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Protective Effects of Rosuvastatin on Kidney in Experimental Hypertension Rats Models

Deneysel Hipertansiyon Oluşturulan Sıçanlarda Rosuvastatinin Böbrek Üzerine Koruyucu Etkileri

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ÖZ

Amaç: Hipertansiyon, son dönem böbrek hastalığına ulaşan hastaların yaklaşık %30'undan sorumludur. Statin tedavisinin böbrek hastalığı gelişme riskini azalttığı bilinmektedir. Bu çalışmada, L-arginin analoğu N-Nitro-L-Arjinin Metil Ester (L-NAME) kullanılarak hipertansiyon oluşturulmuş sıçanların böbrek dokusunda kaspaz-3 ve fibrillin1 (FBN1) üzerindeki değişikliklere bakılarak, hipertansiyonun bu moleküller üzerinde oluşturduğu değişiklikleri rosuvastatinin ne şekilde etkilediği araştırıldı.
Araçlar ve Yöntem: Çalışmada, 200-220 g ağırlığında 18 adet Wistar Albino erkek sıçan kullanıldı. Sıçanlar her grupta 6 hayvan olacak şekilde 3 gruba ayrıldı (1.Kontrol, 2.Hipertansiyon, 3.Rosuvatatin). Hipertansiyon oluşturmak için sıçanlara 7 hafta boyunca nitrik oksit sentaz (NOS) inhibitörü L-NAME içme suyuna karıştırılarak verildi. İkinci hafta sonrasında rosuvastatin (10 mg/kg/gün) 5 hafta boyunca oral gavaj ile verildi. Kan basıncı değerleri 0, 14, 28 ve 42. günlerde tail-cuff yöntemi kullanılarak değerlendirildi. Deney bitiminde tüm sıçanlar dekapite edilerek böbrek dokularında kaspaz-3 ve FBN1 düzeyleri immunohistokimyasal yöntemle değerlendirildi.
Bulgular: Kan basınçları hipertansiyon grubunda kontrolle kıyaslandığında 14, 28, 42. günlerde anlamlı düzeyde yüksek bulundu (p=0.001). Rosuvastatin 28. ve 42. günde anlamlı olmayan bir azalmaya sebep oldu. Kontrol grubuyla kıyaslandığında hipertansiyon grubunda kaspaz-3 (p=0.001) ve FBN1 (p=0.001) immünreaktivitesinin istatistiksel düzeyde anlamlı bir seviyede arttığı görüldü. Hipertansiyon grubu ile kıyaslandığında rosuvastatin verilen grupta ise, kaspaz-3 (p=0.031) ve FBN1 (p=0.030) immünreaktivitesi anlamlı azaldı. Ancak, kontrol grubuna göre rosuvastatin grubunda kaspaz-3 (p=0.036) ve FBN1 (p=0.041) immünreaktivitesinin arttığı izlendi.
Sonuç: Rosuvastatin kan basınçlarını anlamlı olarak düşürmese de, hipertansif böbrekler üzerine koruyucu etkisinde kaspaz-3 ve FBN1'in aracı olabileceği düşünülmektedir.

Anahtar Kelimeler: fibrilin-1; kaspaz-3; l-name; statin

ABSTRACT

Purpose: Hypertension is responsible for approximately 30% of patients who reach end-stage renal disease. Statin is known to reduce the risk of developing kidney disease. In this study, the changes in caspase-3 and fibrillin1 in the kidney tissue of rats with hypertension using L-NAME were investigated along with how rosuvastatin affected the changes caused by hypertension on these proteins.
Materials and Methods: 18 Wistar Albino male rats weighing 200-220 g were used in the study. The rats were divided into 3 groups with 6 animals in each group (1.Control, 2.Hypertension, 3.Rosuvastatin).To induce hypertension, rats were given L-NAME for 7 weeks. After the second week, rosuvastatin was given by oral gavage for 5 weeks. Blood pressure values were evaluated on days 0, 14, 28, 42. At the end of the experiment, all rats were sacrificed and caspase-3 and fibrillin1 levels were evaluated.
Results: Blood pressures were found to be higher in the hypertension group on the 14th, 28th, and 42nd days (p=0.001). Rosuvastatin caused a decrease that was found to be insignificant at 28th, 42nd days. Caspase-3 (p=0.001), fibrillin1 (p=0.001) immunoreactivity were found to be increased in the hypertension group. Compared with the hypertension group, caspase-3 (p=0.031), fibrillin1 (p=0.030) immunoreactivity were decreased in the rosuvastatin group. However, caspase-3 (p=0.036) and fibrillin1 (p=0.041) immunoreactivity was increased in the rosuvastatin group compared to the control group.
Conclusion: Although rosuvastatin didn't significantly decrease blood pressure, it is thought that caspase-3 and fibrillin1 may mediate its protective effect on hypertensive kidneys.

Keywords: caspase-3; fibrillin-1; l-name; statin

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INTRODUCTION

Hypertension, a cardiovascular disease, manifests itself with a continuous increase in systemic arterial blood pressure, causing irreversible changes in the heart and arteries over time, leading to important cardiovascular complications such as acute myocardial infarction, left ventricular hypertrophy, congestive heart failure, stroke, progressive kidney failure, retinopathy, and dissecting aortic aneurysm.[1] It has been reported that vasoactive mediators, endocrine factors, neural reflexes, oxidative stress, and endothelial dysfunctions in the vessels may be effective in the development of hypertension, as well as functional changes in the sympathetic nervous system and renin-angiotensin-aldosterone system.[2] Despite the presence of various preventive and therapeutic approaches, hypertension is still a disease that maintains its importance worldwide.[3,4] Studies showing that cholesterol-lowering agents can reduce cardiovascular complications have gained momentum and rosuvastatin has anti-inflammatory, antiproliferative, antithrombotic, and antiatherogenic effects, improving endothelial dysfunction by reducing reactive oxygen production and increasing eNOS expression.[5]

Complications related to hypertension are very vital and kidney disease caused by ischemia is reponsible for approximately 30% of end-stage renal disease patients.[6] Various mechanisms have been implicated in the hypertensive kidney (e.g., oxidative stress, apoptosis, inflammation, renin-angiotensin-aldosterone system (RAAS), kidney remodeling, and fibrosis), which impair mitochondrial integrity and function.[5,7] However, the molecular mechanism responsible for kidney damage has not been fully elucidated yet.

Fibrillin1 (FBN1) is a 350-kDa glycoprotein found in many tissues such as fibrillin microfibrils, the ciliary layer of the eye, and the kidney glomerulus. Since it is flexible, the microfibrils of the elastic lamella are also effective in the expansion of the aorta.[8] FBN1 is thought to affect the maintenance of wall integrity of central arteries and is a candidate protein for arterial stiffness.[9,10] Caspase-3, on the other hand, is an effective protein in the apoptotic pathway and is activated in response to cytotoxic drugs and plays a key role in tubular epithelial damage due to renal I/R.[11,12]

In this study, caspase-3 and FBN1 levels in kidney tissue of rats with experimental hypertension given N-Nitro-L-Arginine Methyl Ester (L-NAME) and the effects of rosuvastatin on these proteins were investigated.

MATERIALS and METHODS

Chemicals

L-NAME was purchased from Sigma Aldrich (Inc.St. Louis, MO. U.S.A). Rosuvastatin (Rosuvas 10 mg Bilim Pharmaceuticals Industry and Trade Inc.) was dissolved in distilled water.

Animals and Treatments

The study was approved by Adıyaman University Experimental Animals Ethics Committee (28.04.2022-2022/026). A total of 18 Wistar Albino male rats with 200-220 g weight were housed under constant humidity and temperature conditions. Rats were maintained on a 12-hour light/dark cycle with standard laboratory rat chow. No animal deaths occurred during the study. Experiments were done in line with the Manual for the Care and Use of Laboratory Animals (DHEW Publication (NIH) 8523, 1985).

The rats were divided into 3 groups (6 in each group). For the development of hypertension, L-NAME 40mg/kg/day was given with drinking water.[11,13]

I) Control group: The rats were not exposed to any drug administration; **II) Hypertension group:** L-NAME was administered for 7 weeks; **III) Rosuvastatin group:** L-NAME was for 7 weeks, plus rosuvastatin was given by oral gavage once daily (10mg/kg/day) for the last 5 weeks.[14-16]

Blood Pressure Measurements

The tail-cuff method was used to measure the systolic blood pressure (SBP) (Noninvasive Blood Pressure Measurement System (May, NIBP250) on experimental days 0, 14, 28, and 42. A total of 5 measurements were

made for each rat and then the average of these measurements was taken.[11,13]

Surgical Applications

At the end of the 7th week, the subjects were decapitated. The abdomen and thorax were opened rapidly from the midline by taking blood samples. Kidney tissues were separated for histological studies, the remaining tissues were stored at -80ºC.

Immunohistochemical Analysis

According to the immunohistochemical staining method, the Avidin-Biotin-Peroxidase (ABC) complex was applied with minor changes.[17,18] Sections of 4-6 µm thickness were taken from the tissues blocked with this method and deparaffinized.

Primary antibodies caspase-3 (Rabbit polyclonal IgG, Abcam, ab184787, London, UK) and anti-FBN1 (Rabbit polyclonal IgG, Bios, bs 157R Bio Science, Inc. USA) diluted 1/200 with the Thermo Scientific™ TP-015-HA commercial kits were used.

After AEC Chromogen was applied, staining was done with Mayer Hematoxylin and examined under a light microscope. The prepared preparations were monitored and photographed with Leica DM500 microscope (Leica DFC295).

Histoscore was established based on the prevalence (0.1: <25%, 0.4: 26-50%, 0.6: 51-75%, 0.9: 76-100%), and severity (0:no, +0.5: very little, +1: little, +2: moderate, +3:severe) of immunoreactivity in staining.

Histoscore = prevalence x severity

Statistical Analysis

Statistical analyses were performed using the SPSS software, version 25.0 (IBM Inc., Chicago, IL). Numerical data were given as median (minimum-maximum). The Shapiro-Wilk test was employed to examine whether the variables were normally distributed. The Kruskal-Wallis test was employed for overall comparison between more than two groups. Mann Whitney U test was used after Kruskal Wallis for comparison between paired groups. Statistical differences for blood pressures were calculated with the "one-way ANOVA" test in independent groups. Paired t test was used to evaluate the difference between the values of the same group at different time points. A value of $p<0.05$ was taken statistically significant.

Sample Size

In this study, the G power program ANOVA fixed effects procedure was used to calculate the sample sizes of the groups. When the effect size :0.950, statistical power (1 - β) : 0.90 and the significance level of 0.05 were accepted as bidirectional, the real power was determined as 0.90 and 6 subjects for each group, a total of 18 subjects.

RESULTS

Blood Pressures

SBP significantly increased on day 14 in groups given L-NAME (p=0.001). No significant differences were detected between the 14th, 28th, and 42nd-day blood pressures in these groups. SBP decreased in the rosuvastatin treatment group on days 28 and 42, although not significantly (Table 1).

Table 1. Systolic blood pressure (mmHg) at days 0, 14, 28, and 42.

Groups	0th day	14th day	28th day	42nd day
Control	104.43±6.45	109.43±7.66	106.43±5.44	106±6.11
Hypertension	102.87±7.64	144.14±6.09a	147.12±4.73a	148.37±12.66a
Rosuvastatin	105±8.04	147.17±2.64a	142±3.46a	133.83±9.02a

Data are expressed as mean ± SD. **a.** Significant difference compared to control $p<0.05$.

Histological Findings

As a result of the observation of immunohistochemical staining for Caspase-3 and FBN1 immunoreactivity in kidney tissue under light microscopy;

Caspase-3 immunoreactivity; A statistically significant increase was found in hypertension (Figure 1b) (p=0.001) and rosuvastatin (Figure 1c) (p=0.036) groups when compared to the control (Figure 1a) group. Compared with the hypertension group, caspase-3 immunoreactivity

was statistically significantly decreased in the rosuvastatin group (p=0.031).

Caspase-3 immunoreactivity histoscoresfor all three groups are shown in Table 2.

Table 2. Caspase-3 and FBN1 Immunreactivity histoscore.

Groups	Caspase-3 Immunoreactivity Median (min-max)	FBN1 Immunoreactivity Median (min-max)
Control	0.30 (0.20-0.45)	0.20 (0.20-0.40)
Hypertension	2.70 (1.80-2.70)	2.70 (1.20-2.70)
Rosuvastatin	0.80 (0.45-0.90)	0.60 (0.40-0.90)
P*	<0.001	<0.001

Values are given as median and min-max.
[a] Compared to the control group,
[b] Compared with the hypertension group (p<0.05)
*:Kruskal-Wallis Test

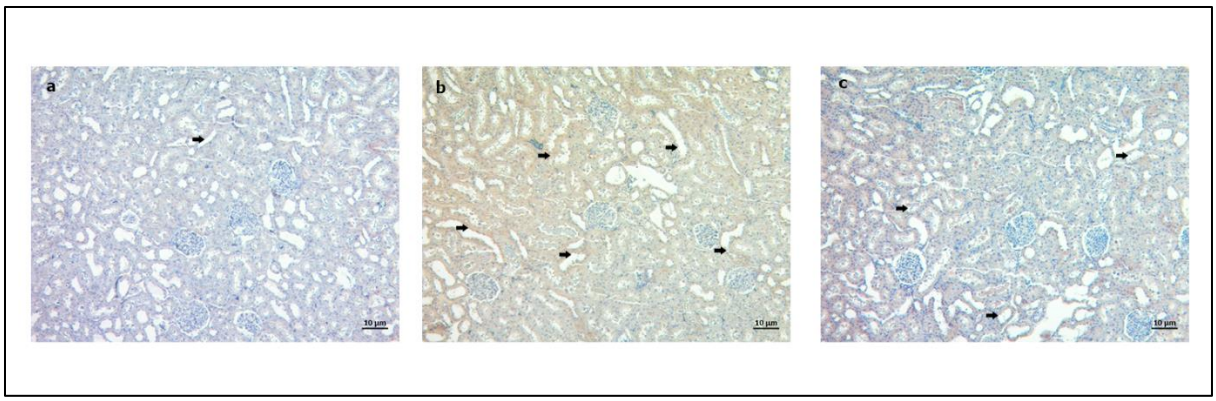

Figure 1. Immunohistochemical staining for Caspase-3 (black arrow) in kidney tissue.
a- Control group b- Increased Caspase-3 immunoreactivity in the hypertension group
c- Decreased Caspase-3 immunoreactivity of the rosuvastatin group

Regarding FBN1 immunoreactivity, it was found to be statistically significantly increased in hypertension (Figure 2b) (p=0.001) and rosuvastatin (Figure 2c) (p=0.041) groups when compared to the control (Figure 2a) group. FBN1 immunoreactivity was statistically significantly decreased in the rosuvastatin group (p=0.030) compared to the hypertension group. FBN1 immunoreactivity histoscores for all three groups are shown in Table 2.

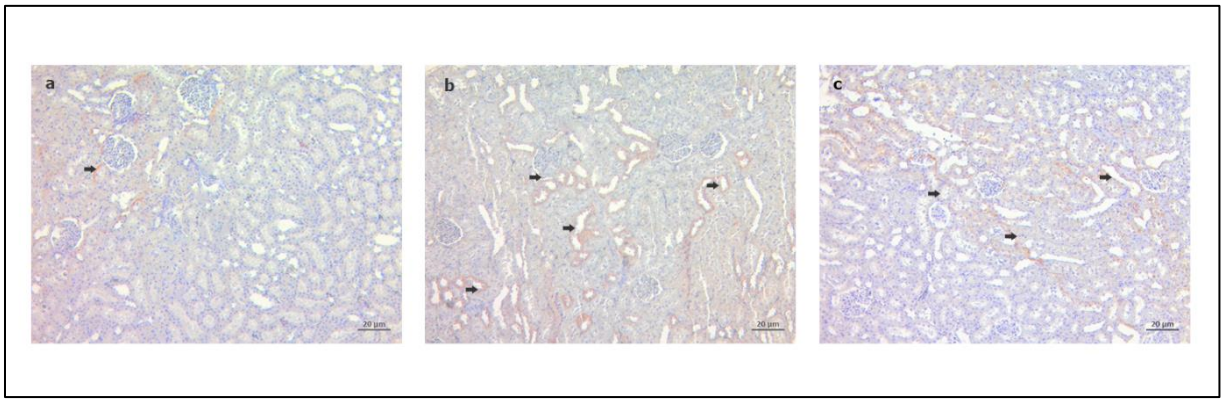

Figure 2. Immunohistochemical staining for FBN1 (black arrow) in kidney tissue.
a- Control group b- Increase in FBN1 immunoreactivity of the hypertension group
c-Decreased in FBN1 immunoreactivity of the rosuvastatin group

DISCUSSION

Despite the presence of various preventive and therapeutic approaches, the fact that hypertension is still a disease that maintains its importance worldwide has accelerated the studies on both the pathophysiology and the search for treatment on this subject.[3,4] It is known that rosuvastatin has beneficial effects in the prevention of oxidative stress, inflammation, and vascular dysfunction caused by ROS. In addition, some statins with hydrophilic properties such as rosuvastatin have been shown to prevent the pathological course of kidney

diseases. In this study, changes in caspase-3 and FBN1 and the effect of rosuvastatin on these proteins were investigated for the first time in the kidney tissue of rats with hypertension by administering L-NAME. The study findings showed that rosuvastatin slightly lowered blood pressure and that caspase-3 and FBN1 might mediate its protective effect on hypertensive kidneys.

It is quite common to use L-NAME, a NOS inhibitor, in the development of hypertension in rats. Since it has a water-soluble structure, it can be given to animals easily with drinking water. In addition to hypertension, long-term and high-dose L-NAME administration also caused vascular and renal pathologies in rats.[19,20] In addition to hypertension due to the activation of the renin-angiotensin system via L-NAME, an increase in oxidative stress occurs as a result of NOS inhibition.[21] In this study, it was observed that blood pressure increased significantly on the 14th, 28th, and 42nd days due to L-NAME administration and hypertension occurred.

In this study, a slight decrease in blood pressure was detected at 28 and 42 days after the administration of rosuvastatin. Consistent with our study, it was observed that blood pressure decreased slightly by giving rosuvastatin 20mg/kg/day for 5 weeks to Wistar rats with hypertension by giving 40mg/kg/day L-NAME. However, unlike our study, it was also shown in this study that rosuvastatin prevented the thickening and elastic fiber increase of the intima and media layers of the thoracic aorta.[14] Blood pressures were reduced in a study using spontaneously hypertensive rats (SHR) with rosuvastatin given at doses of 1, 5, 10, and 20mg/kg/day for 12 weeks and in another study after the first week of treatment with rosuvastatin at a dose of 10mg/kg/day for 3 weeks.[15,16] However, 20mg/kg/day rosuvastatin for 6 weeks given to Wistar rats given 15mg/kg/day L-NAME did not decrease blood pressure and it was predicted that this difference was caused by the hypertension model.[15] Although rosuvastatin significantly lowered blood pressure in SHRs, no significant reduction in blood pressure was observed in the L-NAME-induced hypertension model.

Normally, caspase-3 is a member of the protease family that plays a role in the formation of the apoptotic process and is a necessary protein for the formation of apoptotic cell morphology. Apoptosis (i.e., programmed cell death) is important in the progression to kidney disease that can be activated through extrinsic/intrinsic pathways.[22,23] Extrinsic apoptosis is initiated by caspase-8 activation and extracellular signals that in turn promote caspase-3. Intrinsic apoptosis is a mitochondria-dependent pathway that is activated in response to intracellular injury characterized by mitochondrial membrane permeability and cytochrome-c release into the cytoplasm, triggering caspase-3 activation. This demonstrates the initial role of intrinsic apoptotic pathways in hypertension-induced renal cell loss. Kidneys of hypertensive Dahl/Rapp salt-sensitive rats show elevated apoptosis that is attributed to increased cytochrome-c release, caspase-3, and -9 activation, and severe kidney injury, underlining the role of mitochondria-dependent apoptosis in the pathogenesis of hypertensive nephrosclerosis.[24] In a study measuring the expression of apoptosis biomarkers (e.g., Bcl-2/Bax, cleaved caspase-3, and p-Akt/Ak) conducted to determine whether rosuvastatin has antiapoptosis effects, it was shown that treatment with rosuvastatin elevated Bcl2 expression and decreased Bax expression. As a negative regulator of cell death, the gene-activated Akt was observed to be increased by rosuvastatin, which suppressed apoptosis. In addition, rosuvastatin reduced the expression of cleaved caspase-3, a promoter of apoptosis. With TUNEL staining, these results confirmed that rosuvastatin has an anti-apoptosis effect.[25] Caspase-3 activation is an important step in the progression of apoptosis. Caspase-3 activation is the most important indicator showing the irreversible point in programmed cell death, and the increase in caspase-3 release in the hypertensive kidney in this study confirms the literature. At the same time, in this study, it can be evaluated that caspase-3 may affect the formation of hypertensive nephropathy, as well as that, rosuvastatin exerts its curative effects on hypertensive nephropathy by reducing caspase-3.[26] Studies on this subject have already shown that caspase-3 is positively associated with kidney damage and is an important component in the early phase of apoptotic death of smooth muscle cells, especially in vascular diseases such as atherosclerosis and hypertension, and plays an important role in acute renal failure after I/R.[12,27]

FBN1 is a glycoprotein that functions as a skeleton for the accumulation of tropoelastin, which forms elastic fibers in the arterial wall, and is a protein that is effective in maintaining the wall integrity of the central arteries and on arterial tone.[9,10] Fibrilins are the most important components of 10-12 nm-diameter microfibrils in the extracellular matrix of many elastic and inelastic connective tissues and are also abundant in the lamellae of blood vessels and elastic fibers of elastic organs such as the skin and lungs.[28] Mutations in the FBN1 gene have been associated with aortic stiffness, increased pulse pressure, and aortic root dilatation.[9] In this study, a statistically significant increase in FBN1 immunoreactivity in rat kidney tissues belonging to the hypertension group was an expected finding in this sense and it was evaluated that FBN1 might be effective in the pathophysiology of hypertension. Likewise, the statistically significant decrease in FBN1 immunoreactivity in the treatment group may indicate that rosuvastatin exerts its curative effect on FBN1.

Conclusion

In this study, it was concluded that rosuvastatin did not significantly reduce blood pressure, but caspase-3 and FBN1 might mediate its protective effect on hypertensive kidneys. In this way, it contributed to the explanation of the mechanisms that link hypertension and renal mitochondrial damage and to the development of targeted therapeutic strategies to protect the hypertensive kidney.

Conflict of Interest

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

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Ethics Committee Permission

The study was approved by Adıyaman University Experimental Animals Ethics Committee (28.04.2022-2022/026).

Authors' Contributions

Concept/Design: EO, AT, NK. Data Collection and/or Processing: EO, AT, NK. Data analysis and interpretation: EO, AT, NK. Literature Search: EO, AT, NK. Drafting manuscript: EO, AT, NK. Critical revision of manuscript: EO, AT, NK. Supervisor: EO, AT, NK.

REFERENCES

1. Rauch U, Osende JI, Chesebro JH, et al. Statins and cardiovascular diseases: the multiple effects of lipid-lowering therapy by statins. Atherosclerosis. 2000;153(1):181-189.
2. Suh JW, Choi DJ, Chang HJ, et al. HMG-CoA reductase inhibitor improves endothelial dysfunction in spontaneous hypertensive rats via down-regulation of caveolin-1 and activation of endothelial nitric oxide synthase. J Korean Med Sci. 2010;25(1):16-23.
3. Launer LJ, Masaki K, Petrovitch H, Foley D, Havlik RJ. The association between midlife blood pressure levels and late-life cognitive function. The Honolulu-Asia Aging Study. Jama. 1995;274(23):1846-1851.
4. Kotchen TA, Cowley AW Jr, Liang M. Ushering hypertension into a new era of precision medicine. Jama. 2016;315(4):343-344.
5. Eirin A, Lerman LO. Darkness at the end of the tunnel: Poststenotic kidney injury. Physiology (Bethesda). 2013;28(4):245-253.
6. Collins AJ, Foley RN, Chavers B, et al. Us renal data system annual data report. Am J Kidney Dis. 2013;61(1):e1–e480.
7. Bidani AK, Griffin KA. Pathophysiology of hypertensive renal damage: Implications for therapy. Hypertension. 2004;44(5):595-601.
8. Ramirez F, Pereira L. Mutations of extracellular matrix components in vascular disease. Ann Thorac Surg. 1999;67(6):1857-1858.
9. Kielty CM, Sherratt MJ, Shuttleworth CA. Elastic fibres. J Cell Sci. 2002;115(14):2817-2828.
10. Sherratt MJ, Wess TJ, Baldock C, et al. Fibrillin-rich microfibrils of the extracellular matrix: ultrastructure and assembly. Micron. 2001;32(2):185-200.
11. Ilhan S, Oktar S, Sahna E, Aksulu HE. Salt and nitric oxide inhibition induced hypertension: the role of prostacycline and 8-isoprostane. Clin Exp Hypertens. 2011;33(2):84-88.
12. Kunduzova OR, Escourrou G, Seguelas MH, et al. Prevention of apoptotic and necrotic cell death, caspase-3 activation, and renal dysfunction by melatonin after ischemia/reperfusion. FASEB J. 2003;17(8):872-874.
13. Mutlu E, Ilhan S, Onat E, Kara M, Sahna E. The effects of novokinin, AT2 agonist, on blood pressure, vascular responses, levels of ADMA, NADPH Oxidase and Rho Kinase in hypertension induced by NOS inhibition and salt. Turkish J Med Sci. 2016;46(4):1249-1257.
14. Neto-Ferreira R, Novaes Rocha V, da Silva Torres T, Mandarim-de-Lacerda CA, de Carvalho JJ. Beneficial effects of rosuvastatin on aortic adverse remodeling in nitric oxide-deficient rats. Exp Toxicol Pathol. 2011;63(5):473-478.
15. Susic D, Varagic J, Ahn J, Slama M, Frohlich ED. Beneficial pleiotropic vascular effects of rosuvastatin in two hypertensive models. J Am Coll Cardiol. 2003;42(6):1091-1097.
16. Sicard P, Lauzier B, Oudot A, et al. A treatment with rosuvastatin induced a reduction of arterial pressure and a decrease of oxidative stress in spontaneously

hypertensive rats. Arch Mal Coeur Vaiss. 2005;98(2):804-808.
17. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem. 1981;29(4):577-580.
18. Gurel A, Aydin H, Yalcin A, et al. Cinnamon Protects Kidneys Against Doxorubicin-Induced Nephrotoxicity. Anal Quant Cytopathol Hıstpathol. 2022;43(5):311-319.
19. Zatz R, Baylis C. Chronic nitric oxide inhibition model six years on. Hypertension. 1998;32(6):958-964.
20. Gardiner SM, Kemp PA, Bennett T, Palmer RM, Moncada S. Nitric oxide synthase inhibitors cause sustained, but reversible, hypertension and hindquarters vasoconstriction in Brattleboro rats. Eur J Pharmacol. 1992;213(3):449-451.
21. Sinaiko AR, Steinberger J, Moran A, et al. Relation of body mass index and insulin resistance to cardiovascular risk factors, inflammatory factors, and oxidative stress during adolescence. Circulation. 2005;111(15):1985-1991.
22. Krepela E. Cysteine proteinases in tumor cell growth and apoptosis. Neoplasma.2001;48(5):332-349.
23. Sanz AB, Santamaria B, Ruiz-Ortega M, Egido J, Ortiz A. Mechanisms of renal apoptosis in health and disease. JASN. 2008;19(9):1634-1642.
24. Ying WZ, Sanders PW. Cytochrome c mediates apoptosis in hypertensive nephrosclerosis in dahl/rapp rats. Kidney Int. 2001;59(2):662-672.
25. Qingbo Lv, Wang Y, Li Y, et al. Rosuvastatin Reverses Hypertension-Induced Changes in the Aorta Structure and Endothelium-Dependent Relaxation in Rats Through Suppression of Apoptosis and Inflammation. J Cardiovasc Pharmacol. 2020;75(6): 584-595.
26. Arama E, Agapite J, Steller H. Caspase activity and a specific cytochrome C are required for sperm differentiation in Drosophila. Dev Cell. 2003;4(5): 687-697.
27. Walsh K, Smith RC, Kim HS. Vascular cell apoptosis in remodeling, restenosis, and plaque rupture. Circ Res. 2000;87(3):184-188.
28. Sakai LY, Keene DR, Engvall E. Fibrillin, a new 350-kD glycoprotein, is a component of extracellular microfibrils. J. Cell Biol. 1986;103:2499-2509.