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Quercetin, a Powerful Antioxidant Bioflavonoid, Prevents Oxidative Damage in Different Tissues of Long-Term Diabetic Rats

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ABSTRACT

Objective: The role of oxidant damage on the development of end-organ injuries caused by diabetic rat is well known. The aim of this study is to examine the effect of quercetin, which is a strong antioxidant bioflavonoid, on oxidant damage and antioxidant capacity in various organs in the case of medium-term or long-term diabetic rat.

Material and Methods: Forty-eight male Wistar rats were divided into five groups, namely, control group, diabetic group of 8-weeks, diabetic group of 16-weeks, quercetin treated diabetic group of 8-weeks, and quercetin treated diabetic group of 16-weeks. At the end of the experiment, malondialdehyde levels, superoxide dismutase, catalase, and glutathione peroxidase activities were measured in the lung, aorta, heart, spleen, liver, and kidney tissues.

Results: MDA levels were elevated in all tissues except in the lung in non-treated diabetic groups. Quercetin treatment increased the antioxidant enzyme capacities and considerably reduced oxidant damage.

Conclusion: We suggest that quercetin has a protective effect on the aorta, heart, brain, liver, and kidneys from oxidant damage in the case of medium-term or long-term diabetic rat. It can be argued that quercetin is effective by increasing the antioxidant defence capacity in this process.

Key Words: Quercetin, diabetic rat, oxidative damage, antioxidant enzymes

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Introduction

Oxidative stress has been shown, both in experimental and clinical studies held in recent years, to play a key role in the pathogenesis of many diseases. Oxidative stress is effective on the pathological processes of diseases like cancer, cardio-vascular diseases, rheumatoid arthritis, diabetes mellitus, and neurological disorders such as Alzheimer and Parkinson (1). Oxidative stress has been shown to be effective on both the etiology of DM and the occurrence of DM complications (1, 2). The increased reactive oxygen species (ROS) in DM has various sources such as auto-oxidative glycation, activation of protein kinase C, mitochondrial respiratory chain deficiencies and increased oxidase enzyme activities (3, 4). However, the body has its antioxidant system to prevent ROS production and the probable damages ROS can cause. The most important elements of the intracellular antioxidant defense are superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) enzyme activities (2). It is a well known fact that alterations occur in the antioxidant defense systems in case of DM (1). The most important cause of the increases in the mortality and morbidity rates in DM is end-organ injury. Considering the role of oxidative stress in this process, antioxidant treatment can be expected to reduce/prevent organ damages in DM. It has been shown in recent studies that antioxidant agents such as apocynin, lipoic acid, simvastatin,coenzyme Q10 and acetyl L-carnitine have positive effects in DM (5-9).

Another molecule with a potent antioxidant effect is quercetin, a common flavonoid in Nature. It exists in many nutrients, mostly in red onions, grapes, berries, cherries, broccoli, citrus fruits, tea (*Camelia sinensis*) and capers (10). Quercetin is able to preclude oxidative stress by directly inactivating free radicals, by inhibiting xanthine oxidase and lipid peroxidation, and by affecting antioxidant pathways both *in vivo* and *in vitro* (11-14).

Quercetin, as a potent antioxidant agent, can be expected to reduce the damages in diabetic tissues considering the role of oxidative damage in the occurrence of organ injuries in DM. On the grounds that end-organ injuries occur in the later stage, it is particularly important to understand both the long-term effects of DM and the effects of the treatment administered on oxidative stress in tissues. The aim of this study is to investigate how the extent of oxidative damage and the antioxidant capacity in the lung, aortic, cardiac, brain, liver and renal tissues of rats with DM induced by streptozotocin

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are affected by the administration of quercetin in medium (8 weeks) and long (16 weeks) terms.

Material and Methods

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 of temperature (20±2°C), relative humidity (50±15%), 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 single intraperitoneal injections of streptozotocin (Sigma, St. Louis, MO), prepared in 0.1 mol/L citrate buffer (pH 4.5), 60 mg/kg body wt, following an overnight fasting. The remaining sixteen (vehicle injected) rats served as controls. 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 subcutaneous injections of insulin (Insulatard, Novo Nordisk, Istanbul, Turkey) at a daily dose of 1-3 units in order to avoid ketoacidosis and weight loss. 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 QUER8 and QUER16 groups started to receive quercetin treatment on the third day following the induction of DM. Quercetin (Sigma, St. Louis, MO) dissolved in dimethyl sulfoxide was administered intraperitoneally at a daily dose of 15 mg/kg (15).

The rats were anesthetized by intramuscular injections of a combination of ketamine and xylazine (100 mg/kg and 10 mg/kg, respectively) for 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. Animals were sacrificed by cardiac puncture. The kidney, brain, liver, lung, aorta, spleen and heart tissues were harvested and washed with ice-cold phosphate-buffered saline to remove residual blood. All tissues were kept frozen in liquid nitrogen and stored at -68°C until used.

Biochemical analysis

Tissue homogenization: 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 the supernatant was separated after 20-minute centrifuging at a speed of 3000 rpm. SOD, catalase and GPx 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 (16). Tetramethoxypropane solution was used as the standard. The MDA values determined in this way were expressed as nanomoles per mg protein.

SOD activity was assayed using the nitroblue tetrazolium method of Sun et al. (17) In this method, nitroblue tetrazolium (NBT) is reduced to blue formazan by $O_2^{\cdot -}$, 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 Na_2, 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 and 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 formation of the blue formazan 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 (18). Briefly, the supernatant was diluted 50-fold with phosphate buffer, and 200 ml of the diluted supernatant was added to 2.8 ml of 30 mM H_2O_2. The change in absorbance was read at 240 nm. The rate constant of a first-order reaction (k) was used: $k = (2.3/\Delta t) \times \log(A1/A2)$, where Δt 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/g protein.

GPx activity was measured using Paglia and Valentine's method (19). 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.

The protein amounts in tissue homogenates were determined using Bradford protein assay kit. Fasting blood glucose, serum total protein, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined by an Olympus A800 (Olympus Optical, Tokyo, Japan) autoanalyzer using kits from Olympus.

Statistics

A statistical analysis was performed using SPSS statistical software (version 13.0). The results are expressed as means±SE. The data were not normally distributed, so differences among multiple groups were assessed using the Kruskal-Wallis test. A value of $p<0.05$ was considered to be significant. Post-hoc evaluation to determine the between group differences was

carried out by the Mann-Whitney U-test in which the Bonferroni correction was applied for comparisons within groups. A value of $p<0.00625$ was considered to be significant.

Results

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 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.

Fasting blood glucose, serum total protein, creatinine, ALT, AST and body weight levels can be seen in Table 1. Fasting plasma glucose concentrations were significantly higher in groups having induced DM. Quercetin treatment did not decrease fasting blood glucose values.

It can be clearly seen in Table 2 that MDA levels determined as the indicator for oxidative damage were elevated in all the tissues studied except lung tissue. It was found that long-term diabetes (DM16 group) increased oxidative damage more in the brain and the renal medulla. Quercetin treatment decreased the elevated MDA levels in DM16 group, and even equated those levels with the levels in the CONT group except in brain and cardiac tissues.

SOD enzyme activities can be seen in Table 3. It can be seen that SOD activity in the lung tissue did no change in the groups having induced DM. The aorta SOD activities in DM16 group were increased. No differences in tissues other than the aorta were found between the DM8 and DM16 groups.

Table 1. General characteristics of normal, diabetic and quercetin-treated diabetic animals. Data are the mean±SEM

	CONT (n=16)	DM8 (n=8)	QUER8 (n=8)	DM16 (n=8)	QUER16 (n=8)
Fasting blood glucose (mg/dL)	101.50±9.84	321.37±44.46 *(p=0.0001)	311.75±18.40 *(p=0.0001)	343.50±39.12 *(p=0.0001)	306.87±33.09 *(p=0.0001)
Body weight (g)	251±3	242±6	243±5	240±6	241±5
Total protein (g/dL)	6.12±0.40	6.01±0.29	5.17±0.63	5.82±0.31	5.67±0.27
Creatinine (mg/dL)	0.33±0.02	0.87±0.26 *(p=0.001)	0.97±0.19 *(p=0.002)	1.23±0.03 *(p=0.0001)	0.75±0.13 *(p=0.005)
ALT (IU/L)	45.94±3.99	79.13±12.60	60.50±12.74	96.88±11.52 *(p=0.001)	49.37±5.96 ***(p=0.005)
AST (IU/L)	145.56±13.04	260.25±27.51 *(p=0.002)	165.50±26.04	272.75±27.97 *(p=0.001)	139.12±20.79 ***(p=0.006)

*: significant versus CONT, ***: significant versus DM16. p values are presented in parentheses

Table 2. Effect of 8 and 16 weeks treatment with quercetin (15 mg/kg IP) on MDA levels (nmol/mg protein) in streptozotocin-induced diabetic rats. The MDA levels were measured as a thiobarbituric acid-reactive material. Data are the mean±SEM

	MDA levels				
	CONT (n=16)	DM8 (n=8)	QUER8 (n=8)	DM16 (n=8)	QUER16 (n=8)
Lung	0.75±0.08	0.88±0.09	0.79±0.14	1.05±0.13	1.03±0.24
Aorta	0.97±0.06	2.05±0.56 *(p=0.0001)	1.11±0.20	1.98±0.05 *(p=0.0001)	1.05±0.18 ***(p=0.001)
Heart	0.38±0.08	2.14±0.49 *(p=0.0001)	0.60±0.15 **(p=0.003)	1.71±0.31 *(p=0.0001)	0.74±0.08 *(p=0.002)
Spleen	0.35±0.04	1.38±0.09 *(p=0.0001)	0.39±0.07 **(p=0.001)	1.73±0.10 *(p=0.0001)	0.42±0.03 ***(p=0.001)
Brain	0.33±0.01	1.10±0.06 *(p=0.0001)	0.68±0.11	1.56±0.05 *(p=0.0001) **(p=0.001)	0.68±0.10 *(p=0.003) ***(p=0.001)
Liver	0.45±0.08	1.86±0.50 *(p=0.002)	0.26±0.11 **(p=0.003)	1.26±0.45	0.28±0.09 ***(p=0.005)
Kidney cortex	0.69±0.09	2.42±0.13 *(p=0.0001)	0.49±0.09 **(p=0.001)	2.69±0.19 *(p=0.0001)	0.73±0.11 ***(p=0.001)
Kidney medulla	0.62±0.16	2.08±0.10 *(p=0.0001)	0.92±0.20 **(p=0.001)	2.88±0.25 *(p=0.0001) **(p=0.005)	0.73±0.18 ***(p=0.001)

*: significant versus CONT, **: significant versus DM8, ***: significant versus DM16. p values are presented in parentheses

Long- term DM caused a greater increase in aorta SOD activity than medium-term DM did. SOD activities in spleen, brain, liver and kidney tissues in the 8-week and 16-week DM groups receiving no treatment were decreased.

It can be seen in Table 4 that the aorta and brain catalase enzyme activities did not change in DM induced groups. However we detected an elevated cardiac catalase enzyme activity in DM8 and DM16 groups. Spleen, liver and kidney catalase activities in DM8 and DM16 groups were found to be significantly lower than those in the CONT group. Quercetin treatment significantly elevated enzyme activities in these tissues except the kidney in the QUER16 group. Catalase activity level in the lung tissue in the groups receiving quercetin was significantly higher than those in the DM8 and DM16 groups.

Table 3. Effect of 8 and 16 weeks treatment with quercetin (15 mg/kg IP) on SOD activity (U/mg protein) in streptozotocin-induced diabetic rats. Data are the mean±SEM

	SOD activity				
	CONT (n=16)	DM8 (n=8)	QUER8 (n=8)	DM16 (n=8)	QUER16 (n=8)
Lung	9.55±1.52	4.76±0.95	6.41±0.98	4.67±0.79	7.85±2.26
Aorta	2.36±0.47	3.29±0.55	4.20±0.85	6.35±0.95 *(p=0.0001) **(p=0.006)	3.37±0.76 ***(p=0.006)
Heart	9.68±1.99	20.21±4.72	21.06±1.48 *(p=0.001)	18.75±3.42 *(p=0.002)	21.25±3.28
Spleen	8.82±1.37	3.34±0.37 *(p=0.0001)	6.49±1.06 *(p=0.004)	3.97±0.48	7.71±1.14
Brain	6.12±0.88	2.51±0.61 *(p=0.001)	6.31±0.99 *(p=0.0001)	1.87±0.39	8.10±1.71 ***(p=0.003)
Liver	13.11±1.76	4.28±0.38 *(p=0.0001)	10.99±1.48 **(p=0.001)	4.96±0.76 *(p=0.0001)	12.47±3.64
Kidney cortex	14.51±2.07	6.37±0.84 *(p=0.0001)	15.70±3.60	7.29±1.44 *(p=0.006)	14.82±3.10
Kidney medulla	10.86±1.98	3.93±0.40 *(p=0.001)	11.82±1.19 **(p=0.001)	6.06±0.90 *(p=0.002)	18.68±3.69 ***(p=0.002)

*: significant versus CONT, **: significant versus DM8, ***: significant versus DM16. p values are presented in parentheses

Table 4. Effect of 8 and 16 weeks treatment with quercetin (15 mg/kg IP) on catalase activity (k/g protein) in streptozotocin-induced diabetic rats. Data are the mean±SEM

	Catalase activity				
	CONT (n=16)	DM8 (n=8)	QUER8 (n=8)	DM16 (n=8)	QUER16 (n=8)
Lung	256±91	29±6 *(p=0.0001)	110±24 **(p=0.003)	32±3 *(p=0.0001)	160±48 ***(p=0.001)
Aorta	186±26	205±24	318±115	237±16	296±54
Heart	71±4	149±5 *(p=0.0001)	141±5 *(p=0.0001)	144±21 *(p=0.0001)	140±31
Spleen	1002±56	263±32 *(p=0.0001)	618±69 *(p=0.0001) **(p=0.002)	247±18 *(p=0.0001)	607±40 *(p=0.0001) ***(p=0.001)
Brain	7±2	6±1	5±1	5±1	6±1
Liver	927±128	232±35 *(p=0.0001)	985±104 **(p=0.001)	307±59 *(p=0.0001)	843±86 ***(p=0.001)
Kidney cortex	989±216	267±60 *(p=0.0001)	1140±209 **(p=0.001)	326±89 *(p=0.002)	772±157
Kidney medulla	791±158	117±14 *(p=0.0001)	1392±200 **(p=0.001)	262±77 *(p=0.001)	1205±345

*: significant versus CONT, **: significant versus DM8, ***: significant versus DM16. p values are presented in parentheses

In our study, GPx stands out as the antioxidant enzyme which was least affected by diabetes mellitus (Table 5). Quercetin treatment elevated the levels of GPx activity in the lung and the heart even above those levels in the control group. This impact is very significant particularly in cardiac tissue in QUER16.

Discussion

The results of this study have clearly shown that oxidative stress increased in the studied tissues of the medium and long term diabetic rats, except for lung tissue. Moreover, MDA levels, as the indicator of oxidative damage, were even higher in the brain and renal medulla in long-term DM (DM16 group). As can clearly be seen in the MDA results in QUER8 and QUER16 groups (Table 2), the prevention of oxidative damage in DM by the use of quercetin, a potent antioxidant flavonoid, is one of the most important findings of our study. The improving effects of quercetin treatment on organ functions are seen by observing the changes in levels of ALT, and AST in the QUER16 group.

We found that MDA levels in lung tissues in the DM8 and DM16 groups were not different from those in the control group. There are publications among the few studies done which showed that the MDA level in the diabetic lung tissue either increased or did not change (20, 21). Our more interesting finding about lungs was that, although the MDA concentration did not change, catalase activity decreased in the DM8 and DM16 groups, while GPx activity increased in the QUER8 group. These results are consistent with those Ozansoy et al. (21) obtained in their study. In their study on hamster tracheal cell culture, Shull et al. (22) determined that mRNAs of catalase and GPx, which are natural antioxidants, were expressed in different amounts depending on the type of the stimulus. GPx and catalase use the same substrate, H_2O_2. Baud et al. (23) used the term "suicide substrate" for H_2O_2 in their study because catalase was inactivated in high concentrations of H_2O_2. It was also determined in the same study that GPx was more resistant to being inactivated by H_2O_2 than catalase in the cells in the oligodendrocyte culture. These data elucidate our findings on the oxidant and antioxidant parameters of the lung. However, lungs might still be affected by long term DM even though they do not normally draw attention among DM-affected organs, because they use glucose via transporters stimulated by insulin. Therefore there is need for detailed researches which study the effects of DM on the lungs.

Another remarkable result of our study was that antioxidant enzyme activities in the aorta and heart in the DM8 and DM16 groups increased togather with the increase in the MDA levels. Catalase activities in the heart in the DM8 and DM16 groups and SOD activities in the aorta in the DM16 group were found to be higher than those in the control group (Table 3). The fact that antioxidant enzyme activities in the heart and aorta in diabetic groups were high shows that there is an adaptation against oxidative stress. The increase in SOD and catalase activities also reflect increased production of superoxide anion and hydrogen peroxide. As is known, superoxide anion is transformed into hydrogen peroxide by SOD, which is then broken down into water and oxygen by catalase. Other researchers also reported that in diabetic animals there were different antioxidant enzyme activities in heart and aorta tissues from other tissues. Noyan et al. (24) found SOD and catalase activities in the hearts of diabetic animals to be higher than those in the control group. Similar to our study, their study also indicated that heart MDA levels were higher indiabetic animals. Alıcıgüzel et al. (25) found that catalase activities in the hearts of the animals in early stage diabetic (8-day) and late stage diabetic (56-day) experimental groups were higher than those in the control group, while Cu/Zn SOD activities were lower. Similar results can be seen also in the study done by Hünkar et al. (26) Here MDA levels increased correspondingly as GPx and catalase activities increased both

Table 5. Effect of 8 and 16 weeks treatment with quercetin (15 mg/kg IP) on GPx activity (U/mg protein) in streptozotocin-induced diabetic rats. Data are the mean±SEM

	GPx activity				
	CONT (n=16)	DM8 (n=8)	QUER8 (n=8)	DM16 (n=8)	QUER16 (n=8)
Lung	0.66±0.21	0.63±0.08	1.47±0.28 *(p=0.004) **(p=0.005)	0.47±0.03	1.14±0.33
Aorta	0.28±0.13	0.36±0.11	0.55±0.10	0.25±0.05	0.35±0.06
Heart	0.41±0.11	0.85±0.25	1.10±0.28	0.71±0.17	2.16±0.53 *(p=0.0001)
Spleen	0.48±0.07	0.41±0.08	0.53±0.06	0.44±0.07	0.44±0.03
Brain	0.52±0.07	0.32±0.04	0.58±0.09	0.43±0.04	0.55±0.07
Liver	0.61±0.16	0.77±0.09	0.67±0.10	0.78±0.13	0.68±0.11
Kidney cortex	0.47±0.12	0.97±0.27	0.52±0.13	0.81±0.20	0.62±0.09
Kidney medulla	0.30±0.10	0.44±0.14	0.70±0.36	0.25±0.04	0.42±0.06

*: significant versus CONT, **: significant versus DM8. p values are presented in parentheses

in the heart and the aorta. The expression of antioxidant enzymes by force of oxidative stress can differ depending on the type of stimulus or cell (22). This suggests that the antioxidant enzyme regulation in the heart and aorta can be different from those in the other tissues tested in our test conditions.

We planned this study with the thought that treatment with antioxidants could have positive results, considering the important role oxidative stress has in the pathogenesis of DM, and we used quercetin as the antioxidant agent. It can be suggested according to our results that quercetin reduces oxidative stress and elevates antioxidant capacity in DM. Quercetin is an abundant flavonoid in plants including those used as food, and it also makes up the backbone of other flavonoids such as rutin (a glycosylated quercetin), hesperidine and naringenin (10). The average daily intake varies between 10 and 100 mg, depending on nutritional habits. However, it is clear that the doses we used in this study cannot be reached by normal intake with nutrients. A 70 kg person needs to consume about 7 kgs of apple in order to reach the dose used in our study (15mg/kg/day) (10).

In this study, we did not use a group of healthy animals which were administered quercetin only. Results obtained from studies in which quercetin, which is now in the stage of being accepted as a nutraceutical, was administered to normal animals, clearly show that it does not affect oxidative/antioxidative parameters in healthy animals (27-30). 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 (10). In a recent review where 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 (31).

Quercetin, due to having such a wide spectrum, has been investigated in many recent researches, and there are also researches which investigated the effects of quercetin on the DM. In their study, Sanders et al. (28) administered a 2-week quercetin treatment starting after the induction of DM. In this study, in which it was found that oxidative damage increased only in liver tissues in the DM group which could not be prevented by treating with quercetin, and that levels of oxidative damage were not different in the other tissues from those in the control group, the durations of both DM and the treatment protocol were shorter than those in our study. However in their study Anjeneyulu and Chopra used a longer DM+treatment protocol (4+4 weeks) and showed that oxidative damage increased in renal tissue, and that quercetin treatment both prevented this increase and reinforced antioxidant capacity (29). In our study we investigated quite a large group of tissues and it was also clearly seen in our study that there was a significant increase in antioxidant capacity (particularly in SOD and catalase activities) in the groups treated with quercetin. It is well known that quercetin reduces oxidative damage by acting as a free radical scavenger (10), but there is little information about its effects on the regulation of antioxidant enzymes. However, a recent study showed that quercetin plays a role in the modulation of antioxidant enzymes in liver cells (32). It was found in this study that the expressions of SOD, catalase and GPx mRNA changed in the presence of a cytokine mixture consisting of human recombinant interleukin 1b, tumor necrosis factor a and interferon g. Quercetin was also shown to affect NF-kB activation, which is closely related to antioxidant enzyme expression (33). It is clear that the relationship between quercetin and antioxidant enzyme regulation is a complex process in which several mediators are involved. It is highly probable that quercetin can be used as a supportive treatment element in treating certain diseases in the future, due to its reducing power on oxidative stress, and there is need for further studies to fully understand its effects on the antioxidant defense system of the body.

To conclude, according to the data obtained in this study, it is possible to suggest that quercetin prevents oxidative damage increase due to DM in various tissues in medium and long terms. Considering the key role oxidative stress plays in the occurrence of end-organ damages/injuries in DM, one of the most important findings of our study is the antioxidant effect of quercetin in medium and long term DM. In addition to this effect of quercetin, we found that it also causes a substantial increase in antioxidant enzyme activities. By completely revealing its connection with antioxidant enzyme regulation via further researches, quercetin may become one of the most important supporting agents in preventing DM complications.

Conflict of Interest
No conflict of interest was declared by the authors.

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