QUERCETIN CAUSES TO DECREASE PROXIMAL TUBULES APOPTOTIC CELLS IN STREPTOZOTOCIN-INDUCED DIABETIC RAT

Zahedi Afshin¹, Ahmadnezhad Behnam², Khaki Arash³*

¹Department of Pathology, Rasht Branch, Islamic Azad University, Rasht, Iran.
²Department of Pathology, Sciences & Research Branch, Islamic Azad University, Tehran, Iran.
³Department of Pathology, Tabriz Branch, Islamic Azad University Tabriz, Iran.

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*Corresponding author
Khaki Arash
E-mail: aafzahedi@yahoo.com

ABSTRACT
Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus. Wistar male rat (n=40) were allocated into three groups, control group (n=10) and quercetin (QR) group that received 15mg/kg (IP) QR, (n=10), and Diabetic group that received 55mg/kg (IP) streptozotocin (STZ) (n=20) which was subdivided to two groups of 10; STZ group and treatment group. Treatment group received 55mg/kg (IP) STZ plus 15mg/kg QR, daily for 4 weeks, respectively; however, the control group just received an equal volume of distilled water daily (IP). Diabetes were induced by a single (IP) injection of streptozotocin (55mg/kg). Animals were kept in standard condition. In 28 day after inducing diabetic 5 mL blood were collected for Total Antioxidant Capacity (TAC), Malondialdehyde (MDA) and Oxidized Low density Lipoprotein (Ox-LDL) levels and kidney tissues of Rat in whole groups were removed then prepared for Apoptosis analysis by TUNEL metho. Apoptotic cells significantly decreased in group that has received 15mg/kg (IP) quercetin (P<0.05) in comparison to experimental groups (P<0.05). Since in our study 15mg/kg (IP) quercetin have significantly Preventive affect on kidney cells damages by reducing number of Apoptotic cells in kidney, so it seems that using it can be effective for treatment in Diabetic Rat.

Key words: Apoptosis, diabetic, quercetin, streptozotocin, proximal tubules, rat

INTRODUCTION
Quercetin is a flavonoid widely distributed in nature. The name has been used since 1857, and is derived from quercetum (oak forest), after Quercus. Diabetes is a chronic disease due to has hyperglycemic. Hyperglycemic in long time have side effect in other tissues especially in liver. Attention has been paid to the search of effective drugs in the field of Traditional Chinese Medicine (TCM). Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular diseases (Tappy and Minehira, 2001). Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus (Baynes and Thorpe, 1999; Wolff et al., 1991). Although the mechanisms underlying the alterations associated with diabetes mellitus are presently not well understood, hyperglycemia lead patients to increased oxidative stress because the production of several reducing sugars (through glycosylation and the polychol pathway) is enhanced (Palmeira et al., 2001). These reducing sugars can easily react with lipids and proteins (nonenzymaticglycation reaction), increasing the production of reactive oxygen species (ROS) (Palmeira et al., 2001). Diabetes is the most common endocrine disease that leads to metabolic abnormalities involving regulation of carbohydrate metabolism. In addition to imbalanced carbohydrate metabolism, yet another major concern in diabetes is increased oxidative stress. Increased production of free radicals or ROS formation may induce oxidized LDL (Ox-LDL), which is key step in the sequence of events leading to atherosclerosis.
Sustained hyperglycemia and increased oxidative stress, are the major players in the development of secondary complications in diabetes. These abnormalities produce pathologies including vasculopathies, neuropathies, ophthalmopathies and nephropathies, among many other medical derangements (Sexton and Jarow, 1997). The balance of ROS and antioxidant are major mechanisms in preventing damage by oxidative stress. Therefore, the dietary supplement of antioxidants such as vitamins, flavonoids has been used to prevent the occurrence of many chronic diseases (Peluso, 2006). Many herbal such as: Barberry, Estragon, Rhus cotus, Cinnamonum zelanicum, Hypericum perforatum and onion known has been show as anti diabetic effects and has been used to patient treatment. Quercetin is a well-known flavonoid and a strong antioxidant and long-term treatment of STZ-diabetic animals and it has been shown to reduce oxidative stress (Mahesh and Mcnern, 2004). We plant to study the effect of quercetin as a protective on kidney cells apopiosis.

METHODOLOGY

Animals

Forty adult Wistar albino male rats were 8 weeks old and weighing 250±10g, they were obtained from animal facility of Pasteur institute of Iran. Male rats were housed in temperature controlled rooms (25°C) with constant humidity (40-70%) and 12h/12h light/dark cycle prior to use in experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care. The experimental protocol was approved by the Animal Ethical Committee in accordance with the guide for the care and use of laboratory animals prepared by Tabriz Medical University. All Rats were fed a standard diet and water. The daily intake of animal water was monitored at least one week prior to start of treatments in order to determine the amount of water needed per experimental animal. Thereafter, the rats were randomly selected and divided into control (n=10) and quercetin (QR) group that received 15mg/kg QR (IP), (n=10), and Diabetic group that received 55mg/kg (IP), streptozotocin (STZ) (n=10) which was subdivided to two groups of 10, STZ group and treatment group. Treatment group received 55mg/kg (IP) STZ plus15mg/kg QR (IP), the control group just received an equal volume of 1cc distilled water daily (IP). Diabetes were induced by a single intra peritoneal (1P) injection of streptozotocin (STZ, Sigma- U.S.A.) in 0.1 M citrate buffer (pH 4.0) at a dose of 55 mg/kg body weight. Quercetin (QR) injections were continued to the end of the study (for 4 weeks), (Coskun et al., 2005).

Induction of experimental type 1 diabetes

Experimental type 1 diabetes was induced in rats by intra peritoneal (1P) injection of 55 mg/kg streptozotocin (STZ) in distilled water. Control rats were received distilled water only.

Blood glucose determination

Blood samples were collected from the tail vein. Basal glucose levels were determined prior to STZ injection, using an automated blood glucose analyzer (Glucometer Elite XL). Sample collections were then made 48 h after STZ injection and blood glucose concentrations were determined and compared between groups. Rats with blood glucose concentrations above 300 mg/dl were declared diabetic and were used in the experimental group. One week after the induction of experimental diabetes, protocol was started.

Quercetin preparation

Quercetin powder was obtained from Sigma Chemical Company (St. Louis, MO, USA). It was dissolved and diluted with 29% glycerol in 0.9% normal saline, mixed vigorously and stored in a dark bottle at 4°C. The quercetin solution was freshly prepared each week.

Surgical procedure

In the 28th day, (at the end of the treatment period), the rats were killed with diethyl ether, and kidney tissues in control and experimental groups were immediately removed.
Table I. Statistic analysis Dunnett (one side) shows significant differences between experimental groups in comparison to control group (P<0.05)

<table>
<thead>
<tr>
<th>Groups</th>
<th>control (n=10)</th>
<th>Quercetin (15mg/kg(IP)) (n=10)</th>
<th>STZ (55 mg /kg (IP)) (n=10)</th>
<th>Quercetin +STZ 55mg /kg(IP)streptozotocin1usi15mg/kg Quercetin (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal Tubules Apoptotic cells (TAC) (nmol/mL)</td>
<td>2.05±0.21</td>
<td>1.02±2.12</td>
<td>9.05±0.10*</td>
<td>5.11±0.11*</td>
</tr>
<tr>
<td>(nmol/mL)</td>
<td>0.70±0.03</td>
<td>0.75±0.03*</td>
<td>0.32±0.04*</td>
<td>0.61±0.05*</td>
</tr>
<tr>
<td>(MDA)</td>
<td>0.25±0.04</td>
<td>0.30±0.212*</td>
<td>4.1±0.06*</td>
<td>1.1±0.08*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.

* P-value less than 0.05 was considered significant and it is written in the parentheses, (compared with the control group).

**TUNEL analysis of apoptosis**

The *in-vivo* DNA fragmentation was visualized by TUNEL method (Huang et al., 1995). Briefly, dewaxed tissue sections were predigested with 20 mg/ml proteinase K for 20 min and incubated in phosphate buffered saline solution (PBS) containing 3% H2O2 for 10 min to block the endogenous peroxidase activity. The sections were incubated with the TUNEL reaction mixture, fluorescein-dUTP (*in situ* Cell Death Detection, POD kit, Roche, Germany), for 60 min at 37°C. The slides were then rinsed three times with PBS and incubated with secondary anti-fluorescin-POD-conjugate for 30 min. After washing three times in PBS and diaminobenzidine-H2O2 (DAB, Roche, Germany) chromogenic reaction was added on sections and counterstained with hematoxylin.

As a control for method specificity, the step using the TUNEL reaction mixture was omitted in negative control serial sections, and nucleotide mixture in reaction buffer was used instead. Apoptotic cells were quantified by counting the number of TUNEL stained nuclei per Proximal Tubules, cross sections. Cross sections of 100 kidney tissues per specimen were assessed and the mean number of TUNEL positive apoptotic cells per cross-section was calculated.

**Measurement of Serum Total Antioxidant Capacity (TAC)**

TAC was measured in serum by means of a commercial kit (Randox Co-England). The assay is based on the incubation of 2, 2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) with a peroxidase (methemoglobin) and hydrogen peroxide to produce the radical cation ABTS+, which has a relatively stable blue-green color, measured at 600 nm. The suppression of the color is compared with that of the Trolox, which is widely used as a traditional standard for TAC measurement assays, and the assay results are expressed as Trolox equivalent (nmol/L), (Feng et al., 2001).

**Measurement of serum MDA**

Tissue MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDAformed/mL. Plasma MDA concentrations were determined with spectrophotometer. A calibration curve was prepared by using 1,1',3,3'-tetramethoxypropane as the standard (Quintanilha et al., 1982).

**Statistical analysis**

Statistical analysis was done using the ANOVA for comparison of data in the control group with the experimental groups.
The results were expressed as mean ± S.E.M (standard error of means). P-value less than 0.05 were considered significant and are written in the parentheses.

RESULT AND DISCUSSION

Amount of proximal tubules apoptotic among kidney cells

Number of Proximal Tubules Apoptotic cells colored brown, in diabetic group was (9.05±0.10) and in quercetin received diabetic group and control group was (5.11±1.11) and in quercetin was (1.02±2.12) and in control group was (2.05±0.21) respectively. These changes was significant as p value less than 0.05 (P<0.05).

Results of total blood anti oxidant capacity

Amount of total blood anti-oxidant capacity in control group was (0.70±0.03 mmol/ml) and in experimental groups was 0.75±0.03, 0.32±0.04 and 0.61±0.05 mmol/mL respectively. Statistic analysis Dunnett (one side) shows significant differences between experimental groups in comparison to control group(P<0.05) (Table I).

Results of MDA level in blood

MDA level in control group was 0.25±0.04 mmol/L and in experimental groups was 0.30±0.212, 4.1±0.06, 1.1±0.08 mmol/L respectively. Statistic analysis Dunnett (one side) shows significant differences between experimental groups in comparison to control group(P<0.05) (Table I).

Make use of onion and quercetin in diabetic patient treatment has been experimented (Manchba and Achihe, 2007). Investigations show onion and quercetin decrease serum glucose level (Cunha et al., 2008) but this reduction with onion has been significant (Mohsen et al., 2008). Also quercetin decreases oxidative stress and blood vessels damage in diabetic rats (Atsushi et al., 2008; Gusbro et al., 2001). Other investigations show quercetin increases the level of blood insulin and serum Ca²⁺ and Mg²⁺ (Lecube et al., 2004). Investigations show liver has an important role in carbohydrate metabolism since it has been responsible for the balance of blood glucose level by means of glycogen and glycolysis therefore impaired hepatic function impairs metabolic homeostasis of glucose (Holstein et al., 2002; Tappy and Michlina, 2001). In the presence of impaired glucose metabolism and occurrence of hyperglycemia, genes involved in fatty acid storage were activated (Baynes and Thorpe, 1999). On the other hand, liver diseases can induce diabetes mellitus. This type of diabetes mellitus is clinically different from that of type II diabetes mellitus since it has been less frequently associated with microangiopathy (Lecube et al., 2004). Insulin resistance occurs in muscular and adipose tissues combined with hyperinsulinemia are pathophysiological bases of diabetes in liver disease (Skibola and Smith, 2000). The etiology of liver disease is important in the incidence of diabetes mellitus since non alcoholic fatty liver disease (NALFD), alcohol, hepatitis C virus (HCV) and hemochromatosis are frequently associated with diabetes mellitus (Lecube et al., 2004). Investigations show liver tissue damage and apoptosis induced by diabetes mellitus increase active O2 species. Flavonoids as an antioxidant factor found in nutrient such as fruit, vegetables, tea and black burgundy grape (Manch et al., 1998). Flavonoids value in daily mail varies from 16 mg to 1000 mg. Quercetin as an important flavonoids found in human mail (Formica and Regelson, 1995). Investigations show quercetin absorbs in small bowel. Useful effect of quercetin 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 (Hertog and Hoffman, 1996; Bors et al., 1977; Dok-Go et al., 2003). One of the quercetin antioxidant mechanism is removal of free radicals such as xanthine superoxide and xanthine oxidase (Mi and Zhang, 2005). Investigations show quercetin in the chicken spermatogonial cell culture not only doesn't have harmful effects but also increases amount of spermatogonial cells and decreases oxidative effects. In this study like previous investigations quercetin decreases malondialdehyde and increases serum anti oxidant capacity (Mi et al., 2007; Chandel et al., 2008; Naziroogi, 2003; Sanders et al., 2001; Drobioza et al., 2009).
CONCLUSION

According to our results it seems quercetin as an anti-oxidant which can protect kidney cells from cell injury by modulating TAC and MDA in diabetic conditions, and it is advised to use it in diabetes diseases.

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REFERENCES


