Protective Effects of Quercetin on Spermatogenesis in Streptozotocin-induced Diabetic Rat

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Abstract

Background: Quercetin is a strong antioxidant and long-term treatment of STZ-diabetic animals and it has been shown to reduce oxidative stress.

Objective: antioxidants have essential effect on spermatogenesis and sperm parameters. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus.

Methods: 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 55 mg/kg (IP) streptozotocin (STZ) (n=20) which was subdivided to two groups of 10; STZ group and treatment group. Treatment group received 55 mg/kg (IP) STZ plus15mg/kg QR, daily for,4 weeks, respectively; however, the control group just received an equal volume of distilled water daily (IP). Diabetes was induced by a single intra peritonea injection of streptozotocin (55 mg/kg). Animals were kept in standard condition. In 28day after inducing diabetic 5cc blood were collected for testosterone, TAC, MDA and Ox-LDL levels and testes tissues of Rat in whole groups were removed and sperm was collected from epididymis then prepared for analysis.

Results: Sperm population, percentage of sperm viability and motility significantly increased in group that has received 15 mg/kg (IP) Quercetin ($p<0.05$) in comparison to control and experimental groups.

Conclusion: Since in our study 15 mg/kg (IP) Quercetin have significantly Preventive effect on Sperm percentage of viability and motility by reducing level of Reactive Oxygen Species (ROS) in serum, so it seems that using it can be effective for sperm healthy parameters in Diabetic Rat.

Keywords: Quercetin, Streptozotocin, Spermatogenesis, Rat

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Introduction

Diabetes has been associated with reproductive impairment in both men and women. About 90% of diabetic patients have disturbances in sexual function, including a decrease in libido, impotence and infertility [1, 2] Due 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 [3]. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus [4, 5]. 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 glycolysis and the polyol pathway) is enhanced [6]. These reducing sugars can easily react with lipids and proteins (nonenzymatic glycation reaction), increasing the production of reactive oxygen species (ROS) [6]. 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. increaesd production of free radicals or ROS formation may induce oxidized LDL (Ox-LDL), which is key step in the sequence of events leading to atherosclosis 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 [7]. The balance of ROS and antioxidant is a major mechanism 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 [8]. 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 [9]. Since streptozotocin causes testicular dysfunction and degeneration under situations of experimentally induced diabetes in animal models [10], it is hypothesized that Quercetin can decreasing STZ harmful effect on testis and sperm parameters by reducing reactive oxygen species (ROS). According to our systematic study on effect of *Allium cepa* and other medicinal plant on spermatogenesis [11] we plant to study the effect of Quercetin as a active component of *Allium cepa* on spermatogenesis to confirm wither the single component could be effective as much as the total extracts.

Materials and Methods

Animals

Forty adult Wistar albino male rats were 8 weeks old and weighing 250 ± 10 g, they were obtained from animal facility of pasture 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

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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=20) 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 was induced by a single intra peritoneal (I.P) 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), [12].

Induction of experimental type 1, Diabetes

Experimental type 1 diabetes was induced in rats by intra peritoneal (I.P) injection of 55 mg/kg streptozotocin (STZ) in distilled water. Control rats were received distilled water, only, [13].

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 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.

Surgical Procedure

In the 28th day, (at the end of the treatment period), the rats were killed with diethyl ether, and the testes in control & experimental groups were immediately removed. The weights of testes in all study groups were recorded.

Sperm analysis

Sperms from the cauda epididymis were released by cutting into 2 ml of medium (Hams F10) containing 0.5 % bovine serum albumin .After 5 min incubation at 37°C (with 5 % CO_2), the cauda epididymis sperm reserves were determined using the standard hemocytometric method and sperm motility was analyzed with microscope (Olympus IX70) at 10 field and reported as mean of motile sperm according to WHO method (WHO). The sperm abnormality was evaluated according to a standard method of Khaki et al [13]. Briefly the smears of sperm suspension were made on clean glass slides and stained with periodic acid-Schiff's reaction hematoxylin. The stained smears were observed under a light microscopic with 40X objective. Sperms were classified into normal and abnormal. The total sperm abnormality was expressed as percentage incidence.

Measurement of Serum Total Antioxidant capacity (TAS)

TAS was measured in serum by means of a

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commercial kit (Randox Co-England). The assay is based on the incubation of 2, 2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) with a peroxidase (methmyoglobin) 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 TAS measurement assays, and the assay results are expressed as Trolox equivalent (mmol/L) [14].

Measurement of Serum MDA

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. A calibration curve was prepared by using 1, 1',3,3'-tetramethoxypropane as the standard [15].

Measurement of Ox-LDL

Oxidized LDL level was measured by using a Mercodia Oxidized LDL ELISA kit (Lot No. 15904; Mercodia, Uppsala, Sweden). Mercodia Oxidized LDL Competitive ELISA is based on the monoclonal antibody 4E6 [16, 17].

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 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.

Results

Results of sperm motility, viability and count

Streptozotocin-induced diabetic model by 55 mg/kg significantly decreased sperm count, motility and viability in diabetic group as compared with those observed in the control and other experimental groups. The sperm concentrations, motility and vitality were (23.81 ± 3.20 and 10.05 ± 6.88 and 43.26 ± 2.33) in STZ group and the corresponding values in QR group were (47.05 ± 5.70, 35.42 ± 6.88 and 67.05 ± 5.11) and the corresponding values in treatment (STZ+QR) group were (42.03 ± 5.20, 30.64 ± 3.01 and 57.25 ± 4.22). However, the sperm concentrations, motility and vitality in control group were (48.68 ± 7.70, 33.75 ± 6.88 and 66.25 ± 4.73). There weren't any significant change in sperm abnormality in experimental groups as compared with control group, (Table 1).

Results of Total Antioxidant capacity (TAC) measurement in Serum

The mean concentration of Total Antioxidant capacity (TAC) showed significant increase ($p<0.05$) in QR group (0.75 ± 0.03) as compared with control group (0.70 ± 0.03) and STZ (0.32 ± 0.04) and STZ+QR (0.61 ± 0.05) groups.

Results of Malondialdehyde (MDA) measurement in Serum

Malondialdehyde (MDA) level showed significant ($p<0.05$) decrease in QR group (0.30 ± 0.212) and control group (0.25 ± 0.04) in comparison to STZ (4.1 ± 0.06) and STZ+QR (1.1 ± 0.08) groups.

Results of Ox-LDL concentration measurement in Serum

Ox-LDL level increased) in STZ (5.6 ± 0.85) and STZ+QR (4.9 ± 0.80) groups as compared with control (3.1 ± 0.05) and QR (3.0 ± 0.45) groups. (Table 1).

Discussion

It is noteworthy that diabetes-related alterations in Leydig cells are also related to

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Table 1: The effect of the 55 mg/kg (IP) streptozotocin, 15 mg/kg (IP) Quercetin, 55 mg/kg (IP) streptozotocin plus 15 mg/kg Quercetin (Treatment), daily for30 days on sperm parameters TAS, MDA, Ox-LDL level of control and experimental groups in the rats.

Groups	Control (n=10)	Quercetin (15mg/kg(IP)) (n=10)	Streptozotocin (55mg/kg (IP)) (n=10)	Treatment 55mg/kg (IP) streptozotocin plus15mg/kg Quercetin (n=10)
Sperm Concentration (total count) (No of sperm/rat$\times 10^6$)	48.68±7.70	47.05±5.70 (0.301)	23.81±3.20 * (0.001)	42.03±5.20 * (0.009)
Motility (%)	33.75±6.88	35.42±6.88 (0.231)	10.05±6.88 * (0.001)	30.64±3.01 * (0.008)
Viability (%)	66.25±4.73	67.05±5.11 (0.403)	43.26±2.33 * (0.007)	57.25±4.22 * (0.009)
Sperm Abnormality (%)	4.22±0.666	4.20±0.618	6.27±0.711	5.12±0.656
Total Antioxidant Capacity (TAS) (nmol/ml)	0.70±0.03	0.75±0.03 * (0.006)	0.32±0.04 * (0.003)	0.61±0.05 * (0.004)
Malondialdehyde (MDA) (nmol/ml)	0.25±0.04	0.30±0.212 * (0.004)	4.1±0.06 * (0.008)	1.1±0.08 * (0.005)
LDL-OX(u/l)	3.1±0.05	3.0±0.45 (0.108)	5.6±0.85 (0.061)	4.9±0.80 (0.055)

Data are presented as mean ± SE.
* P-value less than 0.05 were considered significant and are writing in the parentheses, (compared with the control group).

changes in the pituitary-testicular axis [18, 19, 20, 21, 22]. Thus, this disease induces a decrease in the serum levels of luteinizing hormone (LH), which is responsible for normal Leydig cell function [22, 23]. Diabetic testicular dysfunction might be transient or permanent depending on the degree and duration of the disease. Erectile dysfunction (ED) is a well-recognized complication of diabetes mellitus (DM). The low incidence of diabetes in infertile patients might be the reason for the limited amount of current research [24]. However, an altered testicular axis was noted in experimental studies Seethalakshmi et al. [25] found that testicular weight, sperm count and motility significantly decreased in diabetic rats. Moreover, Cameron et al. [26] defined increasing tubule wall thickness, germ cell depletion and Sertoli cell vacuolization in diabetic human testicular biopsies and in diabetic rats. Results in same study showed that diabetes induces a clear impairment of reproductive performance in rats and tungstate treatment in these diabetic rats leads to a recovery of reproductive performance by increasing the number of Leydig cells [27]. Oxidative stress plays a role in the development of diabetic complications [7]. Oxidative damage was ascertained by measuring the malondialdehyde levels, reactive oxygen species (ROS) generation, alterations in antioxidant defences and specifies the level of oxidized-LDL. Our results showed sperm count and motility are significantly decreased and Expansion of interstitial space with vacuolization and Leydig cells had an abnormal fibroblast-like appearance. The measurement of TAC levels showed marked decrease in streptozotocin-induced diabetic group as compared with those

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seen in the control and other experimental groups and these results were in agreement with Tang et al [28] research that showed testicular injury and apoptosis induced by diabetes are partially attributed to the augmented oxidative stress in testicular tissue. The dietary intake of flavonoids in humans has been estimated to be 16 – 1000 mg/day. Quercetin is regularly consumed by humans as it is the major flavonoid found in human diet [29, 30]. This flavonoid is reported to decrease capillary fragility, to protect against diabetic cataracts, to possess antiviral and antiallergenic activities, to inhibit platelet aggregation and the oxidation of low density lipoproteins, and to act as an anti-inflammatory agent [31]. Quercetin an important flavonoid possesses beneficial effects on health due to its antioxidant function. One mechanism of the antioxidant action of quercetin was involved in scavenging free radicals, such as superoxide radicals generated by xanthine/ xanthine oxidase.Previous studies indicated that reactive oxygen substances may be involved in possible testicular complications in streptozotocin-induced diabetic of rats [32]. Since, in our study quercetin could significantly improve epididymal sperm quality and decrease serum Reactive Oxygen Species (ROS) and ox-LDL in Streptozotocin-induced diabetic rats, and has beneficial effect on antioxidants and decreases the risk of degenerative diseases. We suggest using dietary plants, fruits, vegetables, onion, teas, and red wine rich of flavonoids and Quercetin which could have beneficial effects on diabetic persons and decrease risk of infertility in men.

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