Protective effect of Quercetin on liver damage in Streptozotocin-induced Diabetic Rats

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Abstract:
The negative impact of diabetes on the liver is well recognized. This study was designed to evaluate the hepatoprotective properties of Quercetin in streptozotocin-induced diabetes in rats. Male Wistar rats were made diabetic with a single injection of STZ (40 mg/kg i.p.). Rats were randomly divided into four groups 8 animals each: Group 1, healthy control rats; Group 2 non-diabetic rats treated with 15 mg/kg b.w./day i.p. injection of Quercetin; Group 3, diabetic rats; Group 4, diabetic rats treated with Quercetin (15 mg/kg b.w./day, i.p.) for 8 weeks. Finally, serum ALT, AST, ALP and albumin levels as well as liver MDA contents and activities of GSH-Px were measured to assess hepatic injury. Liver tissues of Rat in whole groups were removed then prepared for Apoptosis analysis. Liver MDA content and serum ALT, AST, ALP and bilirubin levels in Groups 3 were found to be significantly increased as compared to Group 1 (P<0.001) and these parameters in Group 4 were significantly decreased as compared to Group 3 (P<0.001). Liver GSH-Px contents and serum albumin level in Group 3 was significantly decreased as compared to Group 1 (P<0.001) and were found to be significantly increased in Group 4 as compared to Groups 3 (P<0.001). Histopathological examination revealed that diabetes increased apoptotic index in liver tissue while the treatment of diabetic rats with Quercetin was shown to have anti-apoptosis effect. This study showed that Quercetin have hepatoprotective effects in experimentally induced diabetic rats.

Keywords: Apoptosis, Diabetic, Quercetin, Streptozotocin, Liver, Rat

Introduction

Diabetes mellitus is one of the most common endocrine metabolic disorders. It is a chronic disease that characterized by hyperglycaemia (1). Hyperglycemic in long time have side effect in other tissues especially in liver. Liver dysfunctional has seen Indirectly or directly, the liver is a major target of insulin action. The onset of diabetes is accompanied by development of major biochemical and functional abnormalities in the liver, including alterations in carbohydrate, lipid, and protein metabolism, and changes in antioxidant status (2,3,4,5). On the other hand, it was established that hyperglycemia increases mitochondrial reactive oxygen species (ROS) production, which could represent a key event in the development of diabetes complications (6,7). The initial cellular response to high glucose challenge is the generation of ROS, which rapidly induces apoptotic cell death (8). The balance of ROS and antioxidant is a major mechanism in preventing damage by oxidative stress. However, although it may not be possible to completely reverse diabetic complications, antioxidants could be useful in preventing or attenuating the adverse effects of chronic hyperglycemia (9). Therefore, the dietary supplement of antioxidants such as vitamins, flavonoids has been used to prevent the occurrence of many chronic diseases (10,11). Flavonoids are a large group of natural polyphenolic substances widely distributed in the plant kingdom (12). They
are important constituents of the nonenergetic part of the human diet and are thought to promote optimal health, via their antioxidant effects in protecting cellular components against ROS (13). Quercetin (3,5,7,3',4'-pentahydroxy flavon) is one of the most widely distributed flavonoids, present in fruit, vegetables, tea olive oil and many other dietary sources (14). It is a strong antioxidant and it has been shown to reduce oxidative stress (15,16). It has been demonstrated that quercetin exhibits its therapeutic potential against many diseases, including ischemic heart diseases, atherosclerosis, liver fibrosis, renal injury, and chronic biliary obstruction (17,18,19). Because liver is subjected to ROS-mediated injury in diabetes (20), our experiments were performed to investigate the potential protective effects of quercetin treatment on liver oxidative stress.

**Material and Methods**

**Animals:**
This study was conducted on 32 adult Wistar albino male rats 6-8 weeks old, weighing between 170 and 200 g. Animals were housed in the animal laboratory at the medical research center at Benha faculty of medicine. They were housed at room temperature (25°C) and 12h/12h light/ dark cycle. All Rats were fed a standard diet and water.

**Groups of the experiment:**
The animals were randomly divided into 4 groups each consisted of 8 rats as follow:
Group (I) : *Control group* injected with citrate buffer daily, intraperitoneal (IP).
Group (II) : *Quercetin (QR)* group that received 15 mg/kg QR (IP).
Group (III) : *Diabetic group* that received 40 mg/kg streptozotocin (IP).
Group (IV): *Treatment group* received 40mg/kg (IP) STZ plus15mg/kg QR (IP).

**Induction and diagnosis of diabetes mellitus:**
Diabetes was induced by intraperitoneal (ip) injection of a single dose of STZ (40 mg/kg in freshly prepared citrate buffer pH 4.5). The animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. Control rats were injected by the buffer alone (21). Diabetes was verified 72 hours later by measuring blood glucose levels (after an overnight fasting) with the use of glucose oxidase reagent strips. Rats having blood glucose level of ≥ 250 mg/dl were considered to be diabetic.

**Quercetin administration:**
Quercetin (QR) treatment was initiated 5 days after the administration of streptozotocin. Quercetin (QR) injections of 15mg/kg intraperitoneal (IP) (22) were continued daily to the end of the study (for 8 weeks) (23).

**Chemicals used:**
*Streptozotocin drug:
It was purchased from Sigma- Aldrich Company (USA). It is presented in powder form, purity more than 99% to be dissolved in freshly prepared
sodium citrate buffer pH 4.5.
*Sodium citrate buffer pH 4.5:
Preparation of 0.1MCitrate Buffer:
Weigh accurately citric acid 10.5 gm and sodium citrate 14.7 gm. Mix it with
500 ml water. Make up volume to 1000 ml with distilled water.
Adjust pH 4.5 by sodium hydroxide (24).
*Quercetin drug:
Quercetin powder was obtained from Sigma Chemical Company (St. Louis,
MO, USA). It was dissolved and diluted with 20% glycerol in 0.9% normal
saline, mixed vigorously and stored in a dark bottle at 4ºC. The quercetin
solution was freshly prepared each week.(22)

Procedure of the experiments:
At the end of the treatment period, the animals were anesthetized after 12
hour fasting by inhalation of diethyl ether. The animals were fixed on
operating table and the blood samples were taken as follow:

Blood sample collection
A craniocaudal incision of about 2 cm is made, parallel and with slightly to the
left of the sternum through the skin and pectoral muscles to expose the ribs. A
blunt curved forceps is then binged between the 5th and 6th ribs, through the
intercostals muscles. The gap is widened so that the rapidly beating heart
becomes visible, then the blood sample were taken from the right ventricle.

Biochemical assessment
Plasma activities of Alanine Transaminase (ALT), Aspartate Transaminase
(AST), alkaline phosphatase (ALP) and concentration of glucose, albumin
and total bilirubin were determined by a standard automated technique using
Hitachi Analyzer Model 911 and adequate kits from Roche Company
(Switzerland). (25) These were investigated in Banha faculty of medicine at
biochemistry analysis unit.

Tissue preparation
A midline laparotomy was performed to remove the liver. The liver was
dissected and fixed in 10% formalin solution at room temperature. Slices of
liver tissue were processed for histopathological & immunohistochemical
studies.

Immunohistochemical analysis
Paraffin embedded tissue sections of 5Mm were prepared on positively-
charged slides to be stained with antiBCL-X antibody using Biotin streptavidin
immune-peroxidase technique.
Interpretation for immunostaining:
BCL-X was detected as cytoplasmic brown staining in examined tissue.
Stained sections were classified as; Mild intensity for weak brown cytoplasmic
stain. Moderate intensity for moderate brown cytoplasmic stain. Strong
intensity for strong brown cytoplasmic stain. (26)

Measurement of antioxidant activity
The rat's Liver were removed immediately and washed in normal saline and
homogenate 10% prepared in 1.15% w/v of potassium chloride. The
homogenate was centrifuged in 7000 x g for 10 minutes at 4°C and
supernatant were used for measurement of oxidative stress by determination
of malondialdehyde (MDA) as well as estimation of antioxidant enzymes such
as glutathione peroxidase (GSH-Px). (27) Tissue MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDA formed/mL. Plasma MDA concentrations were determined with spectrophotometer (28). Glutathione peroxidase (GSH-Px) activity was measured by NADPH oxidation, using a coupled reaction system consisting of glutathione, glutathione reductase, and cumene hydroperoxide. One unit of enzyme activity is defined as the amount of enzyme that transforms 1 μmol of NADPH to NADP per minute. Results are expressed as units/mg protein (29).

**Statistical analysis**
All data were expressed as mean ± S.D; data were evaluated by the one way analysis of variance. Difference between groups were compared by Student’s t-test with P < 0.05 selected as the level of statistical significance.

**Results**
Results of the effect of daily treatment of Quercetin at a dose of 15mg/kg for 8 weeks on blood glucose levels of experimental rats are presented in Table 1. The Quercetin treatment produced hypoglycemic effect in both normal and diabetic rats after 8 weeks of administration, but this hypoglycemic effect is significant in diabetic group (P<0.001). Table 1 shows the effects of Quercetin treatment on the serum levels of markers of liver injury (ALT, AST, ALP and bilirubin) in diabetic rats. ALT, AST, ALP and bilirubin serum contents in Groups 3 was found to be significantly increased as compared to Group 1 (P<0.001) and these parameters in Group 4 were significantly decreased as compared to Group 3 (P<0.001). The albumin serum level in Group 3 was significantly decreased as compared to Group 1 (P<0.001) and this parameter was significantly increased in Group 4 as compared to Group 3 (P<0.001).

Table 1: Comparison of the effect of Quercetin on blood glucose levels and serum markers of liver tissue injury among the experimental groups (mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose level (mg/dL)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>Total serum bilirubin (Mg/dl)</th>
<th>Albumin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group rats</td>
<td>119±2.49</td>
<td>86±1.55</td>
<td>123±1.83</td>
<td>156±1.45</td>
<td>0.83±0.01</td>
<td>4.37±0.02</td>
</tr>
<tr>
<td>Nondiabetic rats + Quercetin (QR) treatment</td>
<td>113±3.12</td>
<td>86±1.28</td>
<td>124±1.04</td>
<td>157±1.41</td>
<td>0.84±0.02</td>
<td>4.36±0.02</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td>304±5.99 a</td>
<td>181±1.30 a</td>
<td>272±1.67 a</td>
<td>245±2.03 a</td>
<td>1.26±0.1 a</td>
<td>3.14±0.01 a</td>
</tr>
<tr>
<td>Diabetic rats + Quercetin (QR) treatment</td>
<td>127±2.06 b</td>
<td>87±1.60 b</td>
<td>135±1.58 b</td>
<td>181±1.48 b</td>
<td>0.88±0.01 b</td>
<td>4.35±0.02 b</td>
</tr>
</tbody>
</table>
Table 2 shows the effects of Quercetin treatment on antioxidative activity in liver tissue of diabetic rats. MDA contents of the liver tissue in Groups 3 was found to be significantly increased as compared to Group 1 (P<0.001) and liver MDA level in Group 4 were significantly decreased as compared to Group 3 (P<0.001). The GSH-Px contents of the liver in Group 3 were significantly decreased as compared to Groups 1 (P<0.001) and GSH-Px activity were increased in Group 4 as compared to Group 3 (P<0.001).

Table 2: Comparison of the effect of Quercetin treatment on liver MDA and antioxidant enzymes activities (GSH-Px) among the experimental groups (mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Biochemical parameters</th>
<th>Biochemical parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDA (nmol/g protein)</td>
<td>GSH-Px (U/mg protein)</td>
</tr>
<tr>
<td>Control group rats</td>
<td>3.24±0.08</td>
<td>22.76±0.4</td>
</tr>
<tr>
<td>Nondiabetic rats + Quercetin (QR) treatment</td>
<td>3.23±0.01</td>
<td>22.44±0.22</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td>5.37±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.51±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic rats + Quercetin (QR) treatment</td>
<td>4.35±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.65±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*<sup>p</sup><0.001 ; <sup>a</sup>compared to Group 1, <sup>b</sup>compared to Group 3.

Pathologically, liver histological structure was normal in healthy control group (Fig. 1, A). In Group 2 also there were no pathological changes so that hepatic lobular structure seemed quite normal (Fig. 1, B). In group 3, Diabetic rats showed (Fig. 1, C). Finally in group 4, Quercetin treatment of diabetic rats prevented the pathologic changes in the liver (Fig. 1, D).
**DISCUSSION**

Worldwide studies have been done to make use of herbal medicine in different fields of medicine. Based on ancient Persians traditional books use of herbal medicine has positive effect on treatment of different diseases especially on diabetes mellitus (30). Quercetin as an important and main flavonoids found in human mail (31) has an useful effect in human health involves prevention of diabetes induced cataract, reduced blood vessels fragility, anti microbial, anti viral, anti allergy, and anti inflammatory effects and prevention of platelet aggregation (31,32,33,34). In both type 1 and type 2 diabetes mellitus the late diabetic pathological complications are mostly due to excessive elevated production of reactive oxygen species over the capacity...
of their removal by internal enzymatic and non-enzymatic mechanisms (35). Therefore, additional numerous dietary artificial or natural antioxidants may be of great importance in such cases (36). Various natural products have long been used in traditional medical systems for treating diabetes (37). Most of them contain a wide scale of antioxidants with a potent scavenging activity for reactive oxygen species. Therefore, it might be assumed that these products or isolated natural compounds could play a very important role in adjuvant therapy. In current study, Intraperitoneal injection of Quercetin caused significant reductions in blood glucose levels of healthy normal rats. Quercetin also caused significant hypoglycemic effect in diabetic rats. This results coincides with results of Mahesh and Menom (38) or Coskum et al. (39), who found a hypoglycemic effect of quercetin when given to streptozotocin-diabetic rats. It has been shown that, hypoglycemic effect of Quercetin is mediated through stimulation of synthesis and/or release of insulin (23). In the current study, significant decline in serum albumin level and elevations in markers of liver injury (ALT, AST, ALP, and bilirubin) reflect the hepatocytes injury in experimental diabetes. These results are consistent with the findings reported by Ramesh et al (40). The data of our study also revealed that daily treatment with Quercetin markedly improves biochemical parameters of rats with streptozotocin induced diabetes. Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases, alkaline phosphatase, bilirubin, and albumin. Hepatocellular damage causes release of these enzymes into circulation. Increase in serum levels of AST shows hepatic injuries similar to viral hepatitises, infarction, and muscular damages. ALT, which mediates conversion of alanine to pyruvate and glutamate, is specific for liver and is a suitable indicator of hepatic injuries. Increased levels of these enzymes are an indicator of cellular infiltration and functional disturbance of liver cell membranes (41). In addition, ALP is membrane bound and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites (42). On the other hand, bilirubin and albumin values are associated with the function of hepatic cells (43). Return of the above enzymes to normal serum values following Quercetin treatment may be due to prevention of intracellular enzyme leakage resulting from cell membrane stability or cellular regeneration (44). Effective control of bilirubin and albumin shows early improvement of functional and secretory mechanism of hepatic cells. In this study, histopathological evaluation of liver tissues showed liver tissue damage and apoptosis induced by diabetes mellitus of the livers in diabetic rats. With Quercetin treatment in diabetic rats no considerable pathological changes were observed demonstrating the protective effect of Quercetin against hepatic complications of diabetes. In this study, significant reduction of antioxidant enzymes (GSH-Px) activity as well as significant increase in MDA reflects oxidative stress of the liver in experimental diabetes. These results are in line with the findings reported by Khaki et al. (22) Increased oxidative stress in the tissues of streptozotocin diabetic rats was similarly reported. This was said to be a contributory factor in the development of the complications of diabetes (45,46). The data of our study also revealed that daily treatment of Quercetin markedly improves antioxidant status of liver
tissue of rats with streptozotocin-induced diabetes as GSH-Px significantly increased and MDA level markedly decreased. This indicates that in the presence of Quercetin, there is an improvement in the oxidative stress. This finding is completely in agreement with those of Dias et al (23) who demonstrated antioxidant activity of Quercetin in streptozotocin induced diabetic mice. Liver is one of the most important organs that maintains blood glucose levels within normal limits thus enhancement of blood glucose leads to imbalance of oxidation-reduction reactions in hepatocytes, so that, hyperglycemia through increasing in advanced glycation end products (AGEs) facilities free radicals production through disturbance in ROS production (47). Therefore, it reveals that diabetic hepatic damage is not controllable only by inhibition of hyperglycemia (48). In other words, in early stages of diabetes, tissues injuries are in association with hyperglycemia but its progress is not related to hyperglycemia. Therefore, monitoring of blood glucose levels solely is not sufficient in retarding diabetes complications. Thus, a suitable drug must have both antioxidant and blood glucose decreasing properties (49). One of the Quercetin anti oxidant mechanism is removal of free radical such as xanthine super oxide and xanthine oxidase (50). Therefore suggested, increased use of herbal medicine, fruit, vegetables, onion, tea and black burgundy grape which are full of flavonoids and Quercetin can decrease side effects of diabetes mellitus on liver tissue in diabetic patient complicated with hepatic diseases.

**CONCLUSION**

We observed that Quercetin improved serum biomarkers of liver tissue injury and histopathologic properties of this organ. It is presumed that Quercetin prevents diabetic complications and ameliorates diabetic hepatopathy through its antioxidant potential.

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