Beneficial Effects of Quercetin on Sperm Parameters in Streptozotocin-Induced Diabetic Male Rats

Arash Khaki1,*, Fatemeh Fathiazad2, Mohammad Nouri3, AmirAfshin Khaki4, Navid A. Maleki1, Hossein Jabbari khamnei5 and Porya Ahmadi1
1Department of Veterinary Pathology,Islamic Azad University Tabriz Branch, Iran. 2Department of Pharmacognosy,Tabriz University of Medical Sciences, Iran. 3Department of Biochemistry,Tabriz University of Medical Sciences, Iran. 4 Department of Anatomical Sciences, National Public Health Management Center (NPMC), Tabriz University of Medical Sciences, Iran. 5Department of Mathematical Sciences, University of Tabriz, Iran.

Quercetin (QR) is a strong antioxidant and has been shown to reduce oxidative stress in the long-term treatment of streptozotocin (STZ)-induced diabetes in animal models. Antioxidants have significant effects on spermatogenesis, sperm biology and oxidative stress, and changes in antioxidant capacity are considered to be involved in the pathogenesis of chronic diabetes mellitus. The present study aims to examine the influence of QR on spermatogenesis in STZ-induced diabetes in male Wistar rats. Animals (n = 50) were allocated into five groups: Group 1: Control rats given 0.5 ml of 20% glycerol in 0.9% normal saline. Group 2: Control rats given buffer (pH4.0).Group 3: diabetic controls. Group 4: rats given QR 15 mg/kg/day (i.p.). Group 5: STZ + QR rats. Animals were kept in standard conditions. At the end of the experiment (28th day), blood samples were taken for determination of testosterone, total antioxidant capacity, and levels of malondialdehyde and oxidized low-density lipoprotein. All rats were euthanized, testes were dissected out and spermatozoa were collected from the epididymis for analysis. Sperm numbers, percentages of sperm viability and motility, and total serum testosterone increased significantly in QR-treated diabetic rats (P < 0.05) compared with control groups. In histopathology, degeneration and inflammation in testes cells associated with diabetes were improved and testes weights in the QR-treated diabetic group decreased significantly in comparison with controls (P < 0.05). We conclude that QR has significant beneficial effects on the sperm viability, motility, and serum total testosterone and could be effective for maintaining healthy sperm parameters and male reproductive function in diabetic rats. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: quercetin; streptozotocin; spermatogenesis; rat.

INTRODUCTION

Diabetes is associated with reproductive impairment in both men and women. About 90% of diabetic patients have disturbances in sexual function, including decreases in libido, impotence and fertility (Jiang, 1996; Shi-Liang et al., 2001). In this context, attention has been paid to the search for effective antidiabetic drugs in the field of traditional medicine and phytochemicals. 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 (Davis, 2006). 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). Although the mechanisms underlying the alterations associated with diabetes mellitus are presently not well understood, hyperglycemia causes increased oxidative stress because the production of several reducing sugars is enhanced via glycolysis and the polyol pathway (Palmeira et al., 2001). These reducing sugars can easily react with lipids and proteins (non-enzymatic glycation), increasing the production of reactive oxygen species (ROS) (Palmeira et al., 2001). The major concern in diabetes is increased oxidative stress. Thus, increased production of free radicals or ROS may induce oxidized low-density lipoproteins (Ox-LDL), which are key factors in the sequence of events leading to atherosclerosis. Thus, sustained hyperglycemia and increased oxidative stress are the major players in the development of secondary complications in diabetes. These abnormalities produce a variety of pathologies including vasculopathies, neuropathies, ophthalmopathies, and nephropathies, among many other medical derangements (Sexton and Jarow, 1997). Maintaining a balance between ROS and antioxidants is a major mechanism in preventing damage from oxidative stress. Therefore, dietary supplementation with antioxidants such as vitamins, and flavonoids has been used in attempts to prevent the occurrence of many chronic diseases (Pelu-
soetal, 2006). Quercetin (QR) is a well-known flavonoid and a strong antioxidant derived from the onion, Allium cepa, and it has been shown to reduce oxidative stress in the long-term treatment of streptozotocin (STZ)-induced diabetes in animals (Mahesh and Menon, 2004). Because STZ causes testicular dysfunction and degeneration under situations of experimentally induced diabetes in animal models (Shrilatha and Muralidhara, 2007), we hypothesized that QR might decrease the harmful effects of STZ on testicular and sperm functions by reducing ROS production. Based on our previous study on the effects of Allium cepa on spermatogenesis (Khaki et al., 2009), we aimed to study the role of QR, an active flavonoid component of Allium cepa, in the improvement of testis architecture impairment associated with diabetes.

MATERIALS AND METHODS

Animals. Fifty male, 8-week-old Wistar albino rats weighing 250 ± 10 g were obtained from the animal facility of the Pushture Institute of Iran. Rats were housed in temperature controlled rooms (25°C) with constant humidity (40–70%) and a 12/12 h light/dark cycle prior to use in experimental protocols. All animals were treated in accordance with the Principles of Laboratory Animal Care [NIH]. The experimental protocol was approved by the Animal Ethics Committee in accordance with the guide for the care and use of laboratory animals prepared by Tabriz University of Medical Sciences. All rats were fed a standard diet and water. The daily intake of animal water was monitored at least one week prior to the start of treatments to determine the amount of water needed per experimental animal. Diabetes was induced by a single intraperitoneal (i.p.) injection of streptozotocin (STZ, Sigma-Aldrich, St Louis, MO, USA) in 0.1 M citrate buffer (pH 4.0) at a dose of 55 mg/kg body weight (Mahesh and Menon, 2004). Blood glucose concentration and changes in body weight were monitored regularly.

The rats were divided into five groups comprising ten animals in each group as follows:

Group 1: Control rats given only 0.5 ml of citrate buffer (pH 4, STZ vehicle) daily.
Group 2: Control rats given only 0.5 ml 20% glycerol in 0.9% normal saline (QR vehicle) daily
Group 3: Diabetic control (55 mg/kg, single intraperitoneal injection of STZ)
Group 4: Normal rats given only quercetin (15 mg/kg/day for 28 days)
Group 5: Diabetic rats treated with QR (15 mg/kg/day, started 48 hours after STZ injection).

At the end of the experiment, blood was collected into heparinized tubes, and serum was separated by centrifugation and used for further analysis. All rats were euthanized, testes were dissected out and spermatozoa were collected from the epididymis.

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, Bayer HealthCare, Basel, Switzerland). Samples were then taken 24 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. The experimental protocol was started 48 h after the induction of experimental diabetes.

Serum insulin level. Serum insulin concentrations were determined by using radioimmunoassay kit (Boehringer Mannheim, Germany). The insulin level in serum was expressed in μU/mL.

QR preparation. QR was dissolved in 20% glycerol in 0.9% normal saline, mixed vigorously and stored in a dark bottle at 4°C. The solution was freshly prepared each week.

Surgical procedure. On the 28th day, (at the end of the treatment period), the rats were sacrificed with diethyl ether and the testes in control and experimental groups were removed immediately. The weight of testes was recorded.

Sperm analysis. Spermatozoa from the cauda epididymis were released by cutting the organ into 2 mL of medium (Hams F10) containing 0.5% bovine serum albumin. After 5 min incubation at 37°C (under 5% CO2 in air), the epididymal sperm reserves were determined using the standard hemocytometric method (WHO) and sperm motility was analyzed microscopically (Olympus IX70) [×40 magnification] in 10 fields according to the World Health Organization (WHO, 1992) recommended method. Sperm abnormalities were evaluated according to Khaki et al. (2008). Briefly, sperm smears were made on clean glass slides and stained with periodic acid-Schiff’s reaction plus hematoxylin. The stained smears were observed under a light microscope using a 40× objective. Sperm were classified as normal or abnormal. The total sperm abnormality was expressed as percentage incidence. Sperm viability was performed by the eosin nigrosin staining. One drop of semen was mixed with two drops of 1% eosin Y. After 30 s, three drops of 10% nigrosin were added and mixed well. A smear was made by placing a drop of mixture on a clean glass slide and allowed to air dry. The prepared slide was examined using a phase contrast microscope. Pink-stained dead sperm were differentiated from unstained live sperm, and there numbers were recorded.

Measurement of serum total antioxidant capacity (TAC). TAC was measured in serum using a commercial kit (Randox Laboratories, Crumlin, UK). The assay is based on the incubation of 2, 2’-azino-di-(3-ethylbenzthiazoline sulfonate) (ABTS) with a peroxidase (methymoglobin) and H2O2 to produce the radical cation ABTS+, which has a relatively stable blue-green color measured spectrophotometrically at 600 nm. The suppression of the color is compared with that of Trolox, which is widely used as a standard for TAC measurements and the assay results are expressed as Trolox equivalents (in nmol/mL) (Quintanilha et al., 1982).

Measurement of serum malondialdehyde (MDA). Serum MDA levels were determined by the thiobarbi-
BENEFICIAL EFFECTS OF QUERCETIN ON SPERM

Epididymal sperm count/rat

Body weight (g) 251

Testis weight (g) 1.40

Blood glucose (mg/dl) 135.3

Oxidized low density lipoprotein (U/L) 3.1

Malondialdehyde (MDA) (nmol/ml) 0.25

Total antioxidant capacity (TAC)

Serum testosterone levels (ng/ml) 4.01

Viability(%) 66.25

Motility (%) 33.75

Assay sensitivity per tube was 0.025 ng/mL (Huang et al., 1995).

Histopathology of the testis. The testis was fixed in 10% formalin and embedded in paraffin wax. Five-micron thick sections were prepared and stained with hematoxylin and eosin (H&E). The specimens were examined using an Olympus 3H light microscope.

Statistical analysis. Statistical analysis was done using the ANOVA and test for comparison of data in the control group with the experimental groups. The results are expressed as the mean ± standard error of mean (SEM) and P < 0.05 was considered significant.

RESULTS

Testis weight

The results are listed in Table 1. There was significant decrease in the mean testis weight in the STZ group compared with controls (P < 0.05). There were no significant differences in testis weight between the other groups.

Sperm motility, viability and counts

STZ treatment decreased sperm count, motility and viability significantly in the diabetic group compared

Table 1. The effect of streptozotocin with and without 28 days of treatment with QR on sperm parameters, serum total testosterone, total antioxidant capacity, malondialdehyde and oxidized low-density lipoprotein levels, blood glucose, insulin and testis weights. P values are shown in parentheses in italics.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (n = 10)</th>
<th>Quercetin 15 mg/kg i. p. (n = 10)</th>
<th>Streptozotocin 55 mg/kg i. p. (n = 10)</th>
<th>Treatment Streptozotocin 55 mg/kg (i. p) plus Quercetin 15 mg/kg (i. p) (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis weight (g)</td>
<td>1.40 ± 0.821</td>
<td>1.36 ± 0.821</td>
<td>1 ± 0.05*</td>
<td>1.34 ± 0.30</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>251 ± 0.365</td>
<td>252.3 ± 0.761</td>
<td>199.3 ± 0.831*</td>
<td>231.7 ± 2.7*</td>
</tr>
<tr>
<td>Epididymal sperm count/rat × 10⁶</td>
<td>48.68 ± 7.70</td>
<td>47.05 ± 5.70</td>
<td>38.21 ± 3.50*</td>
<td>4.03 ± 5.20*</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>33.75 ± 6.88</td>
<td>35.42 ± 6.88</td>
<td>10.05 ± 6.88*</td>
<td>30.64 ± 3.01*</td>
</tr>
<tr>
<td>Viability(%)</td>
<td>66.25 ± 4.73</td>
<td>67.05 ± 5.11</td>
<td>43.26 ± 2.33*</td>
<td>57.25 ± 4.22*</td>
</tr>
<tr>
<td>Serum testosterone levels (ng/ml)</td>
<td>4.01 ± 0.50</td>
<td>4.50 ± 0.45</td>
<td>1.60 ± 0.05*</td>
<td>3.20 ± 0.28*</td>
</tr>
<tr>
<td>Total antioxidant capacity (TAC) (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>Malondialdehyde (MDA) (nmol/ml)</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>
<tr>
<td>Oxidized low density lipoprotein (U/L)</td>
<td>3.1 ± 0.05</td>
<td>3.0 ± 0.45</td>
<td>5.6 ± 0.85</td>
<td>4.9 ± 0.80</td>
</tr>
<tr>
<td>Blood glucose(mg/dl)</td>
<td>135.3 ± 0.943</td>
<td>123.9 ± 1.149*</td>
<td>382.6 ± 0.702*</td>
<td>356.4 ± 1.455*</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>24.7 ± 0.423</td>
<td>21.4 ± 0.427*</td>
<td>12.1 ± 0.547*</td>
<td>17.6 ± 0.34*</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SEM.
P < 0.05 was considered significant, compared with the control group.

Copyright © 2010 John Wiley & Sons, Ltd.

with the control and other experimental groups. The sperm concentrations, motility and vitality were 23.81 ± 3.20 rat × 10^6, 10.05 ± 6.88% and 43.26 ± 2.33 %, respectively, in the STZ group. The corresponding values in the QR group were 47.05 ± 5.70 rat × 10^6, 35.42 ± 6.88 % and 67.05 ± 5.11% and the corresponding values in the STZ + QR group were 42.03 ± 5.20 rat × 10^6, 30.64 ± 3.01% and 57.25 ± 4.22%. However, the sperm concentrations, motility and vitality in control group were 48.68 ± 7.70 rat × 10^6, 33.75 ± 6.88 % and 66.25 ± 4.73%. There were no significant changes in the proportions of sperm abnormalities in experimental groups compared with control groups (Table 1).

**Total serum testosterone**

STZ treatment caused a significant decrease in the total serum testosterone level in the diabetic group (STZ) compared with the control, QR and STZ + QR groups. The values were 1.60 ± 0.05, 3.20 ± 0.28 ng/mL, respectively (Table 1).

**TAC in serum**

The mean TAC showed a significant increase (P < 0.05) in the QR group (0.75 ± 0.03 nmol/mL) compared with the control (0.70 ± 0.03 nmol/mL), STZ (0.32 ± 0.04 nmol/mL) and STZ + QR (0.61 ± 0.05 nmol/mL) groups.

**MDA in serum**

The MDA level showed a significant (P < 0.05) decrease in the QR (0.30 ± 0.212 nmol/mL) and control groups (0.25 ± 0.04 nmol/mL) compared with STZ (4.1 ± 0.06 nmol/mL) and STZ + QR (1.1 ± 0.08 nmol/mL) groups.

**Ox-LDL in serum**

Ox-LDL levels increased in the STZ (5.6 ± 0.85 U/L) and STZ + QR (4.9 ± 0.80 U/L) groups compared with control (3.1 ± 0.05 U/L) and QR (3.0 ± 0.45 U/L) groups (Table 1).

**Light microscopy**

Histopathology showed that the cycle of spermatogenesis was normal in the control (Fig. 1Aa) and QR groups (Fig. 1B). Sperm presence in lumen of seminiferous tubule in the QR group, (arrow) (Fig. 1B). However, the testes of all rats in the STZ group showed seminiferous tubule degeneration. This typically involved depletion of germ cells, germ cell necrosis and evidence of cell debris in the lumen and intratubular space fibrosis and Inflammatory cells also (lymphocytes) were present(Fig. 1Ca) and loose arrangement of spermatogonia in seminiferous tubules, atrophied seminiferous tubules, venous congestion and thickening of the tunica albuginea were showed (Fig.1Da). In the STZ + QR group, re-arrangements of germ cells in seminiferous tubules were seen (Fig. 1E).

**DISCUSSION**

Male reproductive function is clearly impaired in diabetes. Diabetes-induced alterations of Leydig cell functions include a decrease in androgen synthesis and in
the total number of these cells (Foglia et al., 1969). Together, these effects impair male libido (Oksanen, 1975). The diabetes-induced alterations of Leydig cells are related to concomitant alterations in the control mechanisms that modulate the proliferation, differentiation and overall function of these cells (Coskun et al., 2005). Furthermore, diabetes-related alterations in Leydig cells are also related to changes in the pituitary–testicular axis (Steger and Rabe, 1997). Thus, this disease induces a decrease in the serum levels of luteinizing hormone (LH), which is responsible for normal Leydig cell function (Benítez and Perez-Diaz, 1985; Steger and Rabe, 1997). Diabetes-induced testicular dysfunction might be transient or permanent depending on the degree and duration of the disease. Erectile dysfunction is a well-recognized complication of diabetes mellitus. Infertility among men with diabetes is a less well-examined problem and their gonadal status is not clearly established. The low incidence of diabetes in infertile patients might be the reason for the limited amount of current research (Altay et al., 2003). However, an altered testicular axis was noted in experimental studies. Seethalakshmi et al. (1987) found that testicular weight, sperm count and motility decreased significantly in diabetic rats. Moreover, Cameron et al. (1985) found increased seminiferous tubule wall thickness, germ cell depletion and Sertoli cell vacuolization in human testicular biopsies from diabetic subjects and in diabetic rats. Results in the same study showed that diabetes induced a clear impairment of reproductive performance in rats, and tungstate treatment led to a recovery of reproductive performance by increasing the number
of Leydig cells (Ballester et al., 2005). Oxidative stress also plays a role in the development of diabetic complications (Sexton and Jarow, 1997). Oxidative damage in rats with STZ-induced diabetes was ascertained in the present study by measuring the MDA levels, ROS generation, alterations in antioxidant defense and the serum level of Ox-LDL. Testicular complications such as tubular atrophy, depletion of germ cells and congestion in veins were noted. Moreover, testicular weight, epididymal sperm reserves and motility were all decreased significantly. There was expansion of the interstitial space with vacuolization and the Leydig cells had an abnormal fibroblast-like appearance. Fibroblastic degeneration appeared in the seminiferous tubules and was increased in the STZ group compared with those observed in the control and other experimental groups. These results are clearly in agreement with other studies (Cameron et al., 1985; Shrilatha and Muralidhara, 2007). Serum total testosterone and TAC levels showed marked decreases in the STZ-induced diabetic group compared with those seen in the control and other experimental groups and these results were in agreement with Tang et al. (2008). The testicular injury and apoptosis induced by diabetes are partially attributed to augmented oxidative stress in testicular tissue. Flavonoids are antioxidant agents widely distributed in dietary plants frequently consumed by humans such as fruits, vegetables, teas and wine (Skibola and Smith, 2000). The dietary intake of flavonoids in humans has been estimated to be 16–1000 mg/day. QR is regularly consumed by humans as it is the major flavonoid found in the human diet (Manach et al., 1998). A number of beneficial effects of QR on human health have been known for some time (Formica and Regelson, 1995; Hertog and Hollman, 1996). It is reported to decrease capillary fragility, to protect against diabetic cataracts, to possess antiviral and anti-allergenic activities, to inhibit platelet aggregation and the oxidation of LDL and to act as an anti-inflammatory agent (Bors et al., 1977). QR as an important dietary flavonoid possesses beneficial effects for human health because of its antioxidant function. One mechanism of the antioxidant action of QR is scavenging free radicals, such as the superoxide radicals generated by xanthine/xanthine oxidase (Dok-Gh et al., 2003). Studies on the effects of QR on oxidative damage in cultured chicken spermatozoidal cells showed that it had no deleterious effects at doses of 1 and 10 μg/mL. QR at 1 μg/mL increased the numbers of spermatozoidal cells and reduced Aroclor-induced oxidative damage in the testes (Mi and Zhang, 2005; Chandel et al., 2008). In the present study, QR decreased the effect of STZ-induced diabetes on serum MDA and enhanced the serum TAC levels. Thus suggests that excessive ROS might have been involved in the damage. In our study QR treatment significantly improved epididymal sperm quality and numbers, and decreased the serum ROS and Ox-LDL levels in STZ-induced diabetic rats. In additional, blood glucose levels showed significant decreased in STZ + QR-treated rats. These results are in agreement with these reported by Vessal et al. (2003). who showed QR, a flavonoid with antioxidant properties brings about the regeneration of the pancreatic islets and probably increases insulin release in streptozotocin-induced diabetic rats. This anti-hyperglycemic effect consequently may alleviate testis cell damages associated with STZ-induced diabetic rats. The present study suggests a beneficial effect of QR probably by its antioxidant and anti-diabetic properties. As this antioxidant flavonoid is known to decrease the risk of degenerative diseases, we suggest that using dietary fruit, vegetables, onion, teas and red wine rich in flavonoids and QR could have beneficial effects on subjects with diabetes. Additional studies should be performed to better understand the mechanism of QR on spermatogenesis.

Acknowledgment

We thank the staff at Islamic Azad University of Tabriz for their help and financial support in the preparation of this manuscript.

REFERENCES


