ulcer were measured under a dissecting microscope (1.8 x). The area of each ulcer lesion was measured by counting the number of small squares, 2 × 2 mm, covering the length and width of each ulcer band. The sum of the areas of all lesions for each stomach was applied in the calculation of the ulcer area (UA) where the sum of small squares × 4 × 1.8 = UA mm^2 then the inhibition percentage was calculated by the following formula:

(Inhibition %) = [(UAcontrol–UAtreated)¸ UAcontrol] ×100, as described by.[14]

The antibacterial effect was examined for the ethanol extract of *Punicagranatum* with the following bacterial strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. The reference antibiotics that employed in the tests were the broad spectrum antibiotics: (MET: metronidazole 5 µg), (NOR: norfloxacin 10 µg) and (FOX: cefoxicitin 30 µg). Disc Diffusion test was performed using nutrient agar bacterial strains by the Kirby-Bauer's disc diffusion method following the National Committee for Clinical Laboratory Standards methods.[15] The sterile petri dishes containing solid and sterile nutrient agar were used. Sterile (6 mm) paper disc was saturated with the sample at a concentration of 5 mg/disc dissolved in DMSO. The dried surface of the nutrient agar plate was streaked. Then, the discs were placed on the petri dish, including the negative control (DMSO) and the antibiotics. The plates were then incubated at 37°C for 18–24 hours. Microbial growth was indicated by measuring the diameter of the zone of inhibition.

Statistical analysis

The statistical analysis was evaluated by using one-way analysis of variance

https://doi.org/10.15218/zjms.2019.038

(ANOVA) with post-hoc test using Bonferroni multiple comparisons in the IBM statistical package for the social sciences (version 23) program. The data were reported as the mean ± S.E; a *P* value of less than 0.05 was considered statistically significant.

Results

Ethanol extract of *Punicagranatum* displayed antibacterial activity against Gram positive bacteria *Staphylococcus aureus*(20.5± 0.2 mm) of inhibition zone at a dose (50µg/mL) as shown in Table 1. The activity against the gram negative bacteria *Escherichia coli* showed the best results at a dose (100µg/mL) with inhibition zone (22.5±0.3 mm). Norfloxacin (10 µg) was the best antibiotic in which both bacteria were sensitive to, as presented in Table 1.

Table 1: *Punicagranatum*'s antibacterial activity measured by the disc diffusion method.

Test groups	Bacterial species	
	E. coli	***Staph. Aureus***
DMSO	N	N
MET	R	R
FOX	R	R
NOR	19.5 ± 0.01	22.5 ± 0.15
PG 12.5 µg/mL	13 ± 0.3	15 ± 0.34
PG 25 µg/mL	12.5 ± 0.4	18 ± 0.07
PG 50 µg/mL	15 ± 0.11	20.5 ± 0.3
PG 100 µg/mL	22.5 ± 0.2	18.5 ± 0.06
P value	0.04	0.003

Values are represented as mean inhibition zone (mm) ± SEM of triplicates; N = no inhibition zone; R = resistant; PG = *Punicagranatum* ethanol extract.

https://doi.org/10.15218/zjms.2019.038

Stomach ulcer gross estimation results showed a significant decrease in the ulcer area of the plant treated and esomerprazole groups in comparison to the ulcer positive group (Table 2 and Figure 1). Subsequently, the percentage of ulcer area inhibition increased in rats pre-treated with *Punicagranatum extracts (P = 0.013).* Moreover, the ulcer positive group produced the lowest gastric mucus content, while animal groups pre-treated with (500 mg/kg) and (250 mg/kg) of *Punicagranatum* revealed significant increase of mucus weight *(P = 0.027)* and stomach acidity (expressed as PH) *(P = 0.033)* with respect to ulcer negative group (esomperazole group).

Table 2: The effect of the *Punicagranatum* extract on the mucus weight, pH of stomach content, ulcer area, and % inhibition of ulcer area in the stomach.

Animal group	Pre-treatment (5 ml/kg)	Ulcer area (mm^2)	Inhibition (%)	Mucus weight (g)	PH (mEq/l)
Vehicle (Ulcer Positive)	Sterilized distilled water	343.2±0.03	…	0.84±0.01	1.5±0.06
Control (Ulcer Negative)	20 Mg/kg Esomperazole	21.6±0.11*	93.71*	1.71±0.90*	5.2±0.06*
Low Dose Plant Extract	250 mg/kg *Puni-cagranatum*	72±0.07*	79.04*	1.12±0.41*	3.17±0.17*
High Dose Plant Extract	500 mg/kg *Punicagranatum*	28.8±0.32*	91.61*	1.42±0.25*	4.5±0.35*
P value	……	0.013	0.041	0.027	0.033

The values are expressed as the mean ± SEM; significance was indicated in comparison to the ulcer positive group. (*) indicates significance tested by Post- Hos/Boferroni analysis.

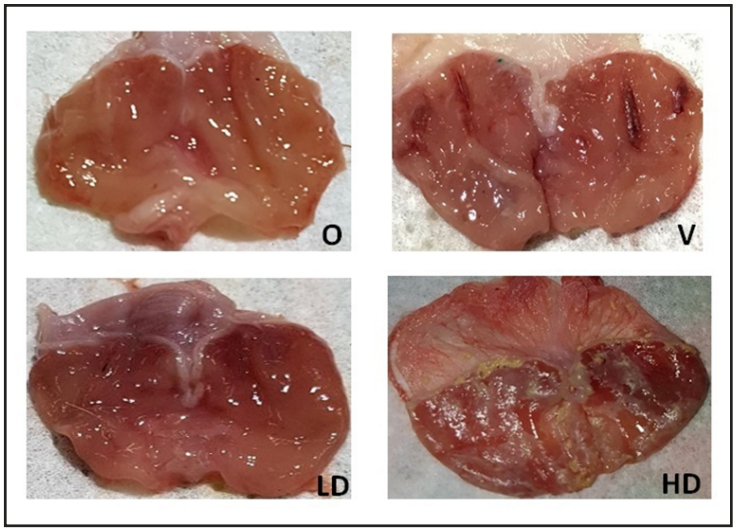

Figure 1: The effect of *Punicagranatum* on the macroscopic appearance of ulcer in rat's stomach. V (Vsehicle/ulcer positive group) had severe injuries to the stomach mucosa, O (Esomeprazole/ ulcer negative group) showed no injuries in the gastric mucosa. LD (250 mg/kg) and HD (500 mg/ kg) doses of *Punicagranatum* extract had less or no disruptions of the surface epithelium in the gastric mucosa in a dose-dependent manner.

https://doi.org/10.15218/zjms.2019.038

Accordingly, TGFß1 levels were higher in *Punicagranatum* treated group in comparison to vehicle/ulcer positive group, and the high dose (500mg/kg) *Punicagranatum* treated group was better than esomeprazole/ulcer negative group as shown in Figure 2.

Discussion

The technique of ethanol-induced stomach mucosal injury is a fast and convenient method of screening plant extracts for their anti-ulcer efficacy, and the gastroprotection level could be defined in terms of reduction or absence in grossly noticeable gastric mucosal lesions. Ethanol is very corrosive to the gastric mucosa, and its mechanism of action on rat gastric mucosa includes apparent necrosis of gastric mucosa and release of leucotrine C4, histamine and as tissue-derived mediators.[16] The results of this study determined that oral administration of *Punicagranatum* extracts significantly protected the stomach mucosal layer from gastric ulcer induced by ethanol, and the stomach mucosal layer protection was enhanced as the doses were increased (Table 2 and Figure 1). The antiulcer effect of *Punicagranatum* might be accredited to several compounds existing in the plant, including punicicacid, anthocyanins, anthocyanidins, ellagic acid ellagitannins (including punicalagins), flavones, estrogenic flavonols, and flavonoids.[17] Previous studies reported the antibacterial activity of different plant parts extracts of *Punicagranatum* which evaluated against several gram negative and grampositive bacteria.[18-20] In the same way, results of this study revealed that *Punicagranatum* exhibited antibacterial effect against gram positive bacteria (*Staph.aureus*) in the dose of50 µg/mL while best results for gram negative bacteria (*E.coli*) were shown in the dose 100 µg/mL. The best antibiotic in which both bacteria were sensitive to was norfloxacin(10 µg) as shown in Table 1. These results were supported by the previously published data in which the antibacterial activity has been explained to be related to the existence of polyphenolics and hydrolyzable tannins in the *Punicagranatum* extract specifically gallagic acid and punicalagin.[21] Moreover, growth factors and their receptors play essential roles in cell migration, proliferation, ulcer healing, and tissue

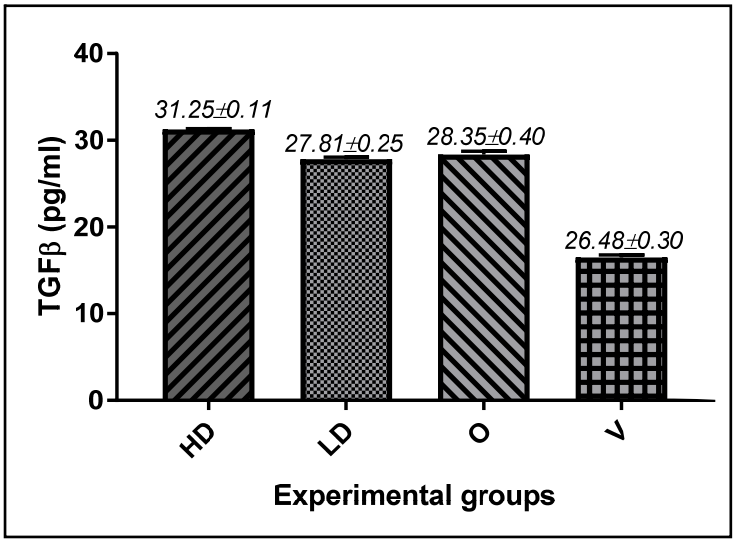

Figure 2: The expression of TGFß1 in different experimental groups; HD indicates high dose (500 mg/kg) *Punicagranatum* treated group, LD indicates low dose (250mg/kg) *Punicagranatum* treated group, V (Vehicle/ulcer positive group) and O (Esomeprazole/ulcer negative group). Data expressed as Mean ± SEM.

https://doi.org/10.15218/zjms.2019.038

injury repair. Transforming growth factorβ (TGFβ) has been proven to participate in the reconstitution of connective tissue, including smooth muscle cells, blood vessels, and afford the extracellular matrix substrate for cell differentiation and migration. Also, the expression of TGFβ was reported to be increased during ulcer healing.[22] Similarly, our results (Figure 2) showed that TGFβ level was increased in *Punicagranatum* treated groups, and the results were better than Esomeprazole treated groups, which indicate ulcer healing and mucosal regeneration.

Conclusion

The anti-ulcer activity of *Punicagranatum* peel extract might significantly protect the stomach mucosa against ethanol-induced gastric damage. The protection was revealed to be dose dependent as determined by the reduction of ulcer lesion areas in the stomach layer, and that protection is most evident at a dose of 500 mg/kg *Punicagranatum* peel extract.

Competing interests

The authors declare no competing interests.

References

1. Mehta R, Lansky E. Breast cancer chemopreventive properties of pomegranate (Punica granatum) fruit extracts in a mouse mammary organ culture. Eur J Cancer Prev 2004; 13(4):345–8.
2. Singh R, Chidambara Murthy K, Jayaprakasha G. Studies on the antioxidant activity of pomegranate (Punica granatum) peel and seed extracts using in vitro models. J Agric Food Chem 2002; 50(1):81–6.
3. César de Souza Vasconcelos L, Sampaio MCC, Sampaio FC, Higino JS. Use of Punica granatum as an antifungal agent against candidosis associated with denture stomatitis. Mycoses 2003; 46(56):192–6.
4. Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmol 2007; 109(2):177–206.
5. Naz S, Siddiqi R, Ahmad S, Rasool S, Sayeed S. Antibacterial activity directed isolation of compounds from Punica granatum. J Food Sci 2007; 72(9):M341-5.
6. Chidambara Murthy K, Reddy VK, Veigas JM, Murthy UD. Study on wound healing activity of Punica granatum peel. J Med Food 2004; 7(2):256–9.
7. Al-Muammar MN, Khan F. Obesity: the preventive role of the pomegranate (Punica granatum). Nutrition 2012; 28(6):595–604.
8. Bhandary SK, Kumari S, Bhat VS, Sharmila K, Bekal MP. Preliminary phytochemical screening of various extracts of Punica granatum peel, whole fruit and seeds. J Health Sci 2012; 2(4):35 –8.
9. Gómez-Caravaca AM, Verardo V, Toselli M, Segura-Carretero A, Fernández-Gutiérrez A, Caboni MF. Determination of the major phenolic compounds in pomegranate juices by HPLC–DAD–ESI-MS. J Agric Food Chem 2013; 61(22):5328–37.
10. Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem 2000; 48(10):4581–9.
11. Ismail T, Sestili P, Akhtar S. Pomegranate peel and fruit extracts: a review of potential anti-inflammatory and anti-infective effects. J Ethnopharmacol 2012; 143(2):397–405.
12. Plumb G, de Pascual-Teresa S, Santos-Buelga C, Rivas-Gonzalo J, Williamson G. Antioxidant properties of gallocatechin and prodelphinidins from pomegranate peel. Redox Report 2002; 7(1):41–6.
13. Mahmood A, Mariod AA, Al-Bayaty F, Abdel-Wahab SI. Anti-ulcerogenic activity of Gynura procumbens leaf extract against experimentally-induced gastric lesions in rats. Journal of Medicinal Plants Research 2010; 4(8):685–91.
14. Al Batran R, Al-Bayaty F, Al-Obaidi MMJ, Abdualkader AM, Hadi HA, Ali HM, et al. In vivo antioxidant and antiulcer activity of Parkia speciosa ethanolic leaf extract against ethanol-induced gastric ulcer in rats. PloS One 2013; 8(5):e64751.
15. Jones RN, Ballow CH, Biedenbach DJ. Multi-laboratory assessment of the linezolid spectrum of activity using the Kirby-Bauer disk diffusion method: Report of the Zyvox® Antimicrobial Potency Study (ZAPS) in the United States. Diagn Microbiol Infect Dis 2001; 40(1):59–66.
16. Abdulla MA, Ahmed KA-A, Al-Bayaty FH, Masood Y. Gastroprotective effect of Phyllanthus niruri leaf extract against ethanol-induced gastric mucosal injury in rats. African Journal of Pharmacy and Pharmacology 2010; 4(5):226–30.
17. Jurenka J. Therapeutic applications of pomegranate (Punica granatum L.): A review. Alternative Medicine Review 2008; 13(2):128.
18. Negi P, Jayaprakasha G. Antioxidant and antibacterial activities of Punica granatum peel

extracts. J Food Sci 2003; 68(4):1473–7.
19. Nair R. Antibacterial activity of Punica granatum in different solvents. Indian Journal of Pharmaceutical Sciences 2005; 67(2):239.
20. Prashanth D, Asha M, Amit A. Antibacterial ctivity of Punica granatum. Fitoterapia 2001; 72(2):171–3.
21. Abdollahzadeh S, Mashouf R, Mortazavi H, Moghaddam M, Roozbahani N, Vahedi M. Antibacterial and antifungal activities of Punica granatum peel extracts against oral pathogens. J Dent 2011; 8(1):1–6.
22. Milani S, Calabrò A. Role of growth factors and their receptors in gastric ulcer healing. Microsc Res Tech 2001; 53(5):360–71