Protective effect of *Punica granatum* peel extract against gastric mucosal erosions induced by ethanol in experimental rabbit models

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Abstract

The present study was designed to detect the gastroprotective effect of ethanolic (96%) extract of *Punica granatum* peel, besides investigation of its activity as hepatoprotective and nephroprotective agent. 1 ml of 80% ethanol/rabbit was given as a single oral dose for induction of gastric erosions and hepato-renal changes in both induction (ethanol group) and treatment groups at the 16 th day of experiment (i.e. following 15 days of daily treatment with pomegranate peel extract).These effects were assessed by examination of some biochemical tests including; blood glucose, triglycerides, renal function test (urea, creatinine), and liver function test (ALT, GGT), in addition to the evaluation of the histopathological changes of the stomach samples. Animals pretreated with *Punica granatum* peel ethanolic extract at a dose of 200 mg/kg/day orally for 15 days showed significant reduction in blood glucose, urea, creatinine, ALT, and GGT where as blood TG was not affected in comparison with the ethanol group ($P \leq 0.05$).On the other hand, microscopic examination of stomach samples of the treatment group revealed regenerated gastric ulcer and lesions, and the distorted superficial gland in the mucosa are regenerated when compared with those observed on the samples of the ethanol 80% group.In conclusion, the obtained data demonstrate that pomegranate peel ethanolic extract is a potent gastroprotective agent and suppresses ethanol 80%-induced gastric damage in rabbits.

Key words / *Punica granatum* peel extract, gastric erosions, ethanol 80%, rabbit

التأثير الواقي لمستخلص قشور ثمار الرمان ضد التسلخات المخاطية المعدية المستحدثة بالايثانول في أرانب التجربة

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الخلاصة

صممت الدراسة الحالية بهدف التحري عن التأثير الواقي للمعدة للمستخلص الايثانولي لقشور ثمار الرمان, الى جانب تقييم فاعلية هذا المستخلص كمادة واقية للكبد والكلية. وقد استخدم 1 مل من الايثانول بتركيز 80% كجرعة منفردة أعطيت لكل ارنب عن طريق الفم لغرض استحداث التسلخات المعدية والتغيرات الكبدية-الكلوية في كلتا مجموعتي الاستحداث (الايثانول فقط) والعلاج وذلك في اليوم السادس عشر من التجربة (اي بعد مضي 15 يوم من المعالجة اليومية بمستخلص قشور الرمان). ان هذه التأثيرات تم تقييمها بواسطة فحص بعض الاختبارات الكيموحيوية والتي اشتملت على كلوكوز الدم, الكليسيريدات الثلاثية, اختبارات وظائف الكلية (اليوريا والكرياتينين), اختبارات وظائف الكبد (الألنين أمينو ترانسفيريز والكاما كلوتامايل ترانزأمينيز) هذا بالإضافة الى تقييم التغيرات النسيجية المرضية لعينات المعدة. ان المعاملة السابقة للحيوانات بالمستخلص الايثانولي لقشور ثمار الرمان بجرعة 200 ملغم/كغم/يوم فمويا ولمدة 15 يوم قد اظهر انخفاضا معنويا في مستويات كلوكوز الدم, اليوريا, الكرياتينين, الألنين أمينو ترانسفيريز والكاما كلوتامايل ترانزأمينيز, في حين لم تتأثر مستويات الكليسيريدات الثلاثية بالمقارنة مع مجموعة الايثانول 80%.من جانب اخر فان الفحص المجهري لعينات المعدة المأخوذة من حيوانات مجموعة العلاج أشارت الى وجود تجديد في القرحة والآفات المعدية وايضا تجديد في الغدد السطحية المشوهة والموجودة في مخاطية المعدة لدى مقارنتها بتلك التغيرات الملاحظة مجهريا على عينات مجموعة الايثانول 80%.وكاستنتاج نهائي, فان محصلة النتائج قد برهنت قوة المستخلص الايثانولي لقشور ثمار الرمان كمادة واقية للمعدة من حيث تقليل التحطيم المعدي المستحدث بواسطة الايثانول 80%.

الكلمات المفتاحية: قشور الرمان ، التسلخات المخاطية المعدية ، الايثانول ، الأرانب .

Introduction

Gastric ulcers are caused due to imbalances between offensive and defensive factors of the gastric mucosa. The antiulcerogenic activity of many plant products is reported due to an increase in mucosal defensive factors rather than

decrease in the offensive factors (1).A number of antiulcer drugs like gastric antisecretory drugs, H2-receptor antagonists, antimuscarinic agents, proton pump inhibitors, mucosal protective agents-carbenoxolone sodium, sucralfate and prostaglandin analogues are available which are shown to have side effects and limitations (2).There are several herbal ayurvedic preparations which have a protective effect against drug-induced gastric mucosal injury (3) Pomegranate is containing natural Phenols: and the most abundant polyphenols in pomegranate juice are the hydrolyzable tannins called ellagitannins formed when ellagic acid binds with a carbohydrate. Pomegranate ellagitannins, also called *punicalagins*, are tannins with free-radical scavenging properties in laboratory experiments (4), Flavonoids and others like vitamin C and B_5 , unsaturated oil (5). Pomegranate peel was traditionally used to cure prostate cancer , prostatic hyperplasia, diabetes, lymphoma, rhinovirus infection, common cold, atherosclerosis, coronary artery disease, infant brain injury, and hemodialysis for kidney disease. Pomegranate has cardiotonic effect and may be effective in reducing heart disease risk factors (6,7,8), it has also antioxidant, antiviral and antibacterial (9), antihypertensive (10), and anti-inflammatory (11).Based on the varied properties of the components of pomegranate peel, we investigated its effect on ethanol-induced gastric mucosal injury. In search for an orally effective treatment of peptic ulcer, this *in vivo* study was designed to evaluate the possible beneficial prophylactic effects of oral monotherapy with ethanolic extract of *Punica granatum* on Ethanol-induced hepato-renal changes and gastric lesions in experimental rabbits.

Materials and methods

Plant material:

Peel of *Punica granatum* (pomegranate) was purchased from local market, dried and powdered before extraction by using of electric grinder.

Animals:

Eighteen healthy, local, domestic rabbits weighing (1500-2000) gm of both sexes were used in this study. They were supplied by the local animal market at Al-Diwaniya city. Rabbits were housed two per cage and were fed standard oxoid pellets also given water *ad libitum*.

Experimental design:

Eighteen rabbits were randomly divided into three groups of six rabbits each:

Group I (Control group): received only saline given orally at a dose of 10 ml/kg body weight.

Group II: received 1 ml of ethanol 80% as a single oral dose/rabbit (12) (Merk-Germany) for induction of gastric ulcer as described below.

Group III: received ethanolic extract of pomegranate peel by stomach tube once daily for 15 days at a dose of 200 mg/kg body weight orally (13).

Preparation of ethanolic extract:

Ethanolic extract of pomegranate peel was accomplished according to the method of Le Grand (14). Briefly 50 gm of powdered plant sample was mixed with 250 ml of 96% ethanol (Merk-Germany). The mixture was kept for 2-5 days in tightly sealed containers at room temperature and shaked several times daily. This mixture was filtered through filter paper to remove the coarse plant materials. Further extraction of the residue was repeated 3-5 times until a clear supernatant extraction liquid was obtained. The filtrates of each tested plant were evaporated to dryness using a rotary evaporator at 40ºC. The final dried samples were weighed and stored at -20ºC until use.

Gastric ulcer induction in rabbits:

One ml of 80% ethanol was used orally to induce gastric ulcer (12). After the experimental period of 15 days, and prior to sacrifice, all the animals were fasted for 24 h (i.e.; after giving the last dose of extract in the fifteenth day). For Group II and Group III animals, 1 ml of 80% ethanol was administered orally (i.e.; in the sixteenth day of experiment and after 24 h from the last extract dose for group III). One hour after the ethanol administration, the animals (group I,II,III) were sacrificed under chloroform

anesthesia and the abdominal cavity was opened; the stomach was removed and placed on the petri dish, then injected via any of gastric orifices with 10 ml of formalin 10% for 10 minutes to enhance easily discarding of food materials. After that, the stomach was incised along the greater curvature and washed gently in running tap water. Finally, it was immersed in formalin 10% to be ready afterward for sectioning.

Method of blood sampling:

At the end of experiment (i.e., one h after administration of ethanol 80% for group II and III), blood samples were taken at most care by heart puncture from all animals in the three groups. 5 ml of blood could be aspirated and put in the anticoagulant coating tube, shacking gently to be used then for haematological assessment of blood glucose, blood urea, blood creatinine, ALT, GGT, and triglycerides by using of Autoanalyzer (Reflotron)® Strips.

Histopathological examination:

The tissue samples were fixed in 10% buffered formalin and processed with paraffin wax. For histopathological examination, 5 µm sections were stained with hematoxylin and eosin. The extent and depth of ulceration and hemorrhage were evaluated (15).

Statistical analysis:

The data were analyzed by student's *t*-test using SPSS (Version 10). The results were expressed as (mean±SE), P values<0.05 were considered statistically significant.

Results

Table (1) and figure (1) show the effect of *P.granatum* peel ethanolic extract on gastric lesions induced by ethanol 80% and biochemical values of the blood in the experimental rabbits. Animals received 1 ml of ethanol 80% as a single oral dose suffered from significant elevation in the levels of urea, creatinine, ALT, and GGT (46±0.36 mg/dl, 0.83±0.003 mg/dl, 96±0.36 IU/l, 53±0.36 IU/l) respectively at ($P \leq 0.05$) in comparison with those of normal saline treated group (control group) that showed the following values; 36.16±2.98 mg/dl, 0.62±0.03 mg/dl, 63.66±3.46 IU/l, 26.33±5.90 IU/l respectively, but no significant changes in the tested values of blood glucose and triglycerides between the ethanol and control groups. The effect of pre-treatment of rabbits with pomegranate peel extract at a dose of 200 mg/kg/day p.o. for 15 successive days on single oral dose of ethanol 80% at the 16 th day of experiment resulted in significant reduction in the values of blood glucose, serum urea, creatinine, ALT, and GGT (104±27.23 mg/dl, 53.5±0.42 mg/dl, 0.67±0.06 mg/dl, 47.66±4.49 IU/l, 18±5.14 IU/l) respectively in comparison with the ethanol group at ($P \leq 0.05$) where as triglycerides level was not significantly affected. In addition, the microscopic examination of the gastric samples of animals in the ethanol group showed saucer-shape erosion and Wedge-shape erosion in the superficial part of mucosa produced by necrosis and destruction of the superficial glandular tissue, besides the superficial gland in the mucosa are distorted, deeply basophilic and necrotic. (figure 4,5). Where as histopathlogical observations showed that, upon pomegranate peel extract pretreatment for the treated group could resulted in regenerated gastric lesion, and the distorted superficial glands in the mucosa are regenerated while some glands still necrotic. (figure 6).

Table (1): Effect of *P. granatum* peel ethanol extract on blood glucose, blood urea, blood creatinine, ALT, GGT, and triglycerides in 80% ethanol-induced gastric lesion model

Parameters / Group	B.Glucose Mg/dl	Urea Mg/dl	Creatinine Mg/dl	ALT U/l	GGT U/l	T.G Mg/dl
Control group (Normal saline)	169.5 ± 11.60 A	36.16 ± 2.98 A	0.62 ± 0.03 A	63.66 ± 3.46 A	26.33 ± 5.90 A	128.5 ± 16.05 A
Induction group (Ethanol 80%)	174 ± 0.36 A	46 ± 0.36 B	0.83 ± 0.003 B	96 ± 0.36 B	53 ± 0.36 B	128 ± 0.36 A
Treatment group (Ethanol 80% and *P. granatum* extract)	104 ± 27.23 B	53.5 ± 0.42 C	0.67 ± 0.06 C	47.66 ± 4.49 C	18 ± 5.14 C	111.16 ± 10.55 A

Different capital letters mean significant changes for vertical values at level ($p<0.05$). (Mean±SE, n=6 animals).

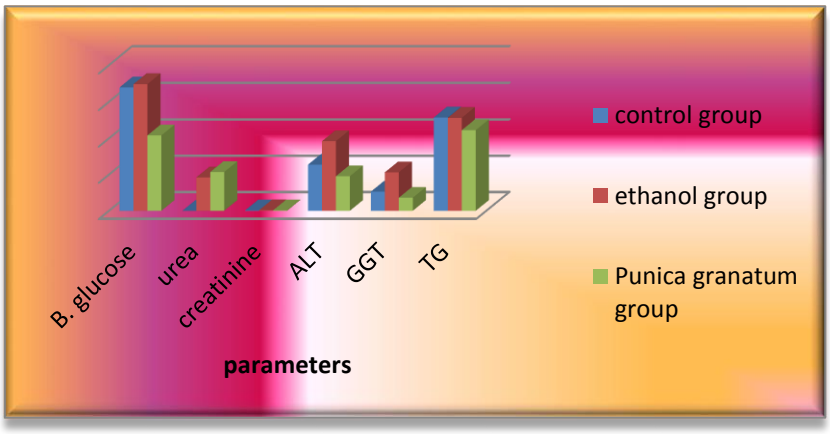

Fig. (1): Effect of *P. granatum* peel ethanol extract on blood glucose, blood urea, blood creatinine, ALT, GGT, and triglycerides in 80% ethanol-induced gastric lesion model.

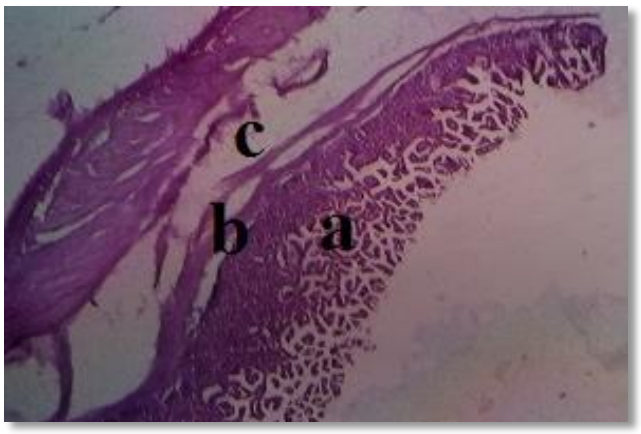

Fig. (2) stomach of control group: mucosa layer (a), submucosa with sparsely distributed collagen fibers (b) and muscularis layer (c). H&E, 40X.

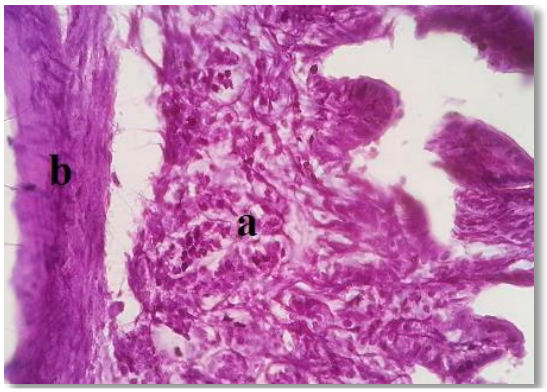

Fig.(3)stomach of control group: mucosa layer, submucosa with collagen fibers.H&E, 400X.

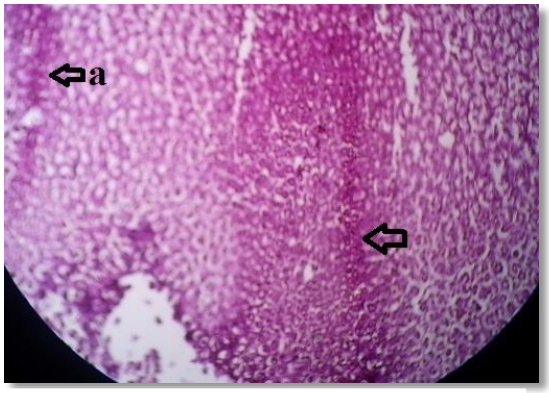

Fig.(4)Stomach of GII gastric lesion-induction group: there is saucer-shape erosion (a) and Wedge-shape erosion (black arrow) in the superficial part of mucosa, H&E, 100X.

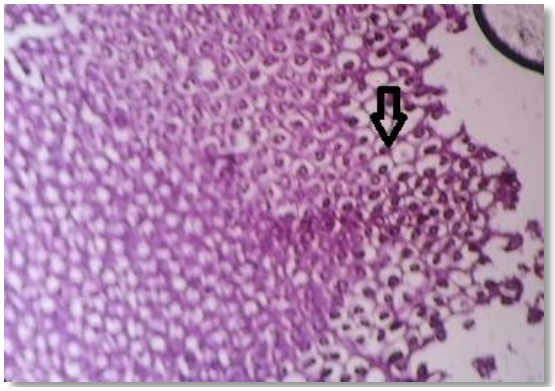

Fig.(5) stomach of Ethanol 80% group: the superficial gland in the mucosa are distorted, deeply basophilic and necrotic (black arrow). H&E, 100X.

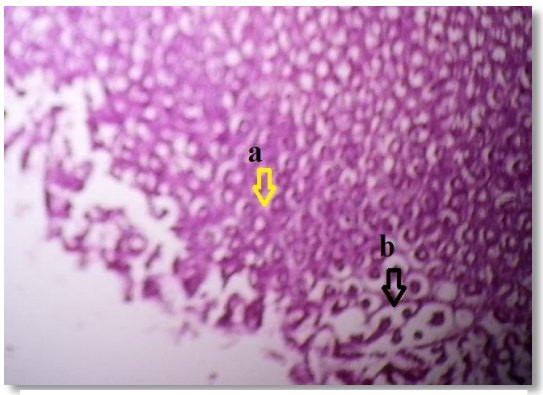

Fig.(6) Stomach of treated group: regenerated gastric ulcer, the distorted superficial gland in the mucosa are regenerated (yellow arrow), while some glands still necrotic (black arrow). H&E, 100X.

Discussion

Ethanol serves as a most common ulcerogenic agent and when given intragastrically to rabbits, it produces severe gastric hemorrhagic erosions (3) .The genesis of ethanol-induced gastric lesions is multifactorial with the depletion of gastric wall mucus content as one of the involved factors (16) and this damage induced by ethanol may be due to mucosal leukotriene release (17). Mucosal blood flow has also been attributed to be an important factor in the damage caused by alcohol and is modulated by prostaglandin (18). Submucosal venular constriction by ethanol and eventual injury is caused due to perturbations of superficial mucosal cells, (17) notably the mucosal mast cells leading to release of vasoactive mediators including histamine, that cause damage to gastric mucosa (19).Ethanol-induced damage to the gastric mucosa is associated also with a significant production of free radicals (oxidative stress) leading to an increased lipid peroxidation and damage. Antioxidants are compounds that prevent the oxidation of essential biological macromolecules by inhibiting the propagation of the oxidizing chain reaction. Keeping in mind the adverse effects of synthetic antioxidants, researchers have channeled their interest in the free radical scavenging activity of some natural antioxidants which are very effective to control the oxidative stress and hence prevent the initiation of disease propagation (20). Pomegranate peel extract has established

antioxidant properties that might have counteracted the oxidant effects of ethanol (21). So, the obtained results are similar to those recorded by Kamel et al., 1999 (22) who found that the pomegranate peel extract could reduce the gastric mucosal damage and lesions induced by intragastrically-given ethanol. Pretreatment with pomegranate peel extract resulted in reduction of blood glucose. Althunibat et al., 2010 (23) found that pomegranate peel methanolic extract has protective role against the oxidative damage in streptozocin-induced diabetic rats. The present study also showed improvement in the renal function tests by reducing the values of blood urea and creatinine in addition to improvement in liver function tests by decreasing the elevated liver enzymes ALT, GGT that were resulted from hepatocytes damage caused by ethanol. Many researchers demonstrated the nephroprotective effect of pomegranate peel extract against ferric nitrilotriacetate (13), cisplatin (24), mercuric chloride (25), gentamicin (26), and CCl4 (27) -induced renal oxidative damage in experimental animals. Besides its activity as hepatoprotective agent (28).Many plant polyphenols, such as ellagic acid, catechins, and chlorogenic, caffeic and ferulic acids act as potent antioxidant, antimutagenic and anticarcinogenic agents (29, 30). Nasr et al., 1996 (31) have reported that pomegranate peel extract contains ellagic acid, ellagitannins and gallic acids. The presence of these polyphenols in the pomegranate peel may be responsible for antiulcerogenic effect of peel extracts (32) that is believed to be related to the astringent property of tannins which are able to bind to protein so as to accelerate the healing of ulcer or trauma (33). As well as, the ability of this plant extract to protect the stomach against ethanol injury suggests that polyphenols participate in enhancing the mucosal barrier. Besides inhibition of the proton pump, polyphenols present free-radical-scavenging properties, a stimulatory effect on prostaglandin E2 and therefore of mucus secretion, the three main components of the gastric mucosal barrier (22). Hence, it can be suggested that the observed nephropreventive, hepatoprotective and gastroprotective activity of pomegranate peel ethanol extract in our study due to the presence of these compounds. In conclusion, we can say that, the high antioxidant and gastroprotective effect of the pomegranate peel extract appeared to be attributed to its high phenolics content. The results reported here raise the possibility that some polyphenols may be useful in gastroduodenal ulcer therapy.

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