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Original Article / Özgün Araştırma

Quercetin, a powerful antioxidant bioflavonoid, attenuates renal dysfunction in long-term experimental diabetes mellitus

Güçlü bir antioksidan bioflavonoid olan quercetin uzun süreli deneysel diabetes mellitustaki böbrek fonksiyon bozukluğunu azaltır

Mustafa EDREMİTLİOĞLU[1], Mehmet Fatih ANDİÇ[2], Derya Beyza SAYIN[3], Oğuzhan KORKUT[4], Üçler KISA[5]

[1]*Physiology Department, School of Medicine, Çanakkale Onsekiz Mart University, Çanakkale, Turkey*
[2]*Educational and Research Hospital, Konya, Turkey*
[3]*Medical Genetics Department, School of Medicine, Kırıkkale University, Kırıkkale, Turkey*
[4]*Pharmacology Department, School of Medicine, Balıkesir University, Balıkesir, Turkey*
[5]*Biochemistry Department, School of Medicine, Kırıkkale University, Kırıkkale, Turkey*

ABSTRACT

Objectives: Diabetes mellitus (DM) can cause serious complications such as nephropathy in long term. One of the factors of diabetic nephropathy pathogenesis is the increase of oxidant stress. The aim of this study is to examine the effect of quercetin, a powerful antioxidant agent, on renal functions in streptozotocin-induced diabetes mellitus and the change in balance of oxidant-antioxidant capacity in this process.

Methods: Five groups of rats were used: Control, DM 8 and 16-week (DM8, DM16), DM 8 and 16-week + quercetin (QUER8, QUER16). Rats in QUER8 and QUER16 were treated with IP quercetin (15 mg/kg/day) until the end of the experiment.

Results: Malondialdehyte increased in the DM8 and DM16 groups. On the contrary, it decreased in the groups which were given quercetin. Accordingly, increase in NADPH oxidase activity, and decrease in superoxide dismutase and catalase activities in diabetic rats were determined. In the QUER8 and QUER16 groups, NADPH oxidase activity decreased while antioxidant enzyme activities increased. Functional kidney parameters were considerably corrupted in the diabetic rats. Sodium and water excretion via urine and plasma creatinine level in the QUER16 group were lower than in the DM16 group.

Conclusion: These findings suggest that the administration of quercetin attenuated renal dysfunction and totally prevented the increase of kidney oxidant damage in DM.

Keywords: Quercetin, Diabetes mellitus, Nephropathy, Oxidative damage, Antioxidant enzymes, NADPH oxidase

ÖZET

Amaç: Uzun süreli diabetes mellitusta (DM) nefropati gibi çok ciddi komplikasyonlar ortaya çıkabilmektedir. Oksidan stres diabetik nefropati patogenezinde rol oynayan faktörlerden biridir. Bu çalışmanın amacı, güçlü bir antioksidan olan quercetinin streptozotosinle oluşturulmuş DM'ta böbrek fonksiyonlarına ve oksidan-antioksidan kapasite arasındaki dengeye etkilerini araştırmaktır.

Yöntem: Kontrol, 8 ve 16 haftalık DM (DM8 ve DM16), 8 ve 16 haftalık DM + quercetin (QUER8 ve QUER16) olmak üzere beş grup sıçan kullanıldı. QUER8 ve QUER16 grubundaki sıçanlara deney süresince periton içi quercetin (15 mg/kg/gün) verildi.

Bulgular: Malondialdehit düzeyleri DM8 ve DM16 gruplarında yüksek bulunmasına karşın quercetin verilen gruplarda azaldı. Diabetik sıçanlarda süperoksit dismutaz ve katalaz aktivitelerinde azalma ve NADPH oksidaz aktivitesinde artış gözlendi. QUER8 ve QUER16 gruplarında ise NADPH oksidaz aktivitesi azalırken, antioksidan enzim aktiviteleri arttı. Fonksiyonel böbrek parametreleri diabetik sıçanlarda önemli ölçüde bozuldu. Oysa, QUER16 grubunda idrarla su ve sodyum atımı ve plazma kreatinin düzeyi DM16 grubundan daha düşüktü.

Sonuç: Bulgularımıza göre, quercetin uygulamasının DM'ta oluşan böbrek fonksiyon bozukluğunu azalttığını ve böbrekteki oksidan hasarı tamamen önlediğini söylemek mümkündür.

Anahtar Kelimeler: Quercetin, Diabetes mellitus, Nefropati, Oksidan hasar, Antioksidan enzimler, NADPH oxidase

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Correspondence to: *Mustafa Edremitlioğlu, M.D. Department of Physiology, School of Medicine, Çanakkale Onsekiz Mart University, Çanakkale, Turkey. e-mail: gymedr@yahoo.com*

INTRODUCTION

One of the most common endocrine-metabolic disorders seen in developed countries is diabetes mellitus (DM). End-organ injuries occurring in DM decrease the quality of life while highly increasing medical costs and mortality rates. One of the most important complications in which end-organ injuries occur is diabetic nephropathy that becomes manifest in the long term. The amount of evidence showing reactive oxygen species (ROS) plays a significant role in the increase of the pathogenesis of diabetic nephropathy[1-4]. Cellular damage begins to occur when the production of ROS exceeds the antioxidant capacity. When this process occurs, macromolecules that exist within the cellular composition such as proteins, lipids and carbohydrates are oxidized and damaged[3]. The antioxidant capacity decreases while the production of ROS increases in DM[5-7]. The elevated levels of ROS in a diabetic kidney come from various enzymatic and non-enzymatic sources, including auto-oxidative glycation, activation of protein kinase C, mitochondrial respiratory chain deficiencies and increased oxidase activities[3,8], whereas decreases in the superoxide dismutase (SOD) and catalase enzyme activities play an important role in the decrease of the antioxidant capacity[5,6]. As a result, the oxidant/antioxidant balance significantly deteriorates in favour of oxidants. In fact, there are studies showing that renal damage is reduced by administering agents that decrease the level of oxidant damage[9-11].

One of the molecules that have been researched in many recent studies due to their antioxidant effect is quercetin. Quercetin is a flavonoid prevalent in nature. Flavonoids are molecules with potent antioxidant qualities. As to quercetin, because it scavenges free radicals[12], and meanwhile also inhibits xanthine oxidase (XO) and lipid peroxidation[13] and affects antioxidant pathways both in vivo and in vitro[14,15], it has been studied on immensely. DM is also among the many disease patterns in which effects of quercetin have been investigated. In Mahesh and Menon's study, a significant fall was observed in the blood glucose levels of the diabetic rats that were given two different doses of quercetin. It was also shown that the plasma lipid peroxidation levels were likewise decreased in both groups (diabetic animals that were given high and low doses of quercetin). In the study mentioned above, quercetin was shown to have also increased the superoxide dismutase and catalase enzyme activities in erythrocytes, which was a remarkable result[16]. In a streptozotocininduced experimental DM model, it was shown that damage in the beta cells of pancreas was reduced and that, as a result, the effect of streptozotocin could not emerge adequately when quercetin was given before the onset of DM[17]. Anjaneyulu and Chopra suggested that quercetin improved renal functions in 8 week-old diabetic rats[18]. The common point of these studies is that they draw attention to the antioxidant activity of quercetin as the cause of its curative effects on DM.

Considering the facts that the increased ROS level plays a major role in the pathogenesis of diabetic nephropathy and that quercetin has antioxidant effects, it appears that quercetin can prevent renal dysfunction caused by DM. The aim of this study is to find out whether long-term (16 weeks) quercetin treatment can prevent the formation of nephropathy and to investigate the change that occurs in the balance between oxidant and antioxidant capacities during the treatment.

METHODS

Reagents: Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co., St. Louis, MO.

Animals

Forty-eight male (age: 2-3 months) Wistar rats were used and allowed to acclimatize for 7 days. The animals were kept in stainless steel cages and maintained under standard laboratory conditions at a temperature of 20 ± 2 °C, relative humidity (50 ± 15%). They were fed 12 h light-dark cycle, standard food pellets and water ad libitum. The rats were randomly divided into five groups:

1- Control group (CONT) (n=16): As we wanted to show the time-related changes in this study, we used two control groups, namely 8 weeks and 16 weeks, each containing 8 rats.

2- Group having DM for 8 weeks (DM8) (n=8)

3- Group having DM for 16 weeks (DM16) (n=8)

4- Diabetic group treated with quercetin for 8 weeks (QUER8) (n=8)

5- Diabetic group treated with quercetin for 16 weeks (QUER16) (n=8)

All experimental protocols were reviewed and approved by the Kırıkkale University Animal Ethics Committee (08-16/26).

Treatment Schedule

DM was induced in thirty two of the rats by a single intraperitoneal (IP) injection of streptozotocin, prepared in 0.1 mol/L citrate buffer (pH 4.5), 60 mg/kg body weight, following an overnight fast. The induction of DM was predicated 3 days later by measuring tail vein blood glucose level using a blood glucometer (AccuChek, Roche Diagnostics, Indianapolis, USA). Animals with a blood glucose level higher than 300 mg/dl were considered diabetic. Diabetic rats were given an injection of insulin subcutaneousy (Insulatard, Novo Nordisk, Istanbul, Turkey) at a daily dose of 1-3 units in order to avoid ketoacidosis and weight loss without normalizing hyperglicemia19. Blood glucose levels were monitored at least once a week in all diabetic rats and occasionally in nondiabetic rats for comparison purposes. The animals in the QUER8 and QUER16 groups were put on quercetin on the third day following the induction of DM. Quercetin dissolved in dimethyl sulfoxide was administered IP

at a daily dose of 15 mg/kg[17,20]. All animals were put into metabolic cages and 24-hour urine samples were collected once in every four weeks.

The rats were anesthetized by intramuscular injections of a combination of ketamine and xylazine (100 mg/kg and 10 mg/kg, respectively) 8 weeks (for DM8, QUER8 groups and half of the control group) and 16 weeks (for DM16, QUER16 groups and the other half of the control group) after the STZ or vehicle application. The kidneys were kept frozen in liquid nitrogen, and blood samples were centrifuged immediately for plasma separation. All samples were stored at -68°C until they were used.

Biochemical Analysis

Tissue homogenization: The renal cortex and medulla were carefully separated. All tissue samples were homogenized in ice cold phosphate buffer (0.5M, pH=7.4). Malondialdehyde (MDA) levels were studied in homogenates after all samples were homogenized.

Then supernatant was separated after a 20- minute centrifuging at a speed of 3000 rpm. SOD, catalase, glutathione peroxidase (GPx), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and XO activities were determined in the separated supernatant.

The MDA levels were measured as a thiobarbituric acid-reactive material. The MDA levels in homogenates were measured spectrophotometrically as described previously[21]. Tetramethoxypropane solution was used as the standard. The MDA values determined in this way were expressed as nanomoles per gram protein in the renal cortex and medulla.

SOD activity was assayed using the nitroblue tetrazolium method of Sun et al[22]. In this method nitroblue tetrazolium (NBT) is reduced to blue formazan by superoxide, which has a strong absorbance at 560 nm. In order to obtain blue formazan, SOD assay reagent was prepared using 0.3 mmole/L xanthine, 0.6 mmole/L EDTA Na2, 150 µmole/L NBT, 400 mmole/L Na_2CO_3, and 1 g/L bovine serum albumin (v/v, 20:10:10:6:3 respectively). Then 2.85 ml SOD assay reagent, 0.1 ml supernatant, 0.05 ml xanthine oxidase (167 U/L) were mixed and incubated for 20 minutes at 25°C. After 20 minutes of incubation, 1 ml 0.8 mmol/L $CuCl_2$ was added to the mixture and the blue formazan formation was assessed by using a spectrophotometer at 560 nm. The SOD activity was expressed as U/mg protein.

Catalase activity was determined using the method of Aebi[23]. Briefly, the supernatant was diluted 50-fold with phosphate buffer, and 200ml of the diluted supernatant was added to 2.8ml of 30mM H_2O_2. The change in absorbance was read at 240nm. The rate constant of a first-order reaction (k) was used: k = (2.3Æt) x log (A1/A2), where Æt is a measured time interval (30s) and A1 and A2 are the absorbances at the initial and final measurement times, respectively. The catalase activity was expressed as k/mg protein.

GPx activity was measured using the Paglia and Valentine's method[24]. The reaction mixture contained 2.65 ml of 50 mmol/l phosphate buffer (pH 7), 0.1 ml of 150 mM glutathione solution, 0.1 ml glutathione reductase (10 mg/ml), 0.1 ml of 3 mM NADPH–Na salt, 0.1 ml 50 mmol/l hydrogen peroxide solution and 0.02 ml of tissue homogenate. The GPx activity was monitored by the decrease in absorbance due to the consumption of NADPH, which absorbs at 340 nm. The GPx activity was expressed as U/mg protein.

NADPH oxidase activity was determined by lucigenin enhanced chemiluminescence (CL)[25] using a multichannel luminometer (OrionII/MPL4 model, Berthold Detection Systems, Pforzheim, Germany). Tissue homogenates were incubated for 5 min at 37°C in 50 mM potassium phosphate, 1 mM EGTA, 100 mM sucrose, 0.23 mM lucigenin and pH 7.0. The background CL of the samples was recorded before the reaction was started with 100 µM NADPH. After an equilibration of 20 s, CL was monitored over 10 min. NADPH oxidase activity was calculated as average counts per minute minus background counts per minute. The results were normalized with respect to the protein levels and expressed as multiplies of control.

XO activity was measured by means of Luminescence[26]. The samples were incubated for 5 min at 37°C in 0.1 M Tris-Cl, 1 mM EDTA, 0.23 mM lucigenin (pH 9•0). Background CL of the samples was monitored before the reaction was started with 50 µM xanthine in 0.1 M Tris-Cl (pH 9). The subsequent phases were determined using the same method used for determining NADPH.

The protein levels in the tissue homogenates were determined using Bradford protein assay kit. Plasma creatinine, sodium, glucose levels and urine creatinine, sodium and albumin levels were determined by an Olympus A800 (Olympus Optical, Tokyo, Japan) autoanalyzer using kits from Olympus.

Functional kidney parameters

Urine flow, fractional excretion of sodium (FENa), fractional excretion of water (FEwater) and glomerular filtration rate (GFR) were calculated using proper formulas[27]. Creatinine clearance was used as an indicator of GFR.

Statistics

A statistical analysis was performed using SPSS statistical software (version 13.0). The results were expressed as means ± SE. The data were not normally distributed, so differences among multiple groups were assessed using the Kruskal-Wallis test and those between two groups with the Mann-Whitney U test. A value of $p < 0.05$ was considered to be significant.

RESULTS

The eight-week and 16-week results of the control group in all parameters were very close to

each other and there was no statistically significant difference between the 8 and 16-week control groups. Therefore, in order to simplify the presentations, the values of these two groups were combined and shown as one group.

Plasma glucose and sodium levels

The fasting plasma glucose concentration in the CONT group was found to be significantly lower other in the four DM-induced groups (Table I). It was observed that quercetin treatment did not decrease the fasting plasma glucose concentration. In contrast, plasma sodium levels in QUER8 and QUER16 groups were found to be close to those in the CONT group, although the levels in DM8 and DM16 were significantly lower than the levels in the CONT group (Table I).

Functional kidney parameters

The GFR values in all groups were decreased dramatically compared to the values in the CONT group (1537 ± 99 µl/min). GFR values were 274 ± 55 µl/min in DM8 group and 174 ± 27 µl/min in the DM16 group. An eight-week quercetin treatment did not cause any increase in GFR values (which were decreased due to DM, 208 ± 44 µl/min), whereas a sixteen-week quercetin treatment was shown to elevate GFR values significantly (434 ± 76 µl/min). However, the GFR values in this group (QUER16) were still lower than the values in the CONT group (Fig 1). Despite these changes in GFR levels, no similar changes were observed in the urine flow (Table I). Urine flow in the DM8, QUER8 and QUER16 groups was found to be lower than the values in the CONT group. However, the urine flow values in the groups receiving quercetin treatment did not present any statistically significant differences as compared to the urine flow values in the DM8 and DM16 groups (p=0.059 DM8 versus QUER8 and p=0.059 DM16 versus QUER16). FENa and FEwater values were also higher in all the four groups than the values in the CONT group (Fig 2, Fig 3). It was observed that distortion in tubular functions becomes evident especially in long-term DM. No differences were observed between the FENa and FEwater values of the DM8 and QUER8 groups. However, the FENa and FEwater values in QUER16 that received quercetin treatment were lower than those in DM16. Plasma creatinine level which is an important indicator of renal functions was elevated in diabetic rats (Table I). However, the plasma creatinine levels decreased due to 16-week quercetin treatment. Daily albumin losses via urine are shown in Figure 4. It could be clearly seen that the 24-hour albumin amounts in the urine samples collected once in every four weeks gradually increased up to the 16th week in all the groups whether receiving quercetin treatment or not. Although urinary albumin losses in animals receiving quercetin were lower than those not receiving, the differences were statistically significant only in the first measurement (week 4).

Oxidative damage

MDA levels in the renal cortex and medulla were determined as the indicator of oxidative damage (Fig 5). MDA levels both in the renal cortex and in the renal medulla were found to be increased approximately fourfold as compared to those in the CONT group. In contrast, MDA levels in the renal cortex and medulla in QUER8 and QUER16 groups were significantly lower than those in diabetic rats not receiving quercetin treatment, and close to those in the CONT group.

Antioxidative enzyme activities (Fig 6, Fig 7)

SOD and catalase activities in both the renal cortex and the renal medulla were lower in the DM8 and DM16 groups than in the CONT group. In contrast, SOD and catalase activities were found to have increased in all the tissues studied in the QUER8 and QUER16 groups. Moreover, the catalase activities in the renal medulla in the QUER8 group were statistically higher than in the CONT group. In particular, cortex GPx activities in the DM8 and DM16 groups were slightly higher than those in the CONT group; however, there were no statistically significant differences between the groups when the medulla and cortex activities were compared. GPx levels (U/mg protein) in groups CONT, DM8, DM16, QUER8 and QUER16 were respectively as follows: 0.51 ± 0.07, 0.97 ± 0.27, 0.81 ± 0.20, 0.52 ± 0.13, 0.62 ± 0.09 in the cortex, 0.31 ± 0.06, 0.44 ± 0.14, 0.25 ± 0.04, 0.70 ± 0.36 ve 0.42 ± 0.06 in the medulla.

Oxidase enzyme activities (Fig 8)

It can be clearly seen in Figure 8 that NADPH oxidase activities in the renal cortex and medulla increased significantly in the DM8 and DM16 groups, while, in contrast, in QUER8 and QUER16, these levels approached the levels in the CONT group. It is also notable that the XO activity in the cortex and medulla was dramatically attenuated due to a sixteen-week quercetin treatment.

Table I : General Characteristics of Normal, Diabetic and Quercetin-Treated Diabetic Animals.

	Fasting blood glucose (mg/dL)	Body weight (g)	Sodium (mEq/L)	Creatinine (mg/dL)	Urine Flow Rate (µL/min)
CONT	101,50 ± 9,84	251 ± 3	140 ± 1	0,33 ± 0,02	19,91 ± 1,34
DM8	321,37 ± 44,46*	242 ± 6	132 ± 2*	0,87 ± 0,26*	11,37 ± 1,17*
DM16	343,50 ± 39,12*	240 ± 6	130 ± 3*	1,23 ± 0,03*	20,08 ± 2,46**
QUER8	311,75 ± 18,40*	243 ± 5	141 ± 2**, ***	0,97 ± 0,19*	15,23 ± 1,46*
QUER16	306,87 ± 33,09*	241 ± 5	137 ± 2***	0,75 ± 0,13*, ***	14,33 ± 1,05*

Data are the mean ± SEM. *: p<0.05 versus CONT, **: p<0.05 versus DM8, ***: p<0.05 versus DM16

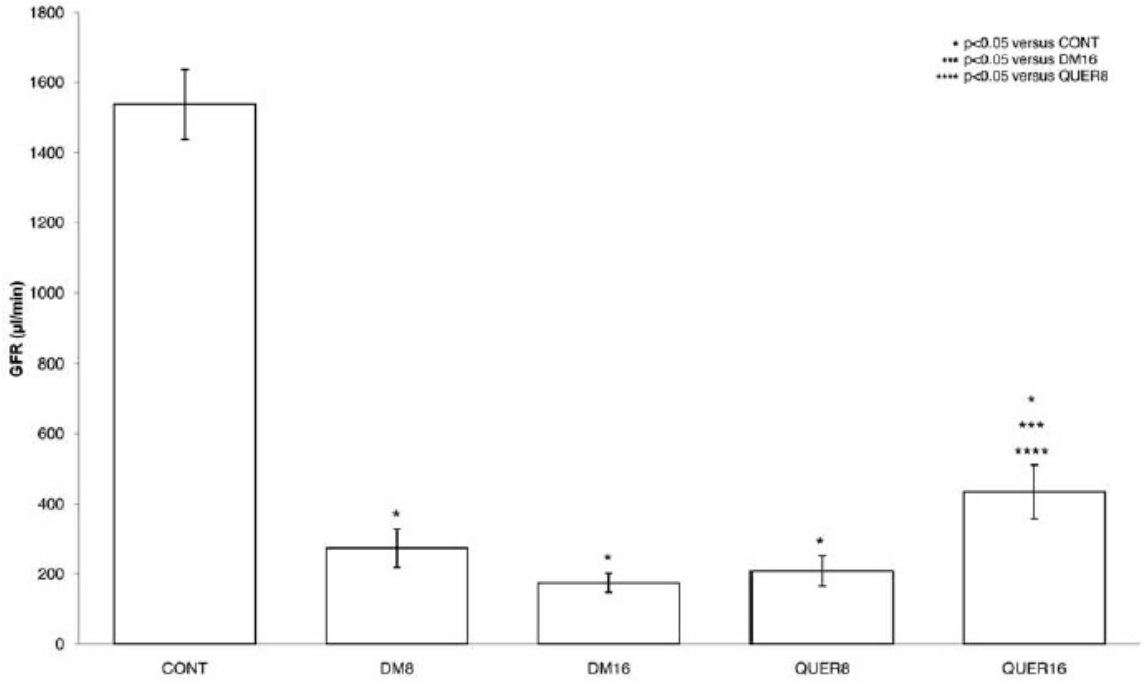

Fig 1 : Effect of 8 and 16 weeks treatment with quercetin (15 mg/kg IP) on GFR values in streptozotocin-induced diabetic rats. Creatinine clearance was used as an indicator of GFR. Bar heights are the means; error bars indicate ± SEM.

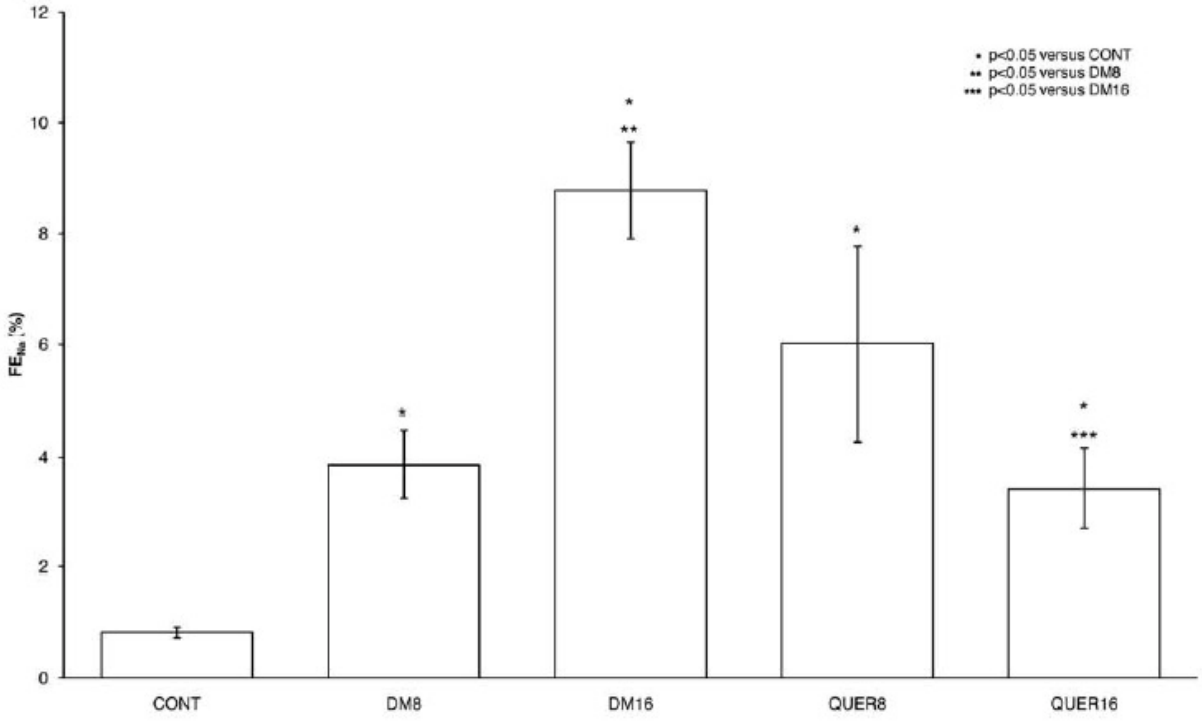

Fig 2 : Effect of 8 and 16 weeks treatment with quercetin (15 mg/kg IP) on FE_{Na} values in streptozotocin-induced diabetic rats. Bar heights are the means; error bars indicate ± SEM.

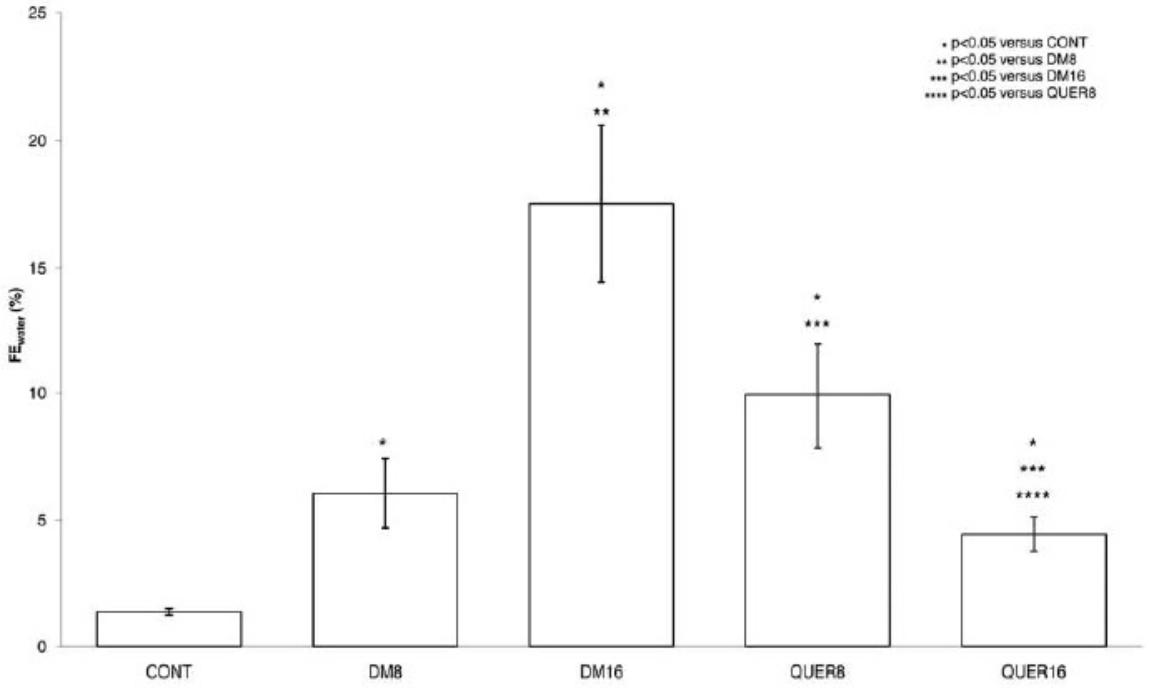

Fig 3 : Effect of 8 and 16 weeks treatment with quercetin (15 mg/kg IP) on FE_{water} values in streptozotocin-induced diabetic rats. Bar heights are the means; error bars indicate ± SEM.

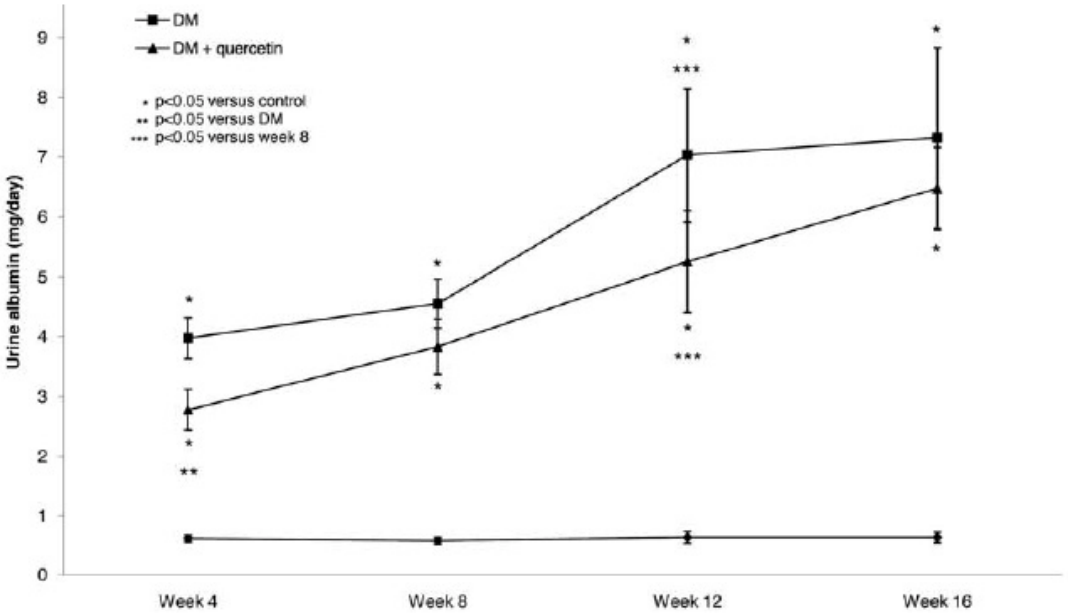

Fig 4 : Effect of quercetin treatment (15 mg/kg IP) on daily albumin loss via urine in streptozotocin-induced diabetic rats, Each point is the means; error bars indicate ± SEM. The values for the 4th and 8th week were obtained from all rats in both the 8 weeks group and the 16 weeks group and they show the average of data relating to16 rats in total. As the rats used in the 8 weeks group were sacrificed at the end of this period, values of 12 weeks group and 16 weeks group show the average of data relating to 8 rats in total.

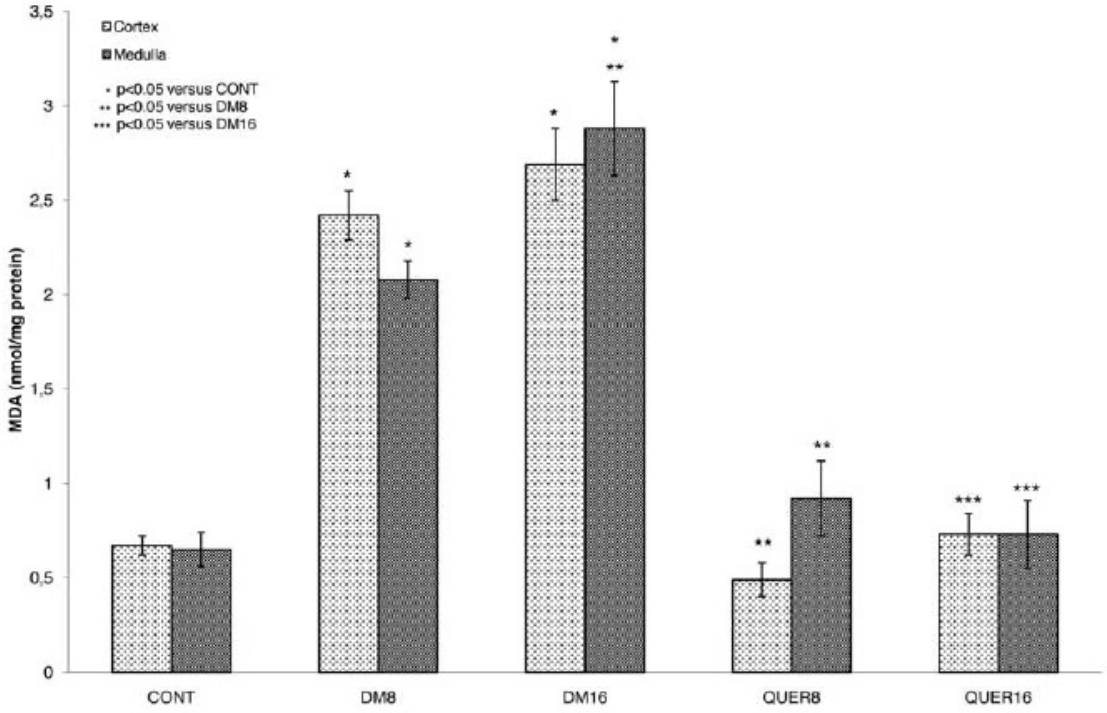

Fig 5 : Effect of 8 and 16 weeks treatment with quercetin (15 mg/kg IP) on kidney cortex and medulla MDA levels in streptozotocin-induced diabetic rats. The MDA levels were measured as a thiobarbituric acid-reactive material. Bar heights are the means; error bars indicate ± SEM.

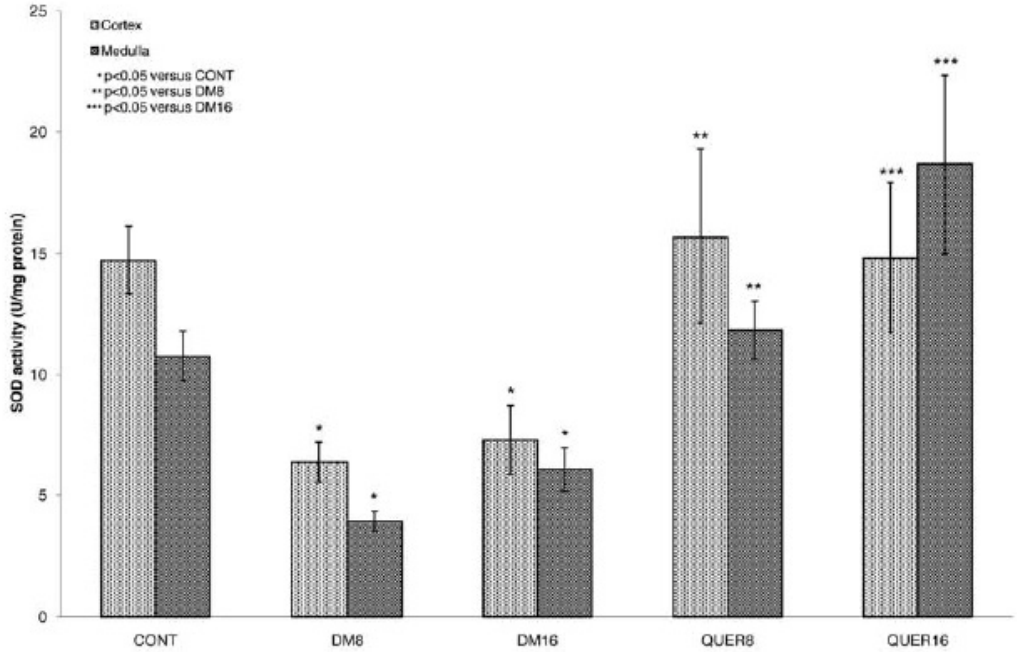

Fig 6 : Effect of 8 and 16 weeks treatment with quercetin (15 mg/kg IP) on the kidney cortex and medulla SOD levels in streptozotocin-induced diabetic rats. Bar heights are the means; error bars indicate ± SEM.

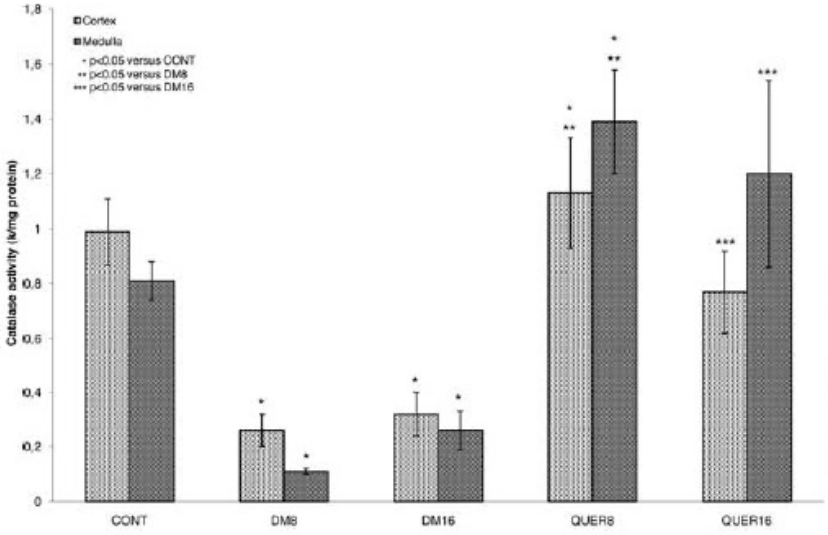

Fig 7 : Effect of 8 and 16 weeks treatment with quercetin (15 mg/kg IP) on kidney cortex and medulla catalase levels in streptozotocin-induced diabetic rats. Bar heights are the means; error bars indicate ± SEM.

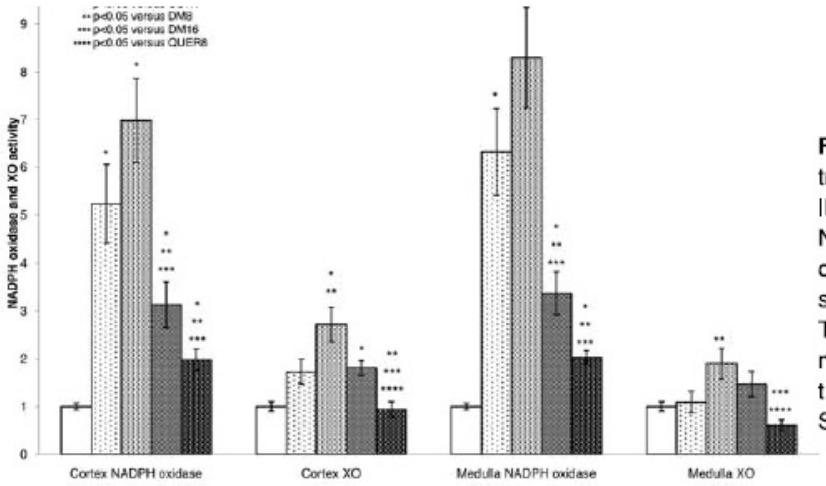

Fig 8 : Effect of 8 and 16 weeks treatment with quercetin (15 mg/kg IP) on the kidney cortex and medulla NADPH oxidase and xanthine oxidase (XO) activity levels in streptozotocin-induced diabetic rats. The results were expressed as multiplies of control. Bar heights are the means; error bars indicate ± SEM.

DISCUSSION

The results of this study have clearly shown that the renal functions impaired because of DM were attenuated by the administration of quercetin. Two different quercetin treatment groups, (8-week and 16-week treatments) were used in this study in order to evaluate the effects dependent on the duration of the treatment. The partially improving effect of quercetin on renal functions occurred more obviously in the longer (16-week) treatment. Both 8-week and 16-week quercetin treatments fully improved oxidant damage causing noticeable changes in the balance between the oxidant and antioxidant systems.

In our study, FENa and FEwater values increased significantly in the DM8 and DM16 groups while GFR values decreased. Furthermore, urine albumin levels also increased significantly. These findings indicate that end-organ injuries occurred in the rats with induced diabetes used in our study. It was observed in our study that quercetin treatment had significant effects on FENa, FEwater and GFR levels. We determined that the progressively elevated FENa and FEwater values decreased significantly after a 16-week quercetin treatment,

yet, the decreases did not reach the values in the CONT group (Figures 2 and 3). Considering these data, it can be said that the impaired tubular functions caused by DM were attenuated by long term quercetin treatment. A similar result can be seen in the GFR values (Figure 1). The GFR values in the QUER16 group were significantly higher than those in the DM16 group, even though lower than those in the control group. This increase in GFR values thus caused the plasma creatinine values in the QUER16 group to be lower than those in the DM16 group. However, the plasma creatinine values in the QUER16 group were still significantly higher than those in the CONT group. It is remarkable that these effects of quercetin on renal functions occurred in the group receiving the 16-week treatment. A previous study showed that an 8-week quercetin treatment ameliorated renal dysfunction caused by DM[18], however we could not obtain the same positive effects before the sixteenth week. As can also be seen in Figure 4, the urine albumin level increased significantly in the fourth week, when we performed our first measurement consequent to the induction of DM. Albuminuria, increased progressively until the 12th week, then followed a stable course until the end of the study. Albuminuria followed a similar course in the animals treated with quercetin as well. According to the results of our study, quercetin treatment did not cause a significant decrease in albuminuria. Considering its attenuating effect on FENa, FEwater and GFR, quercetin can be expected to have a positive effect on albuminuria as well. Quercetin may be able to decrease albuminuria when used throughout protocols longer than 16 weeks and/or in dosages higher than the ones we used.

Although the urine output values in DM8 were lower than those in the CONT group, no such decrease was found in the DM16 group, whereas the GFR values both in DM8 and in DM16 were considerably lower than those in the CONT group. Assessing FEwater values can be a reasonable explanation for this situation. According to this, it is possible to claim that the urine output values in the DM8 and DM16 groups did not exhibit a decrease as dramatically as the GFR values did, a situation which was due to the inadequate tubular reabsorption of water. Thus, considering the FEwater values in the mentioned groups, our argument on this subject is also supported by the fact that while the GFR values in the QUER16 group were significantly higher than the GFR values in the DM16 group, the urine outputs contrarily tended to decrease ($p=0.059$). Given the facts that the FENa values in our study groups with induced diabetes increased and that glucose output with urine increases in DM (a well known fact even though we did not perform our own measurements on this parameter), it may be claimed that the osmotic load increases in the tubular fluid. However, it is a well-known result of tubuloglomerular feedback that GFR consequently rises when the amount of sodium reaching the macula densa increases. As to our study, considering that the GFR decreased, it can be stated that the tubuloglomerular feedback was impaired.

It is well-known that oxidative damage plays an important role in renal dysfunction[28] and the pathogenesis of diabetic nephropathy[1-3]. Thus there are many studies that show oxidative damage increases in DM[4,29-34]. It has been shown that, Type IV collagen, which also exists in glomerular basement membrane, was reported to decrease in DM, however, this decrease was prevented by inhibition of advanced glycation end products with aminoguanidine[35]. In our study too, we observed that MDA levels, as the indicator of oxidative damage, increased significantly in the streptozocotin-induced diabetic rats (Fig 5). It is also well known that the determinant of oxidative damage is the balance between oxidative and antioxidative systems. Antioxidative enzymes play a very important role in preventing the damage in cell components caused by free radicals. SOD, catalase, and GPx are major antioxidative enzymes. Therefore, we not only evaluated the level of oxidative damage, but also studied how the oxidative and the antioxidative systems (SOD, catalase and GPx activities) in the renal tissues were affected by DM or quercetin treatment. We determined significant declines in renal antioxidant activities in the DM8 and DM16 groups. For this purpose we evaluated SOD and catalase activities and found that these activities were significantly attenuated both in the renal cortex and in the medulla. In contrast, DM did not affect GPx activities. Although it is well known that quercetin decreases oxidative damage by being effective as a free radical scavenger[12], much less is known about its effects on the regulation of antioxidative enzymes. Nevertheless, in a recent study it was shown that quercetin played a role in antioxidative enzyme modulation in cultured liver cells[36]. In this study, it was determined that catalase and GPx mRNA expression changed in the presence of a cytokine mixture composed of human recombinant interleukin 1, tumor necrosis factor , and interferon. It was also shown that quercetin affects the NF-B activation which is closely related to antioxidative enzyme expression[37]. It is clear that the relation between quercetin and antioxidative enzyme regulation is a complicated process where many mediators are involved. New studies are needed in order to fully understand the effects of quercetin on the human body's antioxidative defense system as it may be considered for use in the treatment of some diseases in the future, due to its effects on decreasing oxidative stress. Taking into account NADPH oxidase activity in the renal tissue, which is an important source of superoxide, it can be said that the increase in oxidative damage in the DM8 and DM16 groups was not only because of the deficiency in antioxidant defense mechanisms, but the increase in NADPH activity was responsible as well. In our study, we determined that NADPH activity increased progressively in DM both in the renal cortex and in the medulla. We also observed that XO activity, which is one of the important components of ROS production, was elevated in the renal cortex in the DM8 and DM16 groups. These findings clearly showed that the oxidantantioxidant balance was impaired in favor of the oxidant system.

When the results of the oxidative damage are taken into account, kidneys of diabetic patients are essential target organs and should be protected from the effect of ROS. Therefore, reinforcement of the antioxidant capacity seems to be occupying an important place among the novel approaches of treatment that will be used for preventing diabetic nephropathy in the near future. Quercetin has a strong antioxidant activity and is one of the many herbal molecules called flavonoids. Studies on the effects of quercetin on DM[16,18,38,39] have called attention to its antioxidant effect. It is known that quercetin prevents oxidative damage by inhibiting the xanthine dehydrogenase/XO system[12]. In our study too, the decrease in XO activity in the renal cortex and the medulla in diabetic rats that received 16-week quercetin treatment was remarkable. By evaluating NADPH oxidase activities in the renal cortex and the medulla in the QUER8 and QUER16 groups, it can be clearly seen in our results that the inhibitory effect of quercetin on the oxidant system is not merely the XO inhibition. Quercetin attenuated the NADPH oxidase activity in these groups significantly.

NADPH oxidase is an enzyme that consists of two membrane-associated (p22 phox, gp91 phox) and four cytosolic (p47 phox, p40 phox, p67 phox and small GTPase rac) components and plays a major role in the pathogenesis of diabetic nephropathy[40]. NADPH oxidase is present in the proximal tubule and mesengial cells, vascular smooth muscle cells, endothelial cells and fibroblasts in the kidney[25]. In physiological conditions, the amount of superoxide produced by NADPH oxidase is less than the amount of active neutrophils. However, it has been shown that an excessive amount of ROS is produced in the lead of NADPH oxidase[7,41]. In these studies, the impairment in the renal function caused by diabetes was prevented from occurring by the administration of apocynin, an NADPH oxidase inhibitor.

As the information obtained from studies on the role of NADPH oxidase in the pathogenesis of diabetic nephropathy has accumulated, inhibition of NADPH oxidase has begun to be seen as a new approach in the treatment of diabetic nephropathy[42]. It has been recently shown that quercetin inhibited the overexpression of p47phox, the cytoplasmic subunit of NADPH oxidase[43]. This information, when evaluated together with our results, indicates that quercetin can become an important agent in preventing DM complications.

In this study, we did not use a group of healthy animals which were administered quercetin only. Results achieved from studies in which quercetin, which is now in the stage of being accepted as a nutraceutical44, was administered to normal animals, clearly show that it does not affect the kidney functions and oxidative/antioxidative parameters in healthy animals[18,38,39,45-47]. At this point, the toxic effects of quercetin should also be mentioned. While at first it was reported that quercetin had mutagenic effects, subsequent studies showed that it was antimutagenic due to its protective effect against genotoxic agents. Moreover, in 1999 the International Agency for Research on Cancer concluded that quercetin was not one of the carcinogenics for human beings[12]. In a recent review where the results of short and long term human and animal studies were presented in detail, it was concluded that there was not enough evidence regarding quercetin's mutagenic and carcinogenic effects and that it was a dependable agent[48].

To conclude, it is possible to state that quercetin ameliorates renal functions impaired because of DM. The direct relationship between oxidative stress and renal dysfunction was shown[28]. In our study, oxidative damage increased by DM was completely precluded by quercetin. Quercetin clearly caused this effect both by increasing SOD and catalase activities and strengthening antioxidant defense mechanisms, and by inhibiting oxidant systems such as XO and NADPH oxidase. Considering the key role NADPH oxidase activity plays in the pathogenesis of DM complications, one of the most important findings of our study is the inhibitory effect of quercetin on this enzyme activity. Quercetin may become one of the important weapons preventing DM complications, but further data must be obtained from researches on the subject in the future.

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REFERENCES

1. Van Dijk C, Berl T. Pathogenesis of diabetic nephropathy. Rev Endocr Metab Disord 2004;5:237- 48. doi: 10.1023/B:REMD.0000032412.91984.ec
2. Coughlan MT, Mibus AL, Forbes JM. Oxidative stress and advanced glycation in diabetic nephropathy. Ann N Y Acad Sci 2008;1126:190-3. doi: 10.1196/annals.1433.018
3. Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes 2008;57:1446-54. doi: 10.2337/db08-0057
4. Vessal G, Akmali M, Najafi P, Moein MR, Sagheb MM. Silymarin and milk thistle extract may prevent the progression of diabetic nephropathy in streptozotocininduced diabetic rats. Ren Fail 2010;32:733-9. doi: 10.3109/0886022X.2010.486488
5. Ceriello A, Morocutti A, Mercuri F, et al. Defective intracellular antioxidant enzyme production in type 1 diabetic patients with nephropathy. Diabetes 2000;49:2170-7. doi: 10.2337/diabetes.49.12.2170
6. Asaba K, Tojo A, Onozato ML, Goto A, Fujita T. Doubleedged action of SOD mimetic in diabetic nephropathy. J Cardiovasc Pharmacol 2007;49:13-9. doi: 10.1097/FJC.0b013e31802b6530
7. Asaba K, Tojo A, Onozato ML, et al. Effects of NADPH oxidase inhibitor in diabetic

nephropathy. Kidney Int 2005;67:1890-8. doi: 10.1111/j.1523-1755.2005.00287.x

8. Derubertis FR, Craven PA. Activation of protein kinase C in glomerular cells in diabetes. Mechanisms and potential links to the pathogenesis of diabetic glomerulopathy. Diabetes 1994;43:1-8. doi: 10.2337/diabetes.43.1.1
9. Craven PA, DeRubertis FR, Kagan VE, Melhem M, Studer RK. Effect of supplementation with vitamin C or E on albuminuria, glomerular TGF-beta, and glomerular size in diabetes. J Am Soc Nephrol 1997;8:1405-14.
10. Koya D, Lee IK, Ishii H, Kanoh H, King GL. Preventation of glomerular dysfunction in diabetic rats by treatment with d-alpha-tocopherol. J Am Soc Nephrol 1997; 8:426- 35.
11. Melhem MF, Craven PA, DeRubertis FR. Effect of dietary supplementation of alpha-lipoic acid on early glomerular injury in diabetes mellitus. J Am Soc Nephrol 2001;12:124-33.
12. Bischoff SC. Quercetin: potentials in the prevention and therapy of disease. Curr Opin Clin Nutr Metab Care 2008;11:733-40. doi: 10.1097/MCO.0b013e32831394b8
13. Fiorani M, Sanctis R. Menghinello P, Cucchiarini L, Cellini B, Dacha M. Quercetin prevents glutathione depletion induced by dehydro-ascorbic acid in rabbit red blood cell. Free Radic Res 2001;34:639-48. doi: 10.1080/10715760100300531
14. Morand C, Crespy V, Manach C, Besson C, Demigne C, Remesy C. Plasma metabolites of quercetin and their antioxidant properties. Am J Physiol 1998;75:R212- R219.
15. Mapanga RF, Musabayane CT. The renal effects of blood glucose-lowering plant-derived extracts in diabetes mellitus-an overview. Ren Fail 2010;32:132-8. doi: 10.3109/08860220903367585
16. Mahesh T, Menon VP. Quercetin allievates oxidative stress in streptozotocin-induced diabetic rats. Phytother Res 2004;18:123-7. doi: 10.1002/ptr.1374
17. Coskun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. Pharmacol Res 2005;51:117- 23. doi: 10.1016/j.phrs.2004.06.002
18. Anjaneyulu M, Chopra K. Quercetin, an anti-oxidant bioflavonoid, attenuates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol 2004;31:244-8. doi: 10.1111/j.1440-1681.2004.03982.x
19. Osicka TM, Yu Y, Panagiotopoulos S, et al. Prevention of albuminuria by aminoguanidine or ramipril in streptozotocin-induced diabetic rats is associated with the normalization of glomerular protein kinase C. Diabetes 2000;49:87-93.
20. Vessal M, Hemmati M, Vasei M. Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. Comp Biochem Physiol C Toxicol Pharmacol 2003;13C:357- 64. doi: 10.1016/S1532-0456(03)00140-6
21. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
22. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34:497- 500. doi: 10.1016/0003-2697(79)90738-3
23. Aebi H. Catalase. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis. New York, NY: Academic, 1974:673–84.
24. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158-69.
25. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res 1994;74:1141-8.
26. Mohazzab KM, Wolin MS. Sites of superoxide anion production detected by lucigenin in calf pulmonary artery smooth muscle. Am J Physiol 1994;267:L815- 22.
27. Laiken ND, Fanestil DD. Body fluids and renal function. In: West JB, editor. Best and Taylor's Physiological Basis of Medical Practise. Baltimore: Williams and Wilkins, 1990:406-515.
28. Rosas-Rodríguez JA, Valenzuela-Soto EM. Enzymes involved in osmolyte synthesis: How does oxidative stress affect osmoregulation in renal cells? Life Sci 2010;87:515-20. doi: 10.1016/j.lfs.2010.08.003
29. Takenouchi Y, Kobayashi T, Taguchi K, Matsumoto T, Kamata K. Gender differences in vascular reactivity of aortas from streptozotocin-induced diabetic mice. Biol Pharm Bull 2010;33:1692-7. doi: 10.1248/bpb.33.1692
30. Palsamy P, Sivakumar S, Subramanian S. Resveratrol attenuates hyperglycemia-mediated oxidative stress, proinflammatory cytokines and protects hepatocytes ultrastructure in streptozotocin-nicotinamide-induced experimental diabetic rats. Chem Biol Interact 2010;186:200-10. doi. 10.1016/j.cbi.2010.03.028
31. Yargiçoğlu P, Ağar A, Edremitlioğlu M, Oğuz Y, Apaydin C. The effect of cadmium on visual evoked potentials in alloxane-induced diabetic rats: relation to lipid peroxidation. Acta Diabetol 1999;36:197-204. doi: 10.1007/s005920050167
32. Yargicoglu P, Agar A, Edremitlioglu M, Kara C. The effects of cadmium and experimental diabetes on VEP spectral data and lipid peroxidation. Int J Neurosci 1998;93:63-74. doi: 10.3109/00207459808986413
33. Dávila-Esqueda ME, Vertiz-Hernández AA, Martínez- Morales F. Comparative analysis of the renoprotective effects of pentoxifylline and vitamin E on streptozotocininduced diabetes mellitus. Ren Fail 2005;27:115-22. doi: 10.1081/JDI-200042728
34. McCormack AJ, Hak LJ, Finn WF. The effect of aldose reductase inhibition and dietary protein restriction on renal function in experimental diabetes mellitus. Ren Fail 1991;13:267-74.
35. Yavuz D, Bozkurt S, Aydın H, Ersoz Ö, Demirkesen C, Akalın S. Effects of aminoguanidine on dermal collagen structure

and tgf-beta expression in streptozotocin induced diabetic rats. Marmara Med J 2005;18;76-80.

36. Crespo I, García-Mediavilla MV, Almar M, et al. Differential effects of dietary flavonoids on reactive oxygen and nitrogen species generation and changes in antioxidant enzyme expression induced by proinflammatory cytokines in Chang Liver cells. Food Chem Toxicol 2008;46:1555-69. doi: 10.1016/j.fct.2007.12.014

37. Martínez-Flórez S, Gutiérrez-Fernández B, Sánchez- Campos S, González-Gallego J, Tuñón MJ. Quercetin attenuates nuclear factor-kappaB activation and nitric oxide production in interleukin-1beta-activated rat hepatocytes. J Nutr 2005;135:1359-65.

38. Coldiron AD Jr, Sanders RA, Watkins JB 3rd. Effects of combined quercetin and coenzyme Q(10) treatment on oxidative stress in normal and diabetic rats. J Biochem Mol Toxicol 2002;16:197-202. doi: 10.1002/jbt.10035

39. Sanders RA, Rauscher FM, Watkins JB 3rd. Effects of quercetin on antioxidant defense in streptozotocininduced diabetic rats. J Biochem Mol Toxicol 2001;15:143-9. doi: 10.1002/jbt.11

40. Balakumar P, Arora MK, Reddy J, Anand-Srivastava MB. Pathophysiology of diabetic nephropathy: Involvement of multifaceted signalling mechanism. J Cardiovasc Pharmacol 2009;54:129-38. doi: 10.1097/FJC.0b013e3181ad2190

41. Thallas-Bonke V, Thorpe SR, Coughlan MT, et al. Inhibition of NADPH oxidase prevents advanced glycation end product-mediated damage in diabetic nephropathy through a protein kinase C-alphadependent pathway. Diabetes 2008;57:460-9. doi: 10.2337/db07-1119

42. Turgut F, Bolton WK. Potential new therapeutic agents for diabetic kidney disease. Am J Kidney Dis 2010;55:928-40. doi: 10.1053/j.ajkd.2009.11.021

43. Romero M, Jiménez R, Sánchez M, et al. Quercetin inhibits vascular superoxide production induced by endothelin-1: Role of NADPH oxidase, uncoupled eNOS and PKC. Atherosclerosis 2009;202:58-67. doi: 10.1016/j.atherosclerosis.2008.03.007

44. Boots AW, Haenen GR, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. Eur J Pharmacol 2008; 585: 325-37. doi: 10.1016/j.ejphar.2008.03.008

45. Renugadevi J, Prabu SM. Quercetin protects against oxidative stress-related renal dysfunction by cadmium in rats. Exp Toxicol Pathol 2010;62:471-81. doi: 10.1016/j.etp.2009.06.006

46. Galisteo M, García-Saura MF, Jiménez R, et al. Effects of quercetin treatment on vascular function in deoxycorticosterone acetate-salt hypertensive rats. Comparative study with verapamil. Planta Med 2004;70:334-41. doi: 10.1055/s-2004-818945

47. Liu CM, Ma JQ, Sun YZ. Quercetin protects the rat kidney against oxidative stress-mediated DNA damage and apoptosis induced by lead. Environ Toxicol Pharmacol 2010; 30: 264- 71. doi: 10.1016/j.etap.2010.07.002

48. Harwood M, Danielewska-Nikiel B, Borzelleca JF, Flamm GW, Williams GM, Lines TC. A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties. Food Chem Toxicol 2007;45:2179-205. doi: 10.1016/j.fct.2007.05.015