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# Antioxidant effect of pomegranate against streptozotocin-nicotinamide generated oxidative stress induced diabetic rats



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## ABSTRACT

Oxidative stress attributes a crucial role in chronic complication of diabetes. The aim of this study was to determine the most effective part of pomegranate on oxidative stress markers and antioxidant enzyme activities against streptozotocin-nicotinamide (STZ-NA)-induced diabetic rats. Male Sprague-Dawley rats were randomly divided into six groups. Experimental diabetes was induced by a single intraperitoneal injection (i.p), 15 min after the i.p administration of NA. Diabetic rats showed significant increase in plasma glucose level, and the significant decrease in plasma insulin level. The activities of antioxidant enzymes such as total antioxidant status (TAS), superoxide dismutase (SOD), and catalase (CAT) reduced while the levels of biomarkers of oxidative stress such as gamma-glutamyl transferase (GGT), and malondialdehyde (MDA) increased in diabetic control rats as compared to normal control rats. Oral treatment with pomegranate seed-juice for 21 days demonstrated significant protective effects on all the biochemical parameters studied. Besides, biochemical findings were supported by histopathological study. These results revealed that pomegranate has potential protective effect against oxidative stress induced diabetic rats.

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## 1. Introduction

Diabetes mellitus (DM) is the most crucial disease in the endocrine pancreas and cause of death and health problem in the modern society [1]. The main characteristic of DM

includes high level of blood glucose (hyperglycemia), and disordered metabolism lipid, carbohydrate, and protein are caused by insulin deficiency or insulin action or both [2]. Therefore, prolonged exposure to hyperglycemia an imbalance in glucose metabolism increases the generation of free radicals, which reduces abilities of the antioxidant defense system to correct and balance for this overproduction, and places an over exertion on the endocrine system [3].

Free radicals are involved in the pathogenesis of diabetes and the development of diabetic complications [4]. The elevated levels of blood glucose in diabetes are associated with increased lipid peroxidation (LPO), which may

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contribute to long-term tissue damage [5]. Various studies have demonstrated that DM is associated with oxidative stress, and resulting to increased production of ROS, including superoxide radical ( $O_2^\bullet$ ), hydroxyl radical ( $OH^\bullet$ ) and hydrogen peroxide ( $H_2O_2$ ) or reduction of antioxidant defense system [6,7]. There is a lot of evidence that short term and long-term hyperglycemia cause an activation of NADPH oxidase and formation reactive oxygen intermediate (ROI) which are involved in activation of transcription factor such as NF- $\kappa$ B [8]. The endothelium has been demonstrated as one of the major sources of ROI generation. These ROIs are able to induce a thrombogenic transformation of the vessel wall and cause for the endothelial dysfunction observed in diabetes, hypertension, and hypercholesterolemia [9]. In spite of the presentation of many hypoglycemic agents, diabetes and its related complications are still a major medical problem.

Streptozotocin (STZ) has been widely used for inducing diabetes in the experimental animals through its toxic effects on pancreatic  $\beta$ -cells [10]. STZ is noted to cause pancreatic B-cell damage, while NA is administered to partially protect insulin-secreting cells against STZ [11]. This model of diabetes is useful in studies of different aspects of diabetes [12]. The cytotoxic action of STZ is associated with the production of ROS that it causes oxidative stress and oxidative damage in the cells [13]. Lipid peroxidation (LPO) is main marker of oxidative stress. The increased oxidative stress, as determined by indices of increased LPO, depletion of endogenous antioxidant enzymes activities in plasma that they are commonly found in the STZ-NA induced diabetic rats [14].

Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging, neutralize free radicals or their actions, and diminishing oxidative stress [15]. Phytochemicals in fruits and vegetables play one of the important roles in preventing disease that caused by the result of oxidative stress [16]. The formation of ROS is inhibited by an antioxidant system that include non-enzymatic antioxidants (*i.e.* vitamin C, A, E, and glutathione), and enzymatic that means the enzymes regenerating the reduced forms of antioxidant, and ROS scavenging enzymes such as catalase (CAT), superoxide dismutase (SOD) [17]. Epidemiological studies have demonstrated that many phytonutrient of fruits might protect the human body against oxidative stress. The consumption of natural antioxidant phytochemicals was noted to have potential health benefits [18].

Pomegranate fruit has a fascinating history of traditional use as food, folk medicine, and cultural icon, dating back thousands of years [19]. The pomegranate fruit can be divided into three anatomical origins: seeds, peel, and arils, in which have precious compounds in different parts of the fruit. Pomegranate juice is an important product obtained that can be got from arils or from whole fruit. The potent antioxidant capacity of pomegranate and its components has been reported by numerous scientists using multiple *in vitro* assay systems [20]. This activity is largely due to the phenolic content and vitamin C constituents [21]. Pomegranate juice is obtained from arils that was reported to be composed of 85% water, 11% total sugars mostly fructose and glucose, 1.4% pectin, 0.2–1.0% polyphenols,

and organic acids organic acid like ascorbic acid, citric acid and malic acid [22]. Moreover, bioactive compounds such as phenolic compounds and flavonoids, principally anthocyanins [23], and minor compound include fatty acid,  $\alpha$ -tocopherol [24]. Another part of pomegranate fruit is seeds that are a rich source of total lipids. These lipids consist of 12% to 20% of total seed weight that characterized by a high content of polyunsaturated (PUFA) mostly linolenic ( $n-3$ ), linoleic ( $n-2$ ), and other lipids such as punicic acid ( $n-3$ ), stearic acid, oleic acid, and palmitic acid [25,26]. The seeds also contain sugars, protein, crude fibers, minerals, vitamins, pectin, polyphenols and isoflavones [27]. Nowadays, considerable focus has been considered to an intensive search for novel type of antioxidants from numerous plant materials [28]. Phenolic compounds are plant secondary metabolites commonly found in pomegranate with attributed pharmacological properties [29]. Among different kind of phenolic compounds, pomegranate that the class of each phenolic has demonstrated to contribute significantly to its unique biological properties [30,31]. The present study was undertaken to evaluate whether the pomegranate has potential to protect antioxidant status and ameliorate oxidative stress in oxidative stress generated induced diabetic rats. Histopathological study was also carried out to assess the effect of pomegranate on liver cells against STZ-NA damage in rats.

## 2. Experimental procedure

### 2.1. Animals

Forty-eight adult male Sprague-Dawley rats weight (250–280 g) were used in the study, and were obtained from the Animal House at the Faculty of Medicine and Health Sciences (FPSK). The ethics protocol was approved by animal care unit center for the purpose of control and supervision on experimental animals at UPM University, Malaysia (APPROVAL NO, UPM/FPSK/PADS/BR-UUH00502). The animals were housed in standard polypropylene cages with 12-h dark/light cycles at ambient temperature of  $25 \pm 2^\circ\text{C}$ . They were quarantined for one week, prior to experiment to acclimatize them to laboratory condition. The animals were handled in accordance with the Institutional Guidelines for the Care and Use of Animals (ACUC) for experimental purposes. The rats were provided the commercial standard pelleted diet and tap water *ad libitum*. The normal diet (PMI Feeds Inc. Lab Diet #5001) was a commercial laboratory rodent diet recommended for rats, mice and hamsters, with the following approximate chemical composition: protein 24%, fat 5.7%, fiber 6.0%, ash 8.0%, and carbohydrate 58%.

### 2.2. Chemical and drug

STZ was obtained from Sigma Chemical Co. (St. Louis, USA). Nicotinamide (NA) was purchased from Sigma-Aldrich (Co. P.O. Box, St. Louis, MO). Glibenclamide (Glyburide) was prepared from Sigma-Aldrich (Co. P.O. Box G-0639). All other chemicals and reagents were used of analytical grade.

### 2.3. Induction of diabetes in experimental rats

Rats were fasted overnight for 12-h before injection. They were induced by a single intraperitoneal (i.p) injection of STZ (60 mg/kg body weight (b.w.)). STZ was dissolved freshly in 0.05 M citrate buffer, (PH 4.5) for this injection. Nicotinamide was dissolved in normal saline and administered (120 mg/kg, i.p) 15 min before STZ [12]. The development of hyperglycemia was confirmed by the elevated glucose levels in blood, determined at 72 h, and then on day 7 after injection, which was confirmed by measuring fasting blood glucose levels in Rats' blood taken from tail vein. The rats with fasting blood glucose level higher than 126 mg/dl or 7 mmol/L served as diabetic rats and for normal control rats remained 4.2 mmol/L [32]. Glucose measurement was performed with an Accu-Chek glucometer (Roche, Germany).

### 2.4. Preparation of pomegranate for treatment

Ripe sweet red pomegranate fruits were used in this study, were imported from Spain. The fruit was washed, peeled, and the arils were crushed and squeezed with squeezing machine (National Juicer-Blender). The juice is filtered for any water insoluble and stored at  $-18^{\circ}\text{C}$ . The seeds (PS) from the juice preparation were freeze dried at  $-20^{\circ}\text{C}$  separately and ground into powder to produce the second and third treatment. The powder was diluted with distilled water (DW) with 100 mg PS in 1 ml DW and extensively vortex mixed, and then centrifuged at 3000 rpm for 20 min at  $4^{\circ}\text{C}$ . Throughout the study, 1 ml of treatment is force fed by intragastric tube to each rat.

### 2.5. Preparation of Glibenclamide

Sulfonylurea is anti-diabetic drugs such as Glibenclamide (GC), long used in the treatment of non-insulin dependent diabetes mellitus [33]. Research from the antioxidant studies suggests that GC has the potential to counteract the reactive oxygen species mediated oxidative stress [34,35]. Glibenclamide (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) before it was administered.

### 2.6. Experimental design

All rats randomly were divided into six groups (Table 1), each group consists of eight rats that they were placed in the cages (two rats in each cage), and were treated orally with pomegranate seed and juice for a period of 21 days as follow:

**Table 1**  
Type of diet and treatment for the rats according to the group.

Group	Characteristic of rat	Type of treatment	Amount of treatment
NC	Normal control	Distilled water (DW)	1 ml per rat
DC	Diabetic control	Distilled water	1 ml per rat
PJ	Diabetic treated	Pomegranate juice	1 ml PJ per rat
PS	Diabetic treated	Pomegranate seed powder	100 mg PS + 1 ml DW per rat
PSJ	Diabetic treated	Pomegranate seed-juice	1 ml PJ + 100 mg PS per rat
GC	Diabetic treated	Glibenclamide	5 mg/kg b.w.

At the end of the experimental period, the animals were fasted overnight and blood samples were collected from the rats on day 21. The animals were sacrificed under diethyl ether anesthesia, following overnight fasting, and blood sample was collected before sacrifice via intra-cardiac puncture using heparin and sodium citrate as anti-coagulants. The heparinized samples were centrifuge at 3000 rpm for 15 min to separate the plasma and erythrocytes. Then, the plasma samples were transferred into Hitachi cup for storage at  $-80^{\circ}\text{C}$ . Plasma is analyzed by using chemical analyzer. Liver tissues were immediately dissected, washed in ice-cold saline to remove blood. The liver was selected because of high generation of free radical in their tissues.

### 2.7. Biochemical analysis

#### 2.7.1. Determination of plasma glucose and plasma insulin

Plasma insulin was determined by using a commercial kit (Mercodia Rat Insulin ELISA, Catalog No. 10-1250-01) that provides a method for quantitative determination of insulin in rat (Mercodia AB, Sylveniusgatan 8A, Sweden). Plasma glucose levels were estimated using a commercial Glucose Colorimetric Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA).

#### 2.7.2. Estimation of malondialdehyde (MDA)

Lipid peroxidation is estimated indirectly by the measurement of the secondary products, like malondialdehyde (MDA) [36]. MDA level of the plasma was measured by the following procedure in which 0.5 ml of plasma was shaken with 2.5 ml of 20% trichloroacetic acid (TCA) in a 10 ml centrifuge falcon tube. 1 ml of 0.6% thiobarbituric acid (TBA) was added to the mixture, shaken, and warmed for 30 min at  $90^{\circ}\text{C}$  boiling water bath followed by rapid cooling with cold water. The mixture was centrifuged at 3000 rpm for 10 min then it was shaken into a 4 ml of nbutyl-alcohol layer in a separation tube and MDA content in the plasma was determined by spectrophotometer at the absorbance 532 nm against butanol. The results were expressed as  $\mu\text{mol/ml}$  plasma [37].

#### 2.7.3. Estimation of gamma-glutamyl transferase (GGT)

*In vitro* test for quantitative determination of GGT in plasma was used Roche/Hitachi cobas c systems. This GGT colorimetric assay is a modification of the Szasz procedure. GGT catalyzes the transfer of the gamma-glutamyl group from the substrate gamma-glutamyl-3-carboxy-4-nitroanilide, to glycylglycine, yielding 5-amino-2-nitrobenzoate. The change in absorbance at 410/480 nm

is due to the formation of 5-amino-2-nitrobenzoate and is directly proportional to the GGT activity in the sample [38].

#### 2.7.4. Estimation of superoxide dismutase (SOD)

Determination of plasma SOD was done by method of Kakkar et al. [39]. Briefly, 1.35 ml of double distilled water, 50  $\mu$ l of sodium pyrophosphate buffer (PH 8.3), 0.1 ml of phenazine methosulphate (PMS) and 0.3 ml of nitroblue tetrazolium (NBT) were mixed. 0.2 ml of NADH solution is added to it to initiate the reaction. After incubation at 39 °C for 90s, the reaction was terminated by adding 1 ml of glacial acetic acid. 4 ml of n-butanol was added and the mixture was centrifuged at 4000 rpm for 10 min and the absorbance of the upper butanol layer recorded at 560 nm. For the comparison, corresponding blank was prepared in the same way except addition of the plasma. One unit of SOD activity is defined as that amount of enzyme reaction, which inhibits the rate of reactions, by 50% in 1 min under assay conditions.

#### 2.7.5. Estimation of catalase (CAT)

This assay employs the quantitative sandwich enzyme immunoassay technique by ELISA kit. Antibody specific for CAT has been pre-coated onto a micro-plate.

#### 2.7.6. Estimation of total antioxidant status (TAS)

Total antioxidant status was measured in plasma by Selectera II/E Chemical Analyzer (catalog no. NX 2332; Randox Laboratories Limited, Antrim, United Kingdom) applicable for COBAS MIRA (Hoffmann-La Roche, Basel, Switzerland) and flexor analyzer [40].

#### 2.8. Histopathology study

The liver samples fixed in the 10% formalin which were sliced about 1 cm thick, and placed into the cassettes. Then, the cassettes were put into tissue processor machine, which comprise of dehydration with alcohol, clearing with xylene and wax, following with impregnating process automatically for overnight (14 h). The cassettes

were embedded in molten paraffin, which later cooled down and formed blocks paraffin. Each block was trimmed then sectioned about 5  $\mu$ m by using a microtome. Then thin sections were put in water bath at 45 °C few seconds, fished out, and set on a microscopic glass slide, proceed with hematoxylin and eosin (H&E) staining, followed by mounting with DPX and observed under light microscope for evaluation.

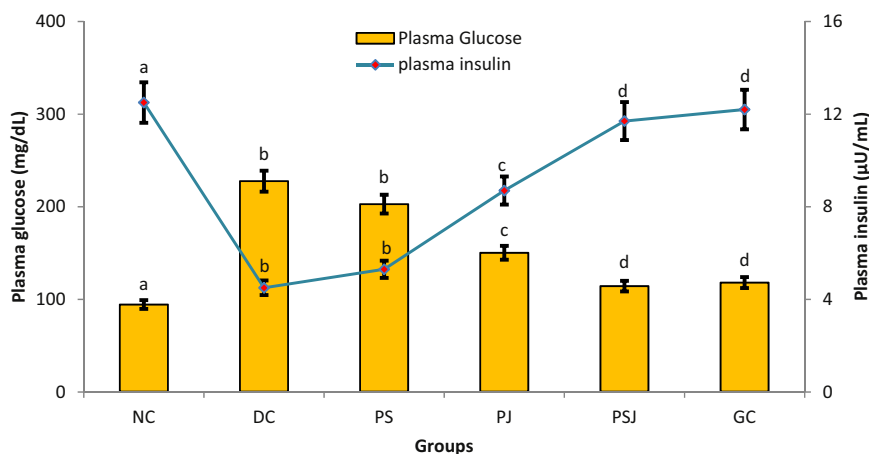
#### 2.9. Statistical analysis

Statistical analysis was performed using SPSS software for windows, version 21 (SPSS, Inc., Chicago, USA). The data was presented as the mean  $\pm$  S.E.M. (standard error of mean). The statistical significant of data analysis has been assessed by one-way analysis of variance (ANOVA) and significant difference among treatment groups were evaluated by Tukey HSD range test. The results were considered statistically significant at  $p$  value less than 0.05.

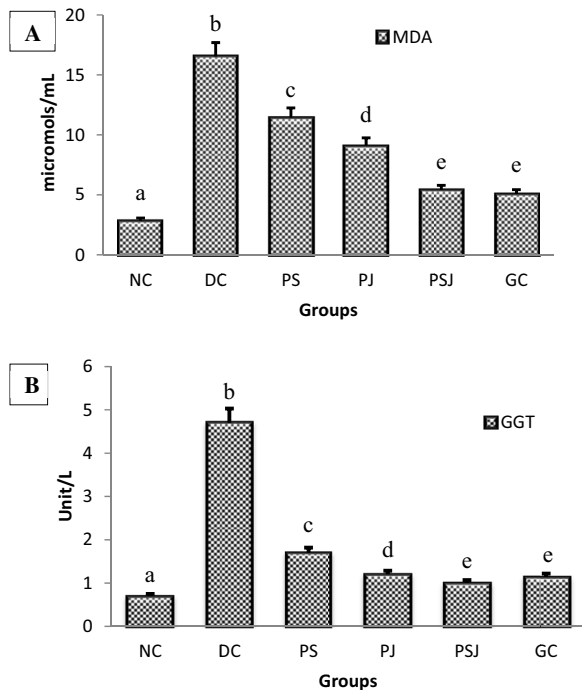
### 3. Results

Fig. 1 shows plasma insulin levels decreased significantly ( $p < 0.05$ ) in the STZ-NA induced diabetic rats, and plasma glucose level significantly ( $p < 0.05$ ) increased compared with the normal control rats. The supplementation of pomegranate and Glibenclamide increased the plasma insulin levels and reduced glucose levels significantly ( $p < 0.05$ ) in the induced diabetic rats. The effect was more pronounced in the PSJ supplemented rats than in the PS or PJ rats.

Plasma levels of MDA and GGT have been demonstrated to be a reliable marker oxidative stress. Fig. 2 shows that these parameters were significantly ( $p < 0.05$ ) increased in diabetic control in comparison with normal control rats. However, treatment of diabetic rats with pomegranate demonstrated a significantly ( $p < 0.05$ ) reversed the concentration of MDA and GGT in the plasma. This reduction more significant ( $p < 0.05$ ) in PSJ than other treatment



**Fig. 1.** Combined changes in the levels of plasma glucose and insulin in normal diabetic rats, Each values is mean  $\pm$  S.E.M. for 8 rats in each group. <sup>a-d</sup>In each bar means different significantly at  $p < 0.05$ . NC: normal control, DC: diabetic control, PS: pomegranate seed, PJ: pomegranate juice, PSJ: pomegranate seed-juice, GC: Glibenclamide.



**Fig. 2.** Effect of pomegranate on plasma MDA (A) and GGT (B) plasma in controls and treatment rats, each value is mean  $\pm$  S.E.M. for eight rats in each group. <sup>a–e</sup>In each bar means different significantly at  $p < 0.05$ . NC: normal control, DC: diabetic control, PS: pomegranate seed, PJ: pomegranate juice, PSJ: pomegranate seed-juice, GC: Glibenclamide.

groups. There was not significantly ( $p < 0.05$ ) difference between PSJ and GC groups.

Table 2 demonstrates the activities of enzymatic antioxidants namely SOD, CAT, and TAS in the plasma of controls and treatment groups. A significant ( $p < 0.05$ ) reduction in the activities of enzymatic antioxidant in STZ-NA administered rats was observed. However, treatment with pomegranate increased the level of enzymatic antioxidant in the plasma. This increases more significant ( $p < 0.05$ ) in PSJ than other treatment groups.

Fig. 3A–D illustrates the micrographs of H&E staining on hepatic tissues in normal control and experimental rats. Normal control shows the same normal hepatic structure found in other mammals. Each lobule is made up of cells (hepatocytes) that appear a network around a central vein and narrow sinusoids. Pathological changes of STZ-NA-induced rats in liver include sinusoidal and central vein dilation, inflammation around central vein, and necrosis

in hepatocytes. These changes decreased in diabetic rats treated with PSJ.

#### 4. Discussion

Diabetes arises from destruction of  $\beta$ -cell due to the toxic effect of STZ, which facilitates preferential uptake into pancreatic  $\beta$ -cells through GLUT2 [41]. There is clear evidence that free radicals play an essential role in the mechanism of DNA damage and cytotoxicity by STZ. It has been reported that STZ causes radicals generation by the xanthine oxidase system of pancreatic cells [42,43], and stimulates  $H_2O_2$  generation. As result, it leads to DNA fragmentation and necrosis in the pancreatic  $\beta$ -cell islets. Therefore, the rate of insulin synthesis is reduced [44]. Injection of NA, a poly-ADP-ribose synthetase inhibitor, protects the  $\beta$ -cells function by preventing the reduction in the level of NAD (nicotinamide adenine dinucleotide); thereby the inhibition of insulin secretion partially reverses and it prevents the aggravation of experimental diabetes following by administration of STZ [45]. This circumstance contributes a number of characteristics similar to type 2 diabetes mellitus.

Pomegranate is rich in antioxidants of the polyphenolic class. The antioxidant level in PJ was discovered to be higher than that in other natural fruit juices such as orange, blueberry, and cranberry, as well as in red wine [46]. These antioxidants showed various biological activities such as eliminating free radicals, inhibiting oxidant and microbial growth [47].

Damages in the pancreatic  $\beta$ -cell clinically associated with the development of diabetes [48]. In this circumstance, hyperglycemia leads to the generation of free radicals, which can exhaust antioxidant defense system, thereby it causes oxidative damage to cells membranes and strengthened susceptibility to LPO [49].

Our present exploration showed a significant increase of plasma LPO levels in diabetic rats. Administration of pomegranate to diabetic rats significantly decreased the levels of MDA. Pomegranate acts as antioxidant by scavenging free radicals that lead to decrease LPO in diabetic rats.

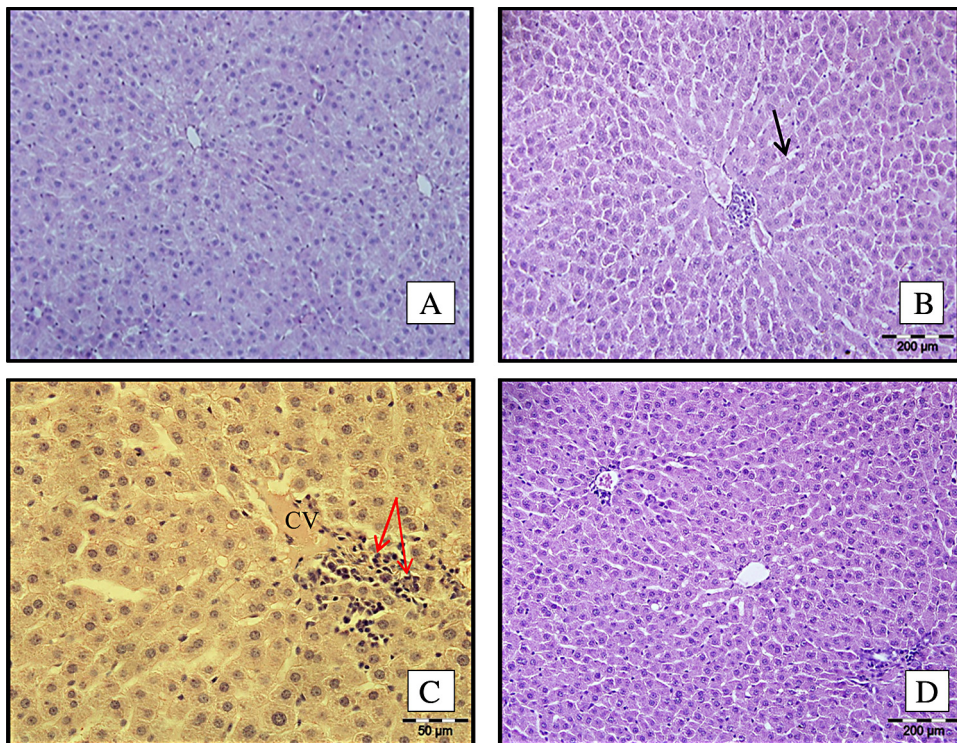
The primary role of GGT ectoactivity is to metabolize extracellular reduced glutathione (GSH), allowing for precursor amino acids to be assimilated and reutilized for intracellular GSH synthesis; in this way, a continuous “GSH cycling” across the plasma membrane occurs in a number of cell types [50]. Thus, ectoplasmic GGT favors the cellular supply of GSH, the most important non-protein antioxidant of the cell [51]. GGT plays a crucial role in antioxidant defense system [52], and cellular GGT is sensitive marker

**Table 2**

The effect of pomegranate on the activities of SOD, CAT, and TAS in controls and treatment.

Parameters	Groups					
	NC	DC	GC	PS	PJ	PSJ
SOD (unit/ml)	34.0 $\pm$ 0.62 <sup>a</sup>	17.2 $\pm$ 0.51 <sup>b</sup>	32.1 $\pm$ 0.95 <sup>c</sup>	20.8 $\pm$ 0.61 <sup>d</sup>	25.1 $\pm$ 0.42 <sup>e</sup>	28.9 $\pm$ 0.8 <sup>f</sup>
CAT (unit/ml)	27.6 $\pm$ 0.90 <sup>a</sup>	11.6 $\pm$ 1.00 <sup>b</sup>	22.0 $\pm$ 1.21 <sup>c</sup>	13.9 $\pm$ 2.63 <sup>d</sup>	19.3 $\pm$ 1.13 <sup>e</sup>	21.3 $\pm$ 1.15 <sup>f</sup>
TAS (mmol/L)	1.19 $\pm$ 0.07 <sup>a</sup>	0.69 $\pm$ 0.03 <sup>b</sup>	0.83 $\pm$ 0.02 <sup>c</sup>	0.73 $\pm$ 0.11 <sup>b</sup>	0.91 $\pm$ 0.01 <sup>d</sup>	1.03 $\pm$ 0.03 <sup>a</sup>

Each value is mean  $\pm$  S.E.M. for eight rats in each group. <sup>a–f</sup>in each row means with different superscript letter significantly at  $p < 0.05$ .



**Fig. 3.** Liver micrograph sections of normal and diabetic rats treated with PSJ. (A) Normal control shows normal architecture of the hepatocytes around the central vein with sinusoidal cords around the central vein and portal tracts (H&E X20). (B) Diabetic section untreated shows dilated sinusoids (black arrow) and dilated central vein (H&E X20). (C) Micrograph with higher magnification shows inflammation (red arrows) around central vein and necrosis in the hepatocytes (H&E X40). (D) Diabetic rats treated with PSJ shows near normal hepatocytes, mild sinusoidal dilatation without any inflammation around central vein when compared to diabetic liver (H&E X20).

and enzyme related to the generation of ROS and oxidative stress. Several previous studies demonstrated that the inverse association between serum antioxidants and GGT was confirmed [53]. A higher consumption of fruit inversely predicts GGT level. Our present finding demonstrated a significant increase in the level of GGT in diabetic groups. While, PSJ acted as antioxidant by scavenging free radicals, as result it can reduce in GGT in diabetic treatment group as same effect of Glibenclamide.

Antioxidant enzymes form the first line of defense against ROS in the cells include SOD, CAT, which play a key role in scavenging the toxic intermediate of incomplete oxidation. A reduction in the activities of these antioxidant enzymes can result in an excess availability of ( $O_2^{\cdot-}$ ) and  $H_2O_2$ , which in turn production  $OH^{\cdot}$ , lead to initiation and propagation of LPO. SOD protects cells against ROS by scavenging ( $O_2^{\cdot-}$ ), which damages the membrane and biological structures [54]. SOD can catalyze dismutation of ( $O_2^{\cdot-}$ ) into  $H_2O_2$  then deactivated to  $H_2O$  by CAT [55]. Thus, SOD can act as a main defense against ROS and prevent further production of free radicals. The activity of SOD was discovered to be lower in diabetic subjects because of inactivation by  $H_2O_2$  or by glycation of the enzyme, which have been noted to occur in diabetes. CAT is a heme-protein, which is present almost in all mammalian cells and is responsible for the reduction of  $H_2O_2$  for protecting cells from highly reactive  $OH$  radicals [56]. The reduction in CAT activity could also result from inactivation by glycation

of the enzyme. Any combination with antioxidant properties might contribute to the partial or total alleviation of oxidative damage. As a result, removing  $O_2^{\cdot-}$  and  $OH^{\cdot}$  radicals are probably one of the most effective defenses against diseases [57]. In our exploration showed that treatment with pomegranate increased the activities of SOD and CAT in diabetic rats when compared with diabetic control rats. In fact, the reactivation in SOD activity promoted by pomegranate may accelerate the dismutation of superoxide to  $H_2O_2$ , which is quickly removed by CAT protecting tissues of diabetic rats against highly reactive and toxic  $OH^{\cdot}$  and consequently preventing the LPO.

Multiple defense systems collectively called antioxidant status that present in the body circumvent damage cell by oxygen free radicals (OFR) [58]. This system comprises of substances that provide stability to free radical by permitting the pairing of electron. Under normal physiological situation, a balance exists between antioxidant activity and ROS. Many factors disturb this equilibrium such as diabetes situation, thereby reducing the cell defense capacity and permitting uncontrolled oxidation of biomolecules [59]. Present study is observed a decrease in antioxidant activity in diabetic, probably due to the depletion of antioxidants. We found that TAS values peaked in PSJ in parallel with GC. This suggests that the antioxidant system can cope efficiently with lipoperoxide production during this critical period, and thus protect against oxidative stress.

In the current study, histopathological observation on the liver of diabetic untreated rats showed dilation of central vein and sinusoid, inflammation and necrosis. These changes are provoked by increase production of highly reactive intermediates of STZ, which are normally detoxified by antioxidant defense system such as SOD and CAT. However, they produced in excess can deplete exogenous antioxidant and react with liver tissues and destroy hepatic cells. The histopathological changes reduced in diabetic rats treated with PSJ with high level of antioxidant. Moreover, histopathological observation support the notion that pomegranate produced significant increase antioxidant enzyme and protected the hepatic tissue in diabetic rats.

From our findings, we infer that pomegranate has ability to ameliorate oxidative stress in plasma STZ-NA induced diabetic rats as evidence by improved glycemic and reduced lipid peroxidation along with improved antioxidant enzymatic status. Moreover, it protect histological changes from peroxidation injury through its antioxidant properties.

### Conflict of interest

The authors declare that they have no conflicts of interest.

### Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

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