The Role of Quercetin in Alleviating the Testicular Oxidative Stress and Apoptosis in Streptozotocin-Induced Diabetes

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Abstract

Objective: The aim of this study was to investigate the effects of quercetin on oxidative stress and apoptosis in testicular tissue of streptozotocin-induced diabetic male rats.

Methods: Thirty-two Wistar type Albino rats were divided into 4 groups. The groups are planned as follows: group 1: control group (only 0.9% sodium chloride, i.p. injection for 30 days, n = 8), group 2: DM group (streptozotocin: 50 mg/kg, freshly dissolved in 0.9% sodium chloride, i.p, n = 8), group 3: DM+QR group (quercetin: 20 mg/kg/day, diluted in 4% ethanol, 30 days, i.p.) (streptozotocin: 50 mg/kg, i.p., single dose, n = 8), group 4: control+QR group (quercetin: 20 mg/kg/day, 30 day, i.p., n = 8). Testicular tissue samples were taken under anesthesia on the 31st day of the study. Malondialdehyde, lipid hydroperoxide, advanced oxidation protein products, total thiol, nonprotein sulfhydryl compounds, and Cu,Zn-superoxide dismutase were determined by spectrophotometric method.

Results: Malondialdehyde and lipid hydroperoxide levels were higher in the DM group compared to the control and DM+QR group. Advanced oxidation protein product levels were lower in the control group than in the control+QR and DM group, while total thiol, nonprotein sulfhydryl levels, and Cu,Zn-superoxide dismutase activity were higher in DM+QR group compared to control and DM groups. In the DM+QR group, a significant decrease was found in the number of apoptotic cells compared to the DM group ($P < .001$).

Conclusion: Quercetin administration can be potentially beneficial and effective in preventing oxidative stress (lipid and protein) damage and apoptosis in the testicles of diabetic rats.

Keywords: Quercetin, experimental diabetes, testicular tissue, oxidative stress, apoptosis

Streptozotosin ile Oluşturulan Diyabette Testiküler Oksidatif Stres ve Apoptozun Hafifletilmesinde Kuersetin'in Rolü

Öz

Amaç: Bu çalışmada, streptozotosin ile diyabet oluşturulan erkek sıçanlarda testis dokusunda kuersetin'in oksidatif stres ve apoptoz üzerindeki etkilerini araştırmak amaçlanmıştır.

Yöntemler: Çalışma kapsamında 32 erkek Wistar Albino sıçan 4 gruba ayrıldı. Grup 1: Kontrol grubu (30 gün boyunca sadece intraperitoneal %0,9 sodyum klorür uygulandı, n = 8), grup 2: Diyabet grubu (DM grubu, %0,9 sodyum klorür çözeltisinde taze olarak hazırlanmış 50 mg/kg streptozotosinin intraperitoneal olarak enjeksiyonu ile oluşturuldu, n = 8), grup 3: Diyabet + Kuersetin grubu (DM+QR grubu, Tek doz 50 mg/kg streptozotosin intraperitoneal enjekte edildi ve 30 gün boyunca %4 etanol çözeltisinde seyreltilmiş 20 mg/kg/gün kuersetin intraperitoneal uygulandı, n = 8), grup 4: Kontrol + Kuersetin grubu (Kontrol +QR grubu, 30 gün boyunca 20 mg/kg/gün kuersetin intraperitoneal uygulandı, n = 8). Çalışmanın 31. gününde anestezi altında testis dokusu örnekleri alındı. Malondialdehit, lipid hidroperoksit, ileri oksidasyon protein ürünleri, total tiyol, protein olmayan sülfhidril bileşikleri ve Cu, Zn-süperoksit dismutaz spektrofotometrik yöntemle belirlendi.

Bulgular: DM grubunda malondialdehit ve lipid hidroperoksit düzeyleri kontrol ve DM+QR grubuna kıyasla yüksek bulundu. Kontrol grubunda ileri oksidasyon protein ürün düzeyleri kontrol+QR ve DM grubuna kıyasla daha düşük bulunurken; DM+QR grubunda total tiyol, protein olmayan sülfhidril düzeyleri ve Cu, Zn-süperoksit dismutaz akivitesi kontrol ve DM grubuna kıyasla daha yüksek bulundu. DM+QR grubunda apoptotik hücre sayısı DM grubuna kıyasla anlamlı derecede azalmış bulundu ($P <$,001).

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Sonuç: Kuersetin uygulaması, diyabetik sıçanların testislerinde oksidatif stres (lipid ve protein) hasarı ve apoptozu önlemede potansiyel olarak faydalı ve etkili olabilir.

Anahtar Kelimeler: Kuersetin, deneysel diyabet, testis dokusu, oksidatif stres, apoptoz

Diabetes mellitus (DM) is a group of endocrine disorders of carbohydrate, fat, and protein metabolism and characterized by hyperglycemia that causes long-term damage to many organs, including the heart, eye, kidney, nervous system, vascular system, and bone. Diabetes mellitus is also one of the most common chronic diseases affecting more than one hundred million people worldwide.[1]

Chronic hyperglycemia can cause reproductive complications. It has been reported that high glucose causes increased oxidative stress and apoptosis in testicular cells and thus contributes to infertility.[2] Although DM is described as a metabolic disorder, it is also important to highlight that it is a state of increased oxidative stress. It is known that in the case of hyperglycemia seen in diabetes, oxidative stress increases, and antioxidants decrease.[3,4] It has been shown that antioxidant therapy in diabetes increases the glycemic index, reduces diabetic complications, and has a protective effect against free radical-induced oxidative stress.[5,6] It is also known that oxidative stress plays an important role in the pathophysiology of diabetes related to male reproductive dysfunction and anomalies.[7-11]

Quercetin (QR) (3,5,7,3′,4′-pentahydroxyl flavone), an important member of the flavonoid family, is one of the most prominent dietary antioxidants.[12] Fat-soluble QR inhibits lipid peroxidation, glutathione peroxidase (GPaz), and xanthine oxidase by directly scavenging radicals such as superoxide ($O^{\bullet}$), lipid alkoxyl (RO•), peroxyl (ROO•), and nitric oxide (NO).[13-15] In addition to its antioxidant effect, it also shows strong anti-inflammatory, anti-ischemic, anti-peroxidative, and anti-apoptotic function by inhibiting NF-κB-linked gene transcription and COX-2 induction.[16,17] It has been reported that QR has protective effects against β-cell damage occurring in diabetes and is a possible effective agent for reducing blood glucose levels in diabetic rats.[15,18] It has also been suggested that QR administration has a protective effect in diabetes by reducing oxidative stress and preserving pancreatic β-cell integrity.[14,15,19]

This study aimed to investigate the effects of QR with known antioxidant properties on apoptosis and oxidative stress in order to prevent lipid and protein damage in testicular tissue exposed to hyperglycemia in rats induced by streptozotocin (STZ). Malondialdehyde (MDA) and lipid hydroperoxide (LPH) levels as markers of lipid peroxidation; advanced oxidation protein products (AOPP) as markers of protein oxidation; total thiol (T-SH), non-protein-SH (NP-SH) levels, and Cu,Zn-superoxide dismutase (Cu, Zn-SOD) activity as an indicator of antioxidant status were measured.

Material and Methods

Experimental animals and procedures

Our study was approved by the decision of Istanbul University Animal Experiments Local Ethics Committee no 2015/36. This work is derived from Eda Buyukcolpan's master's thesis (investigation of the QR effect on apoptosis and oxidative stress in diabetic nephropathy model rats induced by STZ). In this study, adult male Wistar Albino rats weighing 310-410 g were obtained from Istanbul University Laboratory Animal Science Department Production Department. The rats were kept at room temperature of 21°C, in 12 hours light and 12 hours dark, in standard cages measuring 50 × 30 × 30 cm, and 2 separate cages were kept for each of the groups in a well-ventilated room. The rats were fed with standard compressed rat pellet feeds containing 21% protein. The animals had free access to standard rat chow and drinking water. The animals were randomly divided into 4 groups as control groups (*healthy control and control + QR groups*) and diabetes mellitus groups (*DM and DM + QR groups*). *Control group:* It was separated as healthy control group. During the study, only saline (0.9% NaCl) was given intraperitoneally (i.p.) for 30 days (n = 8). *QR group:* QR (sc-206089B, Santa Cruz Biotechology) was administered at a dose of 20 mg/kg/day intraperitoneally for 30 days. This group was separated as a healthy control group to which QR was administered (n = 8). *Diabetes Mellitus (DM) group:* 50 mg/kg STZ (Sigma, St. Louis, MO, USA) freshly dissolved in 0.9% NaCl was administered intraperitoneally as a single dose (n = 8).[20] *DM + QR group:* 50 mg/kg STZ was administered intraperitoneally as a single dose; after the third day, QR dissolved in 4% ethanol was administered intraperitoneally at a dose of 20 mg/kg/day for 30 days (n = 8).[21,22] After creating the groups for the experimental study, blood was taken from the tail veins of the rats in all groups at the beginning of the study, on the 1st, 15th, and 30th days of the study, and blood glucose levels were measured using an eBsensor glucometer device (Blood Glucose Monitoring System, eB-G model, Taiwan) and eBsensor glucostics (Blood Glucose Test Strip, Taiwan). Three days after STZ injection, those with blood glucose levels above 250 mg/dL were considered diabetic and included in the study.

Preparation of tissue samples

Testicular tissues of animals were homogenized with phosphate-buffered solution (PBS, pH = 7.4; 3 × volume of sample) using a homogenizer (Next Advance Bullet Blender Storm 24). The homogenate was centrifuged at 3000 g for 10 minutes to remove debris. The clear upper supernatant was taken, and tissue analyses (MDA, LPH, AOPPs, T-SH, NP-SH, and Cu,Zn-SOD) were performed. All procedures were performed at +4°C throughout the experiments.

Analytical Methods

Lipid peroxidation markers

Measurements of malondialdehyde levels

Malondialdehyde levels were determined by Ohkawa et al[23] method with a minor modification. The reaction mixture was prepared by adding 1.5 mL of 0.75% thiobarbituric acid, 1 mL of 30% trichloroacetic acid, 0.2 mL of 5N hydrochloric acid, and 0.25 mL of tissue homogenates and heated at 100°C for 15 minutes. The mixture was cooled to room temperature, centrifuged (3000 g for 10 minutes), and the absorbance of the supernatant was recorded at 532 nm. 1,1,3,3-Tetramethoxypropane was used as MDA standard. MDA results are expressed as l mol/g wet tissue.

Measurements of lipid hydroperoxide levels

It is based on measuring the absorbance at 365 nm of the color formed by the tri-iodide complex as a result of the reaction between iodide and lipid hydroperoxides. The mixture, containing 0.01 mL butylated hydroxytoluene, 0.1 mL sample, and 1 mL color reagent, was left in the dark for 30 minutes, then centrifuged at 12 000 rpm for 5 minutes, and the supernatant was taken and read spectrophotometrically against the blank at 365 nm. Results are expressed in nmol/mL.[24]

Protein oxidation marker

Measurements of the advanced oxidation protein products levels

Spectrophotometric determinations of AOPP levels were performed using Witko-Sarsat et al[25] method with a minor modification. The linear range of chloramine-T absorbance at 340 nm occurs between 0 and 100 mol/L. Advanced oxidation protein product levels are expressed in mol/L of chloramine-T equivalents.

Indicators of redox status

Measurements of total thiol levels

Total thiol levels were determined by Sedlak and Lindsay[26] method with a minor modification. The standard curve was drawn using 50, 100, 250, and 500 µmol/L reduced glutathione. Total thiol levels of the samples were expressed in µmol/L according to the standard curve.

Measurements of non-protein-SH levels

The concentration of NP-SH groups was determined by Sedlak and Lindsay[26] method with a minor modification. To determine the NP-SH levels, firstly 400 µL 50% HCl was used to remove the proteins in the homogenates, and then T-SH level measurement method was applied using the supernatant (According to Yanar et al[27] method with minor modifications). Non-protein-SH levels of the samples were expressed in µmol/L according to the standard curve.

Measurements of Cu,Zn-superoxide dismutase activity

Cu,Zn-superoxide dismutase activities were measured according to Sun et al[28] with a minor modification. The absorbances were determined spectrophotometrically at 560 nm. According to the calculation that 1 unit of Cu,Zn-SOD activity inhibits xanthine oxidase activity by 50%, the results were expressed as units.

Histopathological evaluations

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling method

The terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) method is a technique that detects DNA fragmentation by labeling the terminal end of nucleic acids. This assay has also been used to confirm apoptosis of testis tissues. Here, the TUNEL assay was performed with the ApopTag Peroxidase In Situ Apoptosis Detection Kit (S7101-KIT; Merck Millipore, Burlington, USA) as previously described. Testis tissues were dissected, fixed in 10% neutral buffered formalin, embedded in paraffin wax, and then cut into 5-µm-thick sections. Briefly, each testis tissue slide was deparaffinized, rehydrated, and treated with proteinase K (20 mg/L) for 15 minutes at room temperature. The endogenous peroxidase was inhibited with 3% hydrogen peroxide for 5 minutes and then the slide was incubated with the TUNEL reaction mixture (TdT) in a humidified chamber at 37°C for 1 hour. Then, the 3,3-diaminobenzidine chromogen was applied, and methyl green was used for counterstaining. The TdT was omitted from the reaction mixture as a negative control.

Apoptotic index

Marked TUNEL-positive apoptotic cells were counted under high-power fields (×40) with a light microscope (Leica DM2500; Leica Camera AG, Wetzlar, Germany).

Cell nuclei stained with methyl green were considered to be healthy, while those with brown nuclear staining were considered TUNEL-positive. All TUNEL-positive cells were counted in 15 randomly selected fields by a researcher who was blinded to the identity of the samples. The average number of cells per unit area for each set of testis tissue specimens in each group was calculated and compared.[29]

Statistical analysis

Statistical analysis was performed by using the statistical package for the social sciences (SPSS) (version 21.0, IBM SPSS Corp., Armonk, NY, USA). The data are expressed as mean–standard deviation. The significance of differences between groups was evaluated using one-way analysis of variance followed by Tukey's post hoc test[30] P value of $< .05$ was considered statistically significant.

Results

Blood glucose levels of rats belonging to all groups were measured at the beginning of the study (day 0), and there was no statistically significant difference ($P > .05$). Seventy-two hours after the STZ injection, blood glucose levels were measured, and it was found that blood glucose levels were significantly higher in the diabetic groups (DM and DM+QR) compared to the control groups (both control and QR groups) ($P < .001$, for all). Similarly, blood glucose levels of the diabetic groups (both DM and DM+QR) were found to be higher than the control (both control and QR groups) on the 15th and 30th day ($P < .001$, for all); differently, at the end of the 30th day, blood glucose levels were found to be statistically significantly lower in the DM+QR group compared to the DM group ($P < .05$) (Table 1). There was no significant difference in the statistical analysis performed between the bodyweight measurements of the groups at the beginning of the study ($P > .05$). At the end of the experiment, a significant decrease was found between the DM and DM+QR groups compared to the healthy control group ($P < .05$, $P < .01$, respectively), but no significant difference was found between the DM and DM+QR groups. In the DM group and in the DM+QR group, a significant decrease was detected in the measurement periods of the 1st day and the 15th day ($P < .05$) (Table 1).

When lipid peroxidation markers were examined as MDA and LPH, QR was significantly decreased in the DM+QR group compared to the DM group ($P < .001$, for both). When non-diabetic rats were compared, it was found that MDA and LPH levels were decreased in healthy animals receiving QR compared to the healthy group ($P < .01$ and $P < .001$, respectively). In addition, it was found that MDA and LPH levels were higher in the DM group compared to both the control ($P < .01$ and $P < .001$) and control+QR group ($P < .001$, for both). While there was no difference in AOPP levels between the DM group and the DM+QR group, it was found that the AOPP levels of both groups were higher than the control+QR group ($P < .001$ and $P < .01$, respectively). Quercetin supplementation was found to reduce AOPP levels in healthy rats ($P < .001$). Total thiol, NP-SH, and Cu,Zn-SOD activity increased significantly in the DM+QR group compared to the DM group and the control group ($P < .001$, for all). In the analysis made

Table 1. Glucose Levels and Body Weights of the Experimental Groups

	Control (n = 8) (mean ± SD)	Control+QR (n = 8) (mean ± SD)	DM (n = 8) (mean ± SD)	DM+QR (n = 8) (mean ± SD)
Glucose				
Day 0	103.28 ± 4.92	105.57 ± 5.68	104.00 ± 4.43	102.71 ± 3.81
Day 3	103.85 ± 1.21	104.85 ± 4.29	505.42 ± 64.67^{a***,b***}	506.00 ± 44.17^{a***,b***}
Day 15	106.71 ± 6.13	110.00 ± 6.16	524.57 ± 34.99^{a***,b***}	520.14 ± 44.17^{a***,b***}
Day 30	107.71 ± 4.92	105.57 ± 5.53	572.71 ± 26.71^{a***,b***}	521 ± 43.79^{a***,b***,c*}
Body weight				
Day 0	347.85 ± 20.98	348.85 ± 14.33	385.00 ± 8.16	385.57 ± 8.01
Day 15	344.42 ± 25.76	320.42 ± 20.00	324.718 ± 42.06	295.28 ± 18.70
Day 30	354.14 ± 23.74	323.71 ± 23.39	303.71 ± 33.47^{a*}	295.28 ± 18.70^{a**}

DM, diabetes mellitus; QR, quercetin. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.
[a]compared to control group; [b]compared to control+QR group; [c]compared to DM group.

Table 2. Biochemical Parameters Belonging to the Groups

	Control (n = 8) (Mean ± SD)	Control + QR (n = 8) (Mean ± SD)	DM (n = 8) (Mean ± SD)	DM + QR (n = 8) (Mean ± SD)
MDA (µmol/L)	12.62 ± 1.43	9.19 ± 1.38^{a***}	20.30 ± 4.52^{a**,b***}	10.89 ± 2.51^{c***}
LPH (nmol/mL)	0.95 ± 0.06	0.74 ± 0.13^{a**}	1.40 ± 0.25^{a***,b***}	0.86 ± 0.11^{c***}
AOPP (µmol/L)	40.93 ± 3.61	28.79 ± 3.17^{a***}	52.56 ± 9.37^{a**,b***}	43.43 ± 10.72^{b**}
T-SH (µmol/L)	62.84 ± 10.13	101.68 ± 25.39^{a**}	46.04 ± 10.13^{a**,b***}	82.04 ± 7.74^{a***,b*,c***}
NP-SH (µmol/L)	11.95 ± 0.66	15.19 ± 0.98^{a***}	9.75 ± 0.61^{a***,b***}	14.14 ± 0.83^{a***,b*,c***}
SOD (U/L)	0.24 ± 0.10	0.67 ± 0.19^{a***}	0.48 ± 0.08^{a***,b*}	0.63 ± 0.10^{a***,c***}

MDA, malondialdehyde; LPH, lipid hydroperoxide; AOPP, advanced oxidation protein products; T-SH, total thiol; NP-SH, non-protein-thiol; SOD, superoxide dismutase; DM, diabetes mellitus; QR, quercetin. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.
[a]compared to control group; [b]compared to control + QR group; [c]compared to DM group.

in healthy controls, it was found that these parameters increased significantly with the use of QR. Total thiol and NP-SH decreased significantly in the DM + QR group compared to the control + QR group ($P < .05$, for both) (Table 2).

According to the data obtained with the TUNEL method, there was no statistical difference between the healthy control and QR applied control groups ($P > .05$). In the seminiferous tubules (↑) of the DM + QR group, a significant decrease was found in the number of TUNEL-positive apoptotic cells compared to the DM group ($P < .001$) (Figures 1 and 2).

Discussion

Today, there are many studies explaining the effects of various diseases on the structure and function of the testicle. In the current study, QR decreased MDA, LPH, and AOPP levels in the diabetes group. Total thiol, NP-SH, and Cu,Zn-SOD activity increased significantly in the DM + QR group compared to the DM group. A significant decrease was found in the number of apoptotic cells, while an increase in seminiferous tubules was observed in TUNEL-positive cells compared to the DM + QR group. We also observed that the reduction in the blood glucose level and body

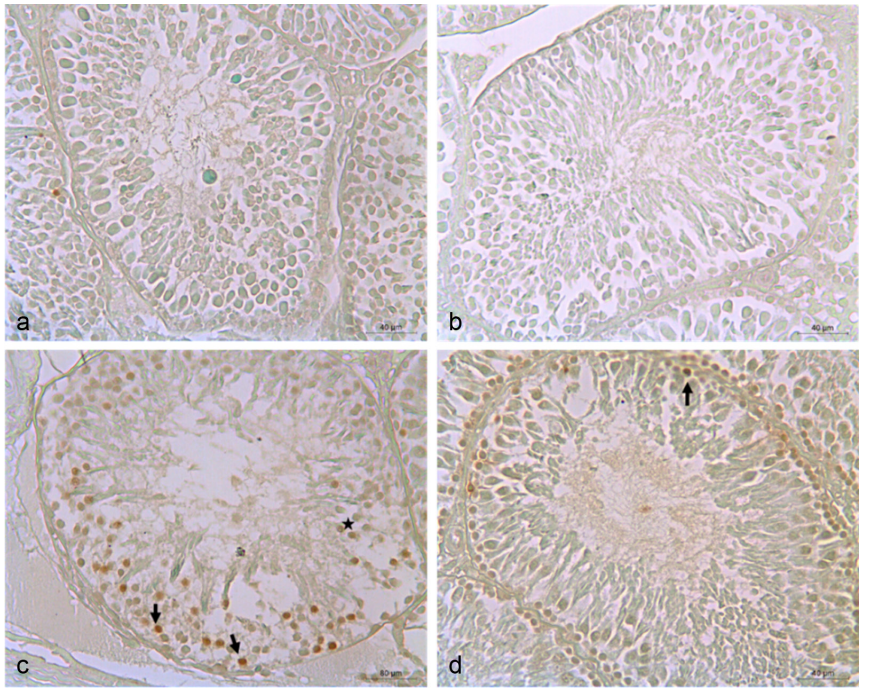

Figure 1. a-d. TUNEL staining images of testicular tissues. (a) control group, (b) control + QR group, (c) DM group, (d) DM + QR. Apoptotic cells are stained brown. Contrast painting: methyl green. TUNEL-positive cells in seminiferous tubules (↑) and cells belonging to spermatogenic series (*). TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling.

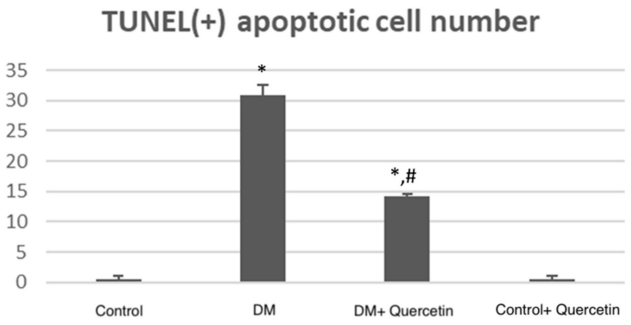

Figure 2. Comparison of TUNEL-positive apoptotic cell counts of testicular tissue in all groups. Values are expressed as mean ± standard deviation (SD). $^{*}P < .001$, compared to the control groups; $^{\#}P < .001$ compared to the DM group. TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling.

weight significantly improved. Quercetin may be a useful nutrient in the treatment for damage of testicular cells by DM.

In many studies, it has been shown that hyperglycemia increases free oxygen radicals and decreases the protective antioxidant capacity, causing oxidative stress, and this has a negative effect on the testicle like other diabetic complications.[31-34] Various studies have reported that the antioxidant effects in DM can be mitigated by the application of antioxidants such as ascorbic acid, melatonin, taurine, or an herbal blend containing extracts from *Musa paradisiaca, Tamarindus indica, Eugenia jambolana,* and *Coccinia indica*.[35-37] Chougala et al[38] showed that QR, a potent dietary antioxidant, improved lysosomal enzyme activities such as N-acetyl-β-D-glucuronidase, N-acetyl-β-D-galactosidase, and acid phosphatase in testes tissue. This effect of QR may be through minimizing damage to lysosomal enzymes through the scavenging ability of reactive oxygen species (ROS). In a 2007 clinical study,[39] increase in oxidative DNA damage was reported in the spermatozoa of diabetic patients compared to non-diabetic controls. This study demonstrated that causative links between diabetes, oxidative stress in the male germline, and oxidative DNA damage are extremely important, both potentially and clinically.

In the current study, MDA, LPH levels as markers of lipid peroxidation and AOPP as markers of protein oxidation increased in the testicular tissue of the diabetic group. Diabetes mellitus increases oxidative stress in the testicular tissue. Significant decreases were found in MDA, LPH, and AOPP levels after QR administration. Kanter et al[40] showed that STZ-induced diabetes resulted in significant increases in MDA levels and induced a significant reduction of the testosterone levels in the testicular tissue. Our results confirm the same as previous studies in diabetic testicular tissue of rats.[38,40-42] DM leads to oxidative stress-associated alterations in the testicle tissue. The large increase in oxidative parameters observed in diabetic animals compared to the control group could explain the increase in oxidative stress. However, the mechanism by which QR causes oxidative stress-mediated changes in MDA, LPH, and AOPP levels needs further elucidation.

In the current study, T-SH, NP-SH, and Cu,Zn-SOD activities increased significantly in the DM + QR group compared to the DM group. Similar to our results, Kanter et al[40] reported that in STZ-induced diabetes, it caused a significant decrease in the antioxidant enzymes such as Cu,Zn-SOD and glutathione peroxidase (GSH-Px) activities in the testicle tissue. Quercetin, a powerful dietary antioxidant, improves the changes in antioxidant parameters in the testicular tissue to various degrees and thus minimizes the damage due to diabetes. Khaki et al[8] reported that QR has significant beneficial effects on sperm viability, motility, and serum total testosterone and could be effective for maintaining healthy sperm parameters and male reproductive function in diabetic rats. Our study and other studies[8,40-43] have shown that enzymes such as Cu,Zn-SOD, GSH-Px, glutathione reductase play an important role in antioxidant defense in order to maintain viable reproductive ability, providing a protective mechanism against oxidative stress.

Oxidative stress and oxidative DNA damage are 2 important factors that induce apoptosis.[44] Zhao et al[45] demonstrated that STZ-induced diabetes caused a series of testicular dysfunctions and functional deficits such as the deterioration of seminiferous tubules and the loss of spermatogenic cells. In another study, the production of ROS and apoptotic cells signiŞcantly increased in the diabetic group compared to the control group.[46] Findings of Khosravi et al[47] showed that apoptosis is enhanced in the testicular tissue following diabetes induction by STZ. In our study, according to the data obtained with the TUNEL method, a significant increase was detected in the number of apoptotic cells in the testicular tissues of the DM and DM + QR groups compared to the control groups. Based on our results, increased oxidative stress in the STZ-induced DM may lead to apoptosis in the testicular tissue of rats. Our results confirm the same as the other results,[31,40,46-50] but a significant decrease was detected in the number of apoptotic cells in the testicular tissues of the diabetic group treated with QR. Similar to our results, Kanter et al[37] indicate that QR administration attenuated diabetes-related testicular dysfunction and histopathologic changes. Quercetin reduced the number

of TUNEL-positive cells and increased PCNA activity in the testis of diabetic rats. Quercetin consequently improved apoptosis in the testis of rats. Similar to our results, Ying et al[51] reported that QR exhibits a protective effect in STZ-induced hyperglycemic Arbor Acre (AA) broilers via decreasing oxidative stress and changing activities of antioxidant enzymes. Yelumalai et al[52] showed that in vivo administration of QR helps to ameliorate the decrease in sperm count, motility, viability, and the increase in sperm with morphological abnormalities in DM. They also showed that QR increases MDA levels and decreases SOD, CAT, and GPx activity in diabetic rat sperm. Collectively, the results of our study show that the antioxidant molecules are depleted due to the increasing amount of ROS, as hyperglycemia induces oxidative stress and apoptosis. It can be stated that as a result of high-glucose exposure, increased lipid peroxidation, protein oxidation, decreased antioxidant effect, and increase in the number of apoptotic cells in testicular tissue, but QR treatment of diabetic group attenuated apoptosis severity and these negative effects. In addition, it was concluded that these results could be a source for further studies.

This study has several limitations. There could have been another diabetic group treated for diabetes appropriately (might be with insulin, oral antidiabetic, etc.) to see the effect of blood glucose regulation on the same parameters. Testosterone levels of the rats could not be measured in the study. Apoptosis parameters such as bcl-2, bax, and caspase-3 could not be examined.

We concluded that QR may have protective effects against the damage of testicular tissue, protect cells from apoptosis, increase antioxidant enzyme level against oxidative stress, and reduce the levels of products formed as a result of oxidative stress. We believe that QR, which stands out with its antioxidant properties in reducing and preventing the effects of diabetic damage, can be useful in creating new treatment options with its antiapoptotic effects. We think that its effects on different diabetic complications at different doses and durations in the testicle tissue can be investigated comprehensively in future studies.

Ethics Committee Approval: Ethics committee approval was received for this study from Istanbul University Animal Experiments Local Ethics Committee (Date: February 20, 2015; No: 2015/36).

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Conflict of Interest: All authors have no conficts of interest to disclose.

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