Effect of quercetin nanoparticles on the kidney of the streptozotocin-induced diabetes in male rats: A histological study and serum biochemical alterations

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Diabetes is directly involved in oxidative stress production. Therefore, this study was conducted to investigate the morphological and functional alterations caused by oxidative stress and to evaluate the antioxidant effect of quercetin nanoparticles (QUNPs) in streptozotocin (STZ)-induced diabetic (type II) rats. Seventy two male albino adult rats were randomly distributed in 6 different experimental groups, with 12 animals per group: Normal Control (NC) group, Positive Control (PC) group received one dose of STZ (60 mg/kg body weight [bw]); QUNPs 10 mg/kg bw/day alone group; QUNPs 10 mg/kg bw/day + one dose of STZ (60 mg/kg bw) group; QUNPs 20 mg/kg bw/day alone group; and QUNPs 20 mg/kg bw/day + one dose of STZ (60 mg/kg bw) group. STZ-diabetic rats were treated with QUNPs (10 and 20 mg/kg bw/day) for 7 weeks to analyze their effects on markers of renal enzymes antioxidant [malondialdehyde (MDA), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx)], total protein and albumin, and also on kidney tissues. The results showed that the particle size of QUNPs is 16.13 nm at flow rate 10 ml/min. QUNPs especially at the dosage of 20 mg/kg bw/day gave results close to normal values observed in NC compared to PC. Also, histopathology of kidney sections for QUNPs 20 mg/kg bw/day + STZ and QUNPs (10 and 20 mg/kg bw/day) alone, appeared similar to NC. It can be concluded that QUNPs could become a promising adjuvant in the treatment of diabetes mellitus and can act as an antioxidant agent.

Key words: Diabetic, streptozotocin, free radical, antioxidant.

INTRODUCTION

Diabetes mellitus (DM) or hyperglycemia is a metabolic disorder that develops from cases of insufficient or absence of insulin release from β-cells (Vardi et al., 2003). Chronic hyperglycemia leads to many complications, such as cardiomyopathy, vascular damage, retinopathy, neuropathy, and nephropathy (Review of World Health Organ Tech Rep Ser., 1985). Streptozotocin (STZ), an antibiotic produced by Streptomyces
achromogenes and used as an agent to induced diabetes, damages the insulin-producing β-cells membranes and results in the depletion of intracellular nicotinamide adenine dinucleotide in islet cells (Kanter et al., 2004; Coskun et al., 2004). Also, it is known to induce diabetic kidney injury with hyperlipidemia, inflammation, and hyperuricemia (Hovind et al., 2009; Tone et al., 2005). Kidney injury is the most common pathological disorder predisposing end-stage renal disease worldwide (Ayodele et al., 2004; Donath and Shoelson, 2011; Murea et al., 2010). Diabetic nephropathy is characterized with progression into glomerulosclerosis, interstitial fibrosis, and tubular atrophy by mesangial expansion and thickening of basement membranes, ultimately resulting in renal failure (Tsao et al., 1999; Voziyan et al., 2002; Feliers et al., 2001). Previous studies proposed a wide variety of mechanisms in the pathogenesis of diabetes, including oxidation of renal glycoproteins by reactive oxygen species (Reddy et al., 2002; Natarajan et al., 2002; Chen et al., 2001).

Oxidative stress is caused by highly toxic components, such as the overproduction of reactive nitrogen and oxygen radicals which interact with the lipid bilayer and produce lipid peroxides of cellular membranes and caused toxicity to all the components of the cells (Tatsuki et al., 1997). Higher oxidative stress is one of the factors for impaired antioxidant defense mechanisms or increased levels of free radicals because it is implicated in the progression and development of diabetic complications (Ceriello, 2000; Saxena et al., 1993). Therefore, in recent years, researchers have developed interest to prevent oxidative damage with high oxidative stress in DM by the role and usage of natural antioxidants. Flavonoids have the capacity to promote β-cell regeneration in islets, normalize blood glucose levels, and normal islets from STZ in rats (Un et al., 2006). Individually, the protective effects of polyphenols and flavonoids may exert in a variety of ways: they may scavenge chelating metal ions, reactive oxygen species, also, scavenging lipid peroxyl radicals to act as a chain-breaking antioxidant, or prevent lipid damage by partition into the lipid bilayer (Plumb et al., 1999; Laughton et al., 1991; Robak and Gryglewski, 1988). Moreover, some studies reported that the activity of flavonoids as antioxidant may be dependent on hydroxylation degree (Plumb et al., 1999; Rice-Evans et al., 1996).

Quercetin (3,3,4,5,7-pentahydroxyflavone, QUE) is a lipid-soluble compound (Figure 1). It reduces lipid hydroperoxide production (Coldiron et al., 2002) and is capable of preventing lipid damage, inhibits biomolecule oxidation, radical scavenging, and alters antioxidant defense pathways in vitro (Candlish and Das, 1996; Morand et al., 1998). QUE is a well-documented bioflavonoid occurring in many foods and is known to be present in higher concentrations in green tea, red wine, broccoli, apples, and onions (Kiviranta et al., 1998; Weisburer, 2000). Previous studies have focused on the QUE beneficial properties, namely, anticarcinogenic properties, antioxidant, anti-inflammatory, antiproliferative, and antibacterial (Weisburer, 2000).

On the other hand, QUE is a challenging molecule to be delivered due to its poor water solubility. A water-soluble derivative of QUE has been synthesized but its bioavailability was only 20% (Mulholland et al., 2001) and it has such poor absorption in the gastro-intestinal tract. All these highlight the need for an improved formulation for QUE with enhanced dissolution so that its absorption can be greatly enhanced. Therefore, micro- and nanoparticle preparations are the most important approaches being investigated these days to improve bioavailability (Bilati et al., 2005). Nanoparticles are particularly useful in drug delivery for water-insoluble compounds such as ellagic acid (Bala et al., 2006) and coenzymeQ10 (Hsu et al., 2003), because their size (less than 1000 nm) can increase the absorption and the bioavailability of the delivered drug. Thus, an improved oral formulation of QUE is required with better bioavailability and higher efficacy. Therefore, this study aimed to prepare quercetin nanoparticles (QUNPs) and evaluate the antioxidant effect of QUNPs on renal histopathological and serum biochemical alterations in STZ-induced diabetic (type II) rats.

**MATERIALS AND METHODS**

In this study, QUE (Sigma-Aldrich, Singapore) was used as received. All reagents used were of technical grade. Absolute ethanol (99.5 to 99.8%) was obtained from J.T. Baker (Avantor Performance Materials, Phillipsburg, NJ).

**Preparation of QUNPs**

To prepare QUNPs, magnetic stirring (1000 rpm) was used to mix water and ethanol (volume ratio 35:1, fixed flow rate of 10 ml/min) according to the nano participation technique (Kakran et al., 2012; Abd El-Rahman and Al Jameel, 2014). Then, commercial QUE was dissolved in predetermined concentration (5 mg/ml) of ethanol (the solvent). The syringe was filled with the prepared solution and...
72 animals weighing 170 ± 2 g) were assigned to six groups. Three of the groups were composed of rats that received single dose of STZ (60 g/kg bw) + QUNPs (10 mg/kg bw/day); G3, treated group that received QUNPs (10 mg/kg bw/day) only; G4, treated group that received one dose of STZ (60 g/kg bw) + QUNPs (10 mg/kg bw/day); G5, treated group that received QUNPs (20 mg/kg bw/day) only; G6, treated group that received one dose of STZ (60 g/kg bw) + QUNPs (20 mg/kg bw/day) for 7 weeks. Fresh feed was provided every day; also at the beginning and during the experimental period, the amount of food consumed were weighed and recorded. The heparinized capillary tubes were used to collect the blood samples from the orbital plexus according to Schermer (1967). To obtain serum, samples were centrifuged (1500 ×g for 10 min) and aliquots were withdrawn for storage at -20°C until analysis. Serum biochemical assays

Serum albumin, total protein, and MDA were determined by kits obtained from bio diagnostic company (Dokki, Giza, Egypt). Serum globulin was determined by the following formula:

\[
\text{Serum globulin} = \frac{\text{Total serum protein}}{\text{Serum albumin}}
\]

Serum biochemical assays

Blood sugars, urea, creatinine, uric acid, albumin, total protein, and MDA were determined by kits obtained from bio diagnostic company (Dokki, Giza, Egypt). Serum globulin was determined by the following formula:

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\text{Serum globulin} = \frac{\text{Total serum protein}}{\text{Serum albumin}}
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Statistical analysis

Mean (SEM) was used to express the results. One way analysis of variance (ANOVA) followed by Fischer’s least significant difference (LSD) test was used to measure the intergroup variation. Statistical significance was considered at (P≤0.05). Statistical analysis was done using the Jandel Sigma Stat Statistical Software version 2.0.

Histopathological assay

For microscopic evaluation, the kidneys were first fixed in neutral phosphate buffered formalin solution (10%). After dehydration in an ascending series of ethanol (70, 80, 96, and 100%), the samples tissue was cleared in xylene and embedded in paraffin. Tissue sections (5 μm) were stained with hematoxylin-eosin (H-E). Fields (10, a minimum) for each kidney slide were examined and assigned for severity of changes by pathologist blinded to the treatments of the animals.

RESULTS AND DISCUSSION

Scanning electron microscope (SEM)

Morphology of original QUE and QUNPs were studied using SEM tool. As shown in Figure 2, QUNPs showed a particle size of 16.13 nm at flow rate 10 ml/min. The powder of original QUE (Figure 2a) exhibited particles lacking uniformity in size which was relatively much larger than the QUNPs. While, QUNPs prepared by syringe pump, exhibited less crystallinity, absence of larger particles, and particles uniformity in size (Figure 2b) (Abd El-Rahman and Al Jameel, 2014).

These results indicated that QUE made by syringe pump gave particle size more uniform and significantly smaller than the commercial QUE that was more evidenced in the case prepared sample at 5 mg/ml (lower drug concentration). This behavior can be explained by considering two factors: the concentration influence on the viscosity and the nuclei number formed in the interface of solvent/anti-solvent (Kakran et al., 2012).

Effect of QUNPs on blood sugars, urea, creatinine and uric acid

As shown in Table 1, STZ diabetic rats showed increases in blood sugars, urea and creatinine and decreased uric acid as compared to NC. Treatment of STZ diabetic rats with QUNPs (10 and 20 mg/kg bw/day) resulted in decreased serum blood sugars, urea and creatinine and increased uric acid levels in those treated rats as compared to PC. QUNPs (10 and 20 mg/kg bw/day) alone gave results close to normal values observed in NC. Also, STZ + QUNPs (20 mg/kg bw/day) gave similar results. The antihyperglycemic effect of quercetin might be due to its property of antioxidant, which inhibits the peroxidation of lipid by scavenging the free radicals produced by STZ and prevents oxidative stress induced...
Figure 2. SEM photographs of (a) original quercetin (QUE) and (b) quercetin nanoparticles (QUNPs).

Table 1. Effect of QUNPs on blood sugars, urea, creatinine and uric acid in serum of control and experimental rats.

<table>
<thead>
<tr>
<th>Group/Parameter</th>
<th>Blood sugars (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76.40 ± 4.50</td>
<td>24.70 ± 2.60</td>
<td>1.03 ± 0.048</td>
<td>7.845 ± 0.014</td>
</tr>
<tr>
<td>Diabetic</td>
<td>219.55 ± 3.60</td>
<td>30.02 ± 2.68</td>
<td>1.77 ± 0.20</td>
<td>5.365 ± 0.010</td>
</tr>
<tr>
<td>QUNPs 10</td>
<td>77.62 ± 1.80</td>
<td>21.89 ± 2.17</td>
<td>1.2 ± 0.17</td>
<td>6.335 ± 0.019</td>
</tr>
<tr>
<td>Diabetic QUNPs 10</td>
<td>121.66 ± 3.02</td>
<td>20.36 ± 1.76</td>
<td>1.45 ± 0.30</td>
<td>7.225 ± 0.004</td>
</tr>
<tr>
<td>QUNPs 20</td>
<td>76.73 ± 2.90</td>
<td>22.78 ± 2.11</td>
<td>1.12 ± 0.12</td>
<td>7.803 ± 0.010</td>
</tr>
<tr>
<td>Diabetic QUNPs 20</td>
<td>85.16 ± 3.30</td>
<td>22.04 ± 0.75</td>
<td>1.27 ± 0.12</td>
<td>7.888 ± 0.011</td>
</tr>
</tbody>
</table>

Each value is mean ± SD for twelve rats in each group. Values that have a different superscript letter (a, b, c, d) differ significantly with each other (p ≤ 0.05).

by STZ, and also, helps the surviving β-cells to secrete more insulin and proliferation. Additionally, QUE enhances the sensitivity of insulin, leading to increased glucose utilization by the extrahepatic tissues and thereby decreasing the levels of blood glucose (Babujanarthanam et al., 2010). Vessal et al. (2003) reported that supplementation has proven to be beneficial in decreasing the concentration of blood glucose, promoting the pancreatic islets regeneration and increasing release of insulin in STZ treated diabetic rats; thus exerting its beneficial antidiabetic effects (Formica and Regelson 1995).

This is supported by the previous literature reports where these types of flavonoids enhance release of insulin up to 70% by its effect on function of islet at least in part, metabolism of cyclic nucleotide, and via alteration in Ca^{2+} fluxes (Yen et al., 2009; Hii and Howell, 1985a; Hii and Howell, 1985b). Blood glucose levels were significantly increased after 72 h following STZ injection compared to control group, while all QUE treatment groups had significantly decreased blood glucose concentrations compared to the diabetic group after 30 days (Elbe et al., 2015).

Previous studies reported that the administration of STZ decreased levels of insulin and increased levels of plasma glucose, while treatment with QUE resulted in decreased glucose in plasma and increased levels of insulin. Diabetic groups treated with QUE suspension showed significantly lower glucose levels as well as QUNPs as against diabetic control group (Babujanarthanam et al., 2011; Sinha, 1972).

Serum urea and creatinine levels are the most important indicators of kidney functions. Lu et al. (2007) and Maciel et al. (2013) reported that urea and creatinine levels were increased in diabetic rats as compared to the control group. Also, urea level was increased in diabetic rats when administrated with 5, 25 and 50 mg/kg of QUE than healthy groups treated with the same QUE dosages (P < 0.05).

Effect of QUNPs on total protein, globulin and albumin

Data in Table 2 showed the total protein levels, albumin (A), globulin (G) and A/G ratio after treatment with STZ
(60 mg/kg bw), QUNPs (10 and 20 mg/kg bw) and QUNPs (10 and 20 mg/kg bw) + STZ for 7 weeks. PC group showed decreased levels (P ≤ 0.05) of total protein, albumin, and globulin in relation to NC group. Diabetic rats treated with QUNPs (10 and 20 mg/kg bw) presented increased concentration of total protein, albumin and globulin to near NC levels (P ≤ 0.05) and these results agree with several studies (Maciel et al., 2013; Arya et al., 2014). The albumin level was significantly decreased in the diabetic group, which might be due to albumin leakage due to glomerular basement damage of membrane combined with an increase in the pressure of trans glomerular filtration or impaired reabsorption of tubular (Maciel et al., 2013). Also, the results should have a significant reduction in concentration of serum albumin in diabetic rats, except the group of rats treated with QUE at 50 mg/kg (Naoum, 1999)\(^{(50)}\). However, the QUE treatment significantly increased the serum albumin towards the negative levels, which was reflected in the reduction of kidney damage due to STZ-induced hyperglycaemia. Consistent with our results, Kandasamy and Ashokkumar (2012) reported that in diabetic nephrotoxic rats, flavonoids restore the reduced level of albumin. Thus, QUE has a beneficial pharmacological effect on diabetic, especially at a dose of 50 mg/kg on hepatic, protein levels, diabetic, and functional markers.

### Effect of QUNPs on MDA, CAT, GR and GPx

Oxidative stress is an imbalance between the production of free radicals and antioxidants defense capacity (Hamadi et al., 2012). It is well known that hyperglycaemia increases mitochondrial ROS production and impairs cellular antioxidant enzymes, which could represent a key event in the development and progression of the complications of diabetes (Hamadi et al., 2012; Chang et al., 2012).

Glutathione reductase, catalase and glutathione peroxidase are among those enzymes that metabolize endogenous free radicals and reactive oxygen species, often with the concomitant oxidation of reduced glutathione (GSH) to its oxidized form (GSSG) (Josephy, 1997). Reduced glutathione deficiency is also seen in tumorigenesis aminoaciduria, nephropathy (Meister, 1988) and cataract genesis (Nagasawa et al., 1996; Walsh and Aleo, 1997).

QUNPs significantly increased the serum activity of MDA, CAT, GR and GPx enzymes in normal rats after treatment for 7 weeks (Table 3). On the other hand, STZ had an opposite effect on the activity of serum
malondialdehyde (MDA), catalase (CAT), GR and GPx enzymes, but treatment with QUNPs ameliorated its effect (Table 3). Similar results were noted by Elbe et al. (2015) where they found that QUE was beneficial in reducing diabetes-related alterations. QUE has a free radical scavenger, transfer electrons, chelate metals and superoxide radical inhibitor properties (Vessal et al., 2003; Ferrali et al., 1997). Beneficial effects of QUE are attributed to its antioxidant effects as well as protective effects on β-cell integrity. Also, Vessal et al. (2003) noted its increasing effect on the islets number of Langerhans in pancreas. Also, there is a significant decrease in the plasma level of glucose.

MDA is the most commonly used indicator of lipid peroxidation. Decreases in cellular antioxidant enzymes and increase in tissue level of MDA emphasize oxidative stress. Previous studies reported that QUE treatment significantly decreased diabetes-related oxidative damage in various organs by increasing the activities of antioxidant enzyme but decreasing the levels of MDA (Dias et al., 2005; Sirovina et al., 2013; Edremitioglu et al., 2012).

Babujanarthanam et al. (2011) indicated that QUE decreases the levels of thiobarbituric acid reactive substances (TBARS) in plasma in STZ-induced diabetic rats. $H_2O_2$ may be an important mediator for tissue damage in STZ induced diabetes (Yanardag et al., 2005). CAT protects the cell from oxidative damage induced by $H_2O_2$, because it is localized in the microperoxisomes or the peroxisomes, which catalyzes the decomposition of $H_2O_2$ to water and oxygen (Abolfathi et al., 2012). If it is not decomposed by GSH peroxidase or CAT, it causes production of reactive hydroxyl radicals. Excess amounts of free radicals damage nucleic acids and cellular proteins by attaching to them and causing lipid peroxidation. STZ significantly increased the ROS and significantly decreased the activity of antioxidant enzyme. The activities of plasmatic GSH levels were increased significantly and the activities of antioxidant enzyme [CAT and superoxide dismutase (SOD)] were decreased significantly in STZ-diabetic rats. The diabetic rats treated with quercetin showed an increased in CAT, GSH and SOD activity. Quercetin directly scavenges free radicals and ROS; therefore, it is an important flavonoid which is known to be a potent antioxidant (Annapurna et al., 2009; Boots et al., 2008; Jeong et al., 2012). When treated with 50 mg/kg of QUE, the levels reverted close to normal values observed in control group ($p < 0.05$) (Maciel et al., 2013; Stanley and Menon, 2001). Also, Elbe et al. (2015) reported that in diabetic group, CAT activity was decreased significantly compared with the control group and were significantly increased in treated group compared with the diabetic group. QUE increases CAT activity and reduces lipid peroxidation, thus, it prevents oxidative stress (Elbe et al., 2015; Maritim et al., 1991).

GPx react with GSH, thus, it serves to detoxify peroxides (Sen, 1997). Low GPx activity in diabetic might be due to low GSH content, since GSH is a substrate and cofactor of this enzyme (Dominguez et al., 1998). In the process of converting $H_2O_2$ to water, GPx converts GSH to GSSG, which is reduced back to GSH by GRx (Maritim et al., 2003). Glutathione may contribute to antioxidant defense by networking with the other major antioxidants (Babujanarthanam et al., 2011).

STZ could decrease pancreatic GSH-Px and CAT, but QUE could enhance pancreatic GSH-Px and CAT activity and consequently antagonizes STZ effect on these antioxidant enzymes (Abdelmoaty et al., 2010). These results together suggest that QUNPs is effective to protect kidney against oxidative stress induced by STZ-induced diabetes.

Histopathological studies

As shown in Figure 3, the rat kidney sections were stained with hematoxylin and eosin. The NC rat kidney presents a normal glomerulus surrounded by Bowman’s capsule, distal convoluted and tubules proximal, normal podocytes (Pc) and normal capillaries (CP) (Figure 3A). Figure 3B shows the kidneys of the STZ-treated rats, which present a glomerular hypertrophy (Gh), thickening of the basement membrane and mesangial expansion. Also, degeneration of glomerular capillaries both tubular and proximal convoluted tubule exhibited edematous changes. Figure 3D shows the effect of QUNPs 10 mg + STZ treatment and the findings present less expansion of the glomerulus, features of healing, mildly dilated capillaries, and little damage of proximal and distal tubules. Moreover, the histopathological of kidney sections for QUNPs 20 mg/kg bw/day + STZ and QUNPs (10 and 20 mg/kg bw/day) alone, appeared similar to NC (Figure 3F, C and E).

Our results agree with Elbe et al. (2015) who reported that diabetic group showed severe tubular and glomerular alterations. Cellular swelling, tubular changes including tubular basal membrane thickening, peritubular infiltration, epithelial desquamation, mesangial matrix expansion within glomerulus and capillary and intracytoplasmic vacuolization were obvious. Eddy (1996) and Babujanarthanam et al. (2011) suggested that tubular and glomerular changes were reduced in QUE administered groups. Also, Arya et al. (2014) and Bashir et al. (2014) reported that the diabetic rats treated with QUE demonstrated a recovery of the normal structure of kidney with intact tubules and glomerular epithelial cells.

Conclusion

The morphological and serum biochemical findings suggested that the administration of QUNPs to diabetic rats causes beneficial effect in terms of regeneration of cells in damaged kidney. Thus, it is concluded that
QUNPs possess preventive and curative effect on STZ induced diabetes in rats and can be used as a natural herbal medicine to protect kidney and pancreatic cells.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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