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Therapeutic Effect of *Punica granatum* Peels Extract against Nephropathy Induced by Streptozotocin in Rats

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In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract

The present study investigated the effects of *Punica. Granatum* peels extract on streptozotocin-induced diabetic rats for 21 days, as well as the biochemical, histopathological and immuno-histochemical effects in the kidney tissues. The aqueous methanol extract of *P. granatum* peels was administrated to STZ diabetes rats at doses of (100 and 200 mg/kg), using Amaryl as standard drug. After treatment, blood glucose, serum insulin, urea, creatinine, TNF-α, and Enzymatic scavengers' levels were measured. Diabetic rats given *P. granatum* extract had their fasting blood glucose and kidney antioxidant status reduced in a dose-dependent manner. Rats given *P. granatum* peel extract revealed substantially fewer histopathological observations than the diabetic control group, including tubular dilatation, tubular epithelium necrosis, glomerular atrophy, and interstitial inflammation. Moreover, after the administration of *P. granatum* extract (200 mg/kg), inducible nitric oxide synthase (iNOS) levels dropped significantly. Thus, the anti-hyperglycemic and antioxidant properties of *P. granatum* extract may expand the potential source for diabetes treatment.

Keywords: Anti-hyperglycemic, antioxidant properties; *Punica granatum* peels; streptozotocin-induced, diabetic rats..

1. Introduction

Diabetic nephropathy (DN) is one of the most serious implications of diabetes, and it can potentially lead to renal function failure in complicated cases [1, 2]. Diabetes has been linked to decreased urine concentrations of creatinine and uric acid, as well as increased levels of urea [3]. Since the synthesis of nitric oxide (NO) is crucial for regular glomerular activities, NO is created endogenously in order to maintain systemic blood pressure and renal function [4]. Furthermore, chronic hyperglycemia causes an increase in free radical generation, particularly reactive oxygen species (ROS), which causes protein glycosylation and glucose autoxidation in all tissues [5], as a result, Catalase (CAT), superoxide dismutase (SOD), and glutathione-s-transferase (GST) activity was reduced as a result of the damage to enzymes and cellular organelles [6]. Increased malondialdehyde levels, Are also, caused by an increase in free radicals and a loss in antioxidant defence mechanisms [7]. According to previous research, hyperglycemia-induced production of free radicals appears to be the first event in the activation of all pathways involved in the development of diabetes complications [8, 9].

Due to their efficacy and low incidence of side effects, herbal medications are currently gaining favour in the treatment of diabetes and its complications. Herbal medications have been shown to have anti-diabetic properties in traditional use. The phenolic antioxidant benefits against oxidative stress-mediated diseases are supported by biochemical and clinical evidence [10, 11].

Phenolic antioxidants' pharmacologic effects are typically attributed to their ability to scavenge free radicals, chelate metals, and influence gene expression and cell signaling pathways. [12, 13].

Punica granatum L. (*Punicaceae*), commonly called pomegranate, which is well known in literature as nature's power fruit, is an important source of polyphenols and antioxidants [14-16]. Pomegranates are becoming increasingly popular as a functional food and a source of nutraceuticals [17]. *P. granatum* is a plant used in traditional medicine to treat antitumor, anti-inflammatory, antiviral, antibacterial, antidiarrheal, anti-diabetic, and anti-obesity

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properties have also been demonstrated [18-21]. Fruit peels exhibit the highest level of antioxidant activity, which is in line with their high polyphenol content. [22]. The peels of pomegranates are high in phenolics, ellagitannins, flavonoids, proanthocyanidin compounds, complex polysaccharides, and a variety of minerals [23-25].

The goal of this study was to evaluate the therapeutic effects of *P. granatum* peels extract on hyperglycemia, oxidative stress, and anti-inflammatory effects in an experimental nephropathy diabetic rat model to the drug reference amaryl by assessing biochemical, histopathological and immunohistochemistry changes.

Material and methods

Plant material

Fresh *P. granatum* L. fruits were collected from Upper Egypt (October 2022).

Extraction of *P. granatum* peels extract

Upon separating the peels; they were washed with tap water, cut into small pieces and sun dried until complete dehydration. In a mortar, dried peels were pounded into a fine powder.

After that, the 500 g dry powder was extracted for 72 hours using 300 ml of 70% methanol aqueous solution in a Soxhlet system. In a rotary evaporator operating at 40–50°C, the extract was filtered and concentrated to dryness under reduced pressure, producing 14.5% (w/w) plant extract. The obtained *P. granatum* peels extract was stored at 5°C until usage. Rats were administered 100 and 200 mg/kg of plant extract (had been suspended in 100 mg/1 ml of warm distilled water) orally via stomach tubes.

Animals

Mature Sprague-Dawley rats of either sex weighing 180-200 g were purchased from the Animal House Colony of the National Research Centre (Dokki, Giza, Egypt). They were housed in clean polycarbonate cages in the animal house under standard laboratory conditions, which included 23±1 ◦C, 55± 5% humidity, and a 12-hour light–dark cycle. They were also fed a standard laboratory diet *ad libitum* and had free access to water. The National Regulations of Animal Welfare and the Institutional Animal Ethical Committee (IAEC) were followed when carrying out experiments.

Chemicals

Streptozotocin and diagnostic kits were purchased from Sigma (St. Louis, MO, USA). High analytical grade chemicals and reagents have been acquired from conventional commercial vendors.

Preparation of diabetic rats

Renal damage similar to human diabetic nephropathy was developed in a mouse model of Type 1 diabetes produced by STZ [26]. For the purpose of interpreting therapeutic interventions in preclinical diabetic renal impairment, this model is useful and widely recognized. Therefore, three days after receiving a single intraperitoneal injection of streptozotocin (65 mg/kg b.wt. dissolved in 10 mM citrate buffer pH 4.5), fasting blood sugar levels were assessed in the diabetic groups. The investigation involved rats whose blood glucose level was above 200 mg/dl [27].

Experimental design

Rats were divided into 7 groups each containing six rats (n = 6) were used in this study: Group 1 was normal control; Group 2 was Diabetic control; Group 3 was orally administered *P. granatum* peels extract at a dose of (100 mg/kg); Group 4 was orally administered *P. granatum* peels extract at a dose of (200 mg/kg); Group 5 was diabetic and orally administered amaryl (0.5 mg/kg); Group 6 was diabetic and orally administered *P. granatum* peels extract at a dose of (100 mg/kg); Group 7 was diabetic and orally administered *P. granatum* peels extract at a dose of (200 mg/kg). All measurement of the seven groups was assembled after two and three weeks of treatments.

Blood and Tissue Collection and Preparation

Rats were starved for the whole night and then slaughtered under light ether anesthesia 24 hours after the last treatment dosage. For additional research on the evaluation of oxidative stress markers, the serum was stored frozen at -20°C after the blood samples were obtained and centrifuged at 1500 rpm (4 °C). Part of the kidneys was homogenized (1 g/10 mL ice-cold potassium chloride; 150 mM). The homogenate was then used for the determination of different oxidative parameters. The remaining kidneys received standard procedures for trichrome and haematoxylin & eosin (H&E) staining after being maintained.

Biochemical analysis

After an overnight fast, blood samples were collected from the retro-orbital Venus plexus to measure the amounts of urea, creatinine, and glucose in the serum [28, 29]. Right after the completion of the study, all animals were sacrificed, and kidneys were extracted. A portion of the kidney was homogenized and used to measure the following: (a) lipid peroxidation (LPO), which was established by estimating the amount of malondialdehyde (MDA) using Uchiyama and Mihara's [30] method; (b) glutathione levels (GSH) using Moron et al method [31]; (c) nitric oxide (NO) using Miranda et al. [32]; and (d) catalase (CAT) activity using Aebi's method [33].

Following the manufacturer's instructions, TNF-α in kidney tissue was also measured using a commercially available ELIZA kit (KOMA BIOTECH, Korea).

Serum immune reactive insulin was determined by the radioimmunoassay method using

Amarsham insulin RIA kit; using rat insulin as the standard [34].

Histopathological examination

Under a light microscope, kidneys were taken for histological examination. The kidney specimens from every experimental group that was utilized were drawn out and fixed in 10% phosphate buffered formalin for a whole day. They were then dehydrated in ethyl alcohol that was progressively graded (50–100%), cleaned in xylene, and mounted in neutral gum. Sections of four micrometres in thickness were cut, stained, and examined under a light microscope with hematoxylin and eosin (H&E).

Immuno-histochemical study

All groups' kidney paraffin slices were subjected to immunostaining for iNOS. A primary anti-iNOS (1:100) (DAKO Corp. Denmark) had been utilized for this, followed by biotinylated horse antimouseanti serum, avidin-biotin complex and DAB as the chromogen. iNOS served as the positive control sample. In contrast, the primary antibody application stage was omitted for one of the kidney

specimens, which served as a negative control. The kidney tissues' cytoplasm displayed a dark brown color, signifying a favorable reaction.

Data analysis

Every result is displayed as the means ± the standard error of the means (SE). The least significant difference test (LSD) was used after one way analysis of variance (ANOVA) to compare data between groups. When $p<0.05$, the difference was deemed significant. These statistical tests were performed using Graph Pad Prism® software (version 5).

RESULTS

Biochemical studies

Blood glucose level of diabetic rats (441.20±3.55 and 442.20±12.60 mg /dl) was increased significantly ($p<0.05$) after 2 and 3 weeks respectively, as compared to the control group (99.80±13.13 and 102.00±3.24 mg /dl). The diabetic group's glucose levels were diminished in a dose-dependent manner upon treatment with *P. granatum* peels extract at doses of 100 and 200 mg/kg b.wt. with averages of 154.20±1.00 and 123.60±6.39 mg/dl respectively, after 2 weeks and 132.20±2.44 and 108.40±0.61 mg /dl respectively, after 3 weeks in the two-dose groups (6 & 7) when compared to diabetic control rats. Amaryl-treatment normalized the glucose level (116.80±3.57 and 118.80±2.70 mg/dl) after 2 and 3 weeks respectively, when compared with the diabetic control rats as illustrated in (Fig. 1).

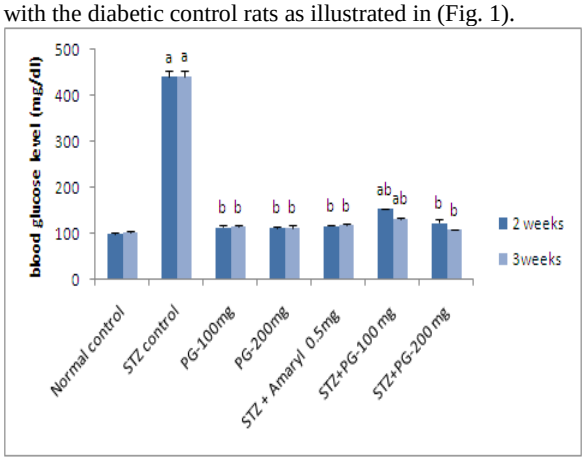

Fig.1: Effect of *P. granatum* at doses (100 and 200mg/kg) on blood glucose level in control and diabetic rats. ANOVA-one way followed by Tukey HSD test for multiple comparisons. [a] Significantly different from normal control at $P<0.05$. [b] Significantly different from STZ control at $P<0.05$.

For the insulin serum levels, the results showed that it decreased significantly in diabetic rats, after 2 and 3 weeks (5.40±0.23 µIU/ml and 4.83±0.33 µIU/ml) respectively, when compared to normal values (56.83±. 2.96 and 53.97±2.54 µIU/ml), while in *P. granatum* treated groups at doses of 100 and 200 mg/kg b.wt., it was determined to be markedly higher as compared to values of the diabetic control rats with the mean values of 14.74±0.21 and 17.89±0.44 µIU/ml after 2 weeks and 15.40±0.32 and 26.63±1.49 µIU/ml after 3 weeks, respectively. In addition, treatment with amaryl increased serum insulin after 2 weeks (52.11±2.80 µIU/ml) and 3 weeks (49.26±2.55 µIU/ml) when compared to diabetic control rats (Fig. 2). The level of serum creatinine in diabetic group was massively higher from 0.32±0.01 mg/dl in the normal values to 0.77±0.03 mg/dl. On the other hand, groups 6 & 7 (groups treated with plant extract at 100 and 200 mg/kg) and group 5 (amaryl treated group) gave the usual range with creatinine values (0.31±0.01, 0.27±0.00 and 0.34±0.02mg/dl, respectively) after 3 weeks when compared to diabetic rats (Table 1).

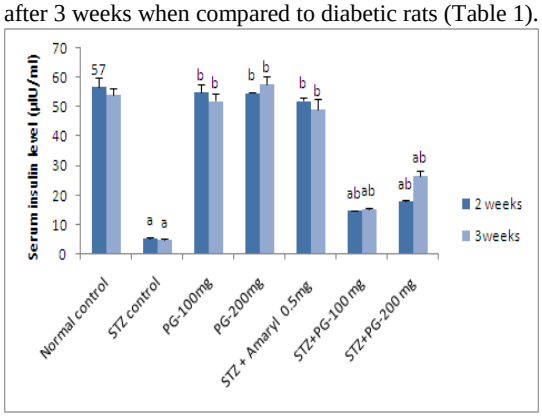

Fig.2: Effect of *P. granatum* at doses (100 and 200 mg/kg) on serum insulin level in control and diabetic rats. ANOVA-one way followed by Tukey HSD test for multiple comparisons. [a]Significantly different from normal control at $P<0.05$. [b] Significantly different from STZ control at $P<0.05$.

Table 1: Effect of *P. granatum* at doses (100 and 200 mg/kg) on serum creatinine and urea levels in control and diabetic rats

		Normal control	STZ control	PG-100 mg	PG-200 mg	Amaryl 0.5mg + STZ	PG-100 mg +STZ	PG-200 mg+STZ
Creatinine (mg/dl)	2 weeks	0.33±0.00	0.76±0.03[a]	0.26±0.00[b]	0.23±0.01[b]	0.36±0.00[b]	0.32±0.01[ab]	0.30±0.05[b]
	3 weeks	0.32±0.01	0.77±0.03[a]	0.26±0.00[b]	0.24±0.01[b]	0.34±0.02[b]	0.31±0.01[b]	0.27±0.00[b]
Urea (mg/dl)	2 weeks	29.07±0.68	66.43±0.30[a]	30.57±5.69[b]	40.14±0.08[b]	37.14±0.39[b]	63.79±0.36[a]	57.93±1.94[a]
	3 weeks	29.43±0.70	67.14±0.71[a]	28.79±5.23[b]	38.57±1.44[b]	37.50±0.39[b]	53.79±0.36[a]	47.71±0.50[ab]

ANOVA-one way followed by Tukey HSD test for multiple. Comparisons, [a] Significantly different from normal control at $P<0.05$. [b] Significantly different from STZ control at $P<0.05$.

The non-treated diabetic group's urea levels were similarly significantly higher (67.14±0.71 mg/dl) than normal control animals. But the urea serum level was significantly reduced in the *P. granatum* extract treatment groups (at 100 and 200 mg/kg b.wt) to 63.79±0.36, 53.79±0.36 and 57.93±1.94, 47.71±0.50 mg/dl, after 2 and 3 weeks respectively, showing a dose dependent effect. However, amaryl had similar effect to reduce urea concentration to be 37.50±0.39 mg/dl after 3 weeks when

compared to diabetic rats (Table 1). In the diabetic group, renal homogenate's MDA concentration significantly elevated by 6 folds comparing to the control rat (89.34±7.36 nmol/g tissue). *P. granatum* significantly reduced MDA level by 28% and 58% respectively, in (100 and 200 mg/kg –treated groups). Moreover, treatment with amaryl caused a significant reduction in MDA level by 83%after 3 weeks as compared with diabetic rats, as shown in Table 2.

After 3 weeks, the diabetic group's NO level increased by 54 percent as compared to the control group. Whereas, *P. granatum* (200 mg/kg b.wt) treated group and amaryl treated group produced a significant reduction in kidney NO level by 43% and 39% respectively, after 3 weeks as compared with diabetic rats (Table 2). When compared to the control group, GSH concentration in diabetic rat renal tissue dropped by 16 %. *P. granatum* extract or amaryl therapy resulted in a 14 % increase in GSH-renal content for both; after 3 weeks as compared with diabetic rats (Table 2). Diabetic rats have considerably reduced the CAT activity in tissue homogenate (0.09±0.02 g/g-tissue) when compared to the control group (0.41±0.01 g/g-tissue) at $p< 0.05$. The CAT activity improved significantly when treated with *P. granatum* at 100 and 200 mg/kg, (0.22±0.00; 0.28±0.02 and 0.25±0.01; 0.31±0.02 g/g-tissue, respectively) compared to the diabetic treated group. Furthermore, the effect of *P. granatum* 100 mg/kg, *P. granatum* 200 mg/kg, and amaryl 0.5 mg/kg on catalyse activity was demonstrated in (Table 2). Also, the results showed that kidney TNF-α content was increased in diabetic groups (20173.57±329.87 and 20499.83±283.60 mg/g-tissue) after 2 and 3 weeks when compared to normal values (13115.38±570.14 and 13387.27±300.47 mg/g-tissue). Kidney TNF-α content was decreased after 2 and 3 weeks in *P. granatum* 100 mg/kg (15899.51±482.85 and 15355.73±36.40 mg/g-tissue); *P. granatum* 200 mg/kg (15410.11±520.91 and13778.79±337.70 mg/g-tissue); amaryl (13708.10±385.77 and 14034.36±501 mg/g-tissue), respectively, in comparison with results of the diabetic control rats (Fig. 3).

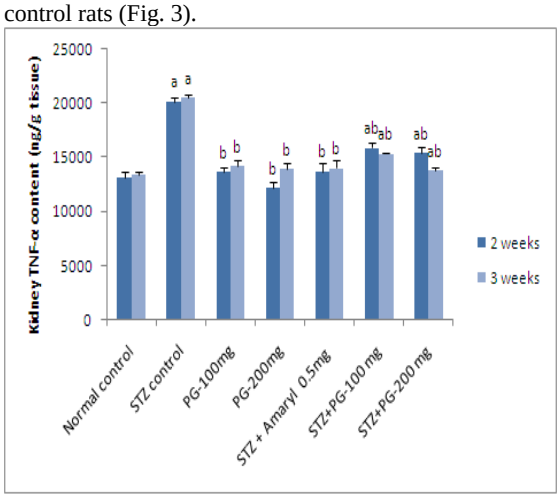

Fig.3: Effect of *P. granatum* at doses (100 and 200 mg/kg) on kidney TNF-α content. ANOVA-one way followed by Tukey HSD test for multiple comparisons. [a]Significantly different from normal control at *P<0.05*. [b] Significantly different from STZ control at *P<0.05*.

Histopathological studies

A histological examination of the control group's normal kidneys showed that the distal and proximal convoluted tubules, as well as the Bowman's capsule (urinary space), encircled the normal glomeruli (Fig. 4A).

Table 2: Effect of *P. granatum* at doses (100 and 200 mg/kg) on oxidative stress in control and diabetic rats

		Normal control	STZ control	PG-100 mg	PG-200 mg	Amaryl 0.5mg + STZ	PG-100 mg +STZ	PG-200 mg +STZ
NO µmol/l	2 weeks	24.96±1.39	39.79±0.19[a]	24.54±1.62[b]	24.33±1.28[b]	25.86±1.78[b]	37.67±0.92[a]	31.64±1.43
	3 weeks	26.83±1.86	41.32±0.55[a]	24.75±1.70[b]	23.28±1.54[b]	25.23±2.27[b]	34.47±2.90[a]	23.72±0.17[b]
MDA (nmol/g tissue	2 weeks	100.33±2.63	669.23±25.70[a]	81.21±4.85 [b]	78.68±7.52 [b]	91.87±4.05[b]	661.54±24.75[a]	642.86±4.40[a]
	3 weeks	89.34±7.36	576.92±9.19[a]	87.80±4.57[b]	81.98±6.13[b]	96.37±3.63[b]	415.60±50.4[ab]	239.78±0.89[ab]
GSH (mg/g tissue)	2 weeks	7.42±0.04	5.67±0.22[a]	7.07±0.33[b]	7.24±0.18[b]	7.03±0.34[b]	6.30±0.24	6.36±0.28
	3 weeks	7.50±0.18	6.31±0.01[a]	7.20±0.30[b]	7.32±0.15[b]	7.22±0.17[b]	7.19±0.02[b]	7.17±0.07[b]
Catalase (U/g tissue)	2 weeks	0.41±0.01	0.09±0.02[a]	0.38±0.00[b]	0.39±0.00[ab]	0.36±0.00[b]	0.22±0.00[ab]	0.25±0.01[ab]
	3 weeks	0.40±0.01	0.10±0.02[a]	0.39±0.01[b]	0.37±0.01[b]	0.37±0.00[b]	0.28±0.02[ab]	0.31±0.02[ab]

ANOVA-one way followed by Tukey HSD test for multiple. Comparisons, [a]Significantly different from normal control at *P<0.05*.
[b]Significantly different from STZ control at *P<0.05*.

There was not a single death among the treated rats administered the plant extract in the successive order in the sections from the groups receiving the dose (100 and 200 mg/kg b.wt). The diabetic group demonstrated atrophy of glomerular, glomerular lobulation, degenerated glomeruli, glomerular capillaries congestion and thickening of the basement membrane with dilation of Bowman's space (urinary space). Histopathological changes showed the renal tubules of diabetic rats exhibiting severe tubular epithelial necrosis and tubular hydropic degenerations. Pyknotic nuclei, vacuolated cytoplasm, and a deletion of brush border were observed in epithelial lining cells. Thickening of the tubular basement membrane and interstitial inflammation with haemorrhage was also noticed (Fig. 4B). Sections of kidneys from group treated with low and high dose of *P. granatum* peels extract (100 & 200 mg/kg b.wt) showed no signs of pathology except interstitial haemorrhage (Fig. 4C & 4D). The diabetic rats treated with amaryl displayed mild degeneration in the lining epithelium with little haemorrhage in between the tubules (Fig. 5A). Groups treated with *P. granatum* low dose (100 mg/kg) 2 weeks showed mild glomerular capillaries congestion, moderate tubular hydropic degeneration, with pyknotic nuclei and interstitial haemorrhage (Fig. 5B). Following administering a high dose of *P. granatum* (200 mg/kg b.wt) for two weeks, the diabetic group experienced healing characteristics equivalent to those of a normal glomerulus as well as the lack of necrotic cells in the proximal convoluted tubule (Fig. 5C). The kidney sections of the diabetic group treated with *P. granatum* low dose (100 mg/kg b.wt) 3 weeks showed almost glomerular lobulation, pyknotic nuclei and

interstitial haemorrhage (Fig. 5D). However, the groups that were treated with *P. granatum* high dose (200 mg/kg b.wt) 3 weeks demonstrated normal with the almost intact glomerulus and normal tubules with, pyknotic nuclei and interstitial haemorrhage was also noticed (Fig. 5E).

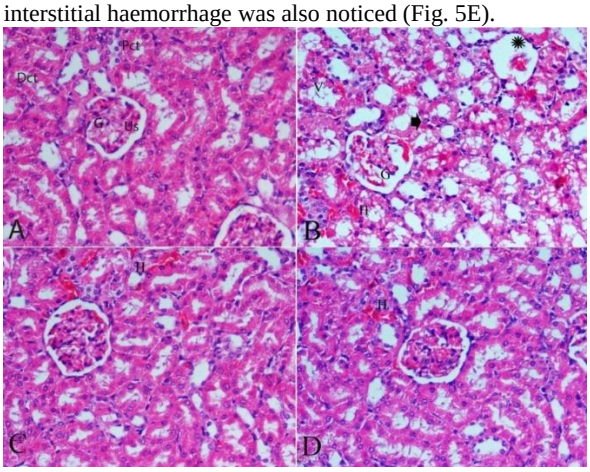

Fig.4: *P. granatum*, amaryl and diabetic rat
A: Normal control of Kidney tissue showing normal cellular architecture (H & E 400×).
B: Kidney of diabetic rat showing degenerative changes (G), glomerular capillaries congestion, the epithelial lining cells were disrupted with pyknotic nuclei (arrowhead), vacuolated cytoplasm (V), and interstitial hemorrhage (H) (H & E 400×). **C:** Kidney sections of *P. granatum* low dose showing normal cellular architecture of glomeruli and tubules with interstitial hemorrhage (H) (H & E 400×). **D:** Kidney sections of *P. granatum* high dose showing normal cellular architecture of glomeruli and tubules with interstitial hemorrhage (H) (H & E 400×).

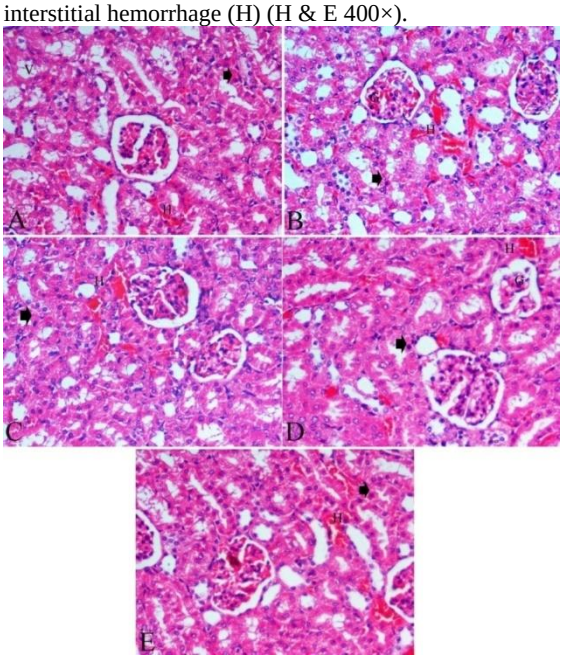

Fig.5: Effect of *P. granatum* (100 & 200 mg/kg) and amaryl (0.5 mg/kg) for 2 weeks and 3 weeks treatment on kidneys structures of rats with STZ-induced diabetes.
A: Kidney of diabetic rat and amaryl (0.5 mg/kg) showing mild change such as glomerular lobulation, pyknotic nuclei (arrowhead), vacuolated cytoplasm (V) and interstitial hemorrhage (H) (H & E 400×). **B:** Kidney of diabetic rat and *P. granatum* low dose (100 mg/kg) for 2 weeks showing mild histo-pathological changes of glomeruli and tubules with pyknotic nuclei(arrowhead)and interstitial hemorrhage (H) (H & E 400×). **C:** Kidney of diabetic rat and *P. granatum* high dose (200 mg/kg) for 2 weeks showing nearly normal cellular architecture of glomeruli and tubules with pyknotic nuclei (arrowhead) and interstitial hemorrhage (H) (H & E 400×). **D:** Kidney of diabetic rat and *P. granatum* low dose (100 mg/kg) for 3 weeks showing minimal histo-pathological changes of cellular architecture except shrinkage of some glomeruli with pyknotic nuclei the epithelial lining cells of tubules (arrowhead) and interstitial hemorrhage (H) (H & E 400×).**E:** Kidney of diabetic rat and *P. granatum* high dose (200 mg/kg) for 3 weeks show in nearly normal cellular architecture of glomeruli and tubules with pyknotic nuclei(arrowhead) and interstitial hemorrhage (H) (H & E 400×).

Immuno-histochemical studies

The immunological response to iNO in control rat slices was negative (Fig. 6A), while; the diabetic group had high levels of iNOS response in glomeruli and tubular cells whereby, positive immuno-histochemical staining of iNOS revealed a strong brown staining (Fig. 6B). Amaryl (0.5 mg/kg) administration for 3 weeks resulted in a minor iNOS reaction in the glomeruli and tubular cells (weak brownish staining) (Fig. 6C). After two weeks of therapy with *P. granatum* extract at a dose of 100 mg/kg b.wt, the expression of iNOS response in glomeruli and tubular cells was reduced (weak brownish staining) (Fig. 6D). Fairly weak immune reactivity for iNOS was observed in treatment with *P. granatum* (200 mg/kg b.wt) for 2 weeks (Fig. 6 E). Upon three weeks of therapy with *P. granatum* extract (100 mg/kg b.wt), iNOS reactivity in glomeruli and tubular cells was found to be minimal (weak brownish staining) (Fig. 6F). *P. granatum* (200 mg/kg b.wt) treatment for 3 weeks revealed in a reduced rate of iNOS reaction in the glomeruli and tubular cells (weak brownish staining) (Fig. 6G).

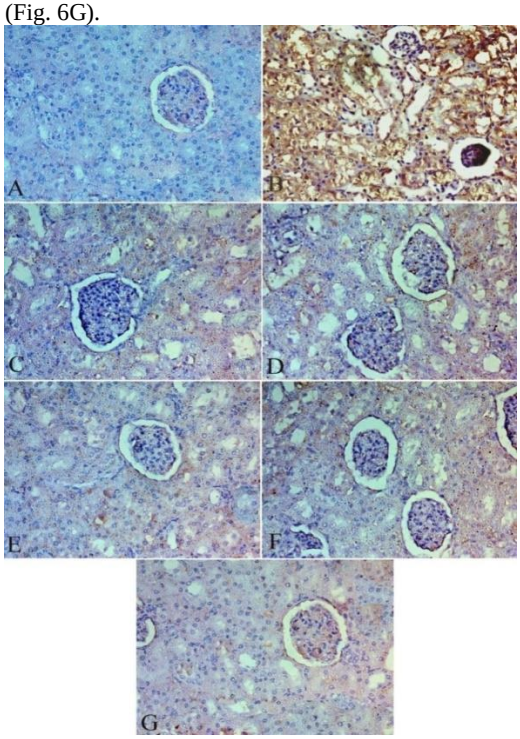

Fig. 6: Effect of *P. granatum* (100 and 200 mg/kg) and amaryl (0.5 mg/kg) for 2 weeks and 3 weeks treatment on kidneys structures of rats with STZ-induced diabetes staining with iNOS

A: Control group showing negative immune reaction of iNOS in the glomeruli and tubules. **B**: STZ- group showing strong positive immune reactivity for iNOS in the kidney tissue (deeply brown staining). **C:** STZ and amaryl showing mild positive immune reactivity for iNOS in kidney tissue (weak brownish staining). **D:** STZ and *P. granatum* (100 mg/kg) for 2 weeks showing week immune reactivity for iNOS in the kidney tissue (weak brownish staining). **E:** STZ and *P. granatum* (200 mg/kg) for 2 weeks showing fairly weak immune reactivity for iNOS in the kidney tissue (weak brownish staining). **F:** STZ and *P. granatum* (100 mg/kg) for 2 weeks showing minimally immune reactivity for iNOS in the kidney tissue (weak brownish staining) **G:** STZ and *P. granatum* (200 mg/kg) for 3 weeks showing very slight immune reactivity for iNOS in the kidney tissue (weak brownish staining) (iNOS immune-histochemistry, hematoxylin counterstain ×400**)**.

DISCUSSION

Result showed that induced diabetic rats (group 2) exhibited severe hyperglycemia, high serum urea, creatinine, and lowered insulin levels, all of which were accompanied by a rise in blood glucose. Previous research indicates that STZ diabetic rats are hyperglycemic and exhibit elevated oxidative stress [35, 36]. This is consistent with the possibility that elevated hepatic glycogenosis, gluconeogenesis in the absence of insulin, or decreased tissue insulin utilization contributed to the rise in blood glucose levels [37]. Furthermore, in the renal tissue homogenate, there was a considerable increase in MDA and NO levels, as well as a reduction in GSH and CAT levels. In the pathogenesis of diabetic nephropathy, oxidative stress has been implicated as a key mediator [38, 39]. Hyperglycemia [40] and rennin-angiotensin system activation [41], both contribute to the formation of reactive oxygen species (ROS). The present study investigated the therapeutic role of that *P. granatum* on renal tissue damage after the induction of DN in rat. The findings showed that *P. granatum* extract has antioxidant and anti-inflammatory activities, which reduced apoptosis in renal proximal tubule cells, alleviating STZ-induced nephrotoxicity and maintaining renal function.

In the current study, diabetic rats were orally administered with *P. granatum* peels extract had significantly lower blood glucose levels than diabetic control rats. This could be ascribed to the *P. granatum* extract's ability to repair existing pancreatic beta cells and enhance glucose transport to peripheral organs, validating the extract's anti-hyperglycemic efficacy as described in the preceding investigations [42, 43]. The hypoglycemic activity of this extract would be comparable to that of the reference medication (amaryl 0.5 mg/kg).

A significantly increase in insulin release was observed after the diabetic rat administration of *P. granatum* extract in a dose dependent manner. The ß cell activation; pancreatic protection, ß cell proliferation, and subsequent release of insulin have been postulated as the fundamental mechanisms of the antidiabetic effect demonstrated by the *P. granatum* extract [44].

Decrease in serum creatinine and urea observed in *P. granatum* extracts treated groups, indicates that the *P. granatum* ameliorated the loss of renal function and glomerular hyper filtration in STZ diabetic rats. Our results are in agreement with [45] who reported that the administration of pomegranate juice and pomegranate peel methanol extract in rats with chronic renal failure induced by adenine caused significant reduction in serum urea and creatinine compared to adenine fed rats, indicating improvement in renal function. In addition, in male rats having ethylene glycol-induced urolithiasis, Renal tissue oxalates, urine oxalate, calcium, and phosphate, serum creatinine, urea, and uric acid were all significantly decreased by a methanol extract of *P. granatum* peel. Such effects are likely related to pomegranate's antioxidant properties, which perform as a scavenger for oxygen radicals generated of oxidative damage [46].

Reactive oxygen species (ROS) are elevated in hyperglycemia and play a part in the development of several problems related to diabetes, such as diabetic nephropathy [47].

Our study showed that the treatment with *P. granatum* extract had enhanced the antioxidant defences of the cell in dose response manner, causing the antioxidant enzyme system (GSH and Catalase, NO level and MAL) in renal homogenate showed better results as well as by the studied treatments with amaryl. As indicated by Derakhshan et al., [48] and Singh et al., [49], *in-vitro* and *in-vivo* studies that the mechanism underlying the scavenging effects of *P. granatum* peels extract, involved inhibiting oxidative stress and increasing the antioxidant enzyme system.

The correlation between DN and inflammation is well-known, and inflammation frequently plays a critical role in the development of DN [50]. The co-administration of *P. granatum* to diabetic rats showed significantly attenuated the changes in inflammatory markers (TNF-α).

All of the above mentioned biochemical results were confirmed by histopathological studies. Most kidney sections exhibited signs of ailments similar to glomerulosclerosis in humans, including thickening of the glomerular membrane, severe tubular necrosis, and arteriolar hyalinization. Chronic glomerulosclerosis and fibrosis, together with a decline in kidney function that results in end-stage renal failure, are the hallmarks of diabetic nephropathy. According to Striker et al. [51] and Harvey et al. [52], hyperfiltration and vasodilation caused by the elevated synthesis of kallikrein and prostaglandin E2 in diabetic kidneys led to glomerular damage. Tubular necrosis, lymphocyte penetration in the interstitial spaces, and loss of brush barrier were reported in most kidney sections in the diabetic STZ group in the present research. In streptozotocin-induced diabetic rats, we also noticed glomerular hypertrophy and Bowman's space dilation. Diabetic glomerular hypertrophy, which occurs as the result of mesangial enlargement, is an early event in the evolution of glomerular disease. *P. granatum* extract administration reduced cell infiltration and enhanced tubular necrosis; it also resulted in a nearly normal Bowman's zone with glomerulus, basement membrane, and capillaries, as well as near-normal kidney architecture. These outcomes are consistent with previous early research that found *P. granatum* possess strong nephron-protective properties against renal damage caused by ferric nitrilotriacetate and ethylene glycol-induced nephrolithiasis [53, 54]. Furthermore, rats that received an aqueous extract of *P. granatum* in addition to gentamicin had less oxidative damage to their kidneys [55].

Moreover, in the current study showed that the diabetic rats receiving *P. granatum* peels extract had reduction in iNOS expression. These results agreement with the study

stated that the Pomegranate juice was found to be active and to have very powerful and unique therapeutic ingredients for nitrite/nitrate scavenging (NOx) [53, 56].

Conclusion

Antioxidants play a function in diabetes nephrotoxicity prophylaxis. It is possible that the presence of natural tannins and polyphenols in the plant extract explains the substantial antioxidant activity of P. granatum against STZ-induced kidney damage in diabetic rats. Consquently, it may be assumed that the aqueous methanol extract of P. granatum peels had an antidiabetic action and antidiabetic nephropathy problems.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

All data generated or analysed in this study are included in this article.

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