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1 # Modulation of renal insulin signaling pathway and antioxidant enzymes with
2 # diabetes: effects of resveratrol

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19 **ABSTRACT**

20 Diabetes mellitus, a disease arising by the deficiency of insulin hormone or its inability of usage,
21 affects carbohydrate, lipid and protein metabolism, and destruct variety of the tissues. A strong
22 antioxidant and anti-inflammatory agent; resveratrol has a high potential to prevent or treat the
23 pathogenesis of diseases. This study was conducted to reveal the relationship between diabetes-
24 induced oxidative stress and tissue inflammation with changes in antioxidant enzymes (*cat, sod,*
25 *gpx,* and *gst*) and the components of insulin signaling pathway (*insulin Rβ, irs-1, pi3k, akt, mtor*)
26 in kidney tissues. Additionally, the effects of resveratrol on these parameters were evaluated. Male
27 Wistar rats were randomly divided into four groups; (1) control/vehicle; (2) control/20 mg/kg
28 resveratrol; (3) diabetic/vehicle; (4) diabetic/20 mg/kg resveratrol. Results demonstrated down-
29 regulation of antioxidant enzymes in the kidney tissues of diabetic rats and this situation was
30 devoted partially to the reduced expression of *nfκb*. Moreover, the components of renal insulin
31 signaling elements were up-regulated in diabetic rats, and resveratrol treatment decreased this
32 sensitization towards the control state. In conclusion, resveratrol improved diabetes-induced renal
33 oxidative stress and inflammation partly due to healing action on renal antioxidant enzymes and
34 insulin signaling pathway components.

35 **Keywords:** diabetes, kidney, oxidative stress, inflammation, resveratrol, insulin signaling

36 **INTRODUCTION**

37 Decreased insulin secretion and/or its responsiveness to the tissues, leading to dysfunctions on
38 protein, lipid and carbohydrate metabolism is characterized as diabetes mellitus [1].
39 Hyperglycemia is the major hallmark of the disease and associated with increased rate of glucose
40 auto-oxidation, non-enzymatic protein glycosylation and increase influx to the polyol pathway [2].
41 These mechanisms initiate oxidative stress and inflammation and might affect many cellular
42 metabolic activities [3]. Prolonged oxidative stress can decrease antioxidant capacity which
43 enhances chronic complications of diabetes [4]. In fact, enzymatic and non-enzymatic defensive
44 mechanisms reduce cellular oxidative stress. Superoxide dismutase (SOD) isozymes; SOD1 and
45 SOD2 neutralizes superoxide radicals in cytoplasm and mitochondria, respectively. Catalase
46 (CAT) decompose hydrogen peroxide to water a function that is shared with glutathione
47 peroxidase (GPx). Glutathione S-transferases (GSTs) catalyze the conjugation of glutathione to a
48 wide range of electrophiles including oxidatively modified compounds.

49 Recent studies have indicated a strong relationship between oxidative stress and steady-state levels
50 of antioxidant enzymes [5,6]. These enzymes are also regulated by two transcription factors;
51 nuclear factor erythroid 2-related factor (Nrf2) and nuclear factor kappa B (NFκB) genes [7].
52 Targets of this two protein are important for the protection since they enhance proinflammatory
53 cytokines and defense against inflammation and oxidative stress [8,9]. Furthermore, the activities
54 or presence of antioxidant enzymes in cells are strongly regulated by transcriptional, translational
55 and post-translational mechanisms as a consequence of changes in the cellular redox potential [6].
56 Resveratrol (3,4,5-trihydroxystilbene) is a phytoalexin found in abundance in red colored fruits
57 such as grapes, peanuts, strawberries, and cherries. It has strong antioxidant, anti-inflammatory,

58 and anti-apoptotic effects [10]. Its role in protection against oxidative damage in the
59 pathophysiology of diabetes has been demonstrated in current studies [5,11–13]

60 Recently, we have publicized that impaired glucose metabolism in the liver tissues leads to adverse
61 effects on the hepatic insulin signaling pathway [12]. Moreover, regulation of main antioxidant
62 enzymes in brain and liver tissues of diabetic rats and effects of resveratrol on these parameters
63 have been demonstrated [4,6]. Based on these findings, to understand molecular alterations in renal
64 insulin signaling pathway and antioxidant systems in diabetes and *in vivo* effects of resveratrol;
65 we hypothesized that diabetes-related modifications in renal tissues could be returned to normal
66 conditions with resveratrol. To make track for the concrete molecular action mechanism of the
67 resveratrol through the regulation of the renal function, the present study was designed to
68 investigate the effects of diabetes and resveratrol on oxidative and inflammatory biomarkers.
69 Additionally, regulation of renal insulin signaling pathway components and antioxidant enzymes
70 in an animal model of streptozotocin (STZ)-induced diabetes are inspected.

71 MATERIALS AND METHODS

72 Animal treatments

73 All animal procedures were approved by the Committee for the Ethical Animal Care (Kobay DHL,
74 2012/45) according to rules of the Guide for the Care and Use of Laboratory Animals as published
75 by the US National Institute of Health (NIH Publication No: 85/23, revised in 1986). Accordingly,
76 male Wistar rats, which were eight-week-old, were housed in temperature-controlled rooms (20 –
77 22°C) with a 12-hours light-dark cycle. They had standard rodent diet (chow pellet) composed of
78 62% starch, 23% protein, 4% fat, 7% cellulose, standard vitamins and salt mixture. One week after
79 acclimation, animals were randomly divided into four groups. The control group (C) (n=12) were

80 injected only vehicle; %10 dimethyl sulfoxide (DMSO) for four weeks. Resveratrol group (RSV)
81 (n=12) were administered with a daily intraperitoneal dose of resveratrol (20 mg/kg/day) in the
82 vehicle throughout the four-week period. Diabetes group (D) (n=12) received a single dose of
83 streptozotocin (STZ) (55 mg/kg) dissolved in 0.05 M citrate buffer (pH: 4.5) and daily vehicle for
84 four weeks. Diabetes+Resveratrol group (D+RSV) (n=9) were given intraperitoneal 20 mg/kg/day
85 resveratrol throughout the four-week period, starting from two days after STZ administration.
86 Fasting blood glucose levels were measured by Accu-check-go (Roche, Germany) glucometer
87 weekly from the blood of tail veins. Animals having blood glucose concentration higher than 200
88 mg/dl were considered as diabetics. At the end of the study period, all rats were decapitated, and
89 kidney tissues were removed. After blotted dry, they were frozen in liquid nitrogen and stored at
90 −85°C until use.

91 **Tissue homogenization and measurement of renal MDA contents**

92 Kidney tissues were homogenized in appropriate medium (50 mM Tris, 150 mM NaCl, 5 mM
93 EDTA, 1% (w/w) Triton X-100, 0.26% (w/v) sodium deoxycholate, 50 mM sodium fluoride, 0.1
94 mM sodium orthovanadate and 0.2 mM PMSF) with TissueRuptor™ homogenizer (Qiagen,
95 USA). After centrifugation at 1500 *g*, protein concentrations of supernatants were determined [14].
96 MDA levels, the end product of lipid peroxidation, were determined by HPLC with Chromsystems
97 Diagnostics (Munich / Germany) kit and HPLC fluorescence detector (Ex: 515 Em: 553 nm).

98 **Determination of gene expressions of antioxidant enzymes, insulin signaling pathway**
99 **components and *nfκb* with real-time polymerase chain reaction**

100 RNeasy total RNA isolation kit (Qiagen, Venlo, Netherlands) was utilized to isolate total RNA
101 from the kidney tissues. Amount and quality of total RNA were determined using

102 spectrophotometry at 260/280 nm and agarose gel electrophoresis. cDNA synthesis was performed
103 with 1 µg of total RNA using commercial first strand cDNA synthesis kit (Thermo Scientific,
104 USA). Gene expression levels of antioxidant enzymes and insulin signaling pathway components
105 were determined with qRT-PCR (LightCycler 480 II, Roche, Germany) as we described in detail
106 previously [12,15]. Nucleotide sequences of pre-validated primer pairs are given in Table 1. The
107 relative expression of genes with respect to the internal control, *gapdh* (glyceraldehyde 3-
108 phosphate dehydrogenase) were calculated with the efficiency corrected advance relative
109 quantification tool provided by the LightCycler® 480 SW 1.5.1 software.

110 **Immunoblot analysis of antioxidant enzymes; CAT, SOD1, SOD2, and insulin signaling**
111 **pathway components; Insulin Rβ, PI3K, AKT1**

112 CAT, SOD1, SOD2, Insulin Rβ, PI3K, AKT1 protein contents were determined by Western blot
113 analysis. Briefly, homogenates containing 50 µg of proteins were separated by SDS-PAGE and
114 electroblotted onto PVDF membranes. Then, blotted membranes were blocked with 5% (w/v) non-
115 fat dried milk and incubated with CAT (Anti-CAT Rabbit IgG, Santa Cruz: sc-50508, 1:500),
116 SOD1 (Anti-SOD1 Goat IgG, Callbiochem: 574597, 1:1000), SOD2 (Anti-SOD2 Rabbit IgG,
117 Santa Cruz, sc-30080, 1/1000), Insulin Rβ (Anti- Insulin Rβ Rabbit IgG, Santa Cruz, sc-711,
118 1/100), PI3K (Anti-PI3K Rabbit IgG, Santa Cruz, sc-423, 1/100), AKT1 (Anti-AKT1 Rabbit IgG,
119 Santa Cruz, sc-8312, 1/100) primary antibodies for two hours. As a cytoplasmic internal control,
120 GAPDH proteins were also labeled with anti-GAPDH Rabbit IgG (Santa Cruz, sc-25778, 1:2000).
121 After primary antibody incubation, horseradish peroxidase (HRP) conjugated secondary
122 antibodies were incubated (1:10,000) for 1 h. Protein bands corresponding to interested proteins
123 were visualized by Clarity^{TM} Western ECL (Bio-Rad Laboratories, Hercules CA, USA) substrate
124 solution. Images of the blots were obtained using the ChemiDoc^{TM} MP Chemiluminescence

125 detection system (Bio-Rad Laboratories, Hercules CA, USA). The relative expression of proteins
126 with respect to GAPDH was calculated using the ImageLab4.1 software.

127 **Determination of antioxidant enzyme activities**

128 Catalase activities were determined using a method in which the rate of H_2O_2 decomposition was
129 followed at 240 nm [16]. Glutathione peroxidase (GPx) activity was determined by measuring the
130 oxidation of NADPH at 340 nm spectrophotometrically [17]. Total GST activity in kidney tissues
131 was determined according to the method [18] in which conjugation of 1-chloro-2,4-dinitrobenzene
132 (CDNB), a common substrate for all GST isozymes, with reduced glutathione is followed at 340
133 nm. Enzyme activities were calculated as U/mg, which reflects the amount of substrate consumed
134 in one minute by one mg protein containing the cytosolic fraction. Superoxide dismutase (SOD)
135 activity was determined as the amount of protein that inhibits pyrogallol auto-oxidation by 50%
136 [19].

137 **Statistical analysis**

138 Data were expressed as mean ± standard error of means and compared for differences using the
139 Statistical Package for Social Sciences version 21.0 (SPSS IBM, Armonk, NY, USA). Statistical
140 comparisons were performed using one-way ANOVA followed by an appropriate posthoc test
141 (Tukey's Honestly Significant Difference). P values <0.05 were considered as statistically
142 significant.

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143 **RESULTS**

144 **Effects of diabetes and/or resveratrol on some metabolic parameters**

145 Weights of diabetic animals were significantly reduced after four weeks of diabetes compared to
146 the control group. Parallel to the increase in fasting blood sugar levels, the amount of glucose in
147 the kidney tissues was also found to be elevated in diabetic rats (43%). When the metabolic effects
148 of diabetes on kidney tissues are examined, MDA levels were significantly ($p<0.05$) increased (2-
149 fold) and resveratrol application to diabetic animals normalizes this situation to the control state.
150 Additionally, significant up-regulation of proinflammatory cytokines; IL-6, IL-8, and TNF-α
151 reflecting the inflammatory state in renal tissues of diabetic rats have been demonstrated
152 previously [13]. In this study, we also confirmed the anti-inflammatory effects of resveratrol since
153 inflammatory markers decreased to the control levels with resveratrol application [13].

154 **Changes in gene expression levels of antioxidant enzymes**

155 Changes in expression levels of *gtsmu*; a biotransformation enzyme, *cat, sod1, sod2, gpx;* main
156 antioxidant enzymes and *nfkb;* an antioxidant response element regulator were measured by qRT-
157 PCR and the results are summarized in Figure 1. Diabetes significantly suppressed the expression
158 levels of major antioxidant enzymes; *cat, gpx, sod-1,* and *gstmu* compared to control group (Figure
159 1A, 1B, 1C and 1E). We can explain one of the causes of this suppression, which occurs at about
160 40% levels, with a significant decrease (about 3-fold) in the *nfkb* that controls the transcription of
161 those enzymes (Figure 1F). In addition, mRNA levels of *sod2*, which is an important mitochondrial
162 enzyme against oxidative stress, did not change significantly with diabetes and/or resveratrol
163 (Figure 1D). Even though, resveratrol treatment to the control group suppressed antioxidant genes;
164 especially *gpx, sod1,* and *gstm* ($p<0.05$), it was not effective enough to normalize the diabetic state

165 to the control values. While resveratrol treatment to the diabetic animals was not effective on gene
166 expression level of antioxidant enzymes, *nfkb* got back to the control levels. The normalization of
167 diabetic *nfkb* expression with resveratrol but not antioxidant enzymes is an indication that other
168 factors other than *nfkb* may be effective in regulating the expression of antioxidant genes.

169 **Changes in protein expression of antioxidant enzymes**

170 Western blot analysis was performed to determine whether changes in the mRNA level of the
171 antioxidant enzymes were reflected in the amount of protein and thus, whether diabetes affects any
172 protein translation. Figure 2 summarizes how the expression levels of CAT, SOD1, and SOD2
173 proteins were regulated with diabetes and/or resveratrol. Accordingly, diabetes significantly
174 suppressed the renal CAT protein, while the SOD1 protein levels were up-regulated (Figure 2B
175 and 2C). Resveratrol, when applied to the control group, significantly increased SOD2 protein (p
176 <0.05) but did not show the same effect to other antioxidant enzymes. As given to the diabetic
177 animals, resveratrol normalized the changes in antioxidant enzymes. In other words, decreased
178 CAT and increased SOD1 protein levels were normalized to the control group. The suppression of
179 CAT protein expression correlates well with the reduction in the amount of mRNA. This shows
180 that diabetes suppresses CAT enzyme at transcription level in renal tissues. The change in the
181 amount of SOD1 mRNA and protein levels with diabetes were contradictory to each other. That
182 is, a significant decrease in mRNA levels was inversely proportional with the up-regulated protein
183 levels indicating a post-translational activation of SOD1 with diabetes. Diabetes did not show a
184 significant effect on SOD2 protein, whereas resveratrol application increased protein expression
185 levels in both control and diabetic groups. This suggests a positive up-regulation of SOD2 with
186 post-translational mechanisms.

187 **Changes in antioxidant enzyme activities**

188 After determining the changes in gene and protein expression levels, enzymatic activities which
189 are the actual modulators of cellular oxidative stress were also determined in this study. Activity
190 results are summarized in Figure 3. Accordingly, CAT activity was significantly suppressed with
191 diabetes in kidney tissues ($p<0.05$). Suppression of CAT activity is strongly correlated with its
192 mRNA and protein expression levels. The changes in other antioxidant enzymes with diabetes
193 were not statistically significant. Moreover, when resveratrol was given to the control animals,
194 GPx, total SOD and total GST activities were down-regulated significantly ($p<0.05$).

195 **Regulation of renal insulin signaling pathway components**

196 Levels of *insulin Rβ*, *irs1*, *irs2, pi3k, akt* and *mTOR* gene expressions in renal tissues were also
197 determined in this study. Accordingly, expression levels of genes which are involved in insulin
198 signal transduction were all up-regulated in the diabetic group (Figure 4A-4E). In addition,
199 resveratrol did not have a significant effect on renal insulin signaling when administered to control
200 animals but reversed all the modifications towards the control values in the diabetic group. Protein
201 expressions of insulin Rβ, PI3K and AKT were also determined by Western blotting (Figure 5).
202 According to results, similar to gene expression levels, the insulin signaling pathway-related
203 proteins were significantly up-regulated by diabetes (Figure 5B, 5C, 5D). This situation suggests
204 the activation of insulin signal transduction elements at the transcription level, resulting in a
205 significant increase in protein expression. Furthermore, resveratrol treatment increased the insulin
206 Rβ as applied to control animals and exerted a normalizing effect on diabetic PI3K levels in kidney
207 tissues. Generally, it affected insulin signaling not at the protein level but at the transcription level.

208

209 **DISCUSSION**

210 Diabetes usually caused by the combination of hereditary and environmental factors results in
211 excessive elevation of blood glucose that enhances the oxidative state in various tissues and
212 damage to the cellular macromolecules, such as lipids, proteins and nucleic acids [2]. Trans-
213 resveratrol exhibits a wide range of biological features, including antioxidant and anti-
214 inflammatory properties and its possible preventative and therapeutic roles in several tissues were
215 determined recently [4,5]. Renal pathologies seem to be the most frequent complications of
216 diabetes and new therapeutic approaches are needed in diabetic nephropathy and chronic kidney
217 diseases. The aim of this study is to analyze the oxidative/inflammatory changes in the kidney
218 tissues of diabetic rats and to determine the changes in antioxidant enzymes together with insulin
219 signal transduction. Consistent with this depiction, we determined the degree of lipid peroxidation
220 in renal tissues, which was enhanced by diabetes. We also recently published the up-regulation of
221 renal inflammatory markers; IL-6, IL-8, and TNF-α in diabetic rats [13]. All these results indicate
222 the oxidized and inflammatory state in renal tissues of diabetic rats. Resveratrol alleviated these
223 biomarkers toward the control levels because of its antioxidant and anti-inflammatory properties
224 and contributed therapeutic effects on diabetic complications [20].

225 Superoxide dismutase is one of the most important antioxidant enzymes that convert superoxide
226 radicals into the hydrogen peroxide. In the presence of transition metals, hydrogen peroxide might
227 turn into hydroxyl radicals known as the most reactive species. However, CAT and GPx enzymes
228 neutralize the hydrogen peroxide into the water in peroxisomes and cytoplasm, respectively. In
229 addition, GSTs also play a role in cleansing toxic intermediates and eliminating harmful products
230 caused by oxidative stress. This study revealed gene expression suppression of antioxidant
231 enzymes in STZ-induced diabetic kidney tissues and this suppression could be partially attributed

232 to the reduction of the redox-sensitive transcription factor; *nfκb*, which particularly affects the
233 transcription of many inflammatory and antioxidant genes [7,21]. This could be due to extensive
234 oxidative conditions dysregulating the initiation machinery of the antioxidant enzymes
235 transcription. Besides, destabilization of mRNA under mild oxidative state could also contribute
236 to the suppression of antioxidant enzymes [22,23]. This apparent decrease in antioxidant enzymes
237 is different from the results that we obtained using brain tissues [4]. In the brain, up-regulation of
238 antioxidant enzymes were devoted as an adaptation process to the moderate increase in the
239 oxidative stress biomarkers. The effects of resveratrol on the recovery of diabetic changes in tissue
240 antioxidant enzymes were revealed in our previous studies in which liver and brain tissues were
241 used [4,6]. Likewise, resveratrol reduced oxidant stress and regulated gene expression of
242 antioxidant enzymes in kidney tissues. Additionally, normalization of *nfκb* expression with
243 resveratrol in diabetic tissues but not antioxidant enzymes designates several other effectors in the
244 regulation of antioxidant genes. Furthermore, enhanced GST enzyme activity due to diabetes could
245 be an adaptation to relieve the pathology of diabetes and oxidative products.

246 Insulin affects the intracellular metabolism by regulating several key proteins starting from its
247 receptor on cellular membrane and line-up until several transcription factors in the nucleus.
248 Activated insulin receptor promotes the insulin receptor substrate family proteins (IRS) which are
249 the adaptors of insulin signal transduction. The downstream signal paths are divided into several
250 branches and among them, PI3K/AKT/mTOR signaling is involved in many cellular processes. In
251 this pathway, activated IRS proteins trigger phosphatidylinositol 3-kinase (PI3K) proteins and its
252 downstream effectors such as protein kinase B (PKB or AKT) and mammalian target of rapamycin
253 (mTOR) proteins. Activation of AKT leads to the phosphorylation of several substrates acting on
254 gluconeogenesis and glycogenolysis. PI3K and AKT are also known to play a role in glucose

255 transporter (GLUT4) translocation [24]. Insulin resistance, a condition often encountered in
256 diabetes, is caused by the down-regulation of all or part of the proteins involved in insulin signaling
257 pathways, or by the inhibition (phosphorylation) of other signaling elements that inactivate the
258 signaling pathways. Recently, we have demonstrated the suppression of insulin signaling
259 components (PI3K/AKT/mTOR) in hepatic tissues of diabetic rats [12]. In this study, we revealed
260 significant up-regulation of insulin signaling elements at both gene and protein levels in kidney
261 tissues. This suggests that renal insulin signal transduction undergoes an activation at transcription
262 level and leads significant increase in their protein levels. This might be due to the need for more
263 active signaling elements in kidney tissues due to lack of insulin and/or its action. Insulin
264 deficiency in diabetes might have stimulated kidney tissues to make them more sensitive to insulin.
265 Resveratrol administration brings the sensitivity of insulin signaling in the kidneys to the normal
266 values at gene expression level. Because, protein levels of insulin signal transduction elements did
267 not change significantly with resveratrol, but the level of gene expression approached to the control
268 levels.

269 Our data point out that STZ-induced diabetes provokes oxidative damage and inflammation in
270 renal tissues and contribute antioxidant enzymes to be regulated at gene, protein, and activity
271 levels. Changes in antioxidant enzymes might contribute the molecular mechanisms associated
272 with oxidative modifications in renal tissues. Additionally, diabetes-induced up-regulation of
273 insulin signaling pathway in association with the activation of inflammatory markers led us to
274 propose that there could be a correlation between insulin signaling and inflammation in renal
275 tissues of diabetic rats. The findings of the present study also revealed that resveratrol may confer
276 beneficial effects on renal functions through its influences on antioxidant enzymes and insulin
277 signaling. Our data are consistent with the large body of literature showing beneficial health effects

278 of resveratrol for therapeutic intervention of diabetes-induced oxidative modifications and thereby
279 the promising results suggest potential therapeutic targets and pathways for further evaluation.

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283 **Author's contributions**

284 GŞ and AB did the experiments. GS designed the study, organized the research, performed the
285 statistical analysis and wrote the paper.

286 **Conflict of interest**

287 The authors declare that there is no conflict of interest associated with this work.

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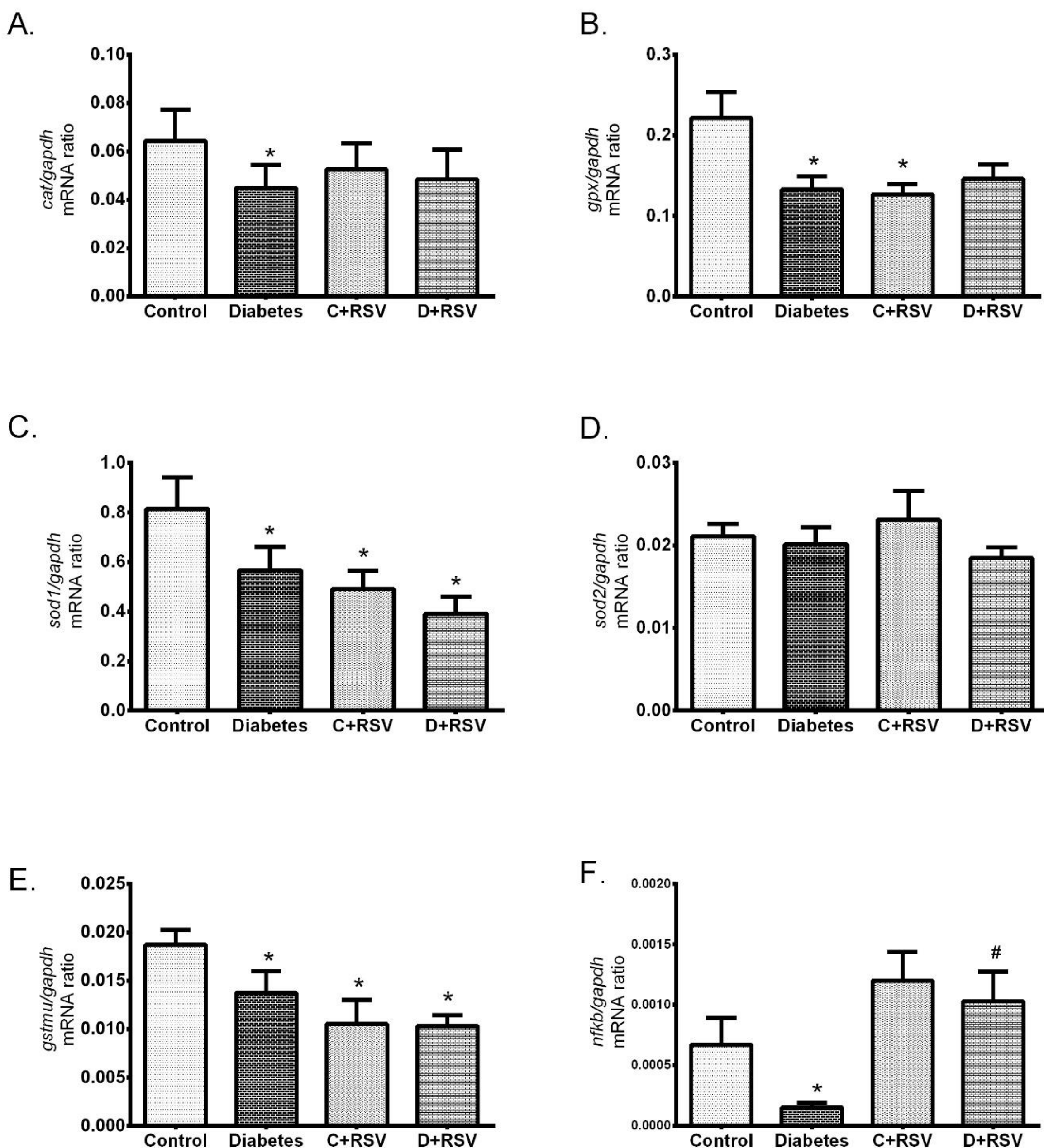
352 **Table 1.** Primer pairs used in the expression analysis of antioxidant enzymes and insulin signal

353 transduction pathway components

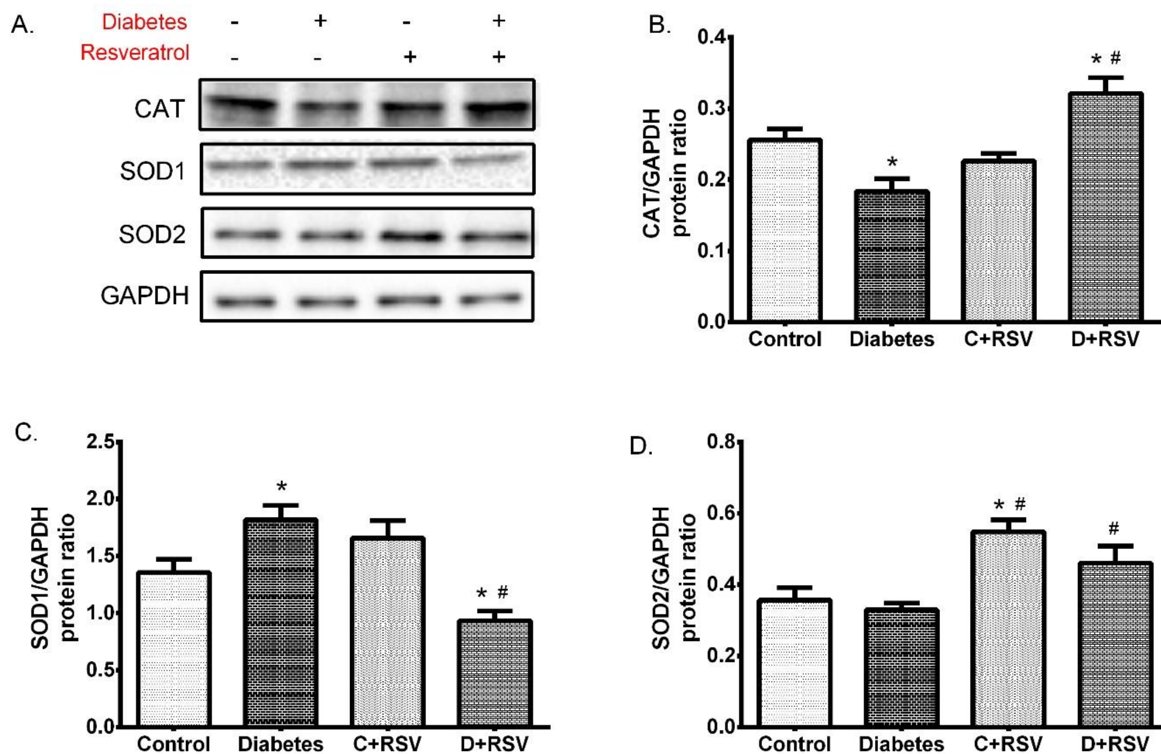
Gene	Forward primer (5'→3')	Reverse primer (5'→3')
cat	GCGAATGGAGAGGCAGTGTAC	GAGTGACGTTGTCTTCATTAGCACTG
gpx1	CCACCACCGGGTCGGACATAC	CTCTCCGCGGTGGCACAGT
sod1	TAGCAGGACAGCAGATGAGT	GCAGAAGGCAAGCGGTGAAC
sod2	GCACATTAACGCGCAGATCA	AGCCTCCAGCAACTCTCCTT
gst-mu	AGAAGCAGAAGCCAGAGTTC	GGGGTGAGGTTGAGGAGATG
nfκb	GGGTCAGAGGCCAATAGAGA	CCTAGCTTTCTCTGAACTGCAAA
insulin rβ	GTGCTGCTCATGTCCTTAGA	AATGGTCTGTGCTCTTCGTG
irs1	GCCAATCTTCATCCAGTTGC	CATCGTGAAGAAGGCATAGG
irs2	CTACCCACTGAGCCCAAGAG	CCAGGGATGAAGCAGGACTA
pi3k	ATGCAACTGCCTTGCACATT	CGCCTGAAGCTGAGCAACAT
akt1	GAAGAAGAGCTCGCCTCCAT	GAAGGAGAAGGCCACAGGTC
mtor	GCAATGGGCACGAGTTTGTT	AGTGTGTTCACCAGGCCAAA
gapdh	TCCTTGGAGGCCATGTGGGCCAT	TGATGACATCAAGAAGGTGGTGAAG

354



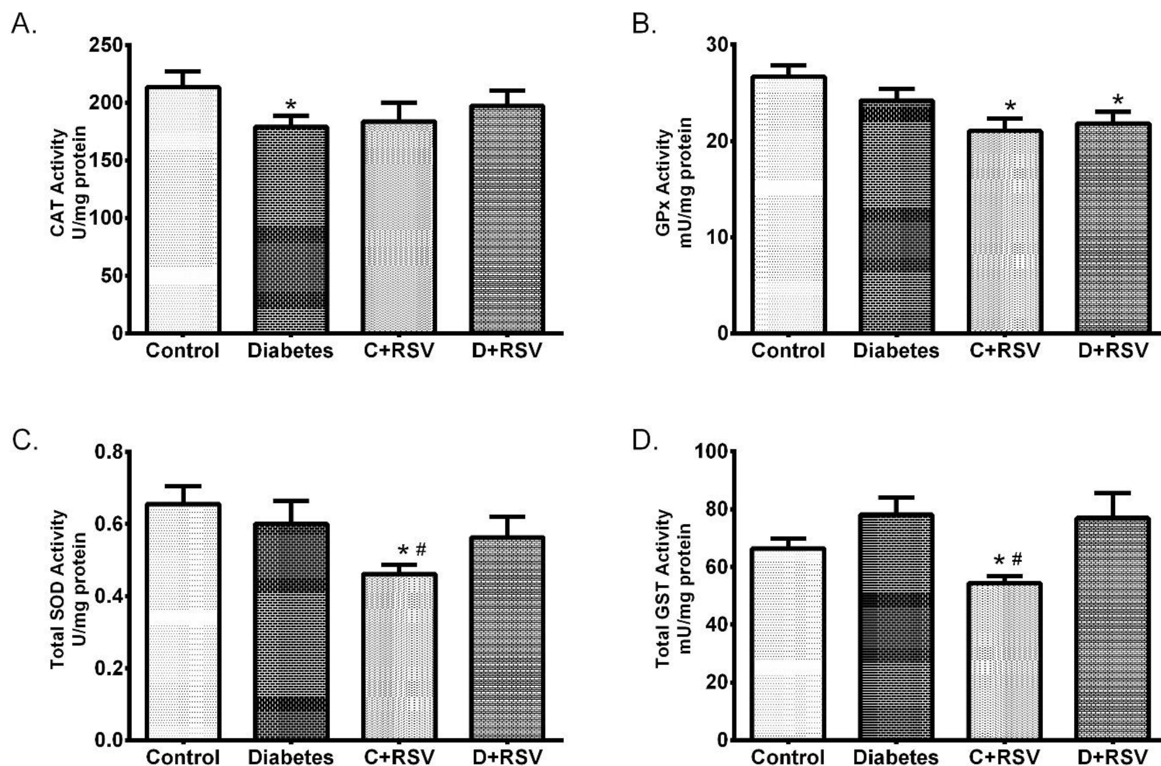
355

356 **Figure 1.** Changes in renal gene expression levels of (**A**) *cat*, (**B**) *gpx*, (**C**) *sod1*, (**D**) *sod2*, (**E**)
357 *gstmu*, (**F**) *nfkb* with diabetes and resveratrol. The data were given with respect to *gapdh* which is
358 used as internal standard. C:Control, D:Diabetes, C+RSV: Resveratrol given control, D+RSV:
359 Resveratrol given diabetic group. *signifies the statistical difference of the data according to the
360 control group ($p<0.05$), and the # sign indicates the statistical difference of the data according to
361 the diabetes group ($p<0.05$).



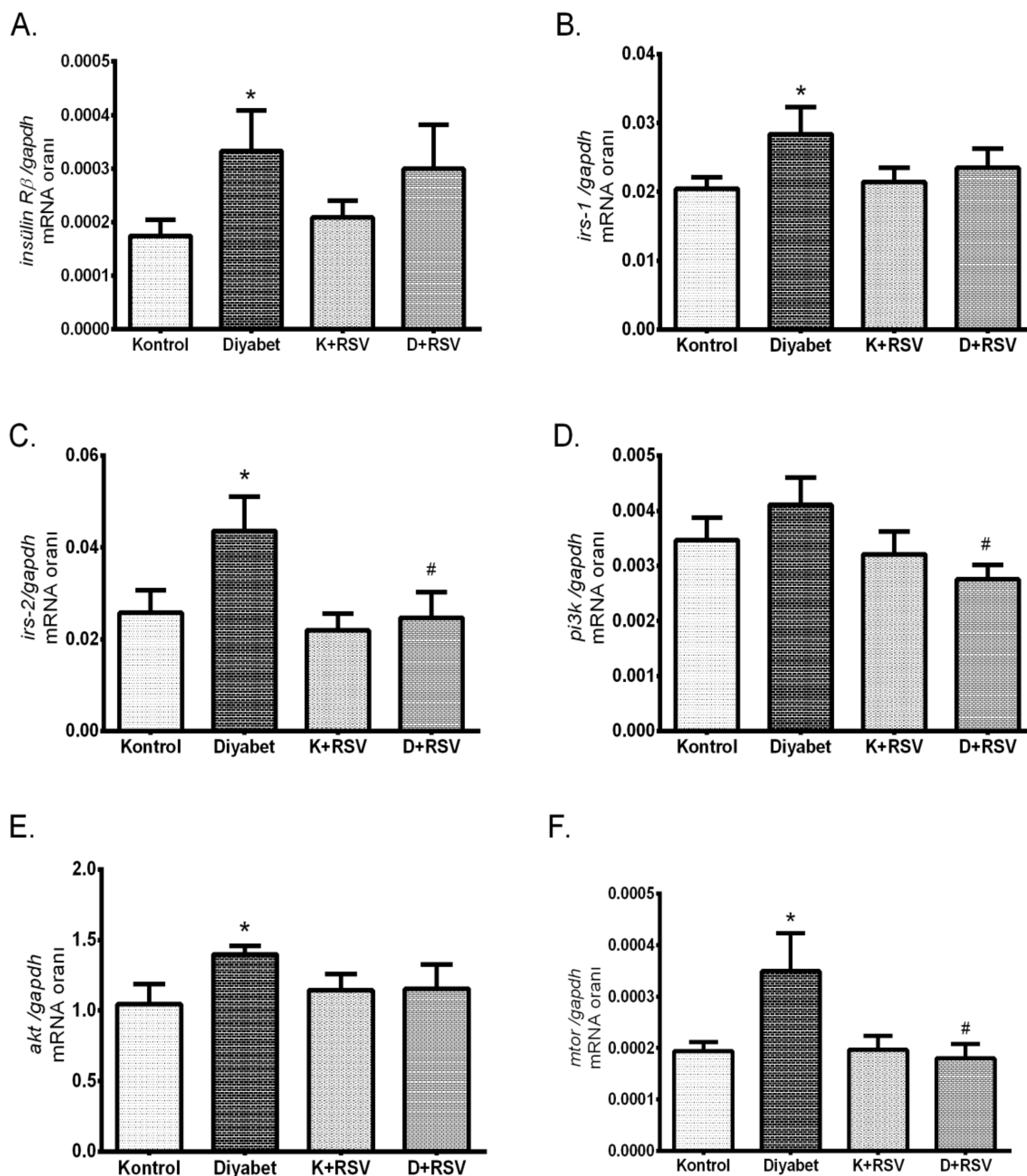
362

363 **Figure 2.** Changes in protein levels of antioxidant enzymes in diabetic and/or resveratrol-treated kidney
364 tissues. Figure demonstrates (**A**) Representative Western blot bands indicating the group averages, (**B**)
365 changes in CAT expression, (**C**) changes in SOD1 expression, (**D**) changes in SOD2 expression levels.
366 Data is given with respect to GAPDH protein. C:Control, D:Diabetes, C+RSV: Resveratrol given control,
367 D+RSV: Resveratrol given diabetic group. *signifies the statistical difference of the data according to
368 the control group ($p<0.05$), and the # sign indicates the statistical difference of the data according
369 to the diabetes group ($p<0.05$).



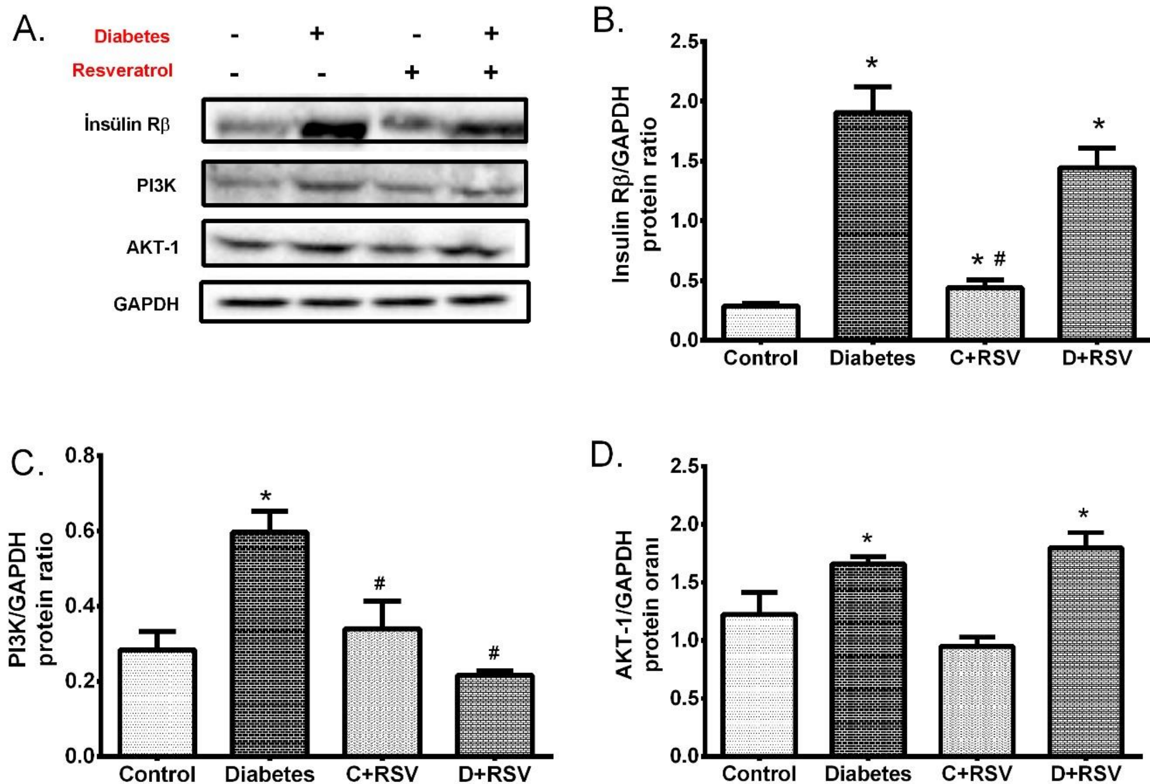
370

371 **Figure 3.** Effects of diabetes and resveratrol on renal (**A**) CAT enzyme activity, (**B**) GPx enzyme
372 activity, (**C**) Total SOD enzyme activity, (**D**) Total GST enzyme activity. C:Control, D: Diabetes,
373 C+RSV: Resveratrol given control, D+RSV: Resveratrol given diabetic group. *signifies the
374 statistical difference of the data according to the control group (p<0.05), and the # sign indicates
375 the statistical difference of the data according to the diabetes group (p<0.05).



376

377 **Figure 4**. Effects of diabetes and resveratrol on renal (**A**) *insulin Rβ,* (**B**) *irs-1,* (**C**) *irs-2,* (**D**) *pi3k,* (**E**)
378 *akt,* (**F**) *mtor* gene expression levels. Data were normalized with *gapdh*. C:Control, D:Diabetes, C+RSV:
379 Resveratrol given control, D+RSV: Resveratrol given diabetic group. *signifies the statistical
380 difference of the data according to the control group ($p<0.05$), and the # sign indicates the statistical
381 difference of the data according to the diabetes group ($p<0.05$).



382

383 **Figure 5.** Changes in renal protein expressions of insulin signaling elements with diabetes and/or
384 resveratrol. (**A**) Representative Western blot bands indicating the group averages. Changes in (**B**)
385 Insulin Rβ, (**C**) PI3K, (**D**) AKT1 protein expressions. Data were normalized with respect to
386 corresponding GAPDH. C:Control, D:Diabetes, C+RSV: Resveratrol given control, D+RSV:
387 Resveratrol given diabetic group. *signifies the statistical difference of the data according to the
388 control group ($p<0.05$), and the # sign indicates the statistical difference of the data according to
389 the diabetes group ($p<0.05$).