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Investigation of Pomegranate Seed Oil Effects on the Renal Function in Alloxan-induced Diabetic Male Rabbits

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Abstract

Diabetes is a chronic disease which has reached pandemic proportions in a lot of countries of the world. In spite of the remarkable development in therapeutics chemistry, the usage of customary drugs is still a common practice for the treatment of diabetes. The current study was designed to investigate the effects of pomegranate seed oil (PSO) on kidney functions in the experimentally-induced diabetes in male rabbits. Diabetes was experimentally introduced by the intraperitoneal injection of alloxan monohydrate (150mg/kg BW). After three days of alloxan injection, samples were taken for the determination of glucose concentration. Serum glucose concentration of 200mg/ml was considered as an indication of animal diabetes. The experimental part was begun after 7 days of alloxan injection.

Thirty two adult male rabbits were arbitrarily separated into four groups and treated daily for 45 days. Group 1 was kept with no treatment, group 2 included animals treated with 30mg/kg BW PSO, group 3 included diabetic animals that received alloxan with a dose of 150mg/kg BW, and, finally, group 4 included animals that received alloxan (150mg/kg BW) and treated with PSO (30mg/kg BW). Fasting blood samples were collected by heart puncture technique after 45days of experiments to assess glucose levels.

Keywords: PSO, DM, renal histopathology, rabbit

التحري عن تأثير زيت بذور الرمان في وظائف الكلى لدى الارانب الذكور

المستحث فيها السكر تجريبيا

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الخلاصة

يعتبر مرض السكر من الأمراض المزمنة التي وصلت لمستوى وبائية في بعض دول من العالم على الرغم من التطور الحاصل في صناعة الأدوية والمكونات الطبية التجارية فما زالت المحاولات مستمرة لعلاج السكر. تهدف الدراسة الحالية للتحري عن التأثيرات المفيدة لزيت بذور الرمان في التغيرات الحاصلة في الكلى نتيجة الإصابة بمرض السكر المستحث تجريبياً في ذكور الأرانب. تم حث مرض السكر بحقن مادة الالوكسان (150 ملغم/ كغم من وزن الجسم) خلال البريتون بعد ثلاثة أيام من الحقن تم اخذ عينات من الدم لقياس

تركيز الكلوكوز و تم اعتبار تركيز السكر 200 ملغم/ مل من الدم في الأرانب المصابة بالسكر . تم بدء التجربة بعد 7 أيام من حقن الالوكسان لإحداث السكر
 تم استخدام 32 من ذكور الأرانب الناضجة بعمر ستة أشهر وكانت أوزانها تتراوح بين (1-2) كغم قسمت عشوائياً إلى أربع مجاميع تم معاملتها يوميا لمدة 45 يوم كما يأتي: مجموعة السيطرة اعتبرت كمجموعة سيطرة سالبة بدون أي معاملة، المجموعة الثانية هي حيوانات تم معاملتها بزيت بذور الرمان بتركيز 30 ملغم / كغم من وزن الجسم وتم اعتبارها مجموعة سيطرة موجبة، المجموعة الثالثة التي تم معاملتها بمادة الالوكسان بتركيز 150 ملغم / كغم من وزن الجسم ، أخيرا المجموعة الرابعة تم إعطاءها مادة الالوكسان بتركيز 150 ملغم / كغم من وزن الجسم مع زيت بذور الرمان بتركيز 30 ملغم / كغم من وزن الجسم تم جمع عينات الدم الصائم عن طريق خزعة القلب بعد 45 يوم من المعاملة

Introduction

Pomegranate (*Pomegranate granatum L.*), from the family Punicaceae, has been traditionally used as a medicinal and ayurvedic fruit for its anti-inflammatory, antimicrobial, cardiovascular protective, anti-diabetic, and anti-obesity properties [1]. Diabetes mellitus is among the most highly significant health challenges with enormous economic burdens to various communities around the world. Chronic hyperglycemia is linked with reactive oxygen species (ROS) production and induced long-term pathological damage, dysfunction, and fail of different organ, especially the kidneys, blood vessels, nerves, heart, and eyes, due to increased oxidative stress. In this study, the effects of PSO on oxidative stress markers, serum biochemical parameters, and pathological features in the kidney and heart tissues of alloxan-induced diabetic rabbits were investigated [2].

The global number of diabetic patient's was 3616 million in 2014, and it is projected to elevate to 552 million by 2030 [3]. Micronutrients, as food supplementation, have widely been improved and used to modulate glycemic problems [4]. Recently, PSO has received considerable (TNF- α) protein induced dysfunction, consequently glucose intake and resistance insulin may improve after administer of punicic acid [5]. There is an evidence that different fractions of pomegranate fruit can be used to treat a wide range of diseases, including hypertension, obesity, and diabetes [6]. PSO may reduce diabetes type 2 risks by ameliorating obesity-induced insulin resistance [7, 8]. PSO was reported to decrease body weight insulin and leptin levels, and type2 diabetes progression, while it enhances glucose tolerance and peripheral insulin sensitivity[9]. This study was designed to investigate the anti-diabetic effects of PSO on the experimentally-induced diabetes mellitus in rabbits.

Materials and Methods

The present study involved 32 male rabbits with an age of 6 months. Their weights were in the range of 1-2 kilogram after adaptation for 45days in the animal house of the College of Science, University of Baghdad. Randomly, the animals were separated into four groups

Group 1 included healthy untreated animals (negative control). Group 2 included animals given PSO (30mg /kg) for 45 days via oral administration by stomach tube (positive control). Group 3 included animals that received a single intraperitoneal (ip) dose of alloxan monohydrate (150 Mg /kg BW) to induce diabetes [10].

4- Group 4 included animals after 7 days of diabetes treated with PSO (30mg /kg BW) for 45 days [11].

Induction of diabetes was achieved by a single IP injection of 150mg/kg BW of monohydrate alloxan (Sigma chemical Co. USA.). The rabbits were subjected to fasting for 12 h before alloxan injection. Pomegranate was given orally for 45 days, after 7 days from alloxan injection. After blood collection via cardiac puncture [12], serum was used for biochemical tests and histopathological examination. ANOVA and LSD were applied to assess the significant differences among mean SAS Data [13].

Results and Discussion

Biochemical parameters

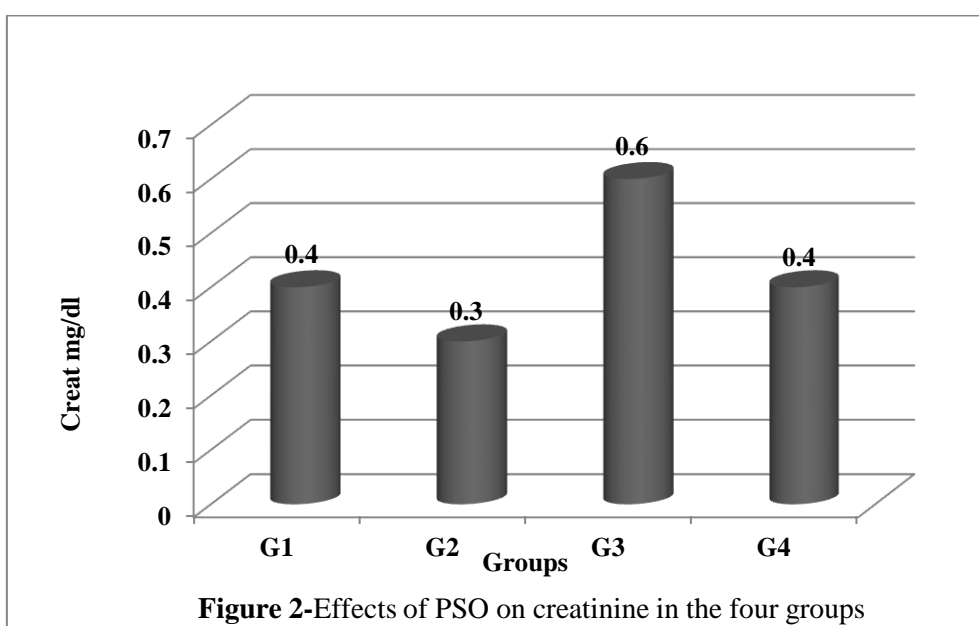
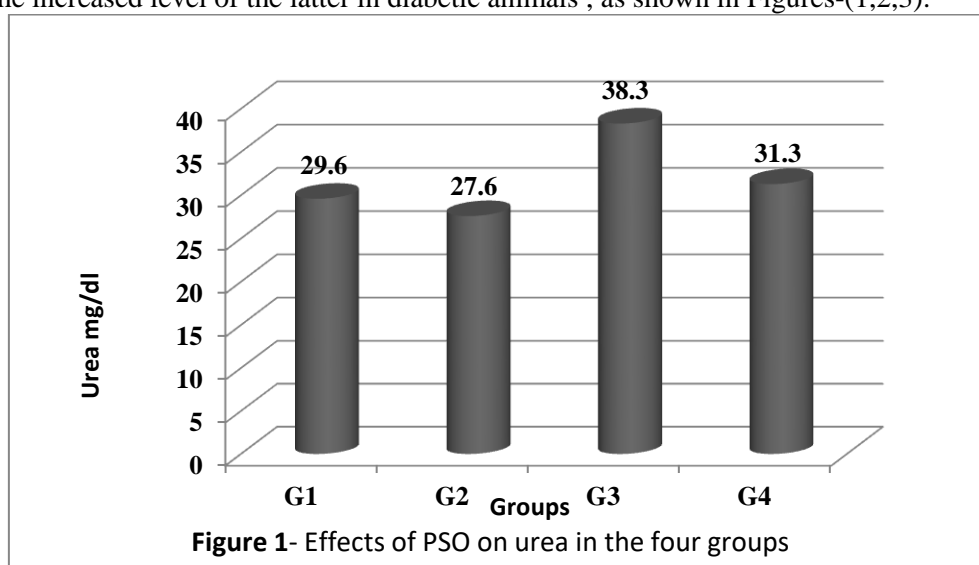
Results of the three biochemical parameters used to assess the effects on the renal functions in the experimental animals are shown in Table-1.

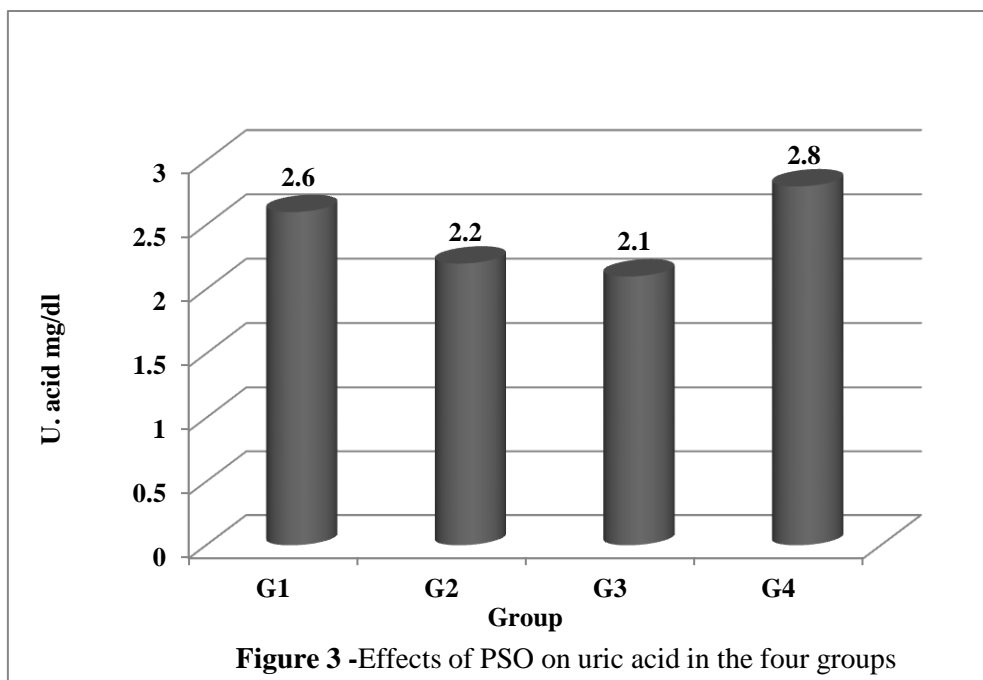
Table 1- Comparison among the different groups of rabbits in terms of renal parameters

Parameters	Mean \pm SE Groups				LSD value
	G1	G2	G3	G4	
Urea mg/dl	29.6 \pm 1.57 B	27.6 \pm 1.16 B	38.3 \pm 2.07 A	31.3 \pm 1.25 B	6.49 *
Creatinine mg/dl	0.4 \pm 0.05 A	0.3 \pm 0.01 A	0.6 \pm 0.07 A	0.4 \pm 0.05 A	0.327 NS
Uric acid mg/dl	2.6 \pm 0.07 A	2.2 \pm 0.05 A	2.1 \pm 0.05 A	2.8 \pm 0.06 A	0.831 NS

* (P<0.05)
Different letters in the same row indicate significant differences

As related to serum urea level, a significant (P<0.05) increase was found in the diabetic group (G3) (38.3 \pm 2.07 mg /dl) along with a significant (P<0.05) decrease in the diabetic group given PSO (G4) (31.3 \pm 1.25mg/dl) as compared to the control groups of G1 (29.6 \pm 1.57mg/dl) and G2 (27.6 \pm 1.16mg/dl) . There was also no significant differences in the levels of creatinine and uric acid, despite the increased level of the latter in diabetic animals , as shown in Figures-(1,2,3).





Histopathological examination

The histological sections of the kidneys of the control group (Figures-(1 and 2) show normal arrangement with no pathological changes of kidney. We observed normal glomerular tissue, tubules, Bowmen's capsule (BC), proximal tubule (PT), distal convoluted tubular (DCT), and macula densa (MD). In the treated groups (G3 and G4), severe vascular degeneration appeared in the renal tubules and glomerular mesangial cells, with mild vascular degeneration in the renal tubules. The medulla showed moderate vascular degeneration with necrosis of renal tubules and inter-tubular vascular congestion Figure-(3 and 4)



Figure 1- Section of renal cortex (Control) showing normal glomerular (G) and renal tubule (RT). H&E .400 xs

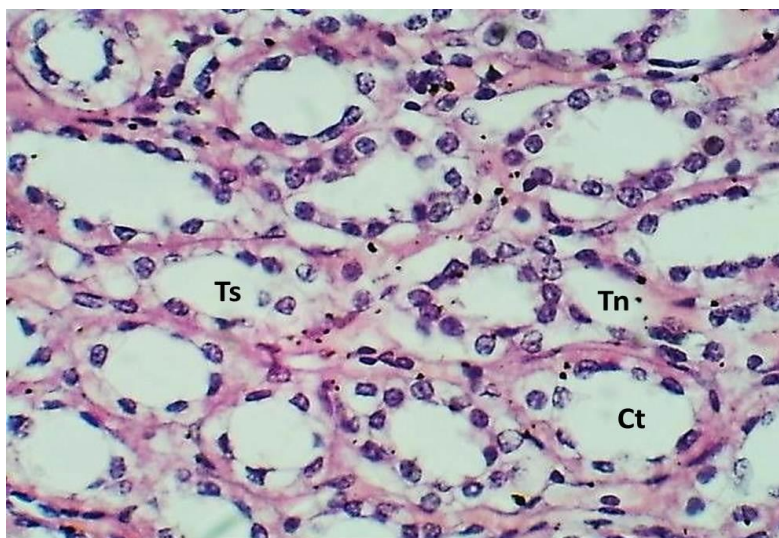


Figure 2-Section of renal medulla (Control) showing normal collecting tubules (Ct), thick segment (Ts), and thin segment of the loop of Henle (Tn). H&E .400 x.

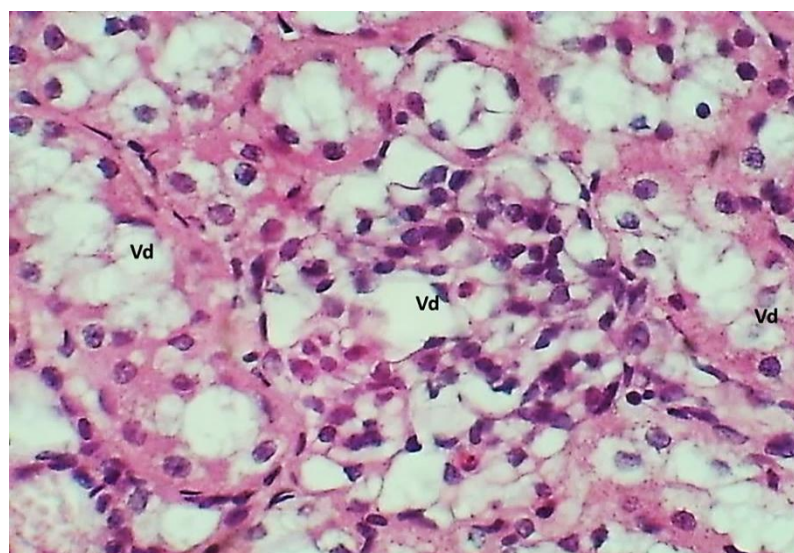


Figure 3-Section of renal cortex of diabetic- induced animal's showing severe vascular degeneration (Vd) of renal tubules and glomerular mesangial cells. H&E .400 x

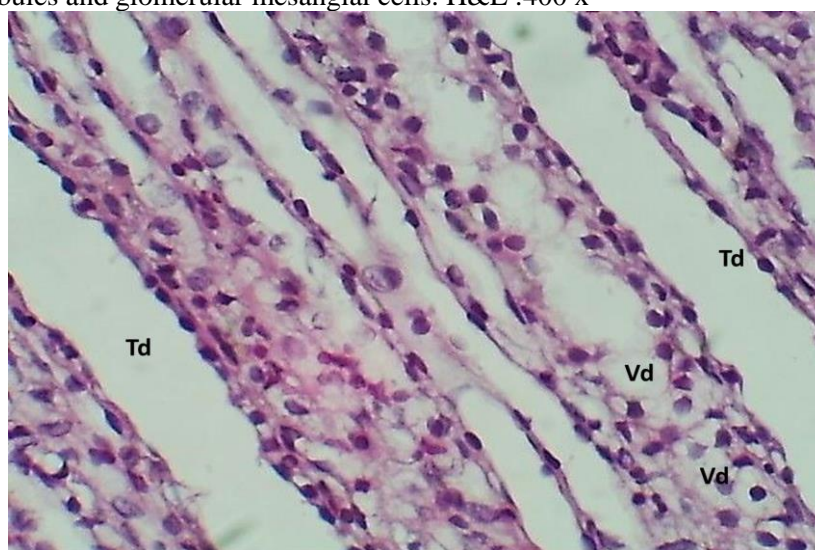


Figure 4-Section the of renal medulla of diabetic- induced animals showing severe vascular degeneration (Vd) of collecting tubules with tubular dilation (Td). H&E .400 xs

Oxidative stress during hyperglycemia is an important factor of complications in patients with diabetes mellitus, which include dyslipidemia and nephropathy. In this study, the alloxan-induced diabetes in the rabbits might cause alterations in the redox potential and generation of reactive oxygen species [14]. Various studies on humans suggested that food with higher intake of fruits and vegetables plays a role in the prevention of a wide range of kidney problems [15]. Diabetes induced the animals could have affected partially the functions of the kidney, as shown by the increased concentration of blood urea, while the statistical analysis did not indicate significant changes in creatinine and uric acid levels in the diabetic induced animals [16]. Nevertheless, urea returned to its normal concentration after the treatment of animals with PSO. This result agrees with that of previous studies which proved the therapeutic role of this plant in some renal diseases. This might be due to effects on certain oxidation mechanisms and reducing the levels of reactive oxygen species, nuclear factor- κ B (NF- κ B), inducible nitric oxide (iNOS), p38-mitogen-activated protein kinases (p38-MAPK), urea, uric acid, and creatinine [17]. In a recent study, PSO could reduce oxidative stress in tissues and blood by increasing the activity of antioxidant enzymes and decreasing ROS production in the mitochondria [18]. The antioxidant action of PSO is due to its contents of tocopherols, flavonoids, conjugated α -linolenic acids (CLn), and punicic acid, which is a conjugated isomer of α -linolenic and polyphenolic acids [19]. Oxidative stress reduction caused by PSO might be due to its contents of metallic chelating agents, single oxygen quenchers, and hydrogen donors [20].

From the histopathological results of the present study, histopathological changes were noticed along with the increase of serum urea level. Some earlier investigations showed that nephropathy diabetes is related to lipid peroxidation in the renal tissue with free radical-induced damage in the cell membranes [21]. The pharmacological and dietary benefits of plants like PSO and their phytonutrients in the prevention of kidney defects have not been well-established yet [22].

Conclusions

Additional *in vivo* and *in vitro* are required to test the claimed hypoglycemic and anti-diabetic actions of plants which has not yet been assessed in terms of the recognition of the active ingredients that may be sources for the development of new medicinal agents.

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