The Effects of Irisin on L-Name Induced Hypertension in Rats

Aydoğan et al. Effect of Irisin on Hypertension

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Abstract

Background: The cause of about 95% of hypertension, an important public health problem, is unknown. Intensive studies are underway to understand the physiopathology of hypertension. Irisin, a newly discovered hormone, has been reported to dilate vascular smooth muscle and also lower blood pressure acutely.

Aims: We aimed at investigating the effects of chronic irisin treatment on blood pressure and renal functions in a hypertension model established by nitric oxide synthase inhibition by L-NAME treatment.

Study design: Animal experimentation

Methods: Male Sprague Dawley rats were divided into 4 groups (n=8). Control (C) and irisin (I) groups received intravenous saline injection, hypertension (HT) and hypertension+irisin (HT+I) groups received 1.5 mg/100 g L-NAME. L-NAME (150 mg/L) was added to the drinking water of the rats in groups HT and HT+I for 3 weeks. In the second week of the experiment, irisin (50 nmol/day) was given to the rats in groups I and HT+I and saline were administered to the C and HT groups rats for two weeks through subcutaneously placed osmotic minipumps. Blood pressure was measured by tail-cuff plethysmography method. The 24-hour urine, blood and both kidneys of the rats were collected on the 21st day of the experiment.

Results: The HT group had elevated systolic, diastolic and mean arterial blood pressure values compared to the C group, with an decreased reduced glutathione levels in tissue and serum, with an increase in serum oxidized glutathione level (p<0.05). Histopathologically, increased tubular injury, cast formation, glomerular sclerosis and peritubular fibrosis levels were observed (p<0.05). Irisin treatment did not cause any significant change in blood pressure, renal functions and injury scores. However, renal NO levels significantly increased and eNOS immunoreactivity was determined to be reduced (p<0.05).

Conclusion: In this study, it was seen that chronic irisin treatment at physiological dose did not decrease blood pressure in experimental HT model. In different experimental models of hypertension, the effects of irisin administration at different doses and periods should be investigated extensively.

Keywords: Hypertension, Irisin, Kidney, Nitric Oxide, Oxidative Stress

Hypertension is commonly observed in society and causes severe mortality and morbidity due to effects on “so called” target organs, including kidneys. Hypertension is one of the major risk factors for development of cardiovascular disease, with nearly 95% of cases being essential hypertension. There are epidemiologic studies in developed and developing countries in recent years showing the prevalence of essential hypertension being in trend of increase in most countries. In many industrialized countries, the prevalence is reported to vary from 25-55% (1). Further, in parallel with the increasing prevalence, the repayment costs for medications used for treatment of target organ injury and comorbid diseases has increased the expenditure for this disease by organizations. Prevention of hypertension will reduce morbidity, mortality and expenditures for diagnostic and
Nitric oxide synthase (NOS) enzymes, commonly found in the body and with a variety of functions, have three different isoforms. These are neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). The NO required for physiologic functions is synthesized by eNOS and nNOS. iNOS causes longer term and higher amounts of NO synthesis compared to the other isoforms, and this increases the severity of the physiologic effects of NO. In the inflammatory process, nearly all cells produce high levels of NO mediated by iNOS. The excess NO transforms into the stable end-products of this radical of nitrite and nitrate or competes with superoxide dismutase enzymes to create peroxynitrite due to interaction with superoxide radicals (3,4).

The new hormone of irisin, so-called myokine, discovered by Boström et al. in 2012, has molecular weight of 12587 kDa and is a polypeptide containing 112 amino acids (5). Irisin is released after exercise by skeletal muscles and is reported to transform white fat cells into brown fat cells (6). Currently, irisin has been shown to be expressed at various levels in skeletal muscles, heart muscle, fat tissue, liver, brain, bone, pancreas, ovaries and kidneys (7). Additionally, irisin plays a protective role against endothelial injury and is reported to reduce blood pressure (8). It has been shown that acute intravenous injection of irisin reduces blood pressure in a dose-dependent manner (0.1, 1 and 10 µg / kg). In this study, it was reported that physiological irisin concentrations (48 and 240 nmol / L) did not decrease blood pressure (9). In contrast, the pharmacological concentrations of irisin in the in vitro study were reported to be 50 to 100 nmol / L (10). However, possible effect of irisin, especially long-term irisin treatment, on blood pressure and hypertension is still unclear.

In this study, we hypothesized that long-term administration of irisin at a low dose of physiological concentration will reduce blood pressure. We aimed to investigate the irisin levels and the effects chronic physiological dose therapy of irisin on blood pressure, oxidative stress, vascular nitric oxide synthase mechanism and renal function in experimental hypertension model induced by L-NAME.

MATERIAL AND METHODS

Animal Ethic

A total of 32 adult male (330-390 g) Sprague Dawley rats were randomly divided into 4 equal groups. Animals were obtained from ……………. University Faculty of Medicine Experimental Animals Unit. All animals were housed in an environment with 12-hour light-12-hour dark period, 55% humidity rate and 21±2 °C temperature and fed with standard feed. Ethical approval was obtained by ………. University Animal Experiments Local Ethics Committee (………..-2015/34).

Experimental design

Control (C) group: Intravenous physiologic serum (1 ml/kg) was administered via the tail vein and then rats in this group were given tap water ad libitum for the duration of the experiment. After 1 week, minipumps were replaced with ALZET 2ML2 osmotic minipumps containing saline for 2 weeks.

Irisin (I) group: Intravenous physiologic serum (1 ml/kg) was administered via the tail vein and then rats in this group were given tap water ad libitum for the duration of the experiment. After 1 week, minipumps were replaced with ALZET 2ML2 osmotic minipumps containing irisin (Phoenix Pharmaceuticals, USA; 50 nmol/day) for 2 weeks. Selected dose of irisin was based on the previous study (9,10)

Hypertension (HT) group: HT groups, a bolus dose was given IV (1.5 mg/100 g of body weight bolus via tail vein) followed by L-NAME ad libitum in the drinking water (150 mg/L) for the duration of the experiment. After 1 week, osmotic minipumps were replaced with ALZET 2ML2 minipumps containing saline for 2 weeks (11).

Hypertension+irisin (HT+I) group: This group, a bolus dose was given IV (1.5 mg/100 g of body weight bolus via tail vein) followed by L-NAME ad libitum in the drinking water (150 mg/L) for the duration of the experiment. After 1 week, minipumps were replaced with ALZET 2ML2 osmotic minipumps containing irisin (50 nmol/day) for 2 weeks.

Preparation and implantation of osmotic mini pumps

The mini osmotic pumps (Alzet 2ML2, Cupertino, CA, USA) were set to 5 µl/hr infusion rate for 14 days. Rats were anesthetized with intramuscular 10 mg/kg xylazine and 50 mg/kg ketamine. Osmotic pumps were surgically implanted under the skin at the back of the neck between the two scapulae of rats.

Blood pressure measurements

Rats in all groups had blood pressure measurements with the indirect tail-cuff plethysmography (MAY NIBP250, Ankara, Turkey) on the tail taken on the 1st, 7th, 14th and 21st days. For each animal, a total of 5 measurements were made at 1-minute intervals, with the highest and lowest 2 measurements removed and the mean of the remaining 3 measurements calculated. Mean blood pressure was calculated with the formula MBP = DBP + (SBP – DBP)/3 (MBP: mean blood pressure, SBP: systolic blood pressure, DBP: diastolic blood pressure).
Termination of Experiment and Collection of Tissue, Serum and Urine Samples

Rats in all groups were held in metabolic cages for the final 24 hours of the experiment and end blood pressure measurements were taken after collecting urine samples. Rats had euthanasia performed with exsanguination after taking blood and both kidneys under 10 mg/kg xylazine and 50 mg/kg ketamine anesthesia. Half of the right kidney was placed in 10% formalin solution for histopathologic investigation, while the other portions were placed in liquid nitrogen for analysis and stored at -80 °C. Blood and urine samples were centrifuged at +4 degrees at 3000 rpm for 10 minutes in a cooled centrifuge and stored at -80 °C.

Biochemical Analysis

Serum urea, creatinine, sodium, potassium levels and alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine kinase (CK) activities and urine creatinine and sodium measurements were performed with an autoanalyzer (Abbott Architect c16000, USA) in ……… University Health Research and Application Center Laboratory. Creatinine clearance was calculated using the standard clearance formula. Fractional sodium excretion was calculated as %.

Irisin levels in serum and urine were determined with the ELISA method (Elabscience, Cat. No: E-EL-R1104). Ox-GSH levels in renal tissue were measured according to kit procedures (Mybiosource, Cat no: MBS752665), while Red-GSH levels were also measured with a kit (Mybiosource, Cat no: MBS724319). The end-product of lipid peroxidation of malondialdehyde (MDA) was measured spectrophotometrically with the color change (pink) due to the interaction with thiobarbituric acid (TBA) in a hot and acidic medium (12, 13). Results are expressed as nmol/g. Nitrate and nitrite were measured according to the method described by Cortas and Wakid (14). Estimation of protein amounts was performed according to the Lowry method (15).

Histological Analysis

For light microscopic investigation, kidneys cut in sagittal plane were fixed in 10% formaline, submerged in paraffin blocks and cut to sections of 5 micron thickness. Sections were stained with the hematoxylin-eosin (HE) method for light microscopy. Preparates were semi-quantitatively assessed for glomerular necrosis, glomerular basal membrane thickening, mesengial matrix widening, tubular hydric degeneration and tubular dilatation. Accordingly pathological changes are scored as 0 no pathology, +1 focal, +2 moderate focal, +3 multifocal and +4 diffuse (16).

Immunohistochemical Analysis

For immunohistochemical analysis, formalin fixed and, in sections prepared from paraffin-embedded tissue 4 mm thick were used. Tissue sections were taken to electrostatic charged slides and dried at 70°C for at least 1 hour. The entire immunohistochemical staining process including deparaffinization and antigen expression was performed in a fully automated immunohistochemistry staining device (Ventana BenchMark Ultra, Ventana Medical System, Tucson, AZ). A biotinylated HRP multimer-based, hydrogen peroxide substrate and a ready-to-use kit containing the 3, 3-diaminobenzidine tetrahydrochloride (DAB) chromogen were used for the process. The immunohistochemical antibody [irisin (Bioss FNDC5 BS-4886-R), iNOS (Spring REF E3744) and eNOS (Neomarkers RB-9279-P)] panel used for diagnosis and differential diagnosis varies according to the cases and cytokeratin. In the opposite dye staining apparatus, the process was terminated by dehydration of the sections defined by hematoxylin and bluing solution, clarifying with xylene and closing with coverslip. All immunopositive cells were evaluated in a random portion of 10 large growth areas (x200). Cells stained for each case were scored and given in percent. Staining prevalence was graded as 0 (0-5%), 1 (6-24%), 2 (25-49%), 3 (50-74%), and 4 (75%). The staining intensity was graded as 0 (negative), 1 (mild), 2 (moderate) and 3 (strong). Two values were multiplied to obtain an immunoreactivity score of 0-300 as described in our previously study (17).

Statistical Analysis

Results were shown as mean ± standard deviation or median (interquartile range). Statistical analysis was performed using the SPSS 18.0 software for Windows. The normal distribution of the quantitative data was examined by Shapiro Wilk test. Although there were 4 groups in the study, no comparison was made between the two groups. Student t test was used instead of ANOVA designs to compare the variables showing normal distribution between Control - Irisin, Control - Hypertension and Hypertension - Hypertension + Irisin groups. Mann Whitney U test was used to compare the variables that do not show normal distribution. P <0.05 was accepted as the limit of statistical significance.

RESULTS

Measurement of oxidative stress markers in renal tissue

Oxidative stress was assessed using oxidative stress markers including MDA and GSH in renal tissue. Reduced GSH in serum (p<0.05) was significantly lower in the hypertension group compared to the control group and oxidized GSH (p<0.01) was significantly higher in the hypertension group compared to the control group. Reduced GSH (p<0.05) and oxidized GSH (p=0.05) in tissue were significantly lower in the hypertension group compared to the control group. The mean and standard deviation data for variables in all groups are shown in Table 1.

Measurement of serum, renal tissue and urine nitric oxide concentration
NO in renal tissue (p<0.05) was significantly higher in the hypertension+irisin group compared to the hypertension group. The mean and standard deviation data for variables in all groups are shown in Table 2. 

**Measurement of serum and urine irisin levels**

There was no significant difference between the serum irisin levels in the groups. Urine irisin (p=0.05) levels were significantly higher in the hypertension group compared to the control group. The mean and standard deviation data for variables in all groups are shown in Table 3.

**Biochemical results**

Serum sodium levels were significantly lower in the hypertension group compared to the control group, with a significant increase in the hypertension+irisin group compared to the hypertension group. Serum ALT levels were significantly higher in the hypertension+irisin group compared to the hypertension group. The mean and standard deviation data for biochemical variables in all groups are shown in Table 4.

**Results of blood pressure measurements**

In the HT group, systolic, diastolic and mean arterial pressure values were observed to statistically increased with L-NAME administration while heart rate reduced (p<0.05). The blood pressure values for all groups are shown in Figure 1.

**Histopathologic and immunoreactivity Results**

Glomeruli and tubules with regular structure were observed. Pathologic findings were not observed in the vein at the vascular pole (common result for C, I, HT and HT+I groups, shown with green arrow). In HT and HT+I groups, mild levels of peritubular fibrosis and sclerosis findings in glomeruli were observed, however, clear histopathologic changes secondary to hypertension were observed (p<0.01). Histopathologic findings for all the groups are shown in Table 2 and Figure 2.

The histopathologic results and mean values for eNOS, iNOS and nNOS values belonging to the groups and tissue irisin immunoreactivity results are shown in Table 6, Figure 3-6.

**DISCUSSION**

In this study, by using L-NAME administration-induced experimental hypertension model; we obtained the following results: in the hypertension group: I) There was no significant difference in serum irisin levels and irisin immunoreactivity of renal tissue. The increase in the urine irisin levels was not significant. II) Histopathological examination of the kidney showed significantly higher tubular damage, glomerular sclerosis, peritubular fibrosis, and cast formation. III) No significant changes in renal function markers were observed. However, serum Na⁺ levels decreased significantly. IV) Serum and renal reduced glutathione levels were significantly lower. A significant increase in serum oxidized glutathione levels was observed. V) Although there was a significant increase in renal eNOS and iNOS immunoreactivity, there was no significant difference in nNOS immunoreactivity. Treatment with irisin did not cause any significant change on the high mean arterial blood pressure. Serum Na⁺ levels decreased significantly in the hypertension group with irisin treatment. In addition, irisin treatment caused a significant increase in renal NO levels.

Hypertension present a significant risk factor for subclinical renal injury in humans and animals. In rats, hypertension development with the NOS inhibitor L-NAME is reported to cause glomerular injury and injury to the interstitial area in kidneys due to vasoconstriction. Administration of L-NAME with different doses and durations to experimental animals induces hypertension. This model is commonly used due to observation of renal complications linked to hypertension similar to humans (18,19).

Histopathological examination of the kidney showed significant increase in tubular damage, peritubular fibrosis, glomerular sclerosis and caste levels in hypertension group. The effect of increased blood pressure on the occurrence of renal damage in different types of hypertension and the relationship between elevated blood pressure and renal dysfunction are different. It is reported that detailed studies are needed to define the relationship between elevated blood pressure, renal damage and renal dysfunction. Although renal injury was significantly increased in our study, no statistically significant difference was observed in renal dysfunction. These findings are consistent with the results of previous studies (19, 20). An increase in systemic blood pressure produces a natriuretic and diuretic effect. As tubular reabsorption is impaired in hypertension, increased Na⁺ excretion has been reported (20). Significant decrease in serum Na levels in the hypertension group of our study may be the result of tubular damage. Our findings on this regard are consistent with previous study results (20). In our study, no significant difference was observed in glomerular functions; L-NAME may be the result of insufficient dosing and administration time.

Oxidative stress plays an important role in the pathophysiology of hypertension. Endogenous and exogenous antioxidants show antihypertensive effect. Free radicals, such as superoxide, reduce the bioavailability of nitric oxide. The main function of eNOS is the production of NO that regulates vasodilation. However, lack or oxidation of L-arginine and tetrahydrobiopterin, separation of the L-arginine-NO pathway reduces NO formation and causes eNOS-mediated superoxide production. Superoxide combines with NO synthesized by eNOS to form peroxynitrite, which promotes eNOS uncoupling and ROS production (21, 22). Hypertension is also known to be associated with disruption of glutathione metabolism. Reduced glutathione levels have an important effect as it
protects proteins and membrane lipids from oxidation. Our protocols aiming investigations for this provided that oxidative stress parameters in the hypertension group significantly decreased in reduced GSH levels in serum and renal tissue, while serum oxide GSH levels were determined to be increased. This data are in accordance with the results of studies inducing hypertension by administering L-NAME to rats with different doses and duration (22). In our study, the increase in renal tissue MDA levels, an end-product of lipid peroxidation used as a marker of oxidative stress, was not significant. This result does not comply with results of some studies in the literature (22). We have no plausible explanation for this except that differences in the L-NAME dose and administration duration.

When NO levels of control and hypertension groups were compared, there was no significant difference. However, the immunohistochemistry results for eNOS and iNOS activity were identified to show a significant increase in the hypertension group compared to the control group. Administration of L-NAME is considered to block NO synthesis, which we assume is due to higher NOS activity in certain tissues ex vivo and higher levels of NOS gene expression. Paradoxically, the effect of L-NAME on NOS expression in vivo appears to vary with treatment duration and tissue being examined. (23). Administration of L-NAME at 40 mg/kg/day for 4 weeks reported to cause increase in eNOS and iNOS expression in cavernous tissue in Sprague-Dawley rats and increased eNOS in heart and kidneys of Wistar rats; however, it did not affect the eNOS expression in brain tissue (24). Additionally, the same L-NAME dose did not affect eNOS or iNOS expression in the aorta of Wistar rats after 5 weeks of treatment, while 7 weeks treatment increased the heart eNOS expression and contrarily reduced the eNOS expression in the brain (25). In our study, increased eNOS expression may be the result of eNOS uncoupling. In addition, the blood pressure lowering effect of peripheral irisin is reported to be very short. Recently, it has been reported that irisin may play an important role in the pathophysiology of cardiovascular diseases including hypertension (9,26,27). In these studies, Zhang and et al (26) administered irisin centrally to the 3rd ventricle at doses of 0.625-2.5 μg / rat and determined increased blood pressure and cardiac contractility. In contrast, injection of peripheral intravenous high-dose irisin (2-8 µg / rat) has been reported to reduce blood pressure in both control and spontaneous hypertensive rats. It has been shown to cause dilatation of mouse mesenteric artery, in vitro. In addition, it is reported that the blood pressure lowering effect of peripheral irisin was very short-lived. This study is important to indicate that different route of irisin applications may cause different effects (26). In another study, bolus injections of irisin (2 minutes) were shown to reduce blood pressure in spontaneous hypertensive rats in dose-dependent (1, 5, and 10 µg / kg) manner. In this study, it was reported that low doses or physiological irisin concentrations (48 and 240 nmol / L) did not decrease blood pressure and did not dilate the mesenteric artery of Wistar rats; they indicated that there is no direct vasodilator effect, although irisin reduces blood pressure in high-dose intravenous administration (9). When the doses used in animal studies are related to doses given to humans, these doses can be very high. Therefore, we wanted to investigate long-term effect of physiological dose of irisin (50 nmol / day). In the current study, it was determined that irisin treatment at physiology dose did not cause any significant change in blood pressure. The reason for this may be insufficient physiological dose of irisin administered. Therefore, we believe that further studies should be performed using pharmacological doses of irisin in different experimental hypertension models. Chen et al (27) shown that circulating irisin levels increased in hypertensive patients compared to control group. They proposed that increased circulating irisin levels might be associated with hypertension and stroke due to hypertension. In our study, we observed a significant increase in renal tissue NO levels in the hypertension group treated with irisin. According to the study of Zhu et al (28) it has been reported that irisin administration to diabetic rats causes NO increase and improves endothelial function (28). The results of this study are similar to our findings.

There are some limitations in our study. First, the physiological dose of long-term irisin was used. Therefore, the effects of the pharmacological dose could not be determined. Second, the dose and duration of L-NAME given to induce hypertension did not impair renal function. Third, the effects of irisin is only investigated in L-NAME-induced experimental hypertension models.

In conclusion, we found that long-term administration of physiological concentrations of irisin does not ameliorate blood pressure in L-NAME-induced hypertension model. In contrast, irisin treatment increased serum sodium levels. In addition, renal NO levels and eNOS immunoreactivity were increased. According to the results of the current studies, irisin may have a double-ended sword effect on blood pressure. Further studies are needed to determine whether the effects of different doses of irisin on the NO-mediated mechanism and whether irisin has therapeutic potential in different hypertension models.

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REFERENCES


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<th>Hypertension (n=8)</th>
<th>Hypertension + Irisin (n=8)</th>
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<tr>
<td>Serum Reduced GSH (ng/mL)</td>
<td>7.48 (0.74)</td>
<td>6.95 (0.27)*</td>
<td>6.88 (0.46)**</td>
<td>6.87 (0.60)</td>
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<td>Serum Oxide GSH (pg/mL)</td>
<td>270.45 ± 89.71</td>
<td>498.42 ± 198.41*</td>
<td>688.18 ± 165.39***</td>
<td>600.95 ± 67.43</td>
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<td>Tissue Reduced GSH (µmol/g)</td>
<td>10.37±0.63</td>
<td>9.94±0.94</td>
<td>9.58±0.43**</td>
<td>9.28±1.30</td>
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<td>Tissue Oxide GSH (µmol/g)</td>
<td>1632.12±608.73</td>
<td>771.27±334.63***</td>
<td>1062.97±297.93**</td>
<td>762.14±443.58</td>
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<td>MDA tissue (nmol/g)</td>
<td>0.23±0.03</td>
<td>0.22±0.03</td>
<td>0.26±0.05</td>
<td>0.25±0.74</td>
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Data are expressed as mean ± standard deviation for normally distributed values. The data are presented in the format median (interquartile range) for non-normally distributed parameters.

*comparisons between the control and irisin group. **comparisons between the control and hypertension group.

*p<0.05  **p<0.01  ***p<0.001
Table 2. Serum, renal tissue and urine nitric oxide concentrations of all groups

<table>
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<td>Serum NO (µmol/L)</td>
<td>7.16±2.86</td>
<td>5.71±1.82</td>
<td>7.90±4.30</td>
<td>11.42±2.73</td>
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<td>Kidney NO (µmol/mg protein)</td>
<td>51.90 (25.79)</td>
<td>42.48 (21.96)</td>
<td>46.07 (23.54)</td>
<td>67.17 (30.08)</td>
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<td>Urine NO (µmol/L)</td>
<td>59.99±17.99</td>
<td>86.33±27.83</td>
<td>72.07±20.29</td>
<td>63.33±20.47</td>
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Data are expressed as mean ± standard deviation for normally distributed values. The data are presented in the format median (interquartile range) for non-normally distributed parameters. *comparisons between the hypertension and hypertension + irisin group. * p<0.05
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<tr>
<td>Serum irisin (pg/mL)</td>
<td>41.34±27.32</td>
<td>30.46±12.85</td>
<td>41.39±20.39</td>
<td>44.96±26.54</td>
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<tr>
<td>Urine irisin (pg/mL)</td>
<td>404.10±134.38</td>
<td>432.14±131.72</td>
<td>542.64±140.64</td>
<td>489.83±159.61</td>
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Data are expressed as mean ± standard deviation for normally distributed values.
Table 4. Biochemical parameters of all groups

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<tr>
<td>Serum Na⁺ (mmol/L)</td>
<td>140.13±10.43</td>
<td>133.50±9.99</td>
<td>125.50±14.92b*</td>
<td>141.13±7.00c*</td>
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<td>Serum K⁺ (mmol/L)</td>
<td>5.33±0.78</td>
<td>4.99±0.65</td>
<td>5.24±0.69</td>
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<td>Serum urea (mg/dL)</td>
<td>32.63±4.81</td>
<td>29.13±5.99</td>
<td>34.88±6.03</td>
<td>35.13±3.87</td>
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<td>Serum creatinine (mg/dL)</td>
<td>0.46±0.04</td>
<td>0.45±0.05</td>
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<td>ALT (U/L)</td>
<td>51.63±11.50</td>
<td>45.13±6.64</td>
<td>45.50±8.72</td>
<td>56.13±7.73c*</td>
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<td>AST (U/L)</td>
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<td>175.00 (58.50)</td>
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<td>CK (U/L)</td>
<td>1830.00±812.25</td>
<td>1543.75±369.58</td>
<td>1642.3±700.19</td>
<td>1653.75±750.13</td>
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<td>Urine creatinine (mg/dL)</td>
<td>91.12±19.85</td>
<td>87.30±14.83</td>
<td>108.67±17.90</td>
<td>103.34±32.27</td>
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<td>Urine Na⁺ (mg/dL)</td>
<td>103.00±50.52</td>
<td>105.38±39.87</td>
<td>128.63±48.07</td>
<td>104.50±30.65</td>
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<td>Creatinine clearance (mL/dk)</td>
<td>1.72±0.42</td>
<td>1.74±0.29</td>
<td>2.02±0.28</td>
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<td>FeNa (%)</td>
<td>0.375±0.20</td>
<td>0.413±0.18</td>
<td>0.436±0.19</td>
<td>0.349±0.09</td>
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Data are expressed as mean ± standard deviation for normally distributed values. The data are presented in the format median (interquartile range) for non-normally distributed parameters.

bcomparisons between the control and hypertension group. ccomparisons between the hypertension and hypertension + irisin group. *p<0.05
Table 5. Histopathologic values of all groups

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<th>Parameters</th>
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<th>Hypertension + Irisin (n=8)</th>
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</thead>
<tbody>
<tr>
<td>Tubular injury score</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>1.00 (1.00)*</td>
<td>1.00 (0.75)</td>
</tr>
<tr>
<td>Peritubular fibrosis</td>
<td>0.25±0.46</td>
<td>0.25±0.46</td>
<td>6.25±4.43**</td>
<td>6.25±4.43</td>
</tr>
<tr>
<td>Glomerular sclerosis</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>10.00 (3.50)**</td>
<td>10.00 (3.50)</td>
</tr>
<tr>
<td>Cast (%)</td>
<td>0.63±0.92</td>
<td>0.50±0.76</td>
<td>1.50±0.53**</td>
<td>1.38±0.52</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation for normally distributed values. The data are presented in the format median (interquartile range) for non-normally distributed parameters. 

*comparisons between the control and hypertension group. * p<0.05 ** p<0.01 *** p<0.001
Table 6. Immunohistochemical levels of all groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=8)</th>
<th>Irisin (n=8)</th>
<th>Hypertension (n=8)</th>
<th>Hypertension + Irisin (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS (%)</td>
<td>219.38±26.52</td>
<td>211.88±23.29</td>
<td>293.75±21.34b***</td>
<td>255.00±27.77c**</td>
</tr>
<tr>
<td>iNOS</td>
<td>30.00 (17.50)</td>
<td>30.00 (17.50)</td>
<td>240.00 (71.25)b**</td>
<td>217.50 (60.00)</td>
</tr>
<tr>
<td>nNOS</td>
<td>262.50±26.59</td>
<td>258.75±15.53</td>
<td>262.50±26.59</td>
<td>251.25±15.53</td>
</tr>
<tr>
<td>Irisin İmmünR</td>
<td>266.25±25.04</td>
<td>255.00±22.68</td>
<td>266.25±25.04</td>
<td>266.25±25.04</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation for normally distributed values. The data are presented in the format median (interquartile range) for non-normally distributed parameters.

bcomparisons between the control and hypertension group. ccomparisons between the hypertension and hypertension + irisin group.

*p<0.05 **p<0.01 ***p<0.001
Figure 1. The mean arterial blood pressure values for all groups. C: The control group. I: Irisin group. HT: Hypertension group. HT + I: Hypertension + irisin group.

Figure 2. Haematoxylin and eosin stained sections of rat kidneys (HEx200). Glomeruli (red arrows) and tubules (blue arrows) with regular structure were observed. In Hypertension and Hypertension + irisin groups, mild levels of peritubular fibrosis and sclerosis findings in glomeruli were viewed. (A) Control group; (B) Irisin group; (C) Hypertension group; (D) Hypertension + irisin group.
Figure 3. Glomerular immunostaining for endothelial nitric oxide synthase (eNOS) in different groups (X200). In all groups widespread strong staining of glomerular capillaries (red arrow) and weak staining of proximal tubules were observed. When the Hypertension and Hypertension + irisin groups were compared, the Hypertension + irisin group was observed to have a significantly reduced level of staining (p<0.01). (A) Control group; (B) Irisin group; (C) Hypertension group; (D) Hypertension + irisin group.

Figure 4. Glomerular immunostaining for inducible nitric oxide synthase (iNOS) in different groups (X200). While the control and B groups were observed to have focal and mild staining [glomeruli (red arrow), tubules (blue arrow)], there was widespread and strong staining in Hypertension and Hypertension + irisin groups (p<0.01). (A) Control group; (B) Irisin group; (C) Hypertension group; (D) Hypertension + irisin group.
Figure 5. Glomerular immunostaining for neuronal nitric oxide synthase (nNOS) in different groups (X200). All groups observed to have similar widespread staining of glomerular capillaries (red arrow) and proximal tubules (blue arrow). (A) Control group; (B) Irisin group; (C) Hypertension group; (D) Hypertension + irisin group.

Figure 6. Glomerular immunostaining for neuronal irisin in different groups (X200). All groups had staining with similar features in glomerular capillaries (red arrow) and distal tubules (blue arrow). (A) Control group; (B) Irisin group; (C) Hypertension group; (D) Hypertension + irisin group.