Arch Clin Exp Med 2018;3(3):179-185. e-ISSN: 2564-6567

DOI:10.25000/acem.452530 Araştırma makalesi / Research article

Renoprotective potential of quercetin in experimental diabetic nephropathy: assesing antiapoptotic and antioxidant effects

Deneysel diyabetik nefropatide quercetin'in renoprotektif potansiyeli: antiapoptotik ve antioksidan etkilerin değerlendirilmesi

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Abstract

Aim: We investigated renoprotective and anti-apoptotic effects of quercetin, a potent bioflavonoid, by evaluating expression of apoptosis-regulatory genes that contribute to the kidney damage caused by diabetes in rats.

Methods: Rats were divided into 4 groups; Control, STZ-induced diabetic, STZ-induced diabetic+Quercetin and Quercetin control. Values of fasting blood glucose, body weight and urine microalbuminuria measured. Immunohistochemistry was performed using bax, bcl-2, caspase-3 antibodies. For apoptosis detection, TUNEL method was applied. Advanced oxidation protein products (AOPP), protein carbonyl oxidation (PCO), malondialdehyde (MDA) and superoxide dismutase (SOD) activity were measured in homogenized kidney tissues.

Results: Blood glucose and microalbuminuria levels were significantly decreased in quercetin-treated diabetic group compared to the untreated-diabetic group (p=0.020 and p=0.003; respectively). MDA, AOPP and PCO levels were significantly decreased (p=0.001, p=0.0001 and p=0.0005; respectively); however, SOD activity were found to increase in quercetin-treated diabetic group (p=0.005). Immunostaining of bcl-2, bax and caspase-3 was decreased compared to the untreated-diabetic group. Apoptotic cells especially increased in the kidney tubuli of untreated-diabetic group and on the contrary, a significant decrease was observed in the group that received a quercetin treatment (p=0.0001).

Conclusion: Our results revealed that antiapoptotic effects of quercetin, which has predominantly antioxidant effects, may be useful in reducing effects of diabetic complications and preventing new complications.

Key words: Experimental diabetic nephropathy, quercetin, kidney, apoptosis, oxidative stress

Öz

Amaç: Sıçanlarda diyabetin neden olduğu böbrek hasarına katkıda bulunan apoptoz düzenleyici genlerin ekspresyonunu değerlendirerek güçlü bir biyoflavonoid olan quercetin'in renoprotektif ve antiapoptotik etkilerini araştırmak.

Yöntemler: Sıçanlar 4 gruba ayrıldı; Kontrol, STZ-diyabetik, STZ-diyabetik + Quercetin ve Quercetin kontrol. Açlık kan şekeri, vücut ağırlığı ve idrar mikroalbüminüri değerleri ölçüldü. İmmünohistokimya bax, bcl-2, kaspaz-3 antikorları kullanılarak gerçekleştirildi. Apoptoz tespiti için TUNEL yöntemi uygulandı. Homojenize böbrek dokularında ileri oksidasyon protein ürünleri (AOPP), protein karbonil oksidasyon (PCO), malondialdehid (MDA) ve süperoksit dismutaz (SOD) aktivitesi ölçüldü.

Bulgular: Tedavi edilmeyen diyabetik gruba kıyasla, quercetin uygulanan diyabetik grupta kan şekeri ve mikroalbüminüri düzeyleri anlamlı olarak azalmıştı (sırasıyla; p=0,020, p=0,003). MDA, AOPP ve PCO seviyeleri anlamlı olarak azaldı (sırasıyla; p=0,001, p=0,0001, p=0,0005), ancak SOD aktivitesinin quercetin uygulanan diyabetik grupta arttığı tespit edildi (p=0,005). Quercetin uygulanan diyabetiklerde, tedavi edilmemiş diyabetik gruba kıyasla bcl-2, bax ve kaspaz-3'ün immün boyanması azaldı. Tedavi edilmeyen diyabetik grubun böbrek tübüllerinde apoptotik hücrelerde belirgin bir artış gözlenirken, quercetin uygulanan diyabetik grupta belirgin bir düşüş gözlendi (p=0,0001).

Sonuç: Antioksidan etkileri olan quercetin'in antiapoptotik etkilerinin, diyabetik komplikasyonların etkilerini azaltmada ve yeni komplikasyonları önlemede yararlı olabileceği sonucuna vardık.

Anahtar kelimeler: Deneysel diyabetik nefropati, quercetin, böbrek, apoptoz, oksidatif stres

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Ethics Committee Approval: The study wass approved by the local ethical authority.
Etik Kurul Onayı: Çalışma lokal etik komite tarafından onaylanmıştır.

Conflict of Interest: No conflict of interest was declared by the author.
Çıkar Çatışması: Yazar çıkar çatışması bildirmemiştir.

This study has been presented as a poster presentation in "15th International Congress of Histochemistry and Cytochemistry".
Bu çalışma "15th International Congress of Histochemistry and Cytochemistry" de poster olarak sunulmuştur.

Financial Disclosure: This work was supported by the Istanbul University Scientific Research Projects Unit (No:52174 and BYP-2017-26104).
Finansal Destek: Bu çalışma İstanbul Üniversitesi Bilimsel Araştırma Projeleri Birimi (No:52174 ve BYP-2017-26104) tarafından desteklenmiştir.

Geliş Tarihi / Received: 09.08.2018
Kabul Tarihi / Accepted: 14.09.2018
Yayın Tarihi / Published: 30.11.2018

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Introduction

Diabetic nephropathy (DN), which is also the chronic complication of Diabetes Mellitus (DM), is characterized by glomerular hypertrophy, proteinuria, decreased glomerular filtration and renal fibrosis that lead to renal dysfunction [1].

Streptozotocin (STZ) is frequently used in different doses to induce DM in experimental animals and the mechanisms of diabetes are attempted to elucidate [2]. STZ has a toxic effect on pancreatic β cells. It gives rise to renal tissue dysfunction and damage caused by insulin deficiency and hyperglycemia; and thereby, it generates the diabetic nephropathy table observed in clinics [2, 3]. STZ, as a free radical source, leads to DNA damage and afterwards, cell death [4]. It is known that free oxygen radicals take place in the pathogenesis of renal damage. It is reported that diabetes accelerates formation of reactive oxygen species (ROS) and increased oxidative stress is associated with irreversible renal damage and DN pathogenesis [5].

Apoptosis, which means programmed cell death, is a physiological process that plays important roles in both persistence of cellular homeostasis and both proliferation and differentiation of cells. The damaged cells or the ones with danger potential are destroyed with apoptosis. It has been reported in different types of cells that increased levels of ROS cause apoptosis [6]. Low levels of ROS function as "redox messengers" that is important for cellular signaling and homeostasis. It is revealed that increased ROS level in kidneys is associated with apoptosis in addition to progression and development of diabetic nephropathy [7]. It is considerably important to perceive changes in apoptosis related genes, oxidative stress biomarkers and antioxidant enzyme activity levels for better understanding of apoptotic mechanism [8].

It is suggested that antioxidants have protective effects against apoptosis [9] Flavonoids, which belong to polyphenol group, have strong antioxidant effect and they are also commonly used for treatment of several diseases. Quercetin (3,5,7,3',4'-pentahydroxyl flavon), which has many useful pharmacological effects like anti-hypertensive, anti-ischemic, anti-carcinogenic, anti-thrombotic, anti-inflammatory in addition to its anti-oxidant effect, is one of the important natural polyphenolic flavonoids [6, 10]. It is also indicated that quercetin has beneficial effects on prevention of ischemia reperfusion [9] and diabetes induced renal damage [11].

We aimed comparatively to investigate the renoprotective and antiapoptotic effects of quercetin by evaluating the expression of apoptosis-regulatory genes that contribute to the kidney damage caused by diabetes in rats.

Material and methods

Animals and protocols

All experiments were approved by the Istanbul University Cerrahpasa Faculty of Medicine Ethics Committee for Animal Experiments (project no:2015/36), and followed the NIH Guide for the Care and Use of Laboratory Animals. Wistar-type-albino male rats weighing approximately 310-410 g were purchased from Istanbul University, Aziz Sancar Institute of Experimental Medicine.

All groups, except the control groups, received a streptozotocin injection (STZ; Sigma, St.Louis, MO,USA,50 mg/kg, freshly dissolved in 0.9 % sodium chloride, i.p.). On the 3rd day after the STZ injection, the rats developing diabetes (above blood glucose levels of 350 mg/dL) [3] were divided into two groups. The first group consisted of untreated-diabetic rats (n=8). Second group of diabetic rats was treated with quercetin (20 mg/kg/day, diluted in 4% ethanol, i.p., 30 days, sc-206089B, Santa Cruz Biotechology (SCBT), n=8). Third control group was treated with quercetin (20 mg/kg/day, n=8). Fourth group was the control group consisted by the non-diabetic rats (for sham injection, 0.9 % sodium chloride, i.p., (n=8). The animals had free access to standard rat chow and drinking water. At the end of the experimental period (31st day), the animals were sacrificed under anesthesia (ketamine–xylazine), and kidney tissue samples removed for histological and biochemical examinations.

Blood glucose levels of all groups were measured using Blood Glucose test strips (Blood Glucose Test-Strip,Taiwan) with a glucometer (eBsensor Blood Glucose Monitoring System, Taiwan eB-G model) in samples obtained from the tail vein. At the 1st,15th, and 30th day of the experiment, all rats from each group were housed in metabolic cages (24-h) for urine obtain. Daily urine volume and microalbuminuria levels were measured from collected urine. Microalbuminuria was measured using Micral-test strips (DIRUI, Urine Analyzer, H11-MA urine strips). Body weights of all animals were measured in a weekly manner. Rats were dissected to obtain the kidneys. The right kidneys from each rat were weighed and recorded.

Biochemical measures

The kidney tissues were homogenized in a fourfold volume of phosphate buffer solution (PBS) using a homogenizer (Next Advance Bullet Blender Storm 24). The homogenate was centrifuged at 3,000×g for 10m to remove debris. Clear upper supernatant was taken, and tissue analyses [(malondialdehyde (MDA), superoxide dismutase (SOD), the advanced oxidation protein products (AOPP), protein carbonyl oxidation (PCO)] were carried out. All procedures were performed at +4°C throughout the experiments.

Measurement of tissue MDA levels: MDA levels were determined as previously described by Ohkawa et al. [12] with a minor modification. The reaction mixture was prepared by adding 0,25ml homogenate into 2,7ml reaction solution (30 % trichloroacetic acid, 0.75 % thiobarbituric acid, 5Nhydrochloric acid, 1:1:1, w/v) and heated at 100 0C for 15 min. The mixture was cooled to room temperature, centrifuged (3,000 g for 10 min), and the absorbance of the supernatant was recorded at 532 nm. 1,1,3,3-tetramethoxypropane was used as MDA standard. MDA results were expressed as μmol/mg wet tissue. The coefficients of intra-and inter-assay variation were 2.9 % (n=15) and 3.5 % (n=15), respectively.

Measurements of superoxide dismutase (Cu, Zn-SOD) activity: Cu, Zn-SOD activity was determined with the method of Sun et al. [13] by inhibition of nitroblue tetrazolium (NBT) reduction, with xanthine/xanthine oxidase used as a superoxide generator. One unit of SOD was defined as the amount of protein that inhibits the rate of NBT reduction by 50 %. The absorbance of each sample was read at 560 nm. SOD activity were expressed as U/mg wet tissue. The coefficients of intra-and inter-assay variation were 2.9 % (n=15) and 3.6 % (n=15), respectively.

Measurements of PCO levels: The kidney tissue PCO levels were determined by a commercially available enzyme-linked immunosorbent assay kit (BioAssay Technology Laboratory, E0870Ra, Shanghai, CHINA). PCO concentrations were expressed ng/mg wet tissue. The absorbances of the samples were measured by spectrophotometer at 540 nm. The coefficients of intra- and interassay variations were 4.3% (n=15) and 5.4% (n=15), respectively.

Measurements of AOPPs levels: Spectrophotometric determinations of AOPPs levels were performed using Witko-Tarsat et al. [14] method. The linear range of chloramine-T absorbance at 340 nm occurs between 0 and 100μmol/L. AOPP levels are expressed in μmol/mg of chloramine-T equivalents.

The coefficients of intra-and inter-assay variation were 2.8% (n=15) and 3.2% (n=15), respectively.

Light microscopy

Kidney tissue samples were fixed in 10% neutral formalin, followed by embedding in paraffin wax and then cut into 5-µm-thick sections. Periodic-Acid-Schiff (PAS) staining was performed.

Immunohistochemistry

Immunohistochemical analysis was performed using both Histostain-Plus Bulk Kits(85-043, Invitrogen) and Ultra Vision Antibody Detection System (LabVision), including mouse monoclonal bcl-2(Sc-7382, SCBT, 1:50 dilution), mouse monoclonal bax (Sc-7480, SCBT, 1:50 dilution) and rabbit polyclonal caspase-3(sc-7148, SCBT, 1:50 dilution) antibodies as described previously [3].

Semiquantitation of immunoperoxidase staining: Immunostaining was evaluated using a Leica DM2500 light microscope (X40 objective, Leica Microsystems, Wetzlar, Germany). Bcl-2, bax and caspase-3 immunostainings were analyzed and scored from 1+ to 3+ (1+refers to weak, while 3+to strong immunopositivity). This analysis was performed in a blind and randomized fashion of all stained sections.

TUNEL method (Terminal deoxynucleotidyl transferase dUTP nick end labeling)

Detection of DNA fragmentation in situ was visualized with the use of the ApopTag Plus Peroxidase In Situ Apoptosis Detection Peroksidase Kit (S7101-KIT,Millipore), as described by the manufacturer. TUNEL assay was performed as described previously [3]. Staining was evaluated using a light microscope after counterstaining with methyl green.

Staining specificity controls

Thymus tissue sections from dexamethasone-treated rats were used as positive control. For negative controls, distilled water was used instead of Tdt enzyme.

Apoptotic index

Marked apoptotic cells were counted under a Leica DM2500 light microscope (X40 mag.). All TUNEL positive cells in randomly selected 12 different unit areas were counted on the cross-sections by a blinded researcher. Average cell per unit area number for each set of specimens in each group was calculated and compared.

Statistical analysis

The all datas of the 5 group of rats were compared using GraphPad Prism 5 software (San Diego, CA, USA) statistical package. The data were expressed as mean±SD. P value of less than 0.05 was considered statistically significant.

Results

Blood glucose level (Bg, mg/dL) and body weights (Bw, g)

At the beginning of the study, there was no statistically significant difference between the blood glucose levels of rats belonging to all groups (p=0.221). 72h after the induction of diabetes, Bg levels in untreated-diabetics were found to be significantly higher than the levels of the controls (p<0.001 for all). There was a significant increase in all diabetics compared to controls on the 15th day. At the end of the experimental period, Bg levels in untreated and quercetin-treated diabetics were higher than the controls (p<0.001 for all). Bg levels of quercetin-treated diabetics were significantly decreased when compared to untreated-diabetics (p=0.02) (Table 1). At the end of the experimental period, body weights of untreated-diabetic and quercetin-treated diabetics were significantly decreased when compared to controls (p=0.006, p=0.0002; respectively) (Table 1).

Microalbuminuria level (mg/L/24 h)

At the 1st and 15th day, there was a significant increase in microalbuminuria levels of either diabetic groups compared to control groups (p<0.01 for all). At the end of experiment, the microalbuminuria levels were lower in the quercetin-treated diabetics compared to the untreated-diabetics (p=0.003) (Table 1).

Daily urine volume (ml/day)

On days 15th and 30th, urine volume of diabetics showed a significant increase compared to controls (p<0.01 for all). At 30th days, a significant decrease was observed in the quercetin-treated diabetics compared to the untreated-diabetics (p=0.01) (Table 1).

Kidney weights (Kw, mg)

Kidney weights were significantly higher in the untreated-diabetics compared to the controls (p=0.006). In the quercetin-treated diabetics were significantly decreased when compared to untreated-diabetics (p=0.006) (Table 1).

MDA, SOD, AOPP and PCO levels

Mean kidney tissue MDA, AOPP and PCO levels of untreated-diabetics were significantly increased when compared with the controls (p=0.0002, p=0.0001, p=0.001; respectively) (Table 2). However; mean MDA, AOPP and PCO levels in the quercetin-treated diabetics were significantly decreased when compared with the untreated-diabetics (p=0.001, p=0.001, p=0.0005; respectively) (Table 2). Mean renal tissue SOD activities of untreated-diabetics were significantly decreased in comparison with the controls (p=0.0001). SOD activities of quercetin-treated diabetics were significantly increased in comparison with the untreated-diabetics (p=0.005) (Table 2).

Table 1: Blood glucose levels (Bg, mg/dL), body weight (Bw, g), Albuminuria level (Alb, mg/L/24 h), daily urine volume (ml/day), kidney weight (Kw, mg) and apoptotic cell count at the end of the experiment.

Groups (n=8)	Bg	Bw	Alb	Urine output	Kw	Apoptotic cell count
Control $^{\mu}$	107.7±4.9	354.1±23.7	0.01±0.008	11.3±3.0	1138±127.4	1.65±0.3
Quercetin $^{\mu}$	105.6±5.5	323.7±23.4	0.01±0.006	11±1.5	1150.7±107.1	1.71±0.3
Untreated-diabetic $^{\mu}$	572.7±26.7 ^{a}	303.7±33.5 ^{c}	0.15±0.004 ^{e}	66.6±7.5^{e}	1337.9±66.1 ^{h}	13.79±3.1^{i}
Diabetic + Quercetin $^{\mu}$	521±43.8 ^{a,b}	295.3±18.7 ^{d}	0.08±0.007^{e,f}	56.6±6.0 ^{e,g}	1155.1±122.3 ^{c}	6.41±1.7 ^{j}
p	^{a}p<0.001 ^{b}p=0.02	^{c}p=0.006 ^{d}p=0.0002	^{e}p <0.01 ^{f}p=0.003	^{g}p=0.01	^{h}p=0.006 ^{c}p=0.006	^{i}p = 0.0001 ^{j}p = 0.0001

$^{\mu}$: mean±SD, ^{a}p, ^{c}p, ^{d}p, ^{e}p, ^{h}p, ^{i}p versus control groups; ^{b}p, ^{f}p, ^{g}p, ^{j}p versus untreated diabetic group.

Table 2: Comparison of kidney tissue levels of MDA, SOD, PCO and AOPP in the four study groups.

Groups (n=8)	MDA[¥]	AOPP[¥]	PCO[β]	SOD[§]
Control[μ]	10.75 ±1.8	21.13±3.6	134.90±16.2	0.82±0.2
Quercetin[μ]	10.57±1.5	24.71±3.5	132.08±18.1	1.07±0.1
Untreated-diabetic[μ]	19.42±2.4[a]	120.77±20.3[c]	166.44±10.5[e]	0.22±0.1[c]
Diabetic + Quercetin[μ]	16.58±1.6[b]	65.85±13.3[d]	133.47±14.9[d]	0.61±0.3[f]
p	[a]p=0.0002 [b]p=0.001	[c]p=0.0001 [d]p=0.0005	[e]p=0.001 [d]p=0.0005	[c]p=0.0001 [f]p=0.005

[¥]: μmol/mg, tissue, [β]: ng/mg, tissue, [§]: U/mg, tissue, [μ]: mean±SD, MDA: malondialdehyde, AOPP: advanced oxidation protein products, PCO: protein carbonyl oxidation SOD: superoxide dismutase, [a]p, [c]p, [e]p versus control groups; [b]p, [d]p, [f]p versus untreated diabetic group.

Histopathological findings

Glomeruli and tubule structures were similar and uniform in controls (Figure 1A-B). In the untreated-diabetics showed tubular degeneration, mesangial matrix thickening in glomeruli and increased of mesangial cells (Figure 1C). In the quercetin-treated diabetics, the renal structural alterations were less distinct compared to those of untreated-diabetics (Figure 1D).

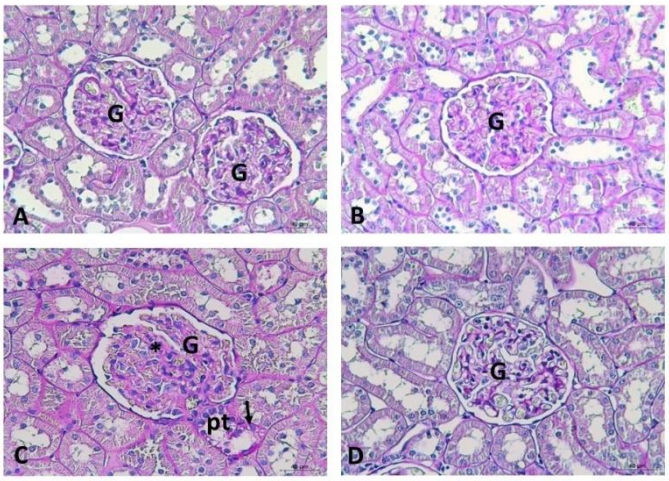

Figure 1. Light photomicrographs of PAS-stained sections of kidney sections. Non-diabetic (A) and quercetin-treated control groups (B). In the untreated-diabetic group showed tubular degeneration (↓), mesangial matrix thickening in glomeruli and increased of mesangial cells (*) (C). In the quercetin-treated diabetic group, the renal structural alterations were less distinct compared to those of untreated diabetic rats (D). (pt: proximal tubuli; G:glomeruli), Bar: 40μm.

Immunohistochemical findings

Bcl-2 and bax

The immunopositiviy of bcl-2 (Figure 2A) and bax (Figure 3A) was weakly observed in the tubules and glomeruli of controls. In untreated-diabetics, bcl-2 (Figure 2C) and bax (Figure 3C) immunopositivity were more prominent in the tubules and glomeruli comparison with that of the controls. In quercetin-treated diabetics, bcl-2 (Figure 2D) and bax (Figure 3D) intensity were weaker than that of the untreated-diabetics.

Caspase-3

Very few caspase-3 immunopositive cells were detected in the glomeruli and tubules in the controls (Figure 4A-B). Immunostaining of caspase-3 was markedly increased in glomeruli and damaged tubuli of the untreated-diabetics (Figure 4C). A significant decrease in the numbers of caspase-3 immunopositive glomeruli was observed in the quercetin-treated diabetics compared to untreated-diabetics (Figure 4D).

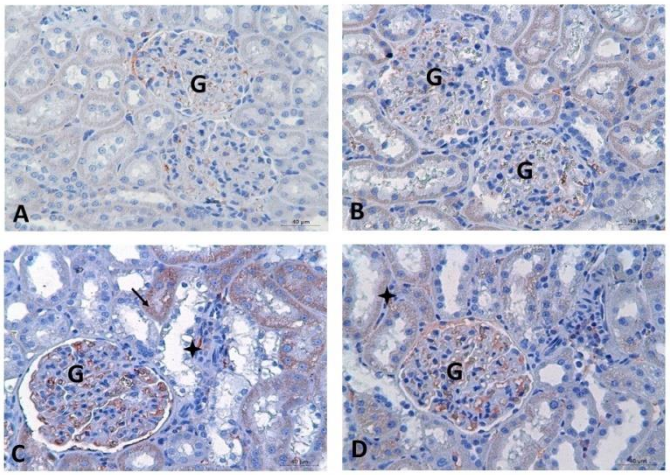

Figure 2. Immunoreactivity of bcl-2. The immunopositiviy of bcl-2 was weakly observed in the tubules and glomeruli of non-diabetic (A) and quercetin-treated (B) control groups. In untreated-diabetic group, bcl-2 immunopositivity were more prominent in the tubules and glomeruli (C). The quercetin-treated diabetic group, showed decreased bcl-2 immunoreactivity in the all sections compared to untreated-diabetic rats (D)(G:glomeruli; ↑:immunopositivity; ✦:interstitial area). Counterstain: Hematoxylin, Bar:40μm.

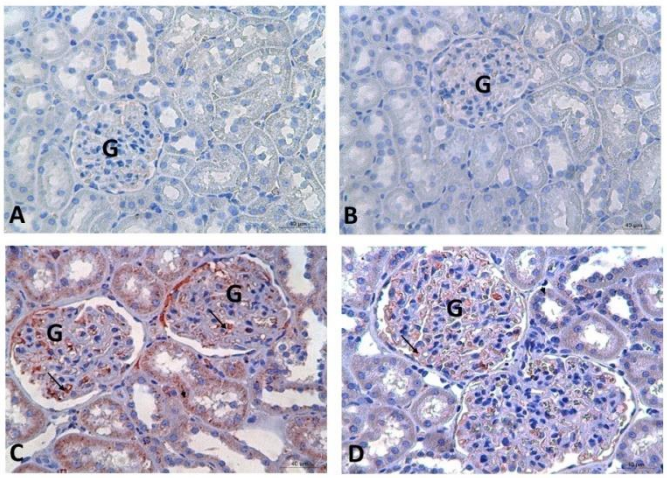

Figure 3: Immunoreactivity of Bax: Non-diabetic (A) and quercetin-treated (B) control groups. Intense cytoplasmic immunostaining of Bax was observed in the glomeruli (↓) (B) and at distal tubuli of untreated STZ-diabetic group (►) (C). In the quercetin-treated diabetic group, weak bax immunoreactivity was observed in the glomeruli (D)(G:glomeruli; ↓:immunopositivity). Counterstain: Hematoxylin, Bar:40μm.

TUNEL method

After staining with TUNEL method, cells with brown staining of nuclei were evaluated as apoptotic. The positive control tissue section (Figure 5A). In the controls, very few apoptotic cells were observed in the cortex, medulla and distal tubuli but no staining was observed in the glomeruli (Figure 5B). However, the number of apoptotic cells increased especially in the medullary area and distal tubuli of the untreated-diabetics compared to the controls (p=0.0001), whereas apoptosis was detected in the cells of the some injured glomeruli (Figure 5C) (Table.1). A significant decrease was also observed in the quercetin-treated diabetics (p=0.0001) compared to untreated-diabetics (Table 1) (Figure 5D).

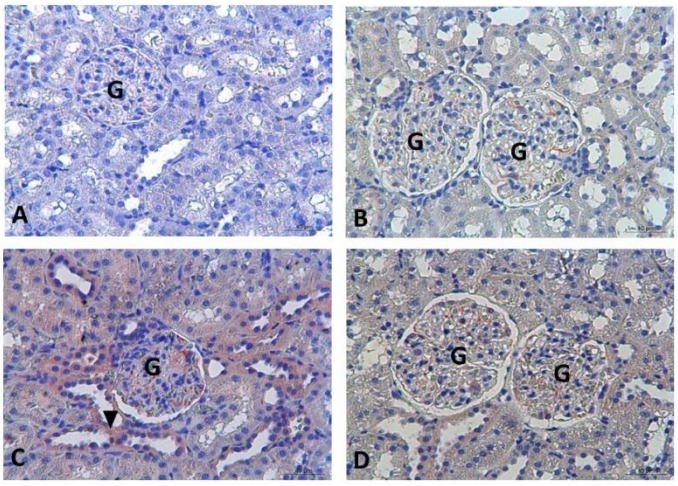

Figure 4. Immunoreactivity caspase-3. The caspase-3 immunostaining was increased in damaged glomeruli and tubuli of untreated diabetic group (thick arrow) (C) compared to controls group (A, B). In the quercetin-treated diabetic group, a significant decrease of caspase-3 immunoreactivity was observed (D). (G: glomeruli;►: immunopositivity). Counterstain: Hematoxylin, Bar: 40µm.

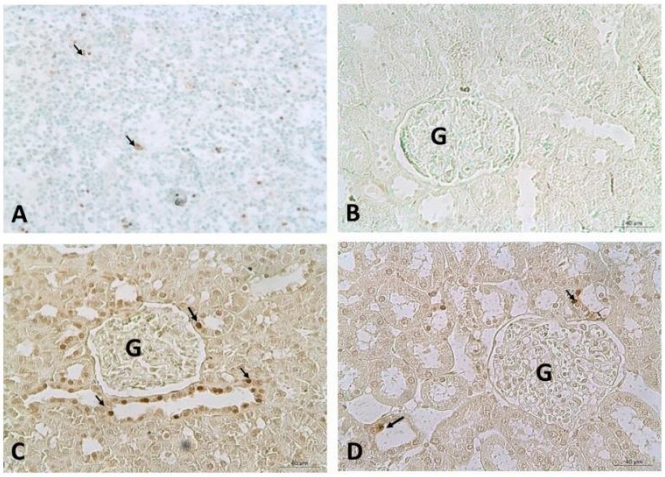

Figure 5. The kidney tissue samples stained with TUNEL method. Arrows refer to apoptotic cell nucleus. Positive control tissue section (A). Control groups demonstrated very few apoptotic cells were in cortex (B). Untreated-diabetic group group showed more apoptotic cells than other groups (C). Quercetin-treated diabetic group showing less few apoptotic cells(D).(G:glomeruli; ↓: apoptotic cell nucleus) Bar:40µm, Counterstain: Methyl green.

Discussion

Diabetes mellitus is a serious chronic and metabolic disease that is common in all countries, leading to impaired carbohydrate, protein and fat metabolism resulting in either insufficient insulin action or loss of insulin action in target tissues [11, 15]. The most common microvascular complication of DM is diabetic nephropathy. Hemodynamic, metabolic and genetic factors as well as oxidative stress play an important role in the pathogenesis of DN [16].

In studies, STZ, a diabetic agent, is administered intraperitoneally in a single dose of 40-60mg/kg due to its specific acute toxicity to pancreatic β-cells to produce experimental diabetes [3, 11]. After STZ injection, the presence or absence of diabetes is determined by observing the blood glucose levels (>250mg/dl) within 2-3 days [3]. Since diabetes is an oxidative stress disorder, antioxidant administration in diabetic animal models has been shown to reduce hyperglycemia caused by STZ. In our study, rats were observed hyperglycemic according to the results of blood glucose measurements performed 72-hours after STZ injection and hyperglycemia was observed during the study. Consistent with studies reporting that antioxidant administration reduced blood glucose level [11], a significant decrease observed in the quercetin-diabetics.

The earliest diagnosis of diabetic nephropathy is possible with the appearance of abnormal microalbuminuria in the urine. Experimental studies have shown that the elevation of the blood glucose is effective at the onset and progression of microalbuminuria [3]. In our study, microalbuminuria results were found to be consistent with expected microalbuminuria levels in diabetic nephropathy model. In the quercetin-treated diabetics were observed decrease in microalbuminuria values with compared the untreated-diabetics. It was assessed in agreement with the decrease in blood glucose levels.

Short and long-term experimental diabetes studies have reported a significant decrease in body weight levels in diabetic rats [11, 17]. In our study, the decrease in body weight of diabetics were found to be consistent with the results of other studies. In addition, some studies reported that antioxidant treatment prevented weight loss in diabetic rats [18, 19]. In the present study, quercetin-treated diabetics displayed less body weight, loss than the untreated-diabetics. This suggests that quercetin may help the maintenance of the body weight by controlling blood glucose level.

There are conflicting research results about the diabetes ans its effect on kidney weight. Tunçdemir et al.[3] reported that diabetic rats showed significantly increased kidney weights due to hypertrophy compared with controls, whereas Elbe et al.[11] reported no difference in kidney weights of diabetics compared to the other groups. In our study, kidney weights of diabetic rats were significantly increased compared to those of controls, and in the quercetin-treated diabetics was significantly decreased compared to untreated-diabetics.

Many studies have reported that hyperglycemia leads to oxidative stress by increased free oxygen radicals and reducing antioxidant capacity, and adverse effects on the kidney, such as in other diabetic complications [11, 18]. Antioxidant enzymes such as SOD separates superoxide and hydrogen peroxide to its components in the cell [20]. It has been reported that SOD enzyme activity is decreased and the MDA level is increased in many tissues of diabetic rats that antioxidant administration increases tissue SOD level and decreases MDA level [20, 21, 22]. Obrosova et al. [23] reported that MDA levels in the renal cortex as well as SOD activity were increased early in diabetes. Dias et al. [22] reported an increase in SOD activity in their diabetic liver studies. We think that this difference may be due to tissue specificity and duration of the disease. In our study, renal tissue SOD enzyme activity in the diabetic group was found to be decreased compared to that in control groups while MDA levels were found to be increased. It has also been suggested that

the SOD levels of quercetin-induced diabetics are increased when compared to the untreated-diabetics and the MDA levels are decreased.

The increased oxidative stress leads to the formation of protein carbonyl derivatives and AOPPs for protein oxidation by reducing the natural antioxidant capacity of the body [24]. In diabetic pancreas, liver and serum, AOPP and PC-related studies [25, 26] as well as there is a study on diabetic kidney tissue with these parameters [27]. Shi et al. [27] reported that the renal tissue AOPP and PC levels of the diabetic group were significantly increased compared to the control group. Quercetin is a powerful antioxidant with the ability to free radical scavenging and inhibit superoxide radicals via xanthine oxidase [20]. Many studies report that oxidative stress due to diabetes is inhibited by quercetin [11, 20]. In our study, AOPP and PC values were found higher in the diabetics compared to the controls, whereas in the quercetin-treated diabetics, it was decreased compared to the untreated-diabetics. Considering the oxidative stress parameters, we can say that quercetin administration significantly reduces oxidative stress.

Histopathological damage is seen everywhere in all over the diabetic kidney [3, 11]. We detected some diabetes-related alterations including mesangial cell enlargement and matrix thickening in the glomerule in addition to tubular basement membrane thickening with degenerations. It was observed that injury observed in glomeruli and tubules in the quercetin-treated diabetics were reduced compared to the untreated-diabetics.

It has also been reported that apoptosis plays a role in the development of oxidative stress as well as in the development of renal damage [6, 28]. Studies on how quercetin affects apoptosis have been conducted with cancer cell lines such as hepatoma, glioblastoma, and osteosarcoma, and results have proven that quercetin induces apoptosis [29, 30]. Kanter et al.[19] reported that the anti-apoptotic effect of quercetin in the diabetic testis. However, we did not find a comprehensive study of the effects of quercetin on apoptosis in the model of diabetic nephropathy. In our study, in the untreated-diabetics, bcl-2 (anti-apoptotic) immunopositivity increased especially in the glomeruli and occasionally in the tubules compared to the controls. Bax (pro-apoptotic) and caspase-3 immunopositivity was found to be associated with an increase in the number of apoptotic cells detected in the renal tissue of diabetics. In the quercetin-treated diabetics, immunopositivity of bcl-2, bax and caspase-3 were significantly reduced compared to the untreated-diabetics, and a significant decrease in the number of apoptotic cells parallel to these findings was found.

In conclusion, in our experimental diabetes model, we observed that quercetin over the dose and duration of use significantly improved blood glucose, microalbuminuria, renal weight, daily urinary excretion and loss of body weight. As a result of histopathological evaluations, we observed that the diabetes specific characteristic morphological changes in the kidney tissues of the untreated-diabetics and the increase in bcl-2, bax, caspase-3 immunopositivity decreased with quercetin administration. We found that the increase in the number of apoptotic cells in the tissue sections of the diabetic group was significantly reduced by quercetin administration. In addition, we have reported that quercetin-treatment increases the activity of MDA, the lipid peroxidation product, the activity of antioxidant enzyme, SOD, in contrast attenuates the oxidative stress markers AOPP and PCO compared with the untreated-diabetics.

In experimental diabetic nephropathy model, the flavonoid quercetin, may have protective effects against damages caused by diabetes in kidney tissue during the applied dose and time, may act on apoptotic regulatory proteins to protect the cells from apoptosis by increasing the level of antioxidants (Figure 6).

We believe that the antiapoptotic effects of quercetin, which is predominantly antioxidant, may be useful in reducing the effects of diabetic complications and preventing new complications. We suggest that the effects on different diabetic complications at different doses and durations can be extensively investigated in future studies.

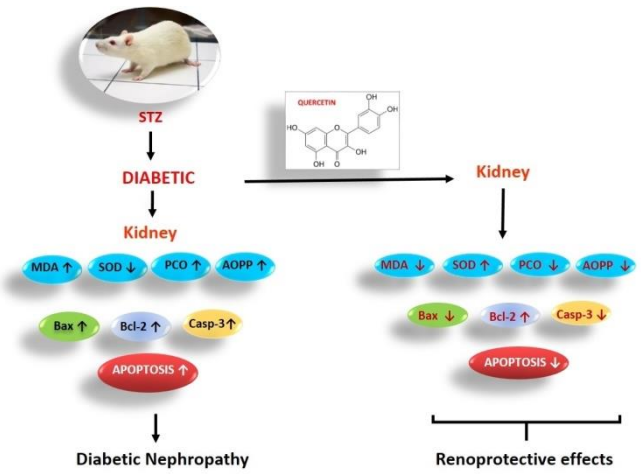

Figure 6. Protective effects of quercetin on diabetic nephropathy in rats. The quercetin may have protective effects against damages caused by diabetes in kidney tissue, may act on apoptotic regulatory proteins to protect the cells from apoptosis by increasing the level of antioxidants in experimental diabetic nephropathy model.

Acknowledgement

This work was supported by the Istanbul University Scientific Research Projects Unit. Project No:52174 and BYP-2017-26104.

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