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Effects of Resveratrol on the Kidney in Rats with Streptozotocin Induced Diabetic Nephropathy

Streptozotosin ile Diyabetik Nefropati Oluşturulan Sıçanlarda Resveratrolün Böbrek Üzerindeki Etkileri

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ABSTRACT

Objective: Diabetic nephropathy is one of the most significant causes of end-stage renal failure and is a common microvascular complication of diabetes (D). Resveratrol (RSV), a natural compound found in grape skins and red wine, has potent antioxidant properties. This study aimed to evaluate the effects of RSV in a streptozotocin (STZ)-induced diabetic rat model.

Methods: Animals were divided into four groups: control, RSV, D, and D + RSV. The diabetic group received a single intraperitoneal dose of STZ (65 mg/kg). After 2 weeks, rats with basal blood glucose levels >250 mg/dL were considered diabetic. RSV(10 mg/kg/day) was administered orally by gavage for 8 weeks. Metabolic analyses were conducted throughout the study. At the study's end, transmission electron microscopy and immunohistochemical analyses were performed. Additionally, the left kidney was isolated and suspended in an organ bath to study the functional changes without damaging the renal artery.

Results: In the study, increased transforming growth factor-beta, fibronectin and inducible nitric oxide synthase immunoreactivity, which are markers of D-induced renal degeneration, were partially

ÖZ

Amaç: Diyabetik nefropati, son dönem böbrek yetmezliğinin en önemli nedenlerinden biridir ve diyabetin (D) yaygın bir mikrovasküler komplikasyonudur. Üzüm kabuğu ve kırmızı şarapta bulunan doğal bir bileşik olan resveratrol (RSV), güçlü antioksidan özellikler gösterir. Bu çalışmada, streptozotosin ile oluşturulan D sıçan modelinde RSV'in diyabetik nefropati üzerindeki etkilerinin değerlendirilmesi amaçlanmıştır.

Yöntemler: Hayvanlar kontrol, RSV, D ve D + RSV olmak üzere dört gruba ayrıldı. D gruplara tek bir intraperitoneal doz streptozotosin (65 mg/kg) uygulandı. İki hafta sonra, bazal kan glukoz seviyeleri 250 mg/dL'nin üzerinde olan sıçanlar D olarak kabul edildi. RSV (10 mg/kg/gün) 8 hafta boyunca gavaj yoluyla oral olarak uygulandı. Çalışma boyunca metabolik analizler yapıldı. Çalışmanın sonunda, transmisyon elektron mikroskopisi ve immünohistokimyasal analizler yapıldı. Ek olarak, sol böbrek izole edildi ve renal artere zarar vermeden fonksiyonel değişiklikleri incelemek için bir organ banyosunda askıya alındı.

Bulgular: Çalışmada, D'nin neden olduğu böbrek dejenerasyonunun belirteçleri olan artmış transforme edici büyüme faktör-beta fibronektin ve indüklenebilir nitrik oksit sentaz immünoreaktivitesinin

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reduced by RSV treatment. In group D, decreased endothelial nitric oxide synthase uptake (weak immune reactivity) was observed, whereas this uptake increased with RSV treatment (moderate immune reactivity). Furthermore, both angiotensin II and phenylephrine responses were reduced in group D treated with RSV. Vasodilator responses to acetylcholine were also reduced in this group.

Conclusion: RSV may protect against diabetic nephropathy by modulating key pathways involved in renal degeneration and vascular function and may have potential as a therapeutic agent for slowing disease progression.

Keywords: Resveratrol, diabetic nephropathy, proinflammatory cytokines, transmission electron microscope, renal vascular responses

RSV uygulaması ile kısmen azaldığı tespit edildi. D grupta endotelyal nitrik oksit sentaz tutulumunda azalma (zayıf immün reaktivite) gözlenirken, bu tutulum RSV tedavisi ile arttı (orta derecede immün reaktivite). Ayrıca, RSV ile tedavi edilen D grupta hem anjiyotensin II hem de fenilefrin yanıtları azaldı. Asetilkoline verilen vazodilatör yanıtlar da bu grupta azaldı.

Sonuç: RSV, böbrek dejenerasyonu ve vasküler fonksiyonda rol oynayan anahtar yolakları modüle ederek diyabetik nefropatiye karşı koruma sağlayabilir ve hastalığın ilerlemesini yavaşlatmada terapötik bir ajan potansiyeli olabileceği düşünülebilir.

Anahtar Sözcükler: Resveratrol, diyabetik nefropati, proinflamatuar sitokinler, transmisyon elektron mikroskobu, renal vasküler yanıtlar

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease with a high prevalence worldwide. It is characterized by hyperglycemia and is associated with a number of critical clinical complications, including nephropathy, retinopathy, neuropathy, and cardiomyopathy (1,2). Diabetic nephropathy (DN) is a microvascular complication that causes end-stage renal failure, impairs patients' quality of life, and ultimately leads to death. DN is observed in 30-40% of patients with type 1 and 2 DM (3,4). DN is characterized by the following pathological features: Glomerulosclerosis, excessive extracellular matrix deposition, glomerular hypertrophy, and basement membrane thickening (5). Hemodynamic changes are important in the pathogenesis of DN. Chronic hyperglycemia induces metabolic changes and dysfunction in endothelial-vascular smooth muscle cells, which in turn causes vascular dysfunction and hemodynamic changes in the kidneys (6). A growing body of evidence indicates that elevated blood glucose levels in DN are the result of a complex interplay between multiple factors, including advanced glycation end products and metabolic and hemodynamic processes, such as the renin-angiotensin system (7). The regulation of dietary habits using a diet based on fruits and vegetables can delay or prevent the progression of DM. Consequently, there has been a surge in interest in foods rich in polyphenols (8). Resveratrol (RSV) (3,4,5-trihydroxy stilbene, respiratuar sinsitiyal virus) is a natural phytoalexin that has been extensively studied in recent years. It is a polyphenolic compound that is primarily found in grains, fruits, vegetables, legumes, and plant-derived beverages, including tea, coffee, and wine (1,9). RSV exhibits a wide range of biological and pharmacological properties, including anti-diabetic, anti-carcinogenic, anti-inflammatory, anti-oxidative, and cardiovascular protective effects (1). The primary objective of DM treatment is to reduce blood glucose levels, enhance insulin sensitivity, and safeguard pancreatic cells (2,10). RSV has been demonstrated to possess anti-diabetic effects, exerting anti-hyperglycemic activity through the stimulation of intracellular glucose transport, a process that occurs independently of insulin (11). Additionally, it has been observed to reduce insulin secretion in the pancreatic cells of isolated rats and to protect pancreatic β cells in diabetic animals (12). Furthermore, it has been demonstrated that RSV can prevent DM-induced kidney damage and mesangial cell proliferation, as well as improve glomerular hypertrophy and mesangial cell glucolipotoxicity. This exerts a beneficial effect on renal function during DM (13). In light of the data presented in the literature, the aim of this study was to investigate the effects of RSV, an important antioxidant, on DN, one of the late-stage complications of diabetes (D), in rats that were made diabetic using streptozotocin (STZ).

MATERIALS AND METHODS

Study Design and Animals

Thirty adult male Wistar albino rats, whose weights varied between 250 and 300 g obtained from Gazi University Laboratory Animal Raising and Experimental Research Center and used in this study. The rats were fed freely in separate cages under a 12-hour dark cycle at temperatures above 24±2 °C with standard rat food and tap water. The relative humidity of the environment was maintained between 30% and 45%. The rats were housed in polycarbonate cages with sawdust underneath. The *in vivo* experiments of the study were carried out in the Gazi University Laboratory Animal Raising and Experimental Research Center and Gazi University Faculty of Medicine Physiology Department laboratory with the permission of the Gazi University Animal Experiments Local Ethics Committee (approval number: G.Ü.ET-11.086, date: 26.09.2011)

Experimental Design

The rats were divided into 4 groups.

1. Control group (C, n=6),
2. RSV group (RSV, n=8),
3. Diabetes group (D, n=8),
4. Diabetes + resveratrol group (D + RSV, n=8).

D was made intraperitoneally (i.p.) using a single dose of 0.1 M (pH:4.5) STZ (65 mg/kg) dissolved in cold citrate buffer (14). Seventy-two hours after STZ administration, rats with a fasting blood glucose >250 mg/dL were considered diabetic. For two weeks following the development of D, the blood glucose of the subjects was measured at specific intervals. During this period, approximately the stabilization of D was ensured. The control groups (C and RSV groups) were administered an injection of i.p. 1 mL of STZ solvent citrate tampon. Following a 2-week adaptation period, for a period of 8 weeks, the RSV groups (R and D + RSV) were administered RSV dissolved in 0,1 M ethanol of 96% at a dose of 10 mg/kg, and the Control and D groups were administered 0.1 M ethanol via oral gavage (15). The body weights, fasting blood glucose values, liquid intake, and urinary discharge of all subjects were noted at the beginning of the study (1st measurement) 2 weeks after the administration of STZ or citrate

tampon (2nd measurement), during the administration of RSV or ethanol, (3rd and 4th measurements), and prior to being sacrificed (5th measurement) by being placed in a metabolic cage mechanism. Glucose, blood urea nitrogen (BUN), calcium, creatinine, potassium, and sodium were examined in blood samples taken from the caudal vein of the rats at the beginning of the study; 2 weeks after the administration of STZ or citrate tampon, and before sacrifice. At the beginning and end of the experiments, glucose, ketone, nitrite/nitrate, leucocyte, bilirubin, albumin, and creatinine levels were analyzed in urine using Siemens Multistix 10 SG urine analysis test strips. 24 hours after the final gavage administration, the rats were sacrificed under intramuscular Rompun + Ketamin (50+60-100 mg/kg) anesthesia by drawing blood from the heart. After the completion of euthanasia, the left kidney was isolated without destroying the renal artery. The right kidney tissue was removed and placed in formalin for histopathological analysis.

Observation of Renal Vascular Responses

Following the development of nephropathy in the isolated perfused rat kidney, the left kidney was isolated without destroying the renal artery to study the functional changes. The solution was perfused using Krebs Hanseleit solution, hung in an isolated organ bath gassed with a mixture of 95% O2 and 5% CO2 and stabilized for approximately 40 min.

The contraction responses were then examined following the administration of bolus manner phenylephrine (Phe) at doses of 10-8, 10-7, 10-6, and 10-5 M, respectively, waiting for approximately 15 min for stabilization between each dose. After that, following the 20-minute stabilization period, Angiotensin II (Ang II) was administered at doses of 10-9, 10-8, 10-7 M respectively, waiting for approximately 15 minutes for stabilization between each dose. Finally, 20 µl 10-2 M Phe was added to 200 cc Krebs Hanseleit solution because the final concentration would be 10-6 M and perfusion pressure was expected to rise. While the increase continued gradually, as soon as the plateau phase was observed, acetylcholine (ACh) was administered at doses of 10-6, 10-5, 10-4 M, waiting for approximately 15 min for stabilization between each dose, and the contraction and relaxation responses were examined. Drug doses were determined following a comprehensive review of numerous studies in the literature pertaining to vascular responses. Dose ranges that were hypothesized to be suitable for our hypothesis were selected based on this evaluation (16-19).

Histological and Immunohistochemical Analyses

Transmission Electron Microscope (TEM) Study

Tissue samples were dissected into pieces of $1mm^3$ were fixed in 2.5% 0.1 M phosphate buffered glutaraldehyde (pH 7.4) for 2-h. At the end of the fixation period, the tissues were washed with buffer three times and post-fixated for 1-h with 1% osmium tetraoxide. At the end of this period, tissues were dehydrated using a graded alcohol series. Finally, the tissues treated with propylene oxide were embedded in the embedding material prepared using the Araldite CY212 kit. The blocks were polymerized for 48-h in an incubator at 56 °C. The half-thin sections were stained with toluidine blue and examined under a light microscope. The thin sections obtained from the marked regions were stained with uranyl-acetate-lead-citrate and evaluated using a Zeiss EVO 010 transmission attachment scanning electron microscope (20).

Immunohistochemical Method

The tissue samples were fixed in 10% neutral formalin, and paraffin blocks were prepared after a routine light microscope study. For the sections placed on polylysine glass, the avidin-biotin peroxidase immune staining technique, one of the indirect immune-histochemical techniques, was used. Anti-transforming growth factor-beta (TGF-β), anti-inducible nitric oxide synthase (iNOS), anti-endothelial nitric oxide synthase (eNOS), and anti-fibronectin were used as primary antibodies. AEC and DAB were used as chromogens, and hematoxylin was preferred as the background stain. The prepared materials were studied using a Leica DM4000 computer-equipped photo-light microscope. For each antibody, score tables for all groups were prepared with respect to the cortex and medulla in the renal sections. 5 areas were chosen randomly on each glass with a magnification rate of x400 and the density of retention was scored semi quantitatively as follows: 0 (-, no retention), 1 (+, weak immunoreactivity), 2 (++, moderate immunoreactivity), 3 (+++, strong immunoreactivity) (21).

Statistical Analysis

The data were compared with Kruskal-Wallis and Mann-Whitney U nonparametric tests using the SPSS 20.0 statistical program. $P<0.01$ values were considered significant.

RESULTS

Fasting Blood Glucose, Body Weight, Liquid Intake and Amounts of Urinary Discharge

There were no changes in fasting blood glucose levels between the control and RSV groups for 8 weeks. In the diabetic groups (D, D + RSV), after STZ administration, symptoms of hyperglycemia, fasting blood glucose, increased fluid intake and urine output, and decreased body weight were detected.

Blood Biochemistry Findings

In the blood biochemistry analysis, a statistically significant increase in alkaline phosphatase (ALP) and alanine aminotransferase (ALT) levels due to chronic D and in BUN and creatinine levels due to deterioration in renal function were observed in the subjects. There was a decrease in the sodium, potassium, calcium levels. Improvements in these deteriorated parameters were not achieved with RSV treatment.

Immunohistochemical findings of eNOS retention

eNOS retention was found at the cortex and medula levels in the control group. In the glomeruli, retention varying from moderate to strong was observed in the proximal and distal tubules (Figure 1A). The retention in the medullar collecting ducts was cytoplasmic (Figure 1B). In the RSV group, the density of retention in some glomeruli and medullar collecting ducts was relatively decreased compared with the control group (Figures 1C, 1D). In the D group, degenerated tubules were observed in the cortex. Although eNOS immunoreactivity was found to have decreased significantly compared with the previously mentioned groups, moderate retention was observed in some

podocytes (Figure 1E). The retention in the medullar collection ducts decreased significantly compared with the two groups (Figure 1F). In the D + RSV group, eNOS retention in the Bowman capsule, especially at the glomerular level, increased compared with the D group. eNOS immunoreactivity detected in some veins of this group also caught our attention (Figure 1G). Retention in the medulla also increased compared with the D group, and the strong eNOS immunoreactivity in some regions attracted attention (Figure 1H).

Immunohistochemical Findings iNOS Retention

In the Control group, iNOS immune retention varied from weak to moderate in the medulla in some collecting ducts (Figure 2B). In the RSV group, expression was observed to have increased in the proximal and some distal tubules compared with that in the control group (Figure 2C). The retention rate in the medullary region was similar to that of the Control group (Figure 2D). In the D group, glomeruli shrank due to sclerotic changes in the cortex, and degenerated distal tubules drew attention. It was observed that iNOS immunoreactivity increased in the proximal tubules and was weak in the degenerated distal tubules and renal glomeruli (Figure 2E). The retention of duct iNOS in the medula increased dramatically (Figure 2F). In the D + RSV group, iNOS retention in the cortex was the same as that of the D group (Figure 2G), and it was observed to have decreased in the collecting ducts in the medulla (Figure 2H).

Immunohistochemical Findings-TGF-β Retention

In the cortex regions of the control and RSV groups, the retention was observed to be the same and very weak especially in glomeruli (Figure 3A, Figure 3C). While the retention in the medullary regions of the two groups was similar, the presence of strong coloration in some places in the RSV group attracted attention compared with the control group (Figure 3B, Figure 3D). In the D group, TGF-β retention was increased in both the cortex and medulla compared with the aforementioned groups (Figure 3E, Figure 3F). In the D + RSV group, similar to the previously mentioned group, distinct TGF-β expression was detected in degenerated tubules (Figure 3G, Figure 3F).

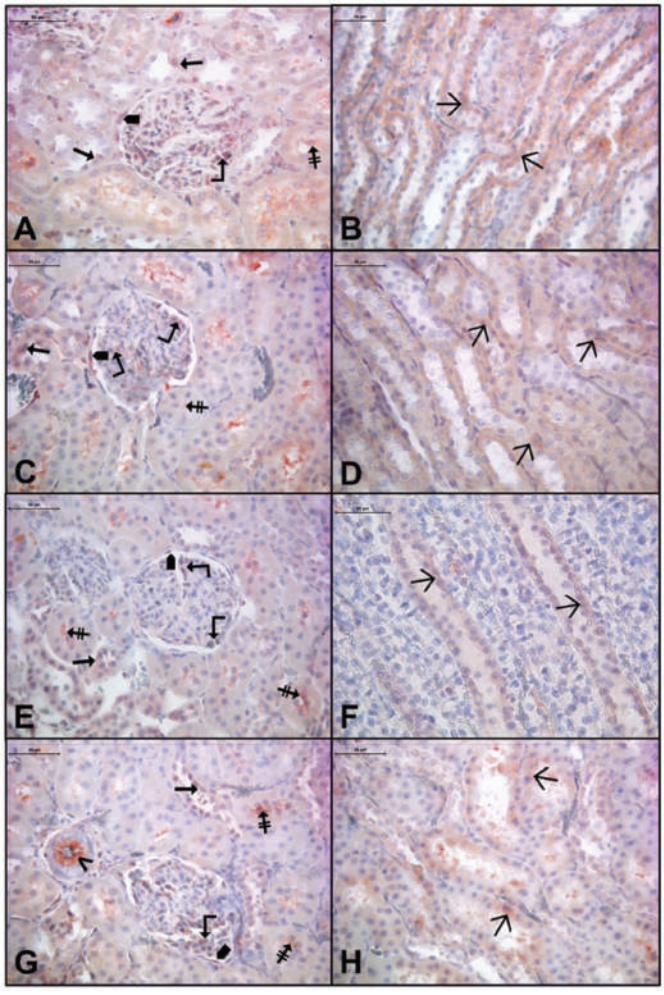

Figure 1. eNOS immunoreactivity; Control Group cortex (A), medulla (B), RSV Group cortex (C), medulla (D), Diabetes Group cortex (E), medulla (F), diabetes + resveratrol group cortex (G), medulla (H) areas.

↳ : Glomerul, ➡: Parietal lobe of Bowman capsule ‡: Proximal tubule, ➡: Distal tubule, ➔: Collector ducts, >: Blood vessel (Immunperoxidase-hematoxylinX400).

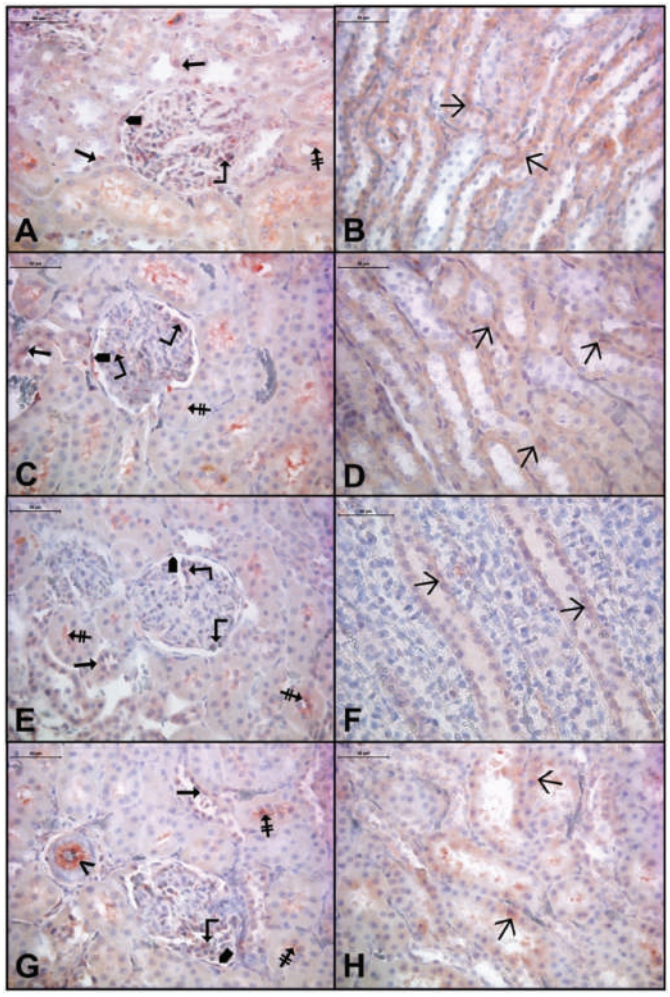

Figure 2. iNOS immunoreactivity; Control Group cortex (A), medulla (B); RSV Group cortex (C), medulla (D); Diabetes Group cortex (E), medulla (F); Diabetes+Resveratrol Group cortex (G), medulla (H) areas. ↳ : Glomerul, ‡: Proximal tubule, ➡: Distal tubule, ➔: Collector ducts, >: Degenerate tubules (Immunperoxidase-hematoxylinX400).

RSV: Respiratuar sinsitiyal virus

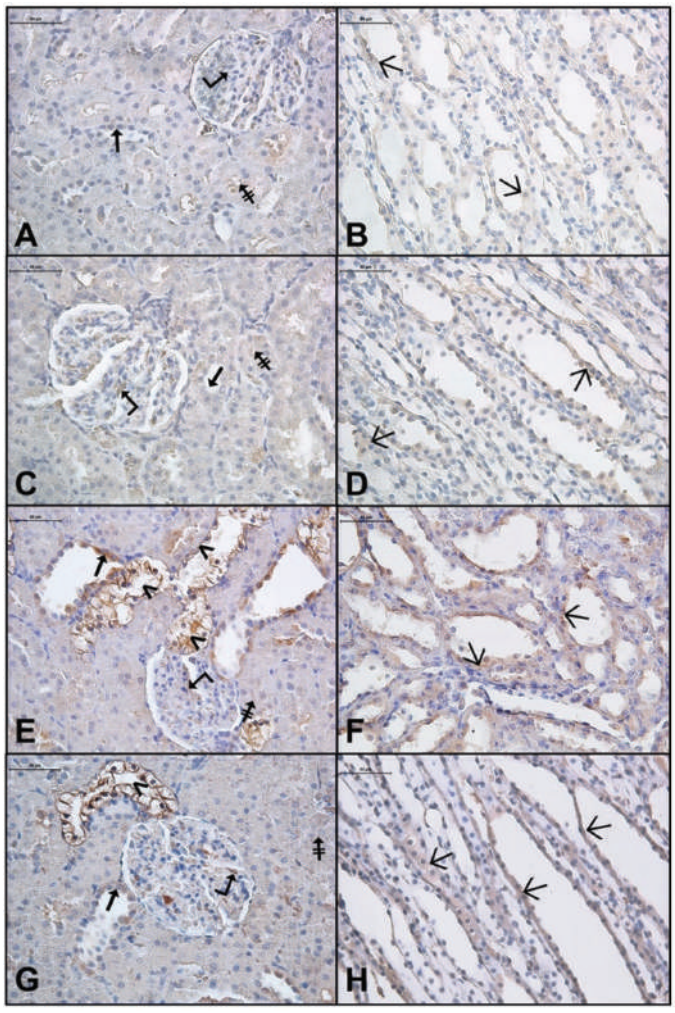

Figure 3. TGF-α immunoreactivity; Control Group cortex (A), medulla (B); RSV Group cortex (C), medulla (D); Diabetes Group cortex (E), medulla (F); Diabetes+Resveratrol Group cortex (G), medulla (H) areas. ↳: Glomerul, ‡: Proximal tubule, ➡: Distal tubule, ➔: Medullar zone, >: Degenerate tubules (Immunperoxidase-hematoxylinX400).

RSV: Respiratuar sinsitiyal virus

Immunohistochemical Findings-Fibronectin Retention

Weak TGF-β expression in the glomeruli was detected in both the control and RSV groups (Figure 4A, Figure 4C). In the RSV group, cells that exhibited strong immune positivity in certain areas compared with the control group attracted attention (Figure 4B, Figure 4D). In the D group, fibronectin retention was increased in the cortex and medulla compared with the other groups. The degenerated tubules observed in this group did not exhibit distinct immunoreactivity (Figure 4E, Figure 4F). In the D + RSV group, fibronectin retention in the cortex region decreased significantly compared with the D group. Moderate fibronectin retention was detected in the glomeruli (Figure 4G). In the medulla, cells that exhibited strong cytoplasmic retention were identified (Figure 4F).

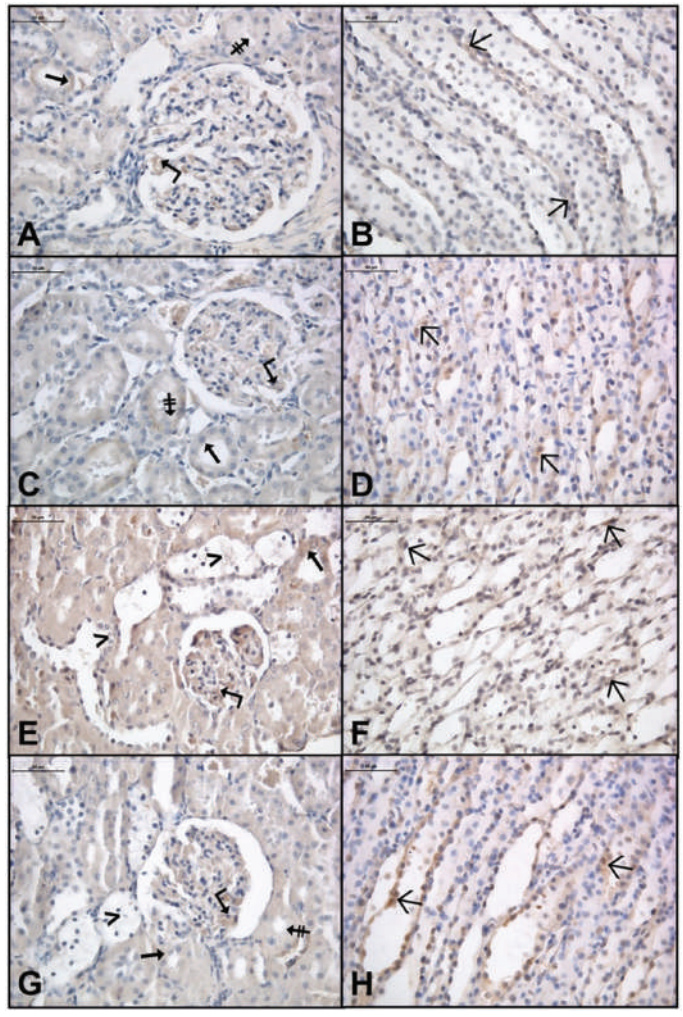

Figure 4. Fibronectin immunoreactivity; Control Group cortex (A), medulla (B), RSV Group cortex (C), medulla (D), Diabetes Group cortex (E), medulla (F); Diabetes + Resveratrol Group cortex (G), medulla (H) areas.

↳: Glomerul, ➡: Parietal lobe of Bowman capsule ‡: Proximal tubule, ➡: Distal tubule, ➔: Medullar zone, >: Degenerate tubules (Immunperoxidase-hematoxylinX400).

RSV: Respiratuar sinsitiyal virus

Transmission Electron Microscopy (TEM) Findings

TEM studies performed, glomerular basal membrane (GBM), mesangium, capillary endothelial cells, and podocyte pedicles were examined. All structures in the Control and RSV-treated groups were normal. However, in the D group, wrinkling of the GBM and irregular thickness were observed. An electron-dense deposit was detected in the mesangial matrix. The loss of podocyte pedicels was observed in many areas. It was also found that capillary endothelial cells had undergone hyperplasia and had a hyperthropic appearance. Large vacuoles were detected in some endothelial cells (Figure 5I). In all sites examined in the D + RSV group, the above-mentioned structures were observed to be preserved. The endothelial cells had normal structures, and few cells had vacuoles. Loss of podocyte

pedicels was not observed. However, wrinkling was still present in some areas. The mesangium had a normal structure, and electron-dense deposits were not observed (Figure 5II).

Renal Vascular Responses

Phe Responses

Although there were no significant differences between the control group and RSV group, there was a significant increase in the D group in the Phe 10-6 and 10-5 doses when compared with the Control group ($p<0.01$). A significant decrease in all Phe doses in the D + RSV group was observed compared with the control group in terms of contraction responses ($p<0.01$) (Figure 6).

Angiotensin II Response

Although there were no significant differences among the Control, D, and RSV groups in the Ang II 10-9 and 10-8 doses, there was a significant increase in the contraction response of the RSV group in the Ang II 10-7 dose ($p<0.01$). A significant decrease was observed in the D+RSV group for all doses of Ang II in terms of contraction responses compared with the control group ($p<0.01$) (Figure 6).

Acetylcholine Responses

When the ACh relaxation responses were studied, a significant decrease in all doses of ACh was observed in the D + RSV group compared with the control group ($p<0.01$). There were no significant differences among the other groups at any dose (Figure 6).

DISCUSSION

DN significantly impairs patients' quality of life by inducing various abnormal physiological and structural changes that lead to renal function deterioration (22). The primary goal of DN treatment is to manage complications and slow the progression of kidney damage. It has been reported that improving abnormal physiological conditions caused by D, such as reducing proteinuria and controlling high blood pressure levels, can slow down the progression of DN (23). In recent years, extensive research has focused on the anti-diabetic and antioxidant properties of RSV in various animal models. Studies have shown that RSV can exert beneficial effects in animals with D, thereby improving the incidence of D. For instance, in similar studies examining STZ-induced D, RSV administration was reported to attenuate weight loss, lower serum glucose, insulin, triglyceride, and free fatty acid levels, and alleviate symptoms such as polyphagia and polydipsia (24,25). Contrary to many findings in the literature, our study did not observe a significant improvement in the characteristic symptoms of hyperglycemia, such as body weight loss, increased fluid intake, and urinary output, with RSV treatment. Blood biochemistry analyses revealed an increase in ALB, ALP, ALT, BUN, and creatinine levels, along with a decrease in sodium levels, in patients with D. These findings supported the deterioration in renal function due to D in our approximately 10-week old diabetic animals, and thus the DN we aimed to make. Furthermore, our study did not detect any significant improvement in the deteriorated

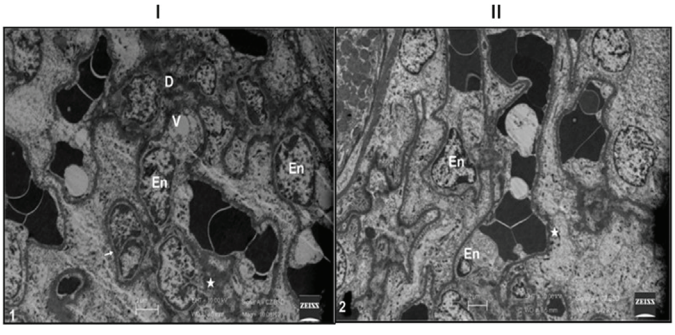

Figure 5. Electron microscobe findings on diabetes group (1) and diabetes + resveratrol group (2) En: capillaries endothelial cells with normal structures observed throughout the tissue, *: consulted GBM, ↑: Podocyte pedicelles (Uranil-acetate-Lead citrate x10000)

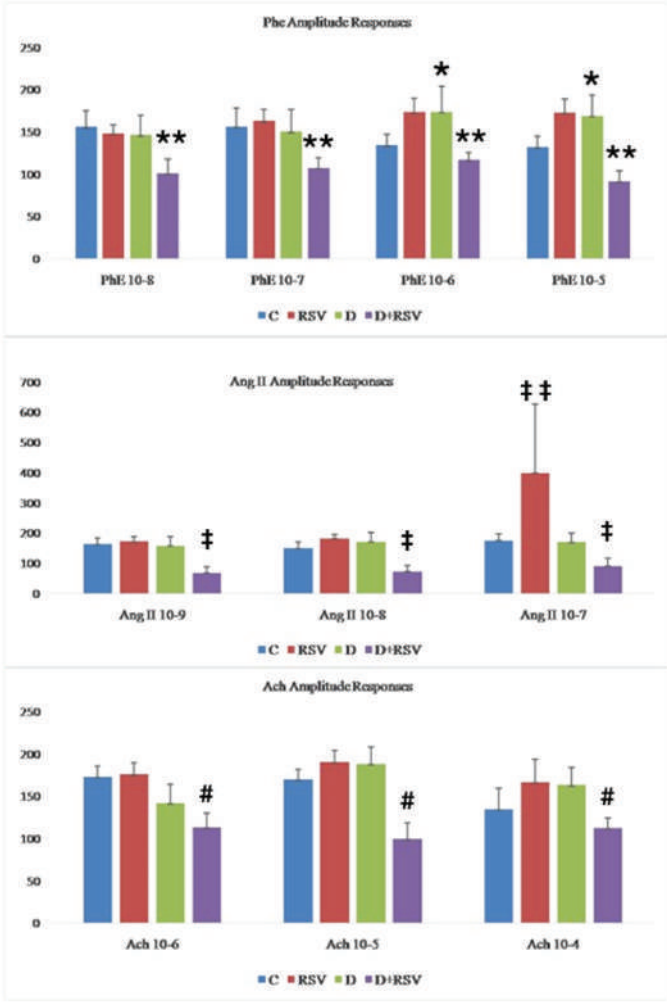

Figure 6. Phenilephrine, Angiotensin II, and Acetlycholine Amplitude Responses in Renal Artery

Phenilephrine;

* $p<0.01$compared with control group (for 10-6,10-5 doses)

** $p<0.01$ compared with the control and diabetes groups (for all doses)

Angiotensin II;

‡ $p<0.01$ compared with the control and diabetes groups (for all doses)

‡‡ $p<0.01$ compared with all groups (for 10-7dose)

Acetlycholine;

# $p<0.01$ compared with the control and diabetes groups (for all doses)

parameters with RSV administration, consistent with several studies in the literature. For instance, a study (26) reported no improvement in elevated aspartate transaminase (AST) and ALT levels in diabetic rats treated with RSV, whereas another study (27) observed no improvement in AST and ALT levels in pigs with metabolic syndrome following RSV treatment. DN is a pathological condition characterized by glomerular hypertrophy, GBM thickening, and an increase in extracellular matrix increase, and results in tubulointerstitial and glomerular fibrosis and sclerosis (28). Podocytes, which surround the capillaries in the Bowman glomeruli, play a crucial role in forming the filtration barrier together with renal endothelial cells (3). Thickening of GBM (29), the decrease in the number of podocytes associated with proteinuria, and loss of podocyte pedicels (30) are among recognized microscopic changes of DN. Our study results regarding the presence of proteinuria in urine and electron microscopy (EM) findings supported DN progression in individuals with D. EM studies revealed locally wrinkled and irregularly thickened GBM and electron-dense deposits in the mesangial matrix of diabetic animals. Contrary to the literature findings, our study did not observe complete recovery although locally wrinkled GBM persisted in the RSV-treated group. However, electron-dense deposits in the mesangial matrix and loss of podocyte pedicels were not observed in the RSV-treated group. Similar studies have reported improvements in GBM thickness, glomerular fibronectin, collagen IV, and TGF-β expression following RSV treatment in animals with D (15,31). In our study, we observed that although the wrinkling appearance in the GBM persisted locally in the RSV-administered diabetic group compared with the diabetic control group, no complete recovery was observed, as reported in the literature. However, RSV administration prevented electron-dense deposits in the mesangial matrix and loss of podocyte pedicels, in contrast to diabetic animals without RSV treatment. This finding is consistent with previous findings in which RSV reduced GBM thickness, mesangial cell numbers, and podocyte loss in diabetic rats. Additionally, diabetic kidneys typically exhibit various morphological anomalies, including tubular cell swelling, endothelial cell vacuolization, and glomerular hypertrophy (32). In our study, RSV-administered diabetic rats showed normal capillary endothelial cells and fewer vacuoles compared with untreated diabetic rats, consistent with studies demonstrating RSV's protective effects against renal hypertrophy (33,34). TGF-β is a multifunctional cytokine implicated in various cellular activities (35) and is one of the main effectors of structural changes in DN (28). TGF-β is known to induce renal hypertrophy, and RSV has been shown to inhibit TGF-β production and decreases collagen levels (36). Immunohistochemical analyses in our study revealed increased TGF-β expression in both the cortex and medullary regions of diabetic kidneys, supporting DN development. Conversely, RSV administration reduced TGF-β expression, consistent with the literature. Furthermore, we observed decreased eNOS and increased iNOS levels in the diabetic group. However, RSV administration increased eNOS levels, consistent with previous studies indicating the potential of RSV to enhance eNOS expression and nitric oxide (NO) production (37,38). In addition to evaluating biochemical and histological parameters, we assessed vascular responses in diabetic rat kidneys. Chronic hyperglycemia induces metabolic changes and dysfunction in endothelial and vascular smooth muscle cells, leading to vascular dysfunction and hemodynamic changes in the kidneys and other organs (6). The endothelium is vital for a variety of physiological functions in the vessel wall, including the regulation of vascular tone. The endothelial vasodilator NO produces various vasoactive mediators that act on vascular smooth muscle cells, such as vasoconstrictor endothelin (39). NO is produced by a mechanism stimulated by hormones such as ACh, bradykinin, and insulin and catalyzed by the nitric oxide synthase (NOS) enzyme. Increasing eNOS activation and expression, one of the NOS isoforms, causes glomerular and functional hemodynamic changes in the diabetic kidney (40). RSV has been reported to protect against ischemia-reperfusion injury in the kidney, heart, and brain (39,41). Our study revealed that RSV administration increased iNOS and eNOS expression in the cortex and medulla of the kidney, suggesting potential vasodilatory effects. These findings are consistent with studies showing RSV-induced renal vasodilation via endothelial-dependent NO production (42,43). In both studies, NOS inhibition or deendothelization partially reversed the vascular relaxation caused by RSV.

The contraction responses formed by both Phe and Ang II depend on the release of Ca^{+2} from the sarcoplasmic reticulum over isotol trisphosphate (IP3) and diacylglycerol within the cell following the stimulation of α1 receptors by Phe and the stimulation of angiotensin receptor 1 receptors by Ang II. In a study examining the cardiovascular effects of RSV, rats were fed corn syrup, which contains high fructose. It was observed that in the thoracic aortic rings of rats, despite the contraction response to Phe and relaxation response to ACh, RSV had a protective effect on the endothelium (44). Our results showed that both the Ang II and Phe responses were significantly reduced in rat kidneys isolated from diabetic rats treated with RSV. It is known that in hyperglycemia, KATP channels are closed. It has been shown that Ca+2 increase in cells is associated with the activation of K+ channels, KATP channel blockers inhibit Ca+2 increase (45), and RSV decreases the sensitivity of smooth muscles to Ca+2 and causes an increase in systolic Ca+2 in the endothelium (46). In our study, the significant improvement in the increased contraction responses mediated by increased Phe and ACh levels in the D group with RSV application also supports the findings in the literature. A study was reported in diabetic rats and showed that noradrenaline and ATP-mediated contraction responses significantly increased in vas deferens tissue, and RSV administration corrected this increase (47). In another study, the effect of RSV on Ca+2 levels in the heart valve endothelium was investigated. It has been stated that the endothelium - dependent relaxant effect of RSV is associated with an increase in Ca+2 levels in endothelial cells, but the inhibitory effect of vascular smooth muscle contraction may occur due to the decrease in Ca+2 levels (46). In our study, decreased vasoconstrictor responses were observed in the D group treated with RSV; inhibition of Ca+2 increase in D suggested that RSV might be due to the reduction of Ca+2 sensitivity of smooth muscles. It is thought that a significant decrease in the vasodilator responses of ACh in kidneys isolated from patients with D treated with RSV may also be through the same mechanism. By preventing an increase in intracellular Ca+2 in hyperglycemia, decreasing the sensitivity of RSV to Ca+2 may cause a decrease in response.

Study Limitations

In this study, we examined the protective effects of resveratrol against DN in an STZ-induced diabetic rat model. However, these findings

are limited to humans. We used a fixed dose of resveratrol (10 mg/kg/day); varying doses and treatment durations might yield more comprehensive results. The exact molecular mechanisms underlying the protective effects of resveratrol have not been fully elucidated. Although we conducted histopathological and functional analyses, advanced imaging and molecular techniques could provide deeper insights. Furthermore, resveratrol's effects were not compared with those of other treatments for DN, which limits our understanding of its relative efficacy.

CONCLUSION

RSV, which we administered to diabetic rats at a dose of 10 mg/kg/day for 8 weeks, did not generally cause a significant improvement in the biochemical findings of D. However, TGF-β, fibronectin, and iNOS immunoreactivity, which increased in line with the kidney degeneration caused by D decreased partially; It was observed that the level of eNOS, which decreased with D, increased. In the diabetic group treated with RSV, both the Ang II and Phe responses and the vasodilator ACh responses decreased in the same group. Based on the findings of our study, we can conclude that RSV does not have a very positive effect on the symptoms of D, as frequently stated in the literature. However, the results of our study are important in terms of demonstrating that RSV may have some protective effects against the negative effects of DN and shedding light on this issue. Since prevention or reduction of disease complications is important both for the patient and in terms of reducing health-related costs, the positive results of RSV on late-stage kidney damage of D in our study suggest that it may be a new treatment option for patients in the coming days, along with other literature information.

Ethics

Ethics Committee Approval: The study was approved by the Gazi University Animal Experiments Local Ethics Committee (approval number: G.Ü.ET-11.086, date: 26.09.2011).

Informed Consent: This study is not applicable because it involves animal subjects.

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Footnotes

Authorship Contributions

Concept: D.T., Ç.Ö., A.B., E.D., Design: D.T., Ç.Ö., S.E., E.D., G.T.K., G.G., Supervision: D.T., Ç.Ö., A.B., S.E., Resources: D.T., Ç.Ö., E.D., G.T.K., G.G., Material: D.T., Ç.Ö., E.D., G.T.K., G.G., Data Collection or Processing: D.T., Ç.Ö., Analysis or Interpretation: D.T., Ç.Ö., A.B., S.E., E.D., G.T.K., G.G., Literature Search: D.T., Ç.Ö., Writing: D.T., Ç.Ö., Critical Review: D.T., Ç.Ö., A.B., S.E., E.D., G.T.K., G.G.

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