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Original scientific paper

The effects of systemic adropin administration on biochemical and morphological effects in diabetic nephropathy: a rat model study

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ABSTRACT

The aim of the study was to investigate the biochemical and morphological effects of systemic adropin administration on renal tissue in streptozotocin-induced diabetic nephropathy in rats. The animals were divided into 4 groups as follows: control, adropin, diabetes, diabetes+adropin. The diabetic groups received a single dose of intraperitoneal streptozotocin (65 mg/kg). The fasting blood glucose level for diabetes was defined as at least 250 mg/dl. Eight weeks after administration of streptozotocin and/or the streptozotocin solvent to the adropin groups, 450 nmol/kg adropin was administered twice daily as an intraperitoneal injection for 10 days. The results of our study indicate that adropin administration has a significant impact on the adverse effects of diabetes on renal tissue. In particular, adropin administration resulted in a notable reduction in the high tissue total oxidant status, glomerular diameter, collagen fiber density, and vascular endothelial growth factor immunoreactivity observed in the diabetic group. These findings suggest that adropin has the potential to be a valuable therapeutic agent in reducing oxidative stress and improving histological and immunohistochemical changes in diabetic nephropathy.

Key words: adropin; diabetes; diabetic nephropathy; oxidative stress; immunohistochemical analyses

Introduction

Diabetes mellitus (DM) encompasses a spectrum of metabolic disorders marked by persistent hyperglycemia, stemming from compromised insulin secretion and insulin resistance. The global incidence of DM is rising, significantly impacting healthcare expenditures worldwide (ZİMMET et al., 2014; PETERSMANN et al., 2019). The primary contributors to morbidity and mortality among individuals with DM are macrovascular complications, including atherosclerosis, peripheral vascular diseases, and coronary artery disease, alongside microvascular complications, such as: nephropathy, neuropathy, and retinopathy (DAS EVCİMEN

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and KİNG, 2007). Diabetic Nephropathy (DN) is one of the most common and serious microvascular complications of DM (HAN et al., 2017). DN is characterized by progressive urinary albumin excretion, increased blood pressure and decreased glomerular filtration rate (FLYVBJERG, 2017). Functional changes are associated with structural changes and ultra-structural changes, such as: apoptosis, thickening of the glomerular basal membrane (GBM), mesangial growth, tubulo interstitial fibrosis, and glomerulosclerosis (FAN et al., 2017; DAİ et al., 2017). In the pathogenesis of DN, inflammatory cytokines, such as: tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), adhesion molecules, enzymes and insulin growth factor (IGF), and transforming growth factor- β (TGF-β), are also known to play a role (DONATE-CORREA et al., 2015). It has been shown that there is an increase in TNF-α levels in diabetic rat kidney tissue, and in the serum and urinary concentrations in patients with DN (LİM and TESCH, 2012; DONATE-CORREA et al., 2015). TNF-α triggers local inflammation by increasing inflammatory cytokine cascades and vascular permeability. In addition, it causes, nuclear factor kappa B (NF-κB) activation. It has cytotoxic properties on glomerular, mesangial, epithelial cells, and can cause renal damage (ELMARAKBY and SULLİVAN, 2012). Another factor whose levels change in DN is vascular endothelial growth factor (VEGF). VEGF plays an important role in endothelial-dependent vasodilation, increased vascular permeability, and the survival, differentiation and proliferation of endothelial cells. In humans, the level of VEGF in the DN decreases in concert with the loss of podocytes, the main VEGF-A producers in the kidneys. In contrast, VEGF-A levels are increased in rodents. This difference is due to the different features in the kidney phenotypes of the two species (MAJUMDER and ADVANİ, 2017). In human and animal studies, it has been demonstrated that the presence of DM increases the activity of oxidant enzymes and reactive oxygen species (ROS) production, while simultaneously decreasing the activity of antioxidant enzymes. Oxidative stress, defined as a state of imbalance between the production of ROS and the antioxidant capacity of the body, has been demonstrated to reduce cell proliferation, cause DNA damage, and is associated with morphological changes in renal tissue in DM. As a consequence of these morphological changes, including thickening of the GBM, tubular basement membrane (TBM) and mesangial tissue, de-hyalinization of afferent and efferent arteri- oles, and glomerulotubular junction abnormalities, functional changes occur, including proteinuria, progressive microalbuminuria and a decreased glomerular filtration rate (BHATTİ and USMAN, 2015). Adropin is a peptide hormone encoded by the energy homeostasis associated (ENHO) gene, generally expressed in the liver, pancreas, heart, renal and central nervous system. It plays a role in insulin sensitivity, energy homeostasis, obesity, and endothelial functions, and stimulates capillary density and angiogenesis (WU et al., 2014; Lİ et al., 2016; BAKA et al., 2016). High plasma lev- els of Adropin reduce insulin resistance and glu- cose intolerance, regulate lipogenesis, and exert an antihepatostatic effect (BAKA et al., 2016). It prevents apoptosis caused by TNF-α, increas- es nitric oxide (NO) release, causes vascular endothelial growth factor receptor 2 (VEGFR2) activation, increases endothelial nitric oxide synthase (eNOS) activity, and protects endothelial barrier function (LOVREN et al., 2010). ZANG et al. (2018) showed that Type 2 DM and obesi- ty reduce adropin levels. The literature states that decreased adropin levels in the liver with obesity cause the deterioration of metabolic homeostasis, including significant insulin resistance and fat- ty liver. Moreover, overexpression of adropin or adropin treatment has been shown to increase glucose utilization, cause hyperinsulinemia, and decrease hepatic steatosis, not only in diet-induced transgenic obese mice, but also in streptozotocin (STZ) induced type 2 diabetic rats (ZANG et al., 2018). Considering the anti-hyperglycemic effects of adropin shown in studies, and its relationship with diseases, the effects of systemic administration of adropin on kidney tissue in DN developing in the diabetes model induced by STZ, we aimed to examine histological (morphologically; GBM thickness, glomerular diameter and collagen fiber density), immunohistochemical (effects on TNF-α

and VEGF immunoreactivities), and biochemical malondialdehyde (MDA), glutathione (GSH) and total oxidant status (TOS) level evaluations.

Materials and methods

Animals, and laboratory conditions. Twen- ty-eight adult male Wistar albino rats, whose weights varied between 200±30 g, obtained from Gazi University Laboratory Animal Raising and Experimental Research Center, were used in this study. The rats were fed freely in separate cages, on a 12 hour dark cycle, above 24±2°C, with stand- ard rat food and tap water. The relative humidity of their environment was kept between 30-45%. The rats were randomly divided into four groups: con- trol group (C, n:7), adropin group (A, n:7), diabetes group (D, n:7), diabetes+adropin group (D+A, n:7).

Induction of diabetes and kidney examination in the study. A single dose of 0,1 M (pH:4.5) STZ (65 mg/kg) dissolved in cold citrate buffer was injected intraperitoneally into the diabetes mod- el group (KO et al., 2018). The control and adro- pin groups were injected with STZ solvent in the same way. After STZ administration, the fasting blood glucose values of the subjects were meas- ured 72 hours after an 8-hour fasting period, and those above 250 mg/dl were included in the diabe- tes group (DE CASTRO et al., 2019; OKESOLA et al., 2020). After the onset of diabetes, a stabili- zation period of 2 weeks was given and during this period, the animals' fasting blood glucose values were checked regularly. All the rats injected with STZ developed diabetes. Development of diabetic nephropathy was observed six weeks after the in- duction of diabetes (SALEH et al., 2011).

Adaptation and adropin treatment. In the control group, eight weeks after the first STZ injection, adropin solvent in 0.9% physiological saline solution was injected intraperitoneally twice a day for 10 days. After the same period, the subjects in the adropin group received adropin treatment at a dose of 450 nmol/kg in 0.9% physiological saline solution twice daily for 10 days. The subjects in the diabetes group did not receive any treatment for 8 weeks after STZ administration. At the end of this period, adropin dissolved in 0.9% physiological saline solution was administered intraperitoneally twice a day for 10 days. Similarly, no treatment was given to the subjects in the diabetes+adropin group for 8 weeks after STZ administration. At the end of this period, adropin at a dose of 450 nmol/kg in 0.9% physiological saline was administered intraperitoneally twice a day for 10 days (AKCİLAR et al., 2016; ALTAMİMİ et al., 2019).

Collection of blood and kidney samples. Twenty-four hours after the last injection in the treatment period, fasting glucose levels were measured, with blood taken from the tail veins of the rats. Afterwards, the rats were sacrificed under intramuscular ketamine (40 mg/kg) and xylazine (5 mg/kg) anesthesia, by taking blood from their hearts. Immediately after euthanasia, the right kidneys were extracted and placed in formalin for histopathological analysis. The left kidney tissues were stored at -80°C for physiological examinations.

Biochemical analyses. GSH was measured using the modified Ellman method. It was expressed as µmol/gr tissue using the GSH 13,600 mol^{-1} cm^{-1} coefficient (AYKAÇ et al., 1985). MDA measurement was made by applying the thiobarbituric acid reaction model. MDA levels were expressed as nmol/gram tissue (CASİNİ et al., 1986). Total oxidant levels were measured with the TOS Assay Kit (RelAssayDiagnostics, Turkey) and the results were given in µmol/L.

Histological analysis. The renal tissues were fixed in 10% formalin and histological slides were prepared according to the standardized pro- tocol, and the sections were cut at 4 µm using an RM 2145 microtome (Leica, Mussloch, Germany) (AKARCA-DİZAKAR et al., 2018). The slides stained with hematoxylin and eosin (H&E) for a diameter of glomeruli (TARLADACALİSİR et al., 2008), Masson's trichrome (MT) for collagen fiber density (WANG et al., 2019) and Periodic acid–Schiff (PAS) stain for GBM thickness (CHANG et al., 2011). After staining, the slides were evaluated using a DM 4000 light microscope and Leica LAS 4.9 v program.

Immunohistochemical analysis. For immunohistochemistry, 4 µm sections were deparaffinized and incubated with pH 6.0 citrate buffer (Lab Vi-

sion, Thermo Scientific, Fremont CA) for antigen retrieval and after that treated with 3% hydrogen peroxide (Lab Vision, Thermo Scientific), then with Ultra V block (Lab Vision, Thermo Scientific). The sections were incubated overnight with primary antibodies. The primary antibodies used were TNF alpha polyclonal antibody (bs-2081R, Bioss) and VEGF-A polyclonal antibody (bs-1957R, Bioss). All antibodies were diluted to 1:100 for incubation for 24h at 4°C in a humid chamber. After washing in phosphate-buffered saline (PBS), secondary antibody, anti-mouse IgG anti-rabbit IgG (TP-125-BN, Lot: PBN100121; Lab Vision, Thermo Scientific) was applied for 10 min. After washing with PBS, the sections were incubated with 3,3′-diaminobenzidine tetrahydrochloride (DAB) solution (90824B; Spring Bioscience, Pleasanton, CA), counterstained with Mayer's hematoxylin (Zymed Laboratories, South San Francisco, CA) and mounted with entellan (Merck, Darmstadt, Germany). TNF-α and VEGF-The staining was analyzed using a computer imaging system (DM 4000, Leica LAS 4.9 v program, Wetzlar, Germany). The quantitative analysis of immunoreactivity (%) in the images was done using Image J software (NIH, USA) (MİLAGRES et al., 2018).

Statistical analysis. The data we obtained in our study were evaluated with the non-parametric Kruskal-Wallis and Mann Whitney U tests using the IBM SPSS 22 statistical program. The results are given as mean and standard deviation.

Results

Tissue total oxidant levels. The diabetes group exhibited a statistically significant increase in total oxidant levels compared to the control and other treatment groups. This elevation in TOS levels is indicative of heightened oxidative stress in diabetic conditions. Remarkably, the administration of adropin significantly reduced the TOS levels in the diabetic group to levels comparable to the control group, suggesting the potent antioxidative effect of adropin in mitigating oxidative stress induced by diabetes (Table 1).

Tissue MDA levels. MDA, a biomarker of lipid peroxidation and oxidative damage, was found to be significantly higher in the tissue samples from the diabetic group compared to the control group. While there was a slight reduction in MDA levels following adropin treatment, this decrease was not statistically significant across the groups (Table 1). This indicates that while adropin may help in reducing lipid peroxidation to some extent, the effect was not substantial enough to show significant differences in MDA levels among the groups.

Tissue GSH levels. GSH, a critical antioxidant, was significantly lower in the diabetic group than in the other groups. However, in the diabetes+adropin group GSH levels were higher than in the diabetes group alone, indicating a trend towards normalization. Despite this upward trend, the differences in GSH levels between the groups did not reach statistical significance. This finding suggests that while adropin has the potential to enhance antioxidant defenses, the variation in GSH levels was not significant enough to demonstrate a statistically meaningful difference (Table 1).

Histological and histomorphological findings. The kidneys of the control and the adropin groups had the typical histological structure. The glomer-

Table 1. Tissue TOS, MDA, GSH levels

	Control	Adropin	Diabetes	Diabetes+adropin
TOS (μmol/L)	19.32±2.81 [a*]	18.92±4.22 [a**]	28.24±8.41	18.71±4.38 [a***]
MDA (nmol/g tissue)	11.31±2.24	19.95±3.05	15.82±4.57	13.62±4.82
GSH (μmol/g tissue)	2.87±0.36	2.97±0.35	2.62±0.3	2.80±0.3

Values are expressed as mean±SD for seven rats in each group

[a] Compared with diabetes group; *P=0.024, ** P=0.017, *** P=0.015

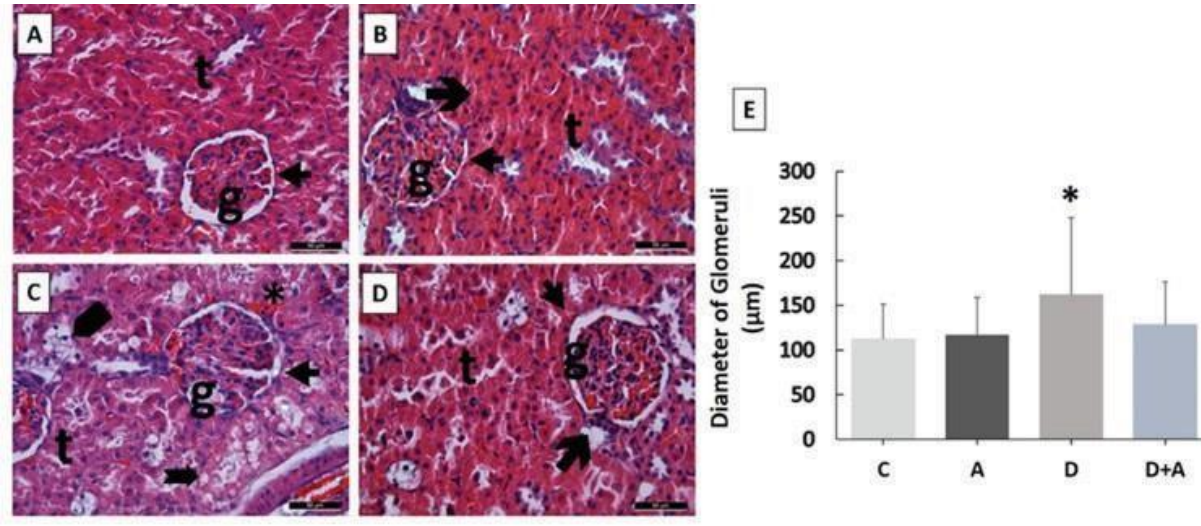

Fig. 1. Histological analysis revealed pathological changes in the kidneys of the experimental groups

Glomeruli (g), tubules (t), renal corpuscle (A), macula densa (A), congestion (*), vacuolization (C), hydropic changes (A) in tubular epithelial cells. (A) C group; (B) A group; (C) D group; (D) D+A group (H&E), Magnification, X400, (E) Diameter of glomeruli. Data are presented as the mean ± standard deviation. *P<0.001 compared with the control and adropin groups.

uli and tubules were normal (Fig. 1A and 1B). In the diabetes group, degenerative changes were observed in the kidneys. Glomerular dilation and congestion were among the findings observed. In addition to hydropic changes and necrosis in tubular epithelial cells, vacuolization, inflammatory cell infiltration, and lipid accumulation were noteworthy. Dilatation and necrosis were observed in some tubules (Fig. 1C). In the diabetes+adropin group, some dilated tubules and hydropic degeneration remained in the cortex, and the typical histological structure of the tubules was observed in the some areas (Fig. 1D).

When the glomerular diameter between groups was evaluated, it was noted that there was a significant increase in glomerular diameter measurement in the diabetes group compared to the control and adropin groups (respectively; P<0.001, and P<0.001) (Fig. 1E). There was no significant difference between the control and adropin groups (P=1.000) (Fig. 1E). Masson's Trichrome stain was used to evaluate the collagen density differences in the kidney tissues of the experimental groups (Fig. 2A-D). No fibrotic changes were observed in the cortex as a result of staining applied to the kidney tissue sections of the control and adropin groups (Fig. 2A and 2B). Fibrotic changes, which are associated with increased collagen fiber density, were observed in glomerular and intertubular areas in the diabetes group. It was noted that the density of collagen in the cortex decreased in the diabetes+adropin group compared to the diabetes group (Fig. 2D and 2E).

The percentage of green areas in the kidney sections of the experimental groups was evaluat- ed using the ImageJ program (Java-based software program, National Institutes of Health). When the data obtained were compared statistically, the density of collagen fiber in the diabetes group was significantly higher than in the other groups: control (P<0.001), adropin (P<0.001) and diabetes+adropin (P<0.001) (Fig. 2E). There was no statistical significance between the adropin and control groups (P=1.000) (Fig. 2E). Moreover, collagen fiber density was found to have diminished significantly in the diabetes+adropin group as compared to the diabetes group (P<0.001) (Fig 2E).

It was noted that after the PAS staining performed to evaluate the GBM thickness of the kidneys (Fig. 3A-D), the basal membrane thickness

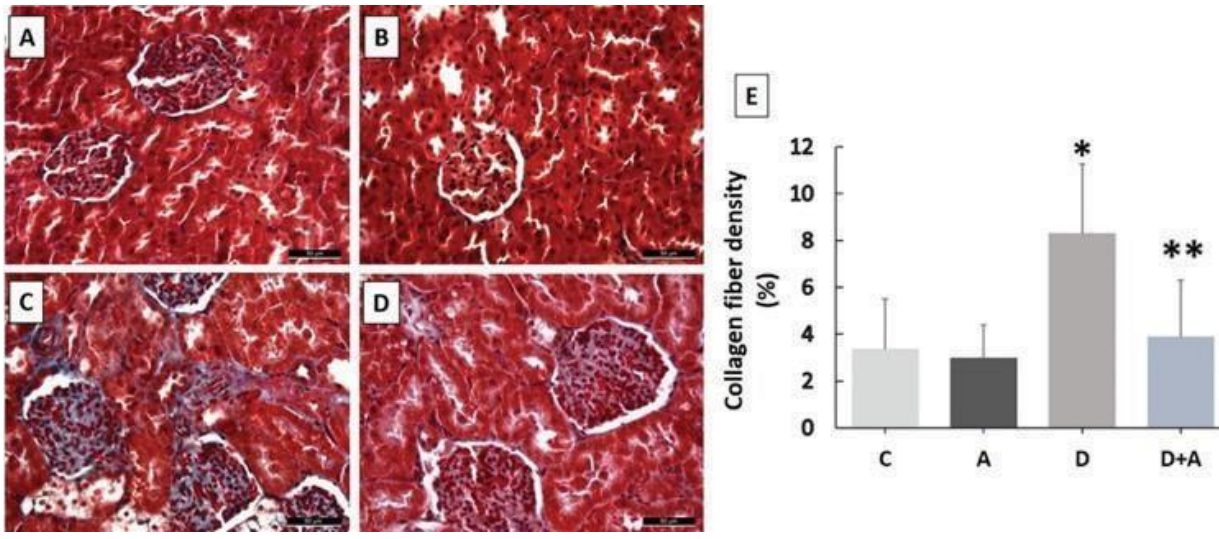

Fig. 2. Representative photomicrographs of Masson's trichrome staining

(A) C group; (B) A group; (C) D group; (D) D+A group (MT, Magnification, X400), (E) collagen fiber density. Data are presented as the mean ± standard deviation. *P<0.001 compared with the other groups. **P<0.001 compared with the diabetic group.

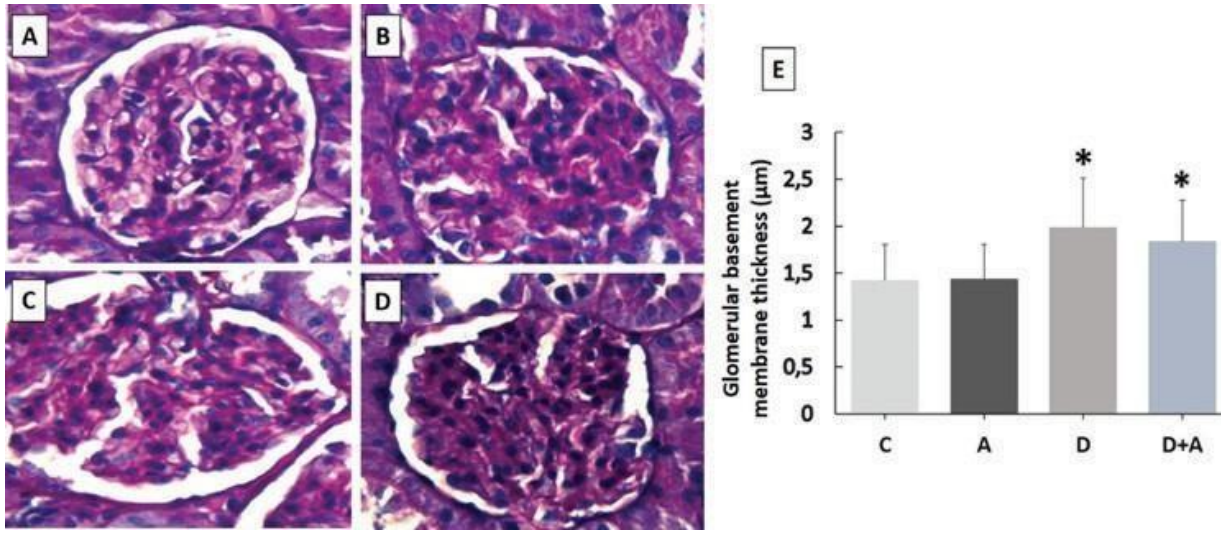

Fig. 3. Representative photomicrographs of PAS staining

(A) C group; (B) A group; (C) D group; (D) D+A group (PAS, magnification, X400), (E) GBM thickness (µm). Data are presented as the mean ± standard deviation. *P<0.001 compared with the control and adropin groups.

in the diabetes group showed a statistically significant increase compared to the control and adropin groups (P<0.001). (Fig. 3E). Similarly, in the diabetes+adropin group, basal membrane thickness was significantly higher than in the control and adropin groups (P<0.001).

Immunohistochemical findings. The TNF-α immunopositivity percentage was significantly higher in the diabetes and diabetes+adropin groups compared to the control and adropin groups (respectively; P<0.001, and P<0.001). It was observed that the percentage of TNF-α immunoreactivity

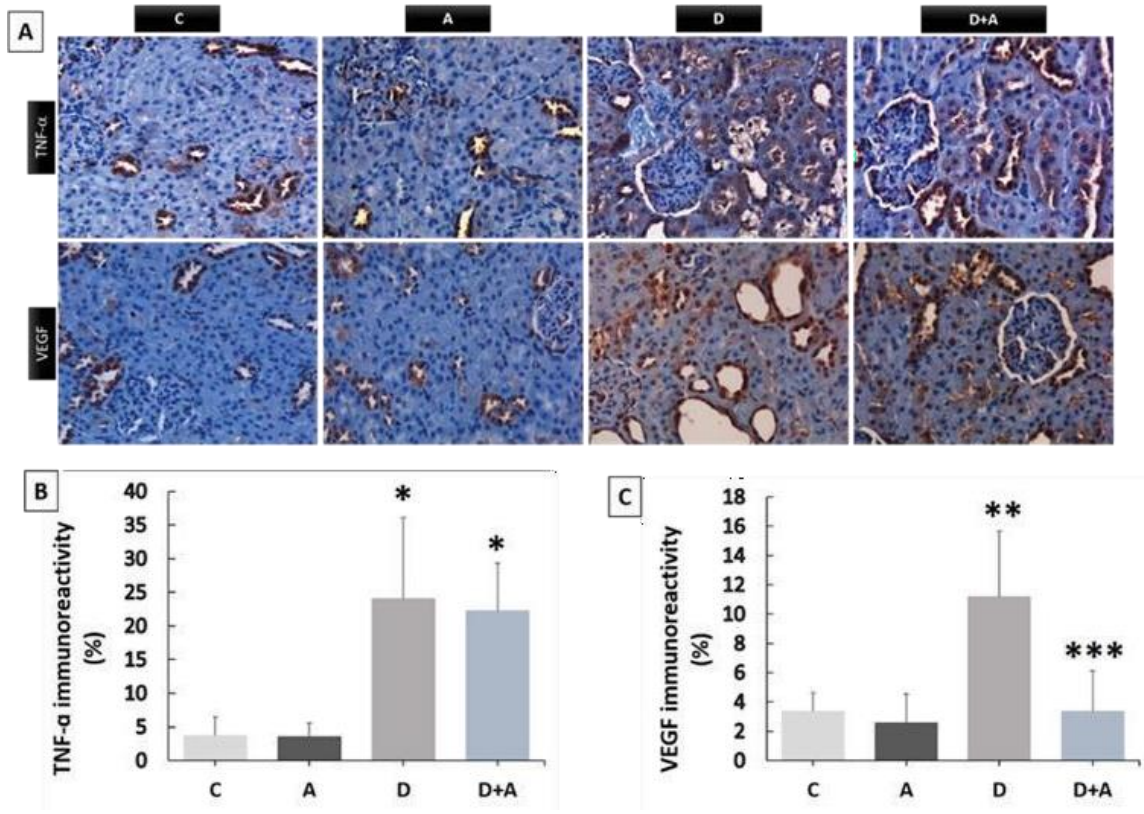

Fig. 4. Representative photomicrographs of immunohistochemistry of TNF-α and VEGF

(A) (DAB & Hematoksilen, Magnification X400). (B) TNF-α immunoreactivity; *P<0.001 compared with the control and adropin groups. (C) VEGF immunoreactivity; **P<0.001 compared with the other groups. ***P<0.001 compared with the diabetes group.

decreased in the diabetes+adropin group compared to the diabetes group, but this decrease was not statistically significant (P=1.000) (Fig. 4A and B). A comparison of the percentage of VEGF immunopositivity between groups revealed a significantly higher percentage of VEGF-positive areas in the diabetes group compared to all other groups: control (P<0.001), adropin (P<0.001) and diabetes+adropin (P<0.001). Lower VEGF immunoreactivity was found in the diabetes+adropin group compared to the diabetes group (P<0.001) (Fig. 4A and 4C).

Discussion

It is well established that oxidative stress and inflammation play a pivotal role in the pathogenesis of diabetes and DN. Oxidative stress impairs cell proliferation, induces DNA damage, and is associated with morphological changes in renal tissue in diabetes (MAGEE et al., 2017; LİGUORİ et al., 2018). In our study, renal tissue TOS levels were found to be significantly increased in the diabetes group compared to the control group, and this finding is consistent with the literature. It was reported that tissue TOS levels increased and antioxidant levels decreased in rats with DN (HAZMAN and BOZKURT, 2015). This is consistent with other literature showing that MDA levels, a biomarker of oxidative stress and lipid peroxidation, were higher and GSH levels were lower in the kidney tissues of STZ-induced diabetes model rats compared to non-diabetic control groups (RODRÍGUEZ-CARRİZALEZ et al., 2014; BARMAN et al., 2018). In our study, MDA levels increased, and GSH lev- els decreased in the diabetes group compared to the control group; however, these changes did not

reach statistical significance. In DN, significant alterations in cytokine levels and structural deterioration in renal tissue are commonly observed. Studies have shown that diabetic kidney tissue exhibits swelling, desquamation, congestion, and microvillus loss in intertubular cells, along with elevated levels of TNF-α in both renal tissue and serum (GUPTA et al., 2015; YANG et al., 2016; MALİK et al., 2017; TUTUN et al., 2019). Consistent with these findings, our study demonstrated that the diabetic group without adropin treatment showed congestion, intertubular inflammation, and significantly higher TNF-α immunoreactivity compared to the control group, indicating impaired venous return. In our study, we created a model of Type 2 DM and then DN with STZ application. The findings from the diabetes group, which did not receive adropin, demonstrate that we were successful in creating DN in accordance with the studies in the literature. One critical factor in the development of DN is VEGF. Podocytes, which are the prima- ry source of VEGF in the kidneys, play a crucial role in the formation and maintenance of the GBM. In the early stages of DN, VEGF levels tend to increase, contributing to endothelial cell damage and increased glomerular permeability. In contrast, in the later stages of DN, VEGF production decreases as podocytes are lost, leading to glomerulosclerosis and a loss of glomerular integrity (MAJUMDER and ADVANİ, 2017; HALLER et al., 2017). Our results are consistent with previous studies showing elevated VEGF levels in diabetic groups compared to controls (HUANG et al., 2015). Morphological changes are also a hallmark of DN. These include glomerular and tubulo-interstitial fibrosis, increased glomerular volume and diameter, arterial hyalinosis, thickening of the GBM, and the formation of Kimmelstiel-Wilson nodules (KİTADA et al., 2016). In line with these characteristics, our study observed dilatation and necrosis in the glomeruli, medulla, and collecting ducts of diabetic rats. Additionally, we noted thickening of the vascular walls, increased glomerular diameter, GBM thickness, hydropic changes in tubule epithelial cells, vacuolization, lipid accumulation, and fibrosis. Some tubules also showed dilatation and necrosis, all indicative of DN. Adropin is a metabolic hormone produced in various organs, including the liver, brain, human umbilical vein and coronary artery endothelial cells, pancreas, and the kidneys. It plays a role in regulating lipid and glucose metabolism. In various studies, it has been demonstrat- ed that adropin levels are decreased in individuals with coronary artery disease, diabetes, metabolic syndrome and obesity. Furthermore, adropin levels are negatively correlated with the severity of coronary artery disease, body-mass index, age, insulin and homocysteine levels (AYDİN et al., 2013; YOSAEE et al., 2017; SATO et al., 2018).

In several studies, the administration of adropin to diabetic groups has been shown to result in a number of beneficial effects. These include an increase in glucose tolerance, an increase in insulin-induced GLUT4 receptor expression, a decrease in insulin resistance, a decrease in serum total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), alanine transaminase, aspartate transaminase, serum glucose and HbA1c values, and a decrease in increased TNF-α and inducible nitric oxide synthase levels in pancreatic tissue (GAO et al., 2015; AKCİLAR et al., 2016). In our study, we observed that systemic adropin administration at a dose of 450 nmol/kg for 10 days, caused a significant decrease in TOS levels, and a decrease in VEGF levels, which increased with diabetes. We did not detect a significant difference in TNF-α levels. In addition to the differences in these molecular levels, we observed a decrease in glomerular fiber density, GBM thickness, and glomerular diameter in diabetic animals treated with adropin compared to the diabetes group and in some areas of the renal cortex of the diabetes+adropin group, tubules with dilated and hydropic changes continued to exist. In contrast, we observed decreased inflammation in the interstitial area and even the standard histological structure of tubules and glomeruli in some areas. One of the most notable findings of our study was the appearance of the collecting ducts located in the medulla, which was close to that of the control group, and the decreased inflammation in the interstitial area. The existing literature on adropin is limited, with studies generally focusing on adropin levels. Our study addresses this gap by examining the potential effects of adropin. Our findings strongly emphasize that

exogenously administered adropin has the potential to significantly ameliorate diabetes-induced damage in kidney tissue. These results have the potential to inform the development of new and effective approaches for the treatment of DN.

Conclusions

Systemic administration of adropin has been demonstrated to significantly reduce the elevated levels of VEGF and TOS associated with diabetes. Additionally, it has been observed to induce a slight decrease in MDA and TNF-α levels, indicating a beneficial effect on diabetes-induced oxidative damage and inflammation. Adropin shows therapeutic effects against inflammatory and morphological changes, such as necrosis, inflammation, fibrosis, thickening of the GBM, and the increased glomerular diameter observed in DN. Evaluating the results of our study, we can say that adropin has positive effects on the increased oxidative stress and pathological structural changes caused by diabetes in renal tissue. Preventing the serious complications associated with DM, improving the quality and duration of patients' lives, and reducing the costs of treatment are crucial. Our study shows the positive effects of adropin application on renal tissue, one of the most commonly affected organs in DM. It suggests that adropin may be a future treatment option for diabetes complications.

Ethics approval

The in vivo experiments in the study were conducted at the Gazi University Laboratory Animal Raising and Experimental Research Center, and the Gazi University Faculty of Medicine Physiology Department laboratory, under the approval of the Gazi University Animal Experiments Local Ethics Committee, with the code number G.U.ET-18.064.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgement

The in vivo experiments of the study were carried out at Gazi University Laboratory Animal Raising and Experimental Research Center and Gazi University Faculty of Medicine Physiology Department laboratory, with the permission of Gazi University Animal Experiments Local Ethics Committee, code number of G.Ü.ET-18.064. We are also thankful to the Gazi University Laboratory Animal Raising and Experimental Research Center in this study.

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SAŽETAK

Cilj rada bio je istražiti sistemsku primjenu adropina na biokemijska i morfološka svojstva bubrežnog tkiva kod dijabetičke nefropatije izazvane streptozotocinom u štakora. Životinje su podijeljene u četiri skupine: kontrolnu skupinu, skupinu kojoj je primijenjen adropin, skupinu koja je imala dijabetes i skupinu s dijabetesom kojoj je dan adropin. Štakori sa dijabetesom primili su pojedinačnu dozu intraperitonealno primijenjenog streptozotocina (65 mg/kg). U uvjetima kad su životinje bile natašte određena je najmanja razina glukoze od 250 mg/dL. Osam tjedana nakon primjene streptozotocina i/ili otopine streptozotocina, u skupinama s adropinom primjenjivano je 450 nmol/kg adropina, dva puta dnevno, intraperitonealno tijekom 10 dana. Rezultati istraživanja pokazali su znakovit utjecaj primjene adropina u slučaju oštećenja bubrežnog tkiva dijabetesom. Primjenom adropina u skupini jedinki s dijabetesom znatno su smanjeni ukupni oksidacijski status tkiva, glomerularni promjer, gustoća kolagenskih vlakana i imunoreaktivnost vaskularnog endotelnog faktora rasta. Ovi rezultati pokazuju da je adropin potencijalno vrijedno terapijsko sredstvo u smanjenju oksidacijskog stresa te poboljšanju histoloških i imunohistokemijskih promjena kod dijabetičke nefropatije.

Ključne riječi: adropin; dijabetes; dijabetička nefropatija; oksidacijski stres; imunohistokemijska analiza