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The protective effects of hesperidin pretreatment on kidney and remote organs against renal ischemia-reperfusion injury

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Abstract. – OBJECTIVE: As an antioxidant and anti-inflammatory agent, Hesperidin was investigated to prove whether it prevents damage to the kidney and lung tissues of rats undergoing renal ischemia-reperfusion injury.

MATERIALS AND METHODS: Four groups of rats were set, including eight subjects each as Group 1 (control), Group 2-RIR (renal ischemia reperfusion), Groups 3 and 4 as pretreatment groups (50 HES, 100 HES).

RESULTS: According to our results, Hesperidin pretreatment improved the biochemical and histopathological parameters in kidney and lung tissues of rats with ischemia-reperfusion injury. Besides, a 100 mg/kg dose of Hesperidin was found to be more beneficial to the rats than 50 mg/kg.

CONCLUSIONS: The study suggests that Hesperidin is protective against renal and lung tissues of rats that underwent ischemia-reperfusion injury.

Key Words:
Hesperidin, Renal ischemia-reperfusion, Injury, Remote organ, Lung.

Introduction

Perfusion of an organ with oxygenated blood is essential for cellular vitality and end-organ functions. Both the reduced blood flow and long duration of ischemia affect the extent of cell dysfunction, injury, and death[1].

Acute kidney injury is a common global challenge with a high risk of mortality and morbidity, leading worldwide researchers to study curative and preventive treatments. One of the leading reasons for acute kidney injury is cellular damage caused by reversible ischemia. Ischemia-reperfusion injury (IRI) has a spectrum of pathology that differentiates between tissues and patients, mainly through local and distant organ damage that progresses to multiple organ failure[2].

Recent studies[3] have shown the distant effects of renal IRI on some organs such as the brain, liver, and lungs through oxidative stress in such ischemic conditions.

When the blood flow decreases, anaerobic metabolism begins in the tissue cells, and thus, cell pH and adenosine triphosphate (ATP) production decreases. This process leads to cellular calcium overload, mitochondrial swelling, and irregularity in mitochondrial cristae[4].

Literature[5-7] summarizes activities of Hesperidin (HES) as antilipemic, calcium channel blocker, capillary antihemorrhagic, antihypertensive-diuretic, anti-inflammatory-analgesic effects, immuno-suppressant activity, antiallergic-antianaphylactic, and antioxidant activity.

In this study, we aimed to investigate the protection of HES against kidney and lung tissues of rats that had undergone ischemia-reperfusion injury.

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Materials and Methods

The experiments of this study were carried out at our University Experimental Animals Research and Application Center after taking the approval of Erzurum Ataturk University Experimental Animals Local Ethics Committee (30.03.2018-55).

Renal IRI Model

We had four rat groups, including eight subjects each (Table I). Group 1 (Control), Group 2 (IRI), Group 3 (IRI+50 mg/kg HES), and Group 4 (IRI+100 mg/kg HES).

In Group 1, no operation was performed. The subjects of Group 2 had 1-hour renal ischemia following reperfusion lasting 24 hours. The subjects of Group 3 had 50 mg/kg HES administration 30 minutes before renal IRI operation. In Group 4, 100 mg/kg HES was administrated 30 minutes before renal IRI operation. HES was solved in the saline. The renal and lung tissues of the rats were taken in all groups.

Groups and Ischemia-Reperfusion Model

Thirty-two rats were weighed (225-270 g) in the current search and randomized into four groups. In Group 1 (control), the back region was shaved, cleaned, and opened with an incision under the anesthesia, then closed again without renal IRI model and any medication. In Group 2 (IRI), the incision area was cleaned with povidone-iodine. The renal arteries and veins were held with an atraumatic microvascular clamp for 1 hour. Later, blood circulation for 24 hours was allowed by opening the clamps in the reperfusion period. The incision was closed with a silk 3/0 suture. When the reperfusion ended, the renal and lung tissues were removed. HES was dissolved in saline and used in Groups 3 (50 mg/kg HES+IRI) and 4 (100 mg/kg HES+IRI) purchased from Sigma Aldrich Co. (St. Louis, MO, USA). It was administered to the rats intraperitoneally (i.p.) at the dose of 50 and 100 mg/kg 30 minutes before the reperfusion. Later as described in Group 2, the IRI model was established. All procedures were performed under anesthesia with 10 mg/kg i.p. xylazine hydrochloride (Rompun®, Bayer, Istanbul, Turkey) and 60 mg/kg i.p. ketamine (Ketalar®, Pfizer, Istanbul, Turkey). Finally, renal and lung tissues were washed and kept frozen until the biochemical analysis when the experiment ended.

Biochemical Analysis of Renal and Lung Tissues

After the renal and lung tissues had been homogenized, all biochemical analyses were made in the homogenized tissues. In tissue samples, malondialdehyde (MDA) levels to define lipid peroxidation status following the method presented by Ohkawa et al[8] were measured. The results were given as μmol/g protein. It was analyzed using the superoxide dismutase (SOD) activity specification protocol detected by Sun et al[9]. SOD activity results of tissue samples were given as U/mg protein. Myeloperoxidase (MPO) activity was measured using a method improved by Bradley et al[10]. The results of MPO activity in the tissues were presented as U/g protein. Total oxidant status (TOS) measurement was made with a commercially available kit (Rel Assay Diagnostics, Gaziantep, Turkey). Total antioxidant status (TAS) value was evaluated with the commercial kit (Rel Assay Diagnostics, Gaziantep, Turkey). TAS and TOS results were presented as nmol/L. The ratio of TOS to TAS was accepted as the oxidative stress index (OSI). OSI value was detected as follows: OSI=[(TOS, μmol H_2O_2 equivalent/L)/(TAS, mmol Trolox equivalent/L)×10].

Immunohistochemical (IHC) Staining

The kidney and lung tissues of rats were fixed in neutral formaldehyde solution for one day, and then these tissues were removed from formaldehyde by washing with tap water. Tissues were blocked in paraffin by undergoing alcohol-xylol follow-up. After the deparaffinization process of the tissues taken on the polylyzed slide, these tissues were kept in 3% H_2O_2 for 10 minutes to inactivate the endogenous peroxidase activity and washed in phosphate buffer solution (PBS). Then, these tissues were preserved in antigen retrieval solution at 500 w for 10 minutes and washed in PBS to reveal the antigens in the tissues. Protein block solution was added to tissues in this process, and these were washed in PBS to prevent nonspecific binding. Nuclear factor-kappa B (NF-κB) (Abcam, Cambridge, UK, Cat. No: ab7971, Dilution: 1/150) was applied as a primary antibody to sections washed with PBS. Next, the procedure specified by expose mouse and rabbit specific horseradish peroxidase-diaminobenzidine (HRP-DAB) detection IHC kit (Abcam, Cambridge, UK, ab80436) was followed. Chromogen was used to 3.3-39; diam-

inobenzidine and counterstained with hematoxylin. Positive cells were examined under a light microscope at 20× magnification.

Statistical Analysis

SPSS 20 (IBM Corp., Armonk, NY, USA) statistical program was used for data analysis. Conformity to normal distribution was examined using the Shapiro-Wilk test. One-way analysis of variance was used for intergroup comparison of quantitative variables, and Tukey post-hoc test was used to determine the difference between groups. Data were given as mean±standard error (SE), and the $p<0.05$ value was considered statistically significant.

Results

Biochemical Results

According to the results, Figure 1 summarizes the levels of oxidant and antioxidant biochemical markers in kidney and lung tissues.

TOS and OSI levels significantly increased in Group 2 in both tissues compared to Group 1. A statistically significant decrease was observed in TOS and OSI levels in the group treated with 50 mg HES compared to Group 2 in both tissues. This significance is also detected between Group 2 and Group 4 in both tissues.

When the SOD concentrations of both tissues were examined, a significant decrease was detected in tissues in Group 1 compared to Group 2. The concentration of SOD in Group 3 significantly increased compared to Group 2. In Group 4, a significant increase in concentration was detected when compared with Group 2.

MDA levels in kidney tissues of Group 2 increased significantly compared to Group 1. A significant decrease was detected in kidney MDA levels of both treatment groups compared to Group 2.

MPO concentration increased significantly in both tissues of Group 2 compared to Group 1. While 50 mg/kg HES application significantly reduced the increase of MPO concentration in kidney tissues of Group 3 compared to Group 2, 100 mg/kg HES application significantly reduced MPO concentration in both tissues of Group 4 compared to Group 2.

When we examined the different application doses of HES, we found the following results: 100 mg/kg HES and 50 mg/kg HES increased the TAS results compared to Group 2, but these results were statistically insignificant. Renal IRI model reduced TAS compared to Group 1, but this result was statistically insignificant. HES application of 100 mg/kg in all tissues decreased the MPO more than 50 mg/kg HES application compared to Group 2, which was statistically significant. Especially in the target lesion, high dose HES was found to be more effective on MPO. 100 mg/kg HES application in tissues decreased the MDA more than 50 mg/kg HES application compared to Group 2. This result was statistically significant for kidneys and insignificant for lungs. HES application of 100 mg/kg in all tissues reduced the decrease of SOD more than 50 mg/kg HES application compared to Group 2. This result was statistically significant. 100 mg/kg HES application in all tissues decreased the OSI more compared to 50 mg/kg HES application compared to Group 2, and this result was statistically significant. 100 mg/kg HES application in all tissues decreased the increase of TOS more than 50 mg/kg HES application compared to Group 2. This result was statistically significant. As a result, 100 mg/kg HES can be said to be more beneficial.

IHC Results

In the IHC staining of the kidney specimens (Figure 2), NF-κB immune positivity was not found in Group 1 (control), which we examined in terms of inflammatory reaction (Figure 2-1a). NF-κB immunopositivity was found to be intense in Group 2 (Figure 2-1b). While NF-κB immunopositivity was observed to be moderate in Group 3 (Figure 2-1c), in Group 4, it was found that NF-κB immunopositivity decreased (Figure 2-1d).

As a result of IHC staining of the lung specimens (Figure 2), NF-κB immunopositivity in terms of inflammatory reaction was not found in Group 1 (Figure 2-2a). NF-κB immunopositivity was found in Group 2 (Figure 2-2b). NF-κB immunopositivity was moderate in Group 3 (Figure 2-2c), while in Group 4 decreased (Figure 2-2d).

Discussion

This is the first study in the literature showing the effects of HES at different doses together in distant organs and target tissues. The fact that it is the only study that examined the responses

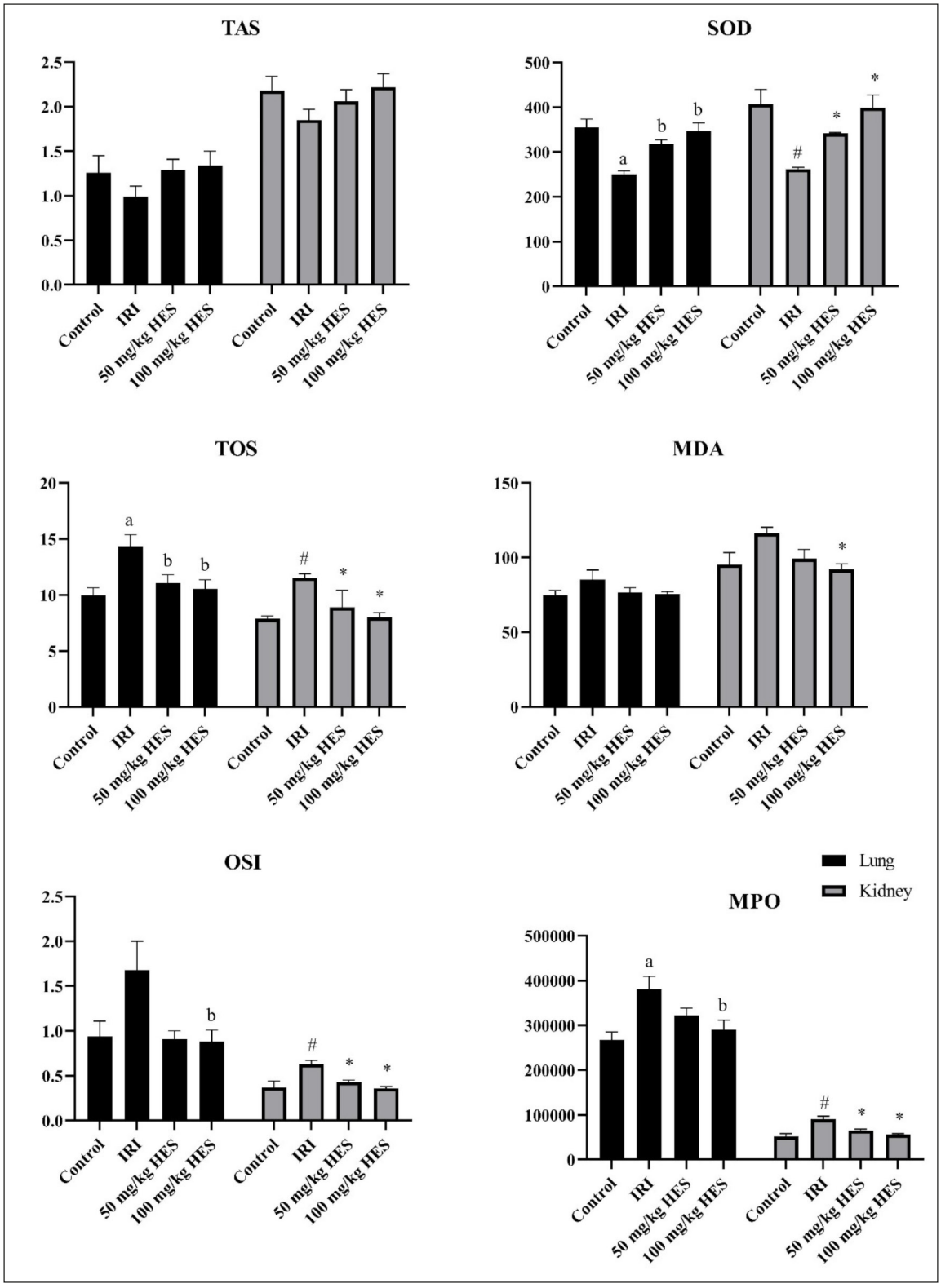

Figure 1. Effects of HES on oxidative stress parameters in IRI-induced lung and kidney injury. All data were presented as mean ± SE. In statistical analysis, Latin letters were used for lung tissue results and symbols for kidney results. a, $^{\#}p < 0.05$ IRI group compared to the control group. b, $^{*}p < 0.05$ HES treated groups compared to IRI group. TAS = Total antioxidant status, TOS = Total oxidant status, SOD = Superoxide dismutase, MDA = Malondialdehyde, OSI = Oxidative stress index, MPO = Myeloperoxidase.

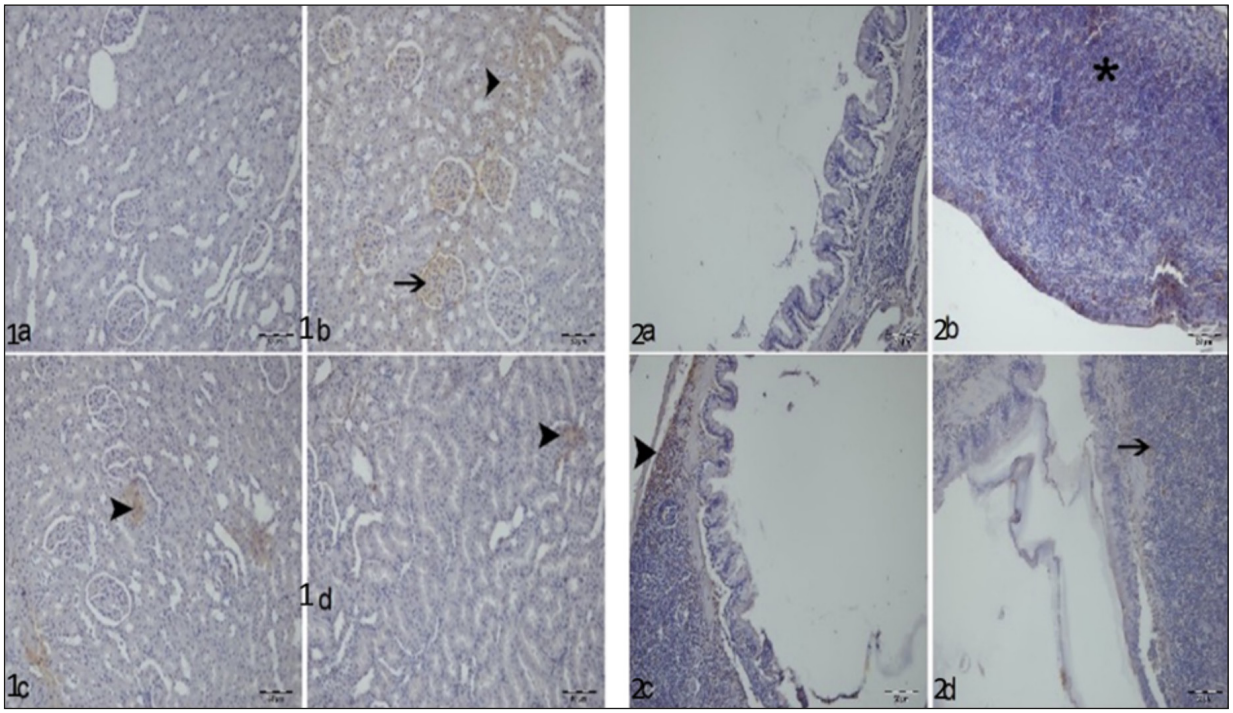

Figure 2. **1a**, Group 1 (control); (**1b**), Group 2 (IRI), intense NF-κB immunopositivity in glomeruli (arrow) and tubules (arrowhead); (**1c**), Group 3 (IRI+50 mg/kg HES), moderate NF-κB immunopositivity in tubules (arrowhead); (**1d**), Group 4 (IRI+100 mg/kg HES), mild NF-κB immunopositivity in tubules (arrowhead), IHC. **2a**, Group 1 (Control); (**2b**), Group 2 (IRI), intense NF-κB immunopositivity in bold-asterisk; (**2c**), Group 3 (IRI+50 mg/kg HES), moderate NF-κB immunopositivity in bold-arrowhead; (**2d**), Group 4 (IRI+100 mg/kg HES), mild NF-κB immunopositivity in bold-arrow, IHC. Magnification: 20×. IRI = Ischemia-reperfusion injury, HES = Hesperidin, IHC = Immunohistochemical, NF-κB = Nuclear factor kappa B.

of HES to oxidant stress both biochemically and histopathologically through the NF-κB pathway increases the importance of this rare study.

In our study, IRI increased TOS and OSI levels in Group 2 compared to Group 1. Still, we found that TOS and OSI were significantly lower in our HES-treated Groups (3 and 4) compared to Group 2, which suggests that HES is beneficial against IRI. The effect of HES on TOS was also decreasing in other studies[11].

The SOD concentration of the lung tissue was significantly lower in Group 2 compared to Group 1. Especially the SOD concentration of the 100 mg/kg HES group was significantly higher than Group 2. HES maintained the SOD amount in Group 4, but it was still lower than Group 1. Another study[12] that examined the antioxidant effect of HES in the lymphoid tissues found similar results with our study through finding that HES prevented SOD reduction in the thymus and spleen. In a study[13] searching HES against γ-irradiation to lungs, it has been shown that oral HES administration protects rats against lung damage, probably by its free radical scavenging and membrane-stabilizing property. There are other studies[14] in which the antioxidant effect of HES was measured *via* SOD.

In line with the literature[15], MDA levels in the kidney were higher in Group 2 compared to the control group showing that IRI increases MDA levels. The significant reduction in MDA levels of HES in treatment groups (3 and 4) compared to Group 2 is consistent with the literature[16].

In our study, the MPO concentration in the kidney tissues of Group 2 was significantly increased compared to the control group. MPO concentration was significantly lower in the 100 mg/kg HES pretreatment group than in Group 2. That MPO reducing the effect of HES is dose-dependent and compatible with the literature[17]. Especially in the target lesion, high dose HES administration was more effective on MPO in our study.

NF-κB plays a role in ischemia-reperfusion events[18]. ROS act as a mediator of NF-κB activation[19]. NF-κB helps predicting the amount of inflammation because NF-κB expression is

increased in inflammatory conditions, and NF-κB plays an antiapoptotic role through complex mechanisms[20]. We found no NF-κB involvement in the control specimens of both tissues in our study (Figure 2-1a, Figure 2-2a), proving the literature that NF-κB is a molecule that is activated when oxidative stress is developed. In our study, NF-κB immunopositivity was found to be intense in the kidney specimens of Group 2 (Figure 2b). There is a positive relationship between NF-κB increase and ischemia in literature[21]. NF-κB immunopositivity of the specimens of both tissues was moderate in Group 3 (Figure 2-1c, Figure 2-2c). NF-κB immunopositivity of Group 4 was lower than Group 3 (Figure 2-1d, Figure 2-2d). Another study[22] proves that HES reduces oxidative stress by showing NF-κB reduction. NF-κB can be activated in cells by various stimuli associated with stress, injury, and inflammation[23,24].

Conclusions

HES demonstrated a reno-protective effect against ischemia-reperfusion damage with its antioxidant, anti-inflammatory, and antiapoptotic properties.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Funding
The study was financially supported by authors.

Informed Consent
Not applicable.

Ethics Approval
The experiments of this study were carried out at our University Experimental Animals Research and Application Center after taking the approval of Erzurum Ataturk University Experimental Animals Local Ethics Committee (30.03.2018-55).

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