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Effect of Pomegranate on Histopathology of Liver and Kidney on Generated Oxidative Stress Diabetic Induced Rats

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Abstract

Oxidative stress has been recommended as a contributory factor in complication and progression of diabetes. The aim of the study was to evaluate the effects of pomegranate on body weight and histopathology of the steptozotocin–nicotiamide (STZ-NA) generated oxidative stress induced diabetic rats. Thirty-two male Sprague-Dawley rats were randomly divided into non-diabetic, diabetic untreated and diabetic treated with pomegranate and Glibenclamide. Experimental diabetes was induced by a single intraperitoneal (i.p) administration of STZ 60 mg/kg body weight (b.w), 15 minutes after the i.p injection of NA (120 mg/kg b.w). Histopathological analyses were conducted on the liver and kidney tissues. While the body weight significantly decreased in the diabetic control group, however it was a significant increase in the diabetic treated group ($P<0.05$). Destruction of the liver architecture of the hepatocytes in the diabetic group showed the signs of necrosis, degeneration, dilatation, and inflammation in the central vein and blood vessels. In the kidney, shrinkage and lesion in Bowman's capsule were observed. Pomegranate acted as an antioxidant thereby preventing oxidative damage in the diabetic liver and kidney.

Keywords: Antioxidants; Diabetes; Oxidative stress; Pomegranate; Streptozotocin

Introduction

Diabetes mellitus (DM) is one of the most crucial chronic disease of the endocrine pancreas. Its main characteristic is unsuitable hyperglycemia and disordered metabolism of the lipid, charbohydrate, and protein that are caused by insulin deficiency or insulin action or both [1], which contributes to rise in free radical generation [2]. Oxidative stress plays a crucial role in chronic complications of diabetes and it is associated with increased lipid peroxidation [3]. Enhanced oxidative stress and changes in antioxidant capacity demonstrated in both clinical and experimental diabetes mellitus were considered the etiology of diabetes complications [4]. Increased glucose level causes slow but significant non-enzymatic glycosylation of protein [5,6]. Modified proteins may be recognized as foreign by the immune system and this triggered the antibody formation [6].

Several in vitro and in vivo studies have illustrated that reactive oxygen metabolites such as, superoxide radical ($O2^{\circ-}$), hydroxyl radical (OH°), and hydrogen peroxide H_2O_2 are important mediators of tissue injury (Fox, 1984). Oxidative stress has been suggested to be a common pathway linking diverse mechanism for the pathogenesis of complications in diabetes [4].

Streptozotocin (STZ) has been widely utilized for induction of diabetes via the toxic effects on pancreatic β-cell in the experimental animals [7]. STZ-induced diabetes in rats had been shown to be associated with functional and/or morphological changes in the kidney and liver [8,9]. High oxidative stress due to constant and chronic hyperglycemia showed in diabetics and experimental models of induced diabetic animal [10]. It causes depletion of the antioxidative defense system, and thus promotes de novo free radicals generation [11]. In vitro studies showed that insulin-secretory response to glucose is attenuated in STZ-NA-induced diabetic rats compered to control group due to reduced β-cell mass as well as metabolic defects. Results of numerous experiments revealed that this model of diabetes is useful in studies of different aspects of diabetes [12].

Many studies were carried out to study the effects of plants on diabetes induced by STZ in many organs of animals [13-18]. Antioxidants contained in dietary plants with fewer side effects that are suitable candidates for prevention or protection of oxidative damage caused by free radical species [19,20]. These phytochemical act as antioxidant, that scavenge free radicals and act as saviors of the cell [21]. The pomegranate fruit has been paid attention for its health benefits because of high levels of antioxidants, pomegranate juice possessed a three-fold higher antioxidant activity than that of green tea, and also higher than those detected in cranberry, grape fruit, and orange juices [22,23]. Edible part of pomegranate fruit is rich in vitamin C [24] and phenolic compounds [25,26], which play as phytochemical antioxidants with potential health related benefits. In recent years, several investigators have attempted to unravel the underlying mechanisms of the beneficial effects of pomegranate. These investigations have focused mainly on the antioxidant, anti-inflammatory, and antibacterial potentials of pomegranate [27-30]. These antioxidants showed various biological activities such as removing free radicals, inhibiting oxidant and microbial growth [31].

Present study on the histological lesion of the liver and kidney of STZ-NA induced diabetic rats compared to control could help in better understanding of the histopathological changes in diabetes mellitus and highlight the protective effects of the fruit.

Experimental Procedure

Animals

Thirty-two adult male Sprague-Dawley rats aged 10–12 weeks (250–280 g) were used in the study and were obtained from the Animal House at the Faculty of Medicine and Science Health (FPSK). The ethics protocol was approved by Animal Care Unit Centre for the Purpose of Control and supervision on experimental animals at UPM University, Malaysia (APPROVAL, NO, UPM/FPSK/PADS/BR-UUH/00502). The animals were placed with 12-h dark/light cycles at ambient temperature of 25 ± 2°C. They were quarantined for one week, prior to starting the experiment to acclimatize them to laboratory conditions. The rats were provided standard food (common rat pelleted diet), and tap water ad libitum. The animals were handled in accordance with the Institutional Guidelines for the Care and Use of Animals for Experimental Purposes.

Protocol for induction of diabetes

STZ (N-nitro derivative of glucosamie) is used to induce diabetes in experimental animals [32]. STZ was freshly dissolved in citrate buffer and administered to overnight fasted rat by a single intraperitoneal (i.p) injection of 60 mg/kg body weight. Nicotinamide (NA) was dissolved in normal physiological saline, and was administered (120 mg/kg b.w.) by i.p injection, 15 min before STZ [33]. The development of diabetes was confirmed by measuring the blood glucose levels in rats taken from the tail vein after 72 hours. Rats with fasting blood glucose >7 mmol/L or 126 mg/dl were considered as diabetic and were used in the present study by American Diabetes Association [34].

Experimental design

The animals were randomly divided into four groups (n=8). Glibenclamide Sulfonylurea is anti-diabetic drugs, long used in the treatment of non-insulin dependent diabetes mellitus. Research from the antioxidant studies suggests that GC has the potential to counteract the reactive oxygen species mediated oxidative stress [11]. Pomegranate juice (PJ) mixed with the (PS) seed powder (1 ml + 100 mg/kg body weight) was administered orally using intragasteric tube for a period of 21 days [27,35], according to the groups below:

- Group I: Normal control, (NC; 1 ml distilled water)
- Group II: Diabetic control (DC; 1 ml distilled water)
- Group III: Diabetic + PSJ (1 ml PJ + 100 mg/kg PS) per rat
- Group IV: Diabetic + Glibenclamide (GC; 5 mg/kg b.w dissolved in DMSO) [36].

At the end of the experimental period, the rats were fasted overnight, anesthetized using diethyl ether, and sacrificed. Liver and kidneys were immediately excised, and kidneys were trimmed of fatty tissue and washed in saline to remove any red blood cells and clots.

Histopathological study

The tissue samples in 10% buffered formalin were sliced to approximately 1 cm thick, and placed into the cassettes. Then, the cassettes are placed a tissue processor machine, which comprise of dehydration with alcohol, clearing with xylene and wax, and impregnating process automatically overnight (14 hours). The cassettes were embedded in molten paraffin, which later cooled down to formed blocks of paraffin.

Each block was trimmed then sectioned about 5 µm by using a microtome. Hematoxylin and Eosin (H&E) dye, which mounted with DPX for microscopic observations.

Statistical analysis

Histopathology changes in diabetic oxidative stress induced animals compared to control non-diabetic induced and treatment group animals' tissue on liver and kidney. Results were analyzed by SPSS version 21. To compare histopathological data (frequency of kidney and liver damages), Pearson's chi-square test was used to discover if there was a relationship between two categorical variables. Results were considered significantly different if $p<0.05$.

Result

Table 1 and Figure 1 demonstrate changes in body weight of controls and treatment groups, in DC group, significantly body weight decreased ($P<0.05$), and in PSJ and GC significantly increased ($P<0.05$) in comparison with the normal control. Histopathological evaluation of the normal liver tissue of the non-diabetic rats demonstrated the normal hepatic structure mammals. Each lobule is made up of radiating plates, strands of cells forming a network around a central vein (Figure 2A1). Liver sections of induced diabetic rats revealed hepatocellular injury observed in loss of normal architecture of the liver, inflammation, dilation in central vein, and sever fibrosis, and leucocytic infiltration around central vein (Figure 2B1). Nucleus of hepatocytes observed pyknosis and binucleated cells (Figure 2C1). Sections of the liver in diabetic rats treated with pomegranate showed hepatic lobules appearing in radiating plates of strands of hepatocytes, and the central vein (Figure 2D1). It significantly ($P<0.05$) the pathological percentage of damages in structure of hepatic sections reduced from 58% in DC to 23% in GC and 20% in PSJ (Figure 3).

Weeks						
Groups	1	2	3	4	5	6
ND	266 ± 8	278 ± 12	289 ± 7	307 ± 10	315 ± 13	327 ± 13[a]
DC	265 ± 5	280 ± 9[a]	264 ± 11	252 ± 10	241 ± 12	233 ± 10[b]
PSJ	270 ± 12	284 ± 12[a]	270 ± 14	262 ± 19	262 ± 18[b]	265 ± 20
GC	269 ± 8	277 ± 8	265 ± 10	255 ± 17[b]	264 ± 17	277 ± 19[a]

Each value is mean ± S.D. for eight rats in each group

a-b with different superscript letter indicates statistically significant different ($p<0.05$)

Table 1: The effect of pomegranate on body weight.

Histopathological evaluation of the normal kidney tissue of the non-diabetic rats demonstrated normal structure of glomerulus surrounded by the Bowman's capsule, distal convoluted tubules and proximal without any inflammatory alterations (Figure 4 A2). The kidneys of untreated diabetic rats showed shrinkage of glomeruli (Figure 4 C2) and tubular inflammation (Figure 4D2).

The groups that were treated with pomegranate demonstrated normal glomerulus, normal basement membrane, and capillaries without any inflammatory cells (Figure 4 B2). It was shown that pathological percentage of damages in structure of kidney section

reduced significantly (P<0.05) from 55.5% in DC to 26% in GC and 18.5% in PSJ (Figure 5).

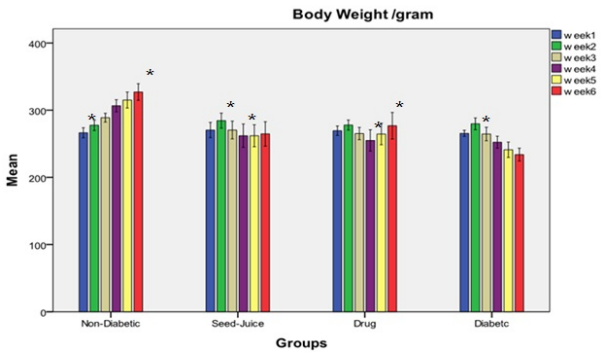

Figure 1: Changes of mean body weight of rats during six weeks before and after treatment according to the groups. The values are mean ± SD for eight rats in each group. * In each bar indicates significantly difference (p<0.05).

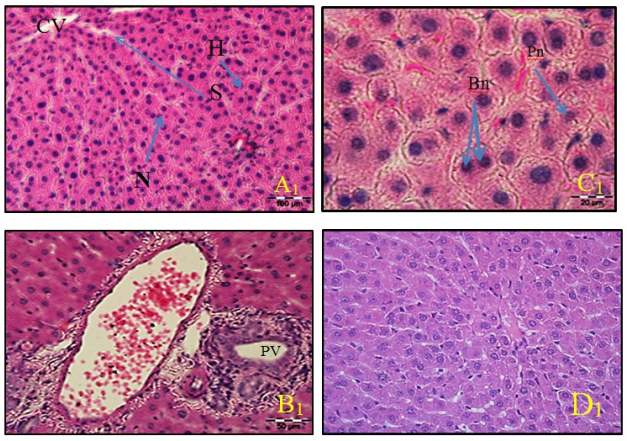

Figure 2: Photomicrographs of histological sections of liver tissue of control and treated rats. (A1) Non-diabetic section of liver showing normal histological structure of hepatocytes (H), central vein (CV), sinusoid (S), nucleus (N), H&E 40X. (B1) Liver of diabetic rat showing loss of the normal architecture with the distended portal vein (PV), fibrosis, leucocytic inflammation, H&E 40X. (C1) Diabetic liver shows binucleated (Bn) pyknosis (Pk) H&E, 100X. (D1) Diabetic rats treated with pomegranate show near normal hepatocytes, mild sinusoidal dilatation around central vein when compared to liver of rat no treated with pomegranate, H&E X40.

Discussion

Studies on diabetes mellitus showed that the occurrence of oxidative stress rises as result of increase in the level of production of free radicals and diminish cells antioxidant capabilities, which all together can cause oxidative stress and tissue damage in diabetic patients [37,38]. These activities of exogenous and endogenous antioxidant, contributes a key role in the defense against free radicals [39]. Streptozotocin (STZ) is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals, and also interferes with cellular metabolic oxidative mechanisms [40].

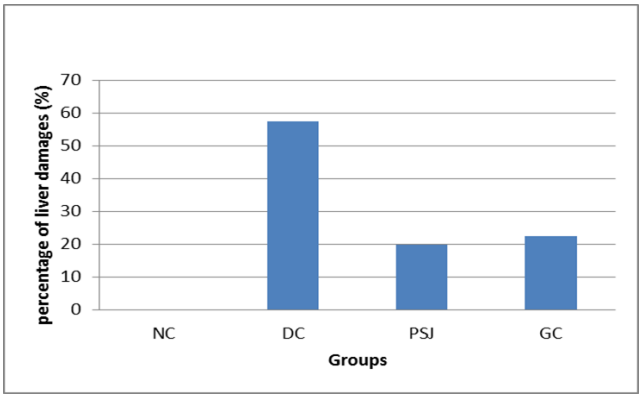

Figure 3: percentage of damages in liver tissue for each group (P<0.05), NC: normal control, DC: diabetic control, PSJ: pomegranate seed and juice, GC: Glibenclamide.

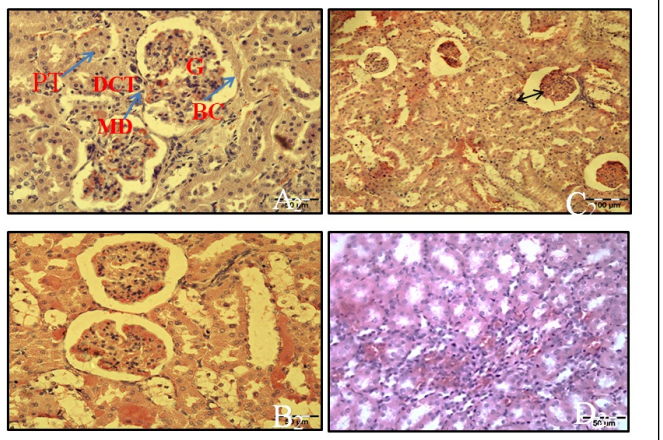

Figure 4: Photomicrographs of histological section of kidney tissue of control and treated rats, (A2) Non-diabetic section of kidney showing normal structure with no pathological changes appearance of kidney, normal glomeruli, and tubules, Bowmen's capsule (BC), glomerulus (G), proximal tubule (PT), distal convoluted tubular (DCT), Macula Densa cell (MD), H&E 40X. (B2) Treated groups with pomegranate show normal glomerulus, normal basement membrane, and capillaries, without any inflammatory cells, STZ-NA-induced diabetic kidney showing shrinkage of tubular (C2) and inflammation (D2), H&E X40.

Animal diabetic models exhibit high oxidative stress due to chronic hyperglycemia that result in depletion of the antioxidant defense system and promotes de novo generation of free radicals [11]. STZ-induced diabetes are characterized by polydypsia, polyuria, weight loss, decreased physical activities and hyperglycemia [41]. Histopathological examinations demonstrated mild to moderate inflammation of the hypotocytes. ROS and lipid peroxidation cause direct damage to hepatocytes by disrupting membranes, protein, and

DNA [42]. In the STZ-induced diabetic animals, were shown to lower levels of activities of endogenous antioxidant enzymes such as SOD and CAT. Subsequently, these reductions can cause tissue degradations [38].

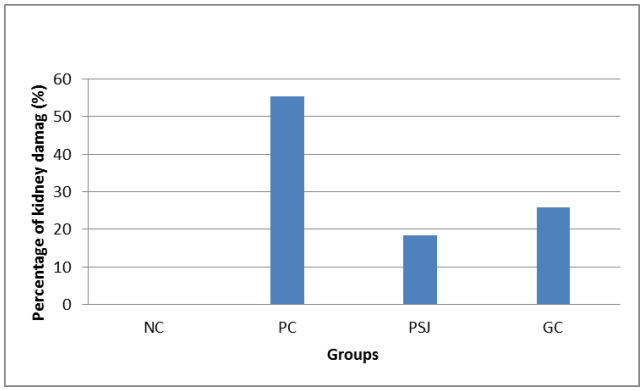

Figure 5: Percentage of damages in kidney tissue for each group (P<0.05), NC: normal control, DC: diabetic control, PSJ: pomegranate seed and juice, GC: Glibenclamide.

Liver and kidney are important organs of storage, detoxification, metabolism, and excretion of many metabolites, so they are particularly vulnerable to oxidative damage [43]. Liver is important organ that its main function is maintaining and control blood glucose with glycogenesis and glycogenolysis. Hepatocyte damage and lipid peroxidation products induce an inflammatory response. The mechanism of liver and kidney destruction because of the oxidative stress involves the secretion of cytokines, mainly tumor necrosis factor TNF-α, interleukin IL-1, and IFN-c [44].

These alterations, might cause an abnormal production of cytokines and growth factors. Subsequently, they facilitate the synthesis of extracellular matrix proteins and the depositions in the glomerular level that finally lead to mesangial expansion, glomerular shrinkage, and glomerular basement thickening. High glucose levels directly increases hydrogen peroxide production in the murine mesangial cells [45], and lipid peroxidation of the glomerulus [46,47]. Therefore, oxidative stress can be the common pathogenic factor for diabetic nephropathy similar to other complications [48]. Diabetic nephropathy is a widespread complication of diabetes mellitus.

Consequently, some positive attributes of pomegranate in this study may be the ability to lower the oxidative stress. High level of antioxidant in pomegranate could boost to quenching of some free radicals inside cells, as well as have the capability to protect kidney and liver tissue from oxidative stress damage.

Conclusion

In the present study, the histopathological observation in diabetic control rats displayed the congestion of portal triad with mild inflammation and remarkable fibrosis near the central vein in the liver, tubular inflammation and glomeruli shrinkage in the kidney. These reactions are provoked by the increased production of highly reactive oxygen species, which are normally detoxified by endogenous antioxidant enzyme in the excessive presentation. The depletion of exogenous antioxidant store can permit the reactive intermediate to react with and destroy the hepatic and renal cells such pathological changes can be in the diabetic rats. Apparently, the treatment with pomegranate can increase antioxidant enzyme, which has ability to ameliorate oxidative stress, and protects the hepatic and renal tissues in diabetic rats.

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