

1 **Makalenin düzeltilmiş tam metni sayfa 3'te başlamaktadır.**

2

3 **Danışman Notları İçin Açıklamalar:**

4 **1. Danışman Notları:**

5 1- Çalışmada kullandığımız insülin miktarları haftada en az bir kez yapılan, her bir sıçana ait kan glikoz  
6 düzeylerine göre belirlendiğinden dozda standart bir uygulama yapmak mümkün olmamıştır. Bizim  
7 kullandımıza benzer hatta daha yüksek olan (4-6 U/gün) doz aralığı örneklerini literatürde bulmak  
8 mümkündür. Bizde bu çalışmaları temel alarak bu uygulamayı yaptık. Bu konudaki örnek  
9 çalışmalardan bazıları şöyledir:

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26 2- Tablolar daha kolay anlaşılabilir şekilde değiştirilmiştir. Parametreler tabloların başına eklenmiş  
27 ve gruplar arasındaki farklar daha kolay görünecek şekilde düzenlenmiştir.

28 3- Yazım hataları düzeltilmiştir.

29

30 **2. Danışman Notları:**

31 1- Giriş bölümü kısaltılarak 524 kelimedenden 403 kelimeye indirilmiştir.

32 2- Sonuçlar bölümünde gerekli değişiklikler yapılmış, tablolarda verilen sayısal değerler çıkarılarak  
33 sadeleştirilmiştir.

34 3- Tablolardaki grupların sırası öneri doğrultusunda CONT, DM8, QUER8, DM16 ve QUER16 olarak  
35 değiştirilmiştir.

36 4-Tartışma bölümü konunun özünü bozmayacak şekilde kısaltılmıştır (Önceki 1781 kelime iken son  
37 gönderilen hali 1438 kelimedir).

38 5- Literatür sayısı 46'dan 33'e düşürülmüştür.

39

40 **3. Danışman Notları:**

41 1- Çalışmanın planlanması aşamasında Etik Kurul'un denek sayısındaki hassasiyetleri dikkate alınarak  
42 power analiz maalesef yapılamamıştır. Buna karşın, kullandığımız gruplar benzer şekilde planlanmış  
43 deneysel çalışmalarda kullanılan denek sayıları göz önüne alınarak belirlenmiştir.

44 2-Çalışmamızda elde ettiğimiz sonuçların istatistiksel analizinde Bonferroni düzeltmesi yapılmıştır.  
45 Buna göre 8 karşılaştırma yapıldığından  $p < 0.00625$  altı anlamlı kabul edilmiştir. Tablolar ve metinde  
46 gerekli düzeltmeler yapılmıştır.

47 3- Tablolarda ve bulgular kısmında önerilen değişiklikler yapılmıştır.

48 4- Tablolara denek sayıları eklenmiştir.

49

50 **Güçlü bir antioksidan bioflavonoid olan quercetin uzun süreli diabetik sıçanların çeşitli**  
51 **dokularındaki oksidan hasarı önler.**

52 **ANAHTAR KELİMELER:** Quercetin, diabetes mellitus, oksidan hasar, antioksidan  
53 enzimler.

54 **Quercetin, a powerful antioxidant bioflavonoid, prevents oxidative damage in different**  
55 **tissues of long-term diabetic rats.**

56 **KEY WORDS:** Quercetin, diabetes mellitus, oxidative damage, antioxidant enzymes.

### 57 **ÖZET**

58 Diabetes mellitusun (DM) neden olduğu son organ hasarlarının gelişiminde oksidan hasarın  
59 oynadığı rol iyi bilinmektedir. Bu çalışmanın amacı orta ve uzun süreli DM ta çeşitli  
60 dokulardaki oksidan hasara ve antioksidan kapasiteye güçlü bir antioksidan bioflavonoid olan  
61 quercetin'in etkisini araştırmaktır.

62 Kırksekiz erkek Wistar sıçan beş gruba ayrıldı:

63 1- Kontrol grubu

64 2- Diabetik 8 haftalık grup

65 3- Diabetik 16 haftalık grup

66 4- Quercetin verilen diabetik 8 haftalık grup

67 5- Quercetin verilen diabetik 8 haftalık grup

68 Çalışma sonunda akciğer, aort, kalp, dalak, karaciğer ve böbrekteki malondialdehit  
69 (MDA)düzeyleri, süperoksit dismutaz, katalaz ve glutatyon peroksidaz aktiviteleri saptandı.

70 Quercetin verilmeyen diabetik sıçanlardaki MDA düzeyleri akciğer dışındaki tüm dokularda  
71 yükseldi. Quercetin antioksidan enzim kapasitelerini artırdı ve oksidan hasar oluşumunu  
72 önemli miktarda önledi.

73 Çalışmamızın sonuçlarına göre, quercetin'in orta ve uzun süreli DM ta aorta, kalp, beyin,  
74 karaciğer ve böbrekleri oksidan hasardan koruyucu etkisi vardır.Quercetin'in bu süreçte  
75 antioksidan savunma kapasitesini artırarak etkili olduğu söylenebilir.

### 76 **ABSTRACT**

77 **Objectives:** The role of oxidant damage on development of end-organ injuries caused by  
78 diabetes mellitus (DM) is well known. The aim of this study is to examine the effect of  
79 quercetin, which is a strong antioxidant bioflavonoid, on oxidant damage and antioxidant  
80 capacity in various organs in case of medium-term or long-term DM.

81 **Materials and Methods:** Forty-eight male Wistar rats were divided into five groups:

82 1- Control group.

83 2- Diabetic group of 8-weeks.

84 3- Diabetic group of 16-weeks.

85 4- Quercetin treated diabetic group of 8-weeks.

86 5- Quercetin treated diabetic group of 16-weeks.

87 At the end of the experiment, malondialdehyde levels, superoxide dismutase, catalase, and  
88 glutathione peroxidase activities were measured in the lung, aorta, heart, spleen, liver, and  
89 kidney tissues.

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

93 **Conclusion:** We suggest that quercetin has a preventive effect on aorta, heart, brain, liver,  
94 and kidneys from oxidant damage in case of medium-term or long-term DM. It can be argued  
95 that quercetin is effective by increasing the antioxidant defense capacity in this process.

96

97 **INTRODUCTION:**

98 Oxidative stress has been shown both in experimental and clinical studies held in recent years  
99 to be playing a key role in the pathogenesis of many diseases. Oxidative stress is effective on  
100 the pathological processes of diseases like cancer, cardio-vascular diseases, rheumatoid  
101 arthritis, diabetes mellitus, and neurological disorders such as Alzheimer and Parkinson.<sup>[1]</sup>  
102 Oxidative stress has been shown to be effective on both the etiology of DM and the  
103 occurrence of DM complications.<sup>[1, 2]</sup> The increased reactive oxygen species (ROS) in DM has  
104 various sources such as auto-oxidative glycation, activation of protein kinase C, mitochondrial  
105 respiratory chain deficiencies and increased oxidase enzyme activities.<sup>[3, 4]</sup> However the body  
106 has its antioxidant system to prevent ROS production and the probable damages ROS can  
107 cause. The most important elements of the intracellular antioxidant defense are superoxide  
108 dismutase (SOD), catalase and glutathione peroxidase (GPx) enzyme activities.<sup>[2]</sup> It is a well  
109 known fact that alterations occur in the antioxidant defense systems in case of DM. <sup>[1]</sup> The  
110 most important reason of the increases in the mortality and morbidity rates in DM is end-  
111 organ injuries. Considering the role of oxidative stress in this process, antioxidant treatment  
112 can be expected to reduce/prevent organ damages in DM. It has been shown in recent studies  
113 that antioxidant agents such as apocynin,<sup>[5]</sup> lipoic acid,<sup>[6]</sup> simvastatin,<sup>[7]</sup> coenzyme Q10,<sup>[8]</sup>  
114 acetyl L-carnitine<sup>[9]</sup> have positive effects in DM.

115 Another molecule with a potent antioxidant effect is quercetin, a common flavonoid in nature.  
116 It exists in many nutrients, mostly in red onions, grapes, berries, cherries, broccoli, citrus  
117 fruits, tea (*Camelia sinensis*), capers.<sup>[10]</sup> Quercetin is able to preclude oxidative stress by  
118 directly inactivating free radicals,<sup>[11]</sup> by inhibiting xanthine oxidase<sup>[12]</sup> and lipid  
119 peroxidation<sup>[13]</sup>, and by affecting antioxidant pathways both in vivo and in vitro.<sup>[14]</sup>

120 Quercetin, as a potent antioxidant agent, can be expected to reduce the damages in diabetic  
121 tissues considering the role of oxidative damage in the occurrence of organ injuries in DM. On  
122 the ground that end-organ injuries occur in the later stage, it is particularly important to  
123 understand both long-term effects of DM and the effects of the treatment administered on  
124 oxidative stress in tissues. The aim of this study is to search how the extent of oxidative  
125 damage and the antioxidant capacity in the lung, aortic, cardiac, brain, liver and renal tissues  
126 of rats with DM induced by streptozotocin are affected by the administration of quercetin in  
127 medium (8 weeks) and long (16 weeks) term.

129

130 **MATERIALS AND METHODS:**

131 **Animals**

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

136 1- Control group (CONT) (n=16): As we wanted to show the time related changes in this  
137 study, we used two control groups, namely 8 weeks and 16 weeks, each containing 8 rats.

138 2- Group having DM for 8 weeks (DM8) (n=8)

139 3- Group having DM for 16 weeks (DM16) (n=8)

140 4- Diabetic group treated with quercetin for 8 weeks (QUER8) (n=8)

141 5- Diabetic group treated with quercetin for 16 weeks (QUER16) (n=8)

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

144 **Treatment Schedule**

145 DM was induced in thirty two of the rats by single intraperitoneal injections of streptozotocin  
146 (Sigma, St. Louis, MO), prepared in 0.1 mol/L citrate buffer (pH 4.5), 60 mg/kg body wt,  
147 following an overnight fasting. Vehicle injected the remaining sixteen rats served as controls.  
148 The induction of DM was predicated 3 days later by measuring tail vein blood glucose level  
149 using a blood glucometer (AccuChek, Roche Diagnostics, Indianapolis, USA). Animals with  
150 a blood glucose level higher than 300 mg/dl were considered diabetic. Diabetic rats were  
151 given subcutaneous injections of insulin (Insulatard, Novo Nordisk, Istanbul, Turkey) at a  
152 daily dose of 1-3 units in order to avoid ketoacidosis and weight loss. Blood glucose levels  
153 were monitored at least once a week in all diabetic rats and occasionally in nondiabetic rats  
154 for comparison purposes. The animals in QUER8 and QUER16 groups were started to receive  
155 quercetin treatment on the third day following the induction of DM. Quercetin (Sigma, St.

156 Louis, MO) dissolved in dimethyl sulfoxide was administered intraperitoneally at a daily dose  
157 of 15 mg/kg.<sup>[15]</sup>

158 The rats were anesthetized by intramuscular injections of a combination of ketamine and  
159 xylazine (100 mg/kg and 10 mg/kg, respectively) 8 weeks (for DM8, QUER8 groups and half  
160 of the control group) and 16 weeks (for DM16, QUER16 groups and the other half of the  
161 control group) after the STZ or vehicle application. Animals were sacrificed by cardiac  
162 puncture. The kidney, brain, liver, lung, aorta, spleen, heart tissues were harvested and  
163 washed with ice-cold phosphate-buffered saline to remove residual blood. All tissues were  
164 kept frozen in liquid nitrogen and stored at -68°C until they were used.

### 165 **Biochemical Analysis**

166 Tissue homogenization: All tissue samples were homogenized in ice cold phosphate buffer  
167 (0.5M, pH=7.4). Malondialdehyde (MDA) levels were studied in homogenates after all  
168 samples were homogenized.

169 Then supernatant was separated after a 20-minute centrifuging at a speed of 3000 rpm. SOD,  
170 catalase and GPx activities were determined in the separated supernatant.

171 The MDA levels were measured as a thiobarbituric acid-reactive material. The MDA levels in  
172 homogenates were measured spectrophotometrically as described previously.<sup>[16]</sup>  
173 Tetramethoxypropane solution was used as the standard. The MDA values determined in this  
174 way were expressed as nanomoles per mg protein.

175 SOD activity was assayed using the nitroblue tetrazolium method of Sun et al.<sup>[17]</sup> In this  
176 method nitroblue tetrazolium (NBT) is reduced to blue formazan by  $O_2^-$ , which has a strong  
177 absorbance at 560 nm. In order to obtain blue formazan, SOD assay reagent was prepared  
178 using 0.3 mmole/L xanthine, 0.6 mmole/L EDTA Na<sub>2</sub>, 150 μmole/L NBT, 400 mmole/L  
179 Na<sub>2</sub>CO<sub>3</sub>, and 1 g/L bovine serum albumin (v/v, 20:10:10:6:3 respectively). Then 2.85 ml SOD  
180 assay reagent, 0.1 ml supernatant, 0.05 ml xanthine oxidase (167 U/L) were mixed and  
181 incubated for 20 minutes at 25°C. After 20 minutes of incubation, 1 ml 0.8 mmol/L CuCl<sub>2</sub>  
182 was added to the mixture and the blue formazan formation was assessed by using a  
183 spectrophotometer at 560 nm. The SOD activity was expressed as U/mg protein.

184 Catalase activity was determined using the method of Aebi.<sup>[18]</sup> Briefly, the supernatant was  
185 diluted 50-fold with phosphate buffer, and 200ml of the diluted supernatant was added to

186 2.8ml of 30mM H<sub>2</sub>O<sub>2</sub>. The change in absorbance was read at 240nm. The rate constant of a  
187 first-order reaction (*k*) was used:  $k = (2.3/\Delta t) \times \log (A1/A2)$ , where  $\Delta t$  is a measured time  
188 interval (30s) and A1 and A2 are the absorbances at the initial and final measurement times,  
189 respectively. The catalase activity was expressed as *k*/g protein.

190 GPx activity was measured using Paglia and Valentine's method.<sup>[19]</sup> The reaction mixture  
191 contained 2.65 ml of 50 mmol/l phosphate buffer (pH 7), 0.1 ml of 150 mM glutathione  
192 solution, 0.1 ml glutathione reductase (10 mg/ml), 0.1 ml of 3 mM NADPH–Na salt, 0.1 ml  
193 50 mmol/l hydrogen peroxide solution and 0.02 ml of tissue homogenate. The GPx activity  
194 was monitored by the decrease in absorbance due to the consumption of NADPH, which  
195 absorbs at 340 nm. The GPx activity was expressed as U/mg protein.

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

## 200 **Statistics**

201 A statistical analysis was performed using SPSS statistical software (version 13.0). The  
202 results are expressed as means  $\pm$  SE. The data were not normally distributed, so differences  
203 among multiple groups were assessed using the Kruskal-Wallis test. A value of  $p < 0.05$  was  
204 considered to be significant. Post-hoc evaluation to determine the between group differences  
205 was carried out by Mann-Whitney U-test in which Bonferroni correction was applied for  
206 comparisons within groups. A value of  $p < 0.00625$  was considered to be significant.

207

208 **RESULTS:**

209 Eight-week and 16-week results of the control group in all parameters were very close to each  
210 other and there was no statistically significant difference between 8 and 16-week control  
211 groups. Therefore, in order to simplify the presentations, the values of these two groups were  
212 combined and shown as one group.

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

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

221 SOD enzyme activities can be seen in Table 3. It can be seen that SOD activity in the lung  
222 tissue didn't change in the groups having induced DM. The aorta SOD activities in DM16  
223 group were increased. No differences in tissues other than aorta were found between DM8  
224 and DM16 groups. Long- term DM caused a bigger increase in aorta SOD activity than  
225 medium-term DM did. SOD activities in spleen, brain, liver and kidney tissues in the 8-week  
226 and 16-week DM groups receiving no treatment were decreased.

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

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

238

239 **DISCUSSION:**

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

248 We found that MDA levels in lung tissues in DM8 and DM16 groups were not different from  
249 those in the control group. There are publications among the few studies done which showed  
250 that MDA level in the diabetic lung tissue either increased<sup>[20]</sup> or did not change.<sup>[21]</sup> Our more  
251 interesting finding about lungs was that although MDA concentration did not change, catalase  
252 activity decreased in DM8 and DM16 groups while GPx activity increased in QUER8 group.  
253 These results are consistent with those Ozansoy et al obtained in their study. In their study on  
254 hamster tracheal cell culture, Shull et al determined that mRNAs of catalase and GPx, which  
255 are natural antioxidants, were expressed in different amounts depending on the type of the  
256 stimulus.<sup>[22]</sup> GPx and catalase use the same substrate, H<sub>2</sub>O<sub>2</sub>. Baud et al used the term “suicide  
257 substrate” for H<sub>2</sub>O<sub>2</sub> in their study because catalase was inactivated in high concentrations of  
258 H<sub>2</sub>O<sub>2</sub>. It was also determined in the same study that GPx was more resistant to be inactivated  
259 by H<sub>2</sub>O<sub>2</sub> than catalase in the cells in oligodendrocyte culture.<sup>[23]</sup> These data elucidate our  
260 findings on oxidant and antioxidant parameters of the lung. However, lungs might still be  
261 affected by long term DM even though they don't normally draw the attention among DM-  
262 affected organs, for the reason that they use glucose via transporters stimulated by insulin.  
263 Therefore there is need for detailed researches studying the effects of DM on lungs.

264 Another remarkable result of our study was that antioxidant enzyme activities in the aorta and  
265 heart in DM8 and DM16 groups increased along with the increase in the MDA levels.  
266 Catalase activities in the heart in DM8 and DM16 groups and SOD activities in the aorta in  
267 DM16 group were found to be higher than those in the control group (Table 3). The fact that  
268 antioxidant enzyme activities in the heart and aorta in diabetic groups were high shows that  
269 there's an adaptation against oxidative stress. The increase in SOD and catalase activities also  
270 reflect increased production of superoxide anion and hydrogen peroxide. As is known,

271 superoxide anion is transformed into hydrogen peroxide by SOD, which then is broken down  
272 into water and oxygen by catalase. Other researchers also reported that in diabetic animals  
273 there were different antioxidant enzyme activities in heart and aorta tissues than in other  
274 tissues. Noyan et al found SOD and catalase activities in the hearts of diabetic animals to be  
275 higher than those in the control group.<sup>[24]</sup> Similar to our study, their study also indicated that  
276 heart MDA levels were higher in diabetic animals. Alicigüzel et al found that catalase  
277 activities in the hearts of the animals in early stage diabetic (8-day) and late stage diabetic  
278 (56-day) experiment groups were higher than those in the control group, while Cu/Zn SOD  
279 activities were lower.<sup>[25]</sup> Similar results can be seen also in the study done by Hünkar et al.  
280 Here MDA levels increased correspondingly as GPx and catalase activities increased both in  
281 the heart and in the aorta.<sup>[26]</sup> The expression of antioxidant enzymes by force of oxidative  
282 stress can differ depending on the type of stimulus or cell.<sup>[22]</sup> This suggests that the  
283 antioxidant enzyme regulation in the heart and aorta can be different from those in the other  
284 tissues tested in our test conditions.

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

295 In this study, we did not use a group of healthy animals which were administered quercetin  
296 only. Results achieved from studies in which quercetin, which is now in the stage of being  
297 accepted as a nutraceutical,<sup>[27]</sup> was administered to normal animals clearly show that it does  
298 not affect oxidative/antioxidative parameters in healthy animals.<sup>[28-30]</sup> At this point, the toxic  
299 effects of quercetin should also be mentioned. While at first it was reported that quercetin had  
300 mutagenic effects, subsequent studies showed that it was antimutagenic due to its protective  
301 effect against genotoxic agents. Moreover, in 1999 International Agency for Research on  
302 Cancer concluded that quercetin was not one of the carcinogenics for human beings.<sup>[10]</sup> In a  
303 recent review where results of short and long term human and animal studies were presented

304 in detail, it was concluded that there was not enough evidence regarding quercetin's  
305 mutagenic and carcinogenic effects and that it was a dependable agent.<sup>[31]</sup>

306 Quercetin, due to having such a wide spectrum, has been searched in many recent researches,  
307 and there are also researches which investigated the effects of quercetin on the DM. In their  
308 study Sanders et al administered a 2-week quercetin treatment starting after the induction of  
309 DM.<sup>[28]</sup> In this study, in which it was found that oxidative damage increased only in liver  
310 tissues in DM group and this damage could not be prevented by treating with quercetin, and  
311 that levels of oxidative damage were not different in the other tissues than those in the control  
312 group, the durations of both DM and the treatment protocol were shorter than those in our  
313 study. However in their study Anjeneyulu and Chopra used a longer DM+treatment protocol  
314 (4+4 weeks) and showed that oxidative damage increased in renal tissue, and that quercetin  
315 treatment both prevented this increase and reinforced antioxidant capacity.<sup>[29]</sup> In our study we  
316 investigated a quite large group of tissues and it was also clearly seen in our study that there  
317 was a significant increase in antioxidant capacity (particularly in SOD and catalase activities)  
318 in the groups treated with quercetin. It is well known that quercetin reduces oxidative damage  
319 by affecting as a free radical scavenger,<sup>[10]</sup> but there is little information about its effects on  
320 the regulation of antioxidant enzymes. However a recent study showed that quercetin plays a  
321 role in the modulation of antioxidant enzymes in liver cells.<sup>[32]</sup> It was found in this study that  
322 the expressions of SOD, catalase and GPx mRNA changed in the presence of a cytokine  
323 mixture consisting of human recombinant interleukin 1 $\beta$ , tumor necrosis factor  $\alpha$  ve  
324 interferon  $\gamma$ . Quercetin was also shown to affect NF- $\kappa$ B activation, which is closely related to  
325 antioxidant enzyme expression.<sup>[33]</sup> It is clear that the relationship between quercetin and  
326 antioxidant enzyme regulation is a complex process in which a lot of mediators are involved.  
327 It is highly probable that quercetin can be used as a supportive treatment element in treating  
328 certain diseases in the future due to its reducing power on oxidative stress, and there is need  
329 for further studies to fully understand its effects on the antioxidant defense system of the  
330 body.

331 To conclude, according to the data obtained in this study it is possible to suggest that  
332 quercetin prevents oxidative damage increased because of DM in various tissues in medium  
333 and long term. Considering the key role oxidative stress plays on the occurrence of end-organ  
334 damages/injuries in DM, one of the most important findings of our study is the antioxidant  
335 effect of quercetin in medium and long term DM. In addition to this effect of quercetin, we  
336 found that it also causes a substantial increase in antioxidant enzyme activities. By

337 completely revealing its connection with antioxidant enzyme regulation via further researches,  
338 quercetin can become one of the most important supporting agents in preventing DM  
339 complications.

340

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425

	<b>CONT</b> (n=16)	<b>DM8</b> (n=8)	<b>QUER8</b> (n=8)	<b>DM16</b> (n=8)	<b>QUER16</b> (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)

427

428 **Table 1.** General characteristics of normal, diabetic and quercetin-treated diabetic animals.

429 Data are the mean ± SEM.

430 \*: significant versus CONT, \*\*: significant versus DM8, \*\*\*: significant versus DM16. p values

431 are presented in parentheses.

432

	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)

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

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

	<b>SOD activity</b>				
	<b>CONT</b> (n=16)	<b>DM8</b> (n=8)	<b>QUER8</b> (n=8)	<b>DM16</b> (n=8)	<b>QUER16</b> (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	21.25±3.28 * (p=0.002)
Spleen	8.82±1.37	3.34±0.37 * (p=0.0001)	6.49±1.06	3.97±0.48 * (p=0.004)	7.71±1.14
Brain	6.12±0.88	2.51±0.61 * (p=0.001)	6.31±0.99	1.87±0.39 * (p=0.0001)	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)

441

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

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

446

## 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

448

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

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

453

	<b>GPx activity</b>				
	<b>CONT</b> (n=16)	<b>DM8</b> (n=8)	<b>QUER8</b> (n=8)	<b>DM16</b> (n=8)	<b>QUER16</b> (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

455

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

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