

Therapeutic effects of hydralazine on renal ischemia-reperfusion injury in rat

Fatemeh Ahmadi,^a Saeed Hajjhashemi,^{*,a} Ali Rahbari,^b Fatemeh Ghanbari^c

^aDepartment of Physiology, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran.

^bDepartment of Pathology, Arak University of Medical Sciences, Arak, Iran.

^cDepartment of Pharmacology, Islamic Azad University, Arak Branch, Arak, Iran.

Corresponding author: Saeed Hajjhashemi (Email: Hajjhashemi@arakmu.ac.ir)

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Objective Renal ischemia-reperfusion (I-R) induces acute kidney injury (AKI). In this study, the effect of hydralazine was investigated on the renal injury induced by the I-R in rats.

Methods AKI was induced with bilateral obstruction of the renal artery and vein for 20 min following 24 h of reperfusion. Hydralazine (5mg/kg) was injected intraperitoneally as post-treatment.

Results Hydralazine significantly increased the levels of renal clearance of creatinine and renal blood flow, while they were decreased by I-R. Also, hydralazine significantly improved levels of serum electrolytes (sodium and potassium) that were impaired by I-R. The tissue malondialdehyde levels were significantly suppressed by hydralazine.

Conclusion According to the results, the post-treatment of hydralazine had a therapeutic effect on renal I-R because of improved ion reabsorption and excretion and increased renal blood flow and glomerular filtration rate and decreased lipid peroxidation.

Key words hydralazine, renal ischemia-reperfusion injury, rat

Introduction

Acute kidney injury (AKI) is a complex clinical disorder simply meaning a sudden loss of kidney function induced by damage to the kidneys that causes structural and functional injury.¹ AKI is diagnosed based on significant increase in serum Cr, oliguria, and anuria.² The causes of AKI are divided into three categories: pre-renal, post-renal, and intrinsic kidney disease. Among these three categories, only intrinsic disorder represents the actual AKI state, while the other two are the result of extra renal disorders, leading to a decrease in glomerular filtration rate (GFR). If the conditions of these two classes are durable, it will eventually lead to cellular damage and intrinsic kidney disease.¹

The ischemia-reperfusion (I-R) model is one of the animal models used for the fundamental and therapeutic studies in AKI.³ I-R activates apoptosis and necrosis. The necrosis is characterized by cellular swelling with a membrane surface rupture. Necrotic cells stimulate the immune system resulting in filtration of inflammatory cells along with the release of cytokines.⁴

Apoptosis activates caspase's complex cascade. In vascular dysfunction, increased vascular permeability, endothelial cell inflammation, activation of the complement system, loss of fluid in the interstitial tissue, vasoconstriction, leukocyte activation, and endothelial-leukocyte reaction result in further damage.⁵

Hydralazine is used as an antihypertensive agent which reduces the contraction responses of a number of contractile agents. Hydralazine is a direct arteriolar vasodilator that controls the pregnancy's high blood pressure with a half-life of 1 h.⁶ Hydralazine activates guanylate cyclase; vasodilation effect does not directly, but indirectly releases endogenous nitric oxide (NO) from endothelial cells, by controlling the prolyl-hydroxylase domain protein and activating the hypoxia-inducible factor (HIF) pathway. Hydralazine also helps to restore the balance between NO and superoxide in endothelial

dysfunction by inhibiting NADPH oxidase.⁷ HIF-1 can increase NO production by multiple mechanisms, including increasing the expression of iNOS and COX4-2.⁸ Considering the characteristics of hydralazine in releasing endogenous NO^{9,10}; in this study, we aim to investigate the effects of hydralazine post-treatment administration on renal injury caused by I-R.

Materials and Methods

This experimental study was performed on 24 Wistar rats weighing 250–300 g. Rats were housed at 12 h of darkness and 12 h of brightness at room temperature of $23 \pm 2^\circ\text{C}$ with free access to the food and water. The experiments were performed on the rats in accordance with the guidelines and regulations and ethical codes approved by the Monitoring Committee for Laboratory Animals of Arak University of Medical Sciences.

The study groups included: (1) control group: did not receive the drug; (2) sham group: under anesthesia, only the kidneys were exposed without any ischemia; (3) I-R group: bilateral renal artery and vein occlusion for 20 min; then with 24 h reperfusion; (4) hydralazine treatment group: bilateral renal artery and vein occlusion for 20 min and then hydralazine (5 mg/kg, NOVAPLUS, USA) + 24 h immediately after reperfusion initiation.^{9–11}

To induce AKI, the animals were weighed first and then sodium thiopental (25 mg/kg; Sandoz, GmbH, Estonia) was injected intraperitoneally into the animal,¹² and after shaving the back hair, 1.5 cm incisions were made on either side of the midline using scissors and forceps. The artery and vein of both kidneys were blocked using a special clamp for 30 min, instantly after the end of the period, the obstruction was removed and the surgical area sutured with silk thread 3-0 and the animals were kept in a separate cage for recovery.¹¹

After recovery, the rats were placed in metabolic cages and the urine was collected for 12 h. Urine specific gravity was

determined. After 24 h of reperfusion, the rats' systolic blood pressure was measured from the tail by using a Power Lab instrument (AD Instruments, Australia) and the mean of systolic blood pressure was calculated for a rat.^{13,14} The rats were anesthetized and a longitudinal cut was created in the abdomen with a blade. After separation of the artery and vein of the left kidney, after stabilization of amount of blood flow, the renal blood flow (RBF) was recorded for 30 min by using flow meter of Power Lab instrument (T402, America)^{15,16} and the mean of blood flow was measured for 30 min. The blood sample was taken from the abdominal aorta by syringe of cold heparin. After plasma extraction, the concentration of creatinine (Cr) and blood urea nitrogen (BUN) was measured in the serum and urine samples by using the Auto Analyzer. (Selectra-XL, Netherlands).¹⁷ The concentration of sodium and potassium were measured by a flame photometer (SEAC-20Fp, Italy). Osmolarity of urine and plasma samples was measured using a Osmometer (Gonotec Osmomat-030, Germany).^{18,19}

Renal clearance of creatinine (C_{Cr}), absolute and relative excretions of potassium and sodium was calculated using the following formula:

$$C_{Cr}(\mu\text{l}/\text{min}/\text{gkw}) = (V^{\circ}/1000 \times U_{Cr})/P_{Cr}$$

Absolute excretion of sodium: $U_{Na} V^{\circ}(\mu\text{mol}/\text{min}/\text{gkw}) = (V^{\circ} \times U_{Na})/1000$.

Absolute excretion of potassium: $U_{K} V^{\circ}(\mu\text{mol}/\text{min}/\text{gkw}) = (V^{\circ} \times U_{K})/1000$.

Relative excretion of sodium: $FE_{Na} = (U_{Na} \times P_{Cr})/(P_{Na} \times U_{Cr}) \times 100$

Relative excretion of potassium: $FE_{K} = (U_{K} \times P_{Cr})/(P_{K} \times U_{Cr}) \times 100$.

Both kidneys separately were removed and weighed, then they were cut into two halves. For the MDA (malondialdehyde) and FRAP (Ferric reducing ability of plasma) experiments, the right kidney was placed in the liquid nitrogen and immediately transferred to the -20°C freezer. The Ohkawa method was used for MDA experiment, indicating the level of lipid peroxidation by MDA. In addition, the Benzie & Strain method was used for the FRAP assay.^{20,21}

For histological study, after removing the left kidney capsule, it was placed in 10% formalin buffer and after fixation, stages of dehydration, clarification, paraffin embedding was performed. After section cutting and preparation of 5-micron sections, slices were mounted and stained with hematoxylin and eosin. After preparation the slides, tissue analysis was performed by an expert pathologist.^{14,16}

The changes in glomerular and tubular and vascular structure were analyzed. Glomerulus diameter and Bowman's space size, percentage of injuries in tubule and glomerulus, the number of red blood cells (RBCs) in glomeruli, the shedding of tubular cells, formation of cast proteins in the lumen, tubular cells necrosis and the formation of vacuoles within the cells were examined.

Based on the severity of injuries in the glomerular and renal parenchyma, the quantity of damage was graded as follows: "Grade 0"; 1–25% damage, "Grade 1"; 25–50% damage, "Grade 2"; 50–75% damage, "Grade 3"; and 75–100% damage, "Grade 4".²²

Finally, the data were presented as the mean \pm standard error of the mean (S.E.M.). For statistical analysis, SPSS software version 25 (SPSS software, Chicago, IL, USA), one-way ANOVA, Tukey test, Kruskal–Wallis multiple comparison test

and Dunnett test were employed at $P \leq 0.05$ as the significance level for statistical analysis.²³

Results

Post-Treatment Effects of Hydralazine on the RBF and Systolic Blood Pressure

The results indicated RBF decreased significantly in the I-R groups (6.42 ± 0.3 ml/min, $P < 0.001$) compared to the control and sham group (8.5 ± 0.2 ml/min, $P < 0.01$). In the hydralazine-treated rats the RBF significantly increased (9.04 ± 0.4 ml/min, $P < 0.01$) compared to the I-R group, while there was no significant difference between the control group and the sham group. The systolic blood pressure did not show a significant difference between the groups (► Fig. 1).

Post-Treatment Effects of Hydralazine on Renal Clearance of Creatinine (C_{Cr}), Absolute Excretion of Sodium ($U_{Na} V^{\circ}$) and Potassium ($U_{K} V^{\circ}$) and Relative Excretion of Sodium (FE_{Na}) and Potassium (FE_{K})

Results showed that renal creatinine clearance was significantly decreased in the I-R groups (0.008 ± 0.005 $\mu\text{l}/\text{min}$ gkw; $P < 0.001$) compared to the control and sham (0.05 ± 0.01 $\mu\text{l}/\text{min}$ gkw). In the hydralazine-treated rats, the renal clearance

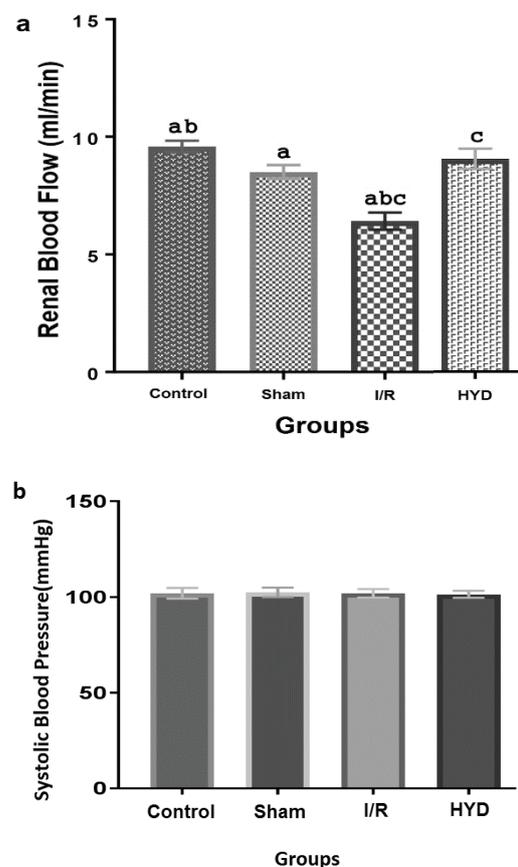


Fig. 1 Comparison of (a) renal blood flow and (b) systolic blood pressure among the groups. Similar characters represent significant differences between the groups. Results are expressed as mean \pm the standard Deviation (SD) for six rats in each group.

Table 1. Comparison of creatinine clearance (CCr), absolute (UNa^V) and relative (FENa) excretions of sodium and absolute (U_k^V) and relative (FE_k) excretions of potassium

Parameters groups	FE _k (%)	FE _{Na} (%)	U _k ^V (μmol/min.gkw)	U _{Na} ^V (μmol/min.gkw)	C _c (μl/min.gkw)
Control	28.6 ± 9.8	0.39 ± 0.3	2.68 ± 0.3	0.56 ± 0.1	0.05 ± 0.01
Sham	42.7 ± 13.8	0.28 ± 0.05	2.16 ± 0.2	0.58 ± 0.1	0.05 ± 0.01
I-R	42.7 ± 13.3 *	0.42 ± 0.2	0.82 ± 0.2 *** +++	0.47 ± 0.1	0.008 ± 0.005 *** +++
HYD + I-R	8.6 ± 3.1 * +++ □□□	0.001 ± 0.0004 * □□	0.64 ± 0.1 *** +++	0.4 ± 0.1	0.02 ± 0.009 * □□□ +

$P < 0.001$ ***, $P < 0.01$ ** , $P < 0.05$ * compared to the control group
 $P < 0.001$ +++ , $P < 0.01$ ++ , $P < 0.05$ + compared to the sham group
 $P < 0.001$ □□□ , $P < 0.01$ □□ , $P < 0.05$ □ compared to the I-R group
 Results expressed in mean ± standard deviation (SD) for 6 rats in each group

of creatinine (0.02 ± 0.009 μl/min gkw; $P < 0.05$) significantly increased in Compare to the I-R group (0.008 ± 0.005 μl/min gkw; $P < 0.001$).

The relative excretion of sodium (FE_{Na}) was not increased significantly in the I-R group compared to the control group ($0.42 \pm 0.2\%$ vs. $0.39 \pm 0.3\%$).

The FE_{Na} in the hydralazine group was significantly lower than that of the I-R group (0.001 ± 0.0004 vs. $0.42 \pm 0.2\%$; $P < 0.01$) and control group (0.001 ± 0.0004 vs. $0.39 \pm 0.3\%$; $P < 0.05$). The FE_{Na} control group was not significantly different from the sham group (►Table 1).

In the I-R group the FE_k showed a significant increase compared to the control group. ($47.28 \pm 13.3\%$ vs. $28.6 \pm 9.8\%$; $P < 0.05$). The FE_k significantly decreased in the hydralazine-treated rats compared to the control group ($8.6 \pm 3.1\%$ vs. $28.6 \pm 9.8\%$; $P < 0.05$), sham group ($8.6 \pm 3.1\%$ vs. $42.74 \pm 13.8\%$; $P < 0.001$) and I-R group ($8.6 \pm 3.1\%$ vs. $47.28 \pm 13.3\%$; $P < 0.001$) (►Table 1).

The absolute excretion of sodium (U_{Na}^V) did not show any significant difference among the groups. The U_k^V in the I-R group (0.82 ± 0.2 μmol/min gkw, $P < 0.001$) showed a significant decrease compared to the control and sham groups (2.68 ± 0.3 and 2.16 ± 0.2 μmol/min gkw, respectively), while the hydralazine group (0.64 ± 0.1 μmol/min gkw) did not show any significant difference with I-R group (►Table 1).

Post-Treatment Effects of Hydralazine on Urinary Levels of Sodium ([Na]_p), Potassium ([K]_p), Creatinine ([Cr]_p), and Osmolality (Osmol_p)

The urine sodium concentration in the I-R group was significantly higher than the control group. (28.72 ± 8.4 μmol/ml vs. 65.73 ± 6.9 μmol/ml; $P < 0.001$). The urine sodium concentration in the hydralazine group (28.45 ± 3.7 μmol/ml) was not significantly different from sham (32.33 ± 3.9 μmol/mL) and control groups. The urine sodium concentration decreased significantly in hydralazine group compared to the I-R group ($P < 0.001$).

The urine potassium concentration in the I-R group was significantly lower than the control group (112.9 ± 2.7 μmol/ml vs. 133.63 ± 13.6 μmol/ml; $P < 0.01$). The urine potassium concentration in the hydralazine group was significantly lower than the sham group (45.03 ± 6.3 μmol/ml vs. 120.28 ± 9.13

μmol/ml; $P < 0.001$) and the I-R groups (45.03 ± 6.3 μmol/ml vs. 112.9 ± 2.7 ; $P < 0.001$). The urinary potassium concentration was not significantly different in the I-R group and sham group. The urinary creatinine concentration was not significantly different between the groups.

Urine osmolality in the I-R group was significantly lower than the control group. (908.33 ± 48.12 mOsm/KgH₂O vs. 1515 ± 70.5 mOsm/KgH₂O; $P < 0.001$). Urine osmolality in the hydralazine group was significantly lower than the sham and control groups (1285.33 ± 162.08 mOsm/KgH₂O vs. 1493.83 ± 80.85 mOsm/KgH₂O; $P < 0.01$), whereas significantly increased compared to the I-R group (1285.33 ± 162.08 mOsm/KgH₂O vs. 908.33 ± 48.12 mOsm/KgH₂O; $P < 0.001$) (► Table 2).

Post-Treatment Effects of Hydralazine on Plasma Concentrations of Sodium ([Na]_p), Potassium ([K]_p), Creatinine ([Cr]_p), Urea ([BUN]_p), and Osmolality (Osmol_p)

The results showed that plasma creatinine concentration significantly increased with I-R induction in comparison with control group (1.24 ± 0.35 mg/dl vs. 0.55 ± 0.09 mg/dl; $P < 0.001$) and sham group (1.24 ± 0.35 mg/dl vs. 0.64 ± 0.08 mg/dl; $P < 0.01$). The plasma creatinine concentration in the hydralazine group exhibited significant reduction as compared to the I-R group (0.71 ± 0.1 vs. 1.24 ± 0.35 mg/dl; $P < 0.001$). There was no significant difference between the control and sham groups.

In I-R group, BUN was significantly higher than the control group (47.81 ± 5.9 mg/dl vs. 18.76 ± 2.4 ; $P < 0.001$) and the sham group (47.81 ± 5.9 mg/dl vs. 24.9 ± 3.1 mg/dl; $P < 0.01$). There was no significant difference between the hydralazine group and the I-R group.

Plasma sodium concentration was not significantly different between I-R group and control group (149.23 ± 6.2 μmol/ml vs. 152.1 ± 6.1 μmol/ml; $P < 0.01$). In the hydralazine group, plasma sodium concentration significantly decreased compared to control group (134.35 ± 2.5 μmol/ml vs. 152.1 ± 6.1 μmol/ml; $P < 0.001$), sham group (134.35 ± 2.5 μmol/ml vs. 144.45 ± 6.7 μmol/ml; $P < 0.05$) and I-R group (134.35 ± 2.5 μmol/ml vs. 149.23 ± 6.2 μmol/ml; $P < 0.001$).

Plasma osmolality and potassium concentration were not significantly different among the four groups (►Table3).

Table 2. Comparison of urinary concentrations of sodium ($[Na]_u$), potassium ($[K]_u$), creatinine ($[Cr]_u$) and osmolality ($Osmol_u$) among the groups

Parameters groups	$[Na]_u$ ($\mu\text{mol/ml}$)	$[K]_u$ ($\mu\text{mol/ml}$)	$[Cr]_u$ (mg/dl)	$Osmol_u$ (mosm/KgH ₂ O)
Control	28.72 ± 8.4	133.63 ± 13.6	1.38 ± 0.2	1515 ± 70.5
Sham	32.33 ± 3.9 □□□	120.28 ± 9.1	1.33 ± 0.3	1493.83 ± 80.8
I-R	65.73 ± 6.9 ***	112.9 ± 2.7 **	1.25 ± 0.2	908.33 ± 48.1 *** +++
HYD + I-R	28.45 ± 3.7 □□□	45.03 ± 6.3 *** +++ □□□	1.28 ± 0.2	1258.33 ± 162.08 ** ++ □□□

$P < 0.001^{***}$, $P < 0.01^{**}$, $P < 0.05^*$ compared to the control group
 $P < 0.001^{+++}$, $P < 0.01^{++}$, $P < 0.05^+$ compared to the sham group
 $P < 0.001^{□□□}$, $P < 0.01^{□□}$, $P < 0.05^{\square}$ compared to the I-R group
 Results expressed in mean ± standard deviation (SD) for 6 rats in each group

Table 3. Comparison of plasma concentrations of sodium ($[Na]_p$), potassium ($[K]_p$), creatinine ($[Cr]_p$) and osmolality ($Osmol_p$) among the groups

Parameters groups	$[Na]_p$ ($\mu\text{mol/ml}$)	$[K]_p$ ($\mu\text{mol/ml}$)	$[Cr]_p$ (mg/dl)	$[BUN]_p$ (mg/dl)	$Osmol_p$ (mosm/KgH ₂ O)
Control	152.1 ± 6.1	3.66 ± 0.4	0.55 ± 0.09	18.76 ± 2.4	309.16 ± 30.2
Sham	144.45 ± 6.7	3.79 ± 0.4	0.64 ± 0.08	24.9 ± 3.1	305.66 ± 6.1
I-R	149.23 ± 6.2	4.02 ± 0.5	1.24 ± 0.3 *** +++	41.11 ± 6.5 *** ++	318.83 ± 10.06
HYD + I-R	134.35 ± 5.2 *** + □□□	4.14 ± 0.2	0.71 ± 0.1 □□□	47.81 ± 5.9 *** +	333.66 ± 19.7

$P < 0.001^{***}$, $P < 0.01^{**}$, $P < 0.05^*$ compared to the control group
 $P < 0.001^{+++}$, $P < 0.01^{++}$, $P < 0.05^+$ compared to the sham group
 $P < 0.001^{□□□}$, $P < 0.01^{□□}$, $P < 0.05^{\square}$ compared to the I-R group
 Results expressed in mean ± standard deviation (SD) for 6 rats in each group

Post-Treatment Effects of Hydralazine on MDA and FRAP Levels in Renal Tissue

The results of this study indicated that MDA level per gram of kidney weight (gkw) significantly increased in I-R group (35.68 ± 6.4 $\mu\text{mol/gkw}$; $P < 0.001$) compared to control group (15.33 ± 4.21 $\mu\text{mol/gkw}$) and sham group (20.41 ± 3.3 $\mu\text{mol/gkw}$; $P < 0.001$). There was no significant difference in the MDA level in sham group compared to control group.

The MDA level in hydralazine-treated rats significantly reduce compared to the I-R rats (21.53 ± 4.19 $\mu\text{mol/gkw}$ vs. 35.68 ± 6.4 $\mu\text{mol/gkw}$; $P < 0.001$; ►Fig. 2a). There was no significant difference in the MDA levels of hydralazine group compared to the control and sham groups. FRAP level in the kidney tissue of I-R rats declined significantly compared to the control rats (5.69 ± 0.5 mmol/gkw vs. 8.61 ± 1.06 mmol/gkw; $P < 0.001$) and the sham group (7.59 ± 0.3 mmol/gkw; $P < 0.001$). FRAP levels of the control group was not significantly different from the sham group. FRAP level in the hydralazine-treated rats (7.006 ± 0.6 mmol/gkw) increased but was not significant compared to the I-R rats. FRAP level in the hydralazine group did not differ significantly from that of the control and sham groups (►Fig. 2b).

Post-Treatment Effects of Hydralazine on Histopathological Changes (►Fig. 3)

The results of this study showed extensive renal damage in the I-R rats that were significantly different compared to the control group (Grade 0), necrosis (Grade 3), and vacuolation (Grade 2) of tubular cells, increased of Bowman's space (Grade 1), presence of protein casts within the tubular lumen (Grade 2), scatter of cells into the lumen (Grade 3), reduce number of RBCs glomerular (Grade 1), and glomerular injury (Grade 2) were significantly different from the control group (Grade 0; $P < 0.05$).

In the hydralazine-treated rats, necrosis of the tubular cells (Grade 2) was significantly different in compared to the control and sham groups (Grade 0; $P < 0.05$) and significantly reduce in comparison with the I-R group (Grade 3; $P < 0.05$) (►Fig. 3).

Hydralazine had no significant effects on increased Bowman's space (Grade 1), formation of protein casts (Grade 2), vacuolation (Grade 2), glomerular injury (Grade 2), cell scattering (Grade 2), and reduction in the number of glomerular RBCs (Grade 1) compared to the I-R group (►Table 4).

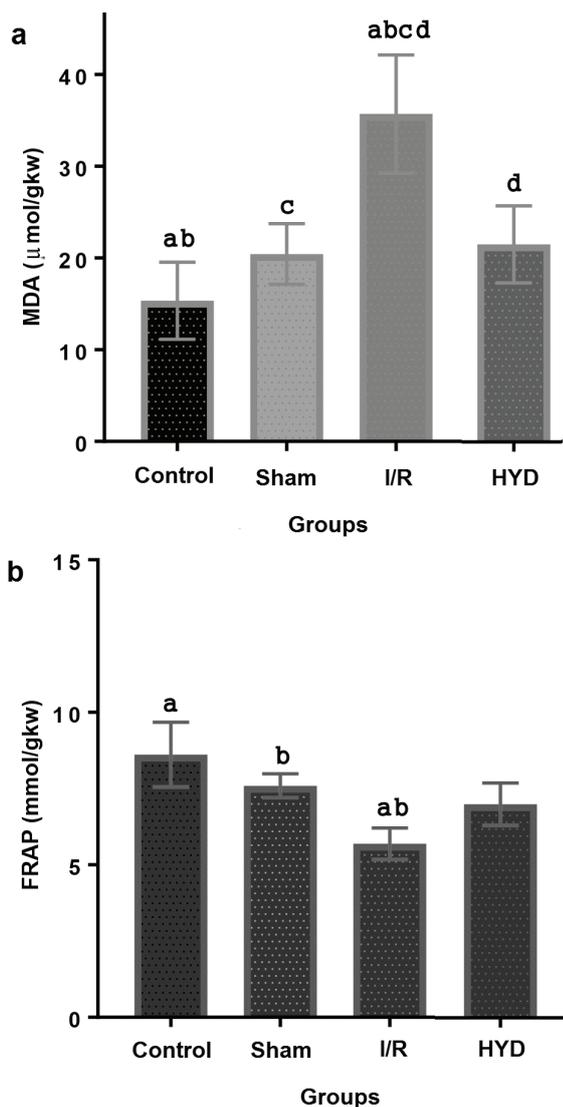


Fig. 2 Comparison of (a) MDA and (b) FRAP levels among the groups. Similar characters represent significant differences between the groups. Results are expressed as mean \pm the standard deviation (SD) for six rats in each group. Results are expressed on per gram kidney weight (gkw).

Discussion

The results of present study showed renal I-R caused AKI that is associated with increased plasma creatinine, BUN and decreased RBF and clearance of creatinine.²⁴ Post-treatment with hydralazine significantly increased the renal clearance of creatinine and RBF and also improved sodium reabsorption and urine osmolality. Post-treatment with hydralazine had a nephroprotective effects on renal I-R. These results may be due to the fact that renal I-R injury leads to a lack of integrity and polarity of the epithelial cells with brush border destruction of the tubule. These changes promote the epithelial cellular downfall and the appearance of protein casts, lumen obstruction, and increased intratubular pressure. These factors caused a reduction in ion reabsorption, which during the reperfusion can lead to tubular obstruction and decreasing of GFR. Injured proximal tubules cannot reabsorbed sodium ions, which activates tubuloglomerular feedback. This feedback

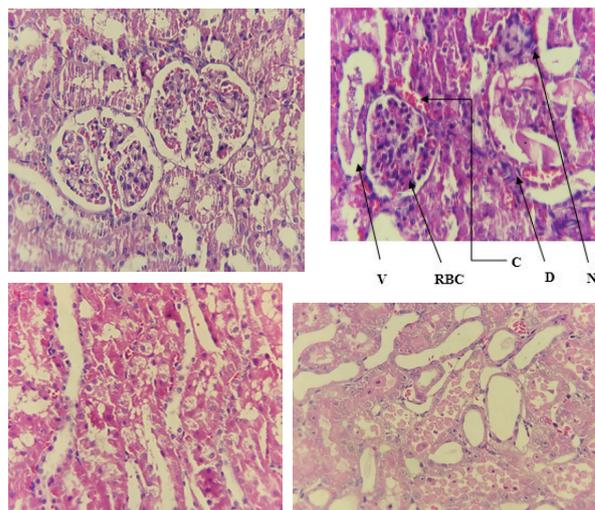


Fig. 3 Comparison of renal histological between different groups. (a) Control group with glomerular and normal tubular structure ($\times 40$); (b) I-R group with tubular cell necrosis, formation of protein casts inside the tubule lumen, cells scattering into the tubule lumen, vacuolation of tubular cells, increased Bowman's space and reduced number of red blood cells in glomerulus ($\times 40$); (c) sham group with tubular cell necrosis ($\times 40$); (d) Hydralazine group with increased Bowman's space, vacuolation of tubular cells and reduced tubular cell necrosis ($\times 40$).

RBC: Red Blood Cell, N: Necrosis, C: Intratubular cast, D: Downfall, V: Vacuolization

significantly caused pre-glomerular arteriole constriction and reduce GFR.²⁵

Hydralazine significantly increased the renal clearance of creatinine rate in the I-R rats. Previous studies indicated that hydralazine increased the formation of NO by the vascular endothelium and elevated intracellular cGMP levels.^{26, 27} Increased intracellular cGMP levels dilated afferent and efferent arterioles vasodilation leading to increase of GFR. In other study, Reetu R. Singh showed that NO increases GFR by increasing in the ultrafiltration coefficient; NO can also reduce TGF and cause constriction of pre-glomerular blood vessels.^{28, 29}

Plasma electrolytes in the I-R rats were impaired in comparison with the control rats. FE_{Na} increased in the I-R rats compared to the control rats but was not significant. FE_K also increased in the I-R group. This increase indicates the injury to the tubular epithelial cells, especially the proximal tubule during I-R.²⁵ Proximal tubules (PT, especially S3), thick ascending limb of Henle's loop (TAL) and external medulla are susceptible to I-R injury.³⁰ In addition, I-R causes brush border destruction with reduced reabsorption of sodium by proximal tubules, impaired expression of tubular sodium transporters and unsuitable regulation of Na^+/K^+ -ATPase expression in the basolateral membranes.^{19, 30} In the I-R injury lead to direct injury of cells responsible for the secretion of potassium in the distal tubules and collecting ducts.³¹ Post-treatment of hydralazine in the I-R rats reduced urinary excretion of sodium compared to the I-R rats. Wu et al. showed the direct inhibitory effect of NO on renal Na^+ transport in proximal tubule and also the renal sympathetic nervous system that stimulated Na^+ transport in proximal tubule. Many studies showed the direct inhibitory effect of NO on Na^+ transport in the isolated tubule segments but not in the intact kidney.³² Hydralazine is not a first-line drug for treating

Table 4. Comparison of necrosis level, protein casts, cell scattering, vacuolation, the reduced number of red blood cells, increased Bowman's capsular space, and glomerular injury

Parameters groups	Cell necrosis	Vacuolation	Increased Bowman's capsular space	Formation of protein casts	Cell scattering	Reduced number of red blood cells	Glomerular injury
Control	0	0	0	0	0	0	0
Sham	1 * □	0 □	1 *	0 □	0 □	0 □	0 □
I-R	3 * +	2 * +	1 *	2 * +	2 * +	1 * +	2 * +
HYD + I-R	2 * + □	2 * +	1 *	2 * +	2 * +	1 * +	2 * +

$P < 0.05^*$ compared to the control group

$P < 0.05^+$ compared to the sham group

$P < 0.05^{\square}$ compared to the I-R group

Results expressed in mean \pm standard deviation (SD) for 6 rats in each group

hypertension due to increased activity of the sympathetic nervous system of the juxtamedullary cells, increased plasma renin activity and salt maintenance.³³ Therefore, the activation of the renin-angiotensin system tends to neutralize the hydralazine vasodilator effect.³⁴ In the cortical collecting duct, NO inhibits basolateral membrane potassium conductance.³⁵

Urine osmolality was significantly decreased in the I-R rats compared to the control group because of impairment in the ability of the renal urine concentration. Inappropriate regulation of the AQP and Na⁺ transporter proteins in renal tubules and decreasing the expression of Na⁺/K⁺-ATPase in the thick descending limb of the external medulla that causing changes in sodium and water regulation under the influence of I-R injury.¹⁹ In the hydralazine-treated rats, urinary osmolality was significantly more than that of the I-R rats. Previous studies have shown that hydralazine reduces urinary volume due to water and sodium retention.³⁶

In I-R rats, MDA levels significantly increased and FRAP levels significantly decreased in the kidney tissue. Previous studies have shown that I-R causes an imbalance between reactive oxygen species (ROS) sources and free radicals scavenger (reduced glutathione peroxidase, catalase, and superoxide dismutase).³⁷ During the reperfusion phase, the oxygen free radicals caused lipid peroxidation which indicated AKI and necrosis.³⁸ The MDA levels in the hydralazine-treated rats decreased compared to the I-R group and FRAP levels were increased compared to the I-R group. Previous studies have shown that treatment with hydralazine reduces the level of lipid peroxidation in myocardium in rats with spontaneous hypertension.³⁹ The antioxidant effects of hydralazine are via the formation of ROS scavenger and inhibitor of O₂-generation and peroxynitrite.⁴⁰

In the present study, blood pressure was not significantly different among the groups. Regulation of blood pressure is via the short- and medium-term mechanisms, and the kidneys are long-term regulators of blood pressure that regulated blood volume via the renin-angiotensin system and function of aldosterone hormone.

In this study, in the I-R rats the RBF rates showed a significant decrease compared to the control and sham groups, which can be due to the fact that increased renal vascular

resistance (RVR) can be a vascular response to ischemia. Increase of RVR may activate vasoactive factors, ROS and inflammatory pathways that can affect renal perfusion. Activation of the sympathetic nervous system, the renin-angiotensin system, prostaglandins, and endothelin A are main vasoconstrictor agents reducing RBF under I-R injury.⁴¹ In the sham group, the RBF was significantly lower than the control group, which could be due to the effects of anesthesia and surgical stress. Mercatello et al. has shown that anesthesia drugs influenced renal function not only directly, but also through changes in function of cardiovascular and endocrine system. Many barbiturates tend to reduce RBF.⁴² In the hydralazine-treated rats, the RBF was significantly increased in compare to the I-R rats. Previous studies indicated that hydralazine significantly reduces RVR and increased cardiac output which ultimately increases RBF.^{43, 44} The cellular mechanism of vasodilatory effect of hydralazine is not clearly determined, but it may be related to its calcium binding ability that is required for smooth muscle contraction of vessels, inhibiting IP₃-induced calcium release from sarcoplasmic reticulum, and inhibiting myosin phosphorylation in arterial smooth muscle cells.²⁷ Elkayam Uri et al. has shown that stimulating the production of endogenous NO in the kidney can consider as a therapeutic target for increasing RBF in patients with heart failure.⁴⁵

In the present study, histopathological studies showed that compared to the control group, the I-R group induced vacuolation of tubular cells, degeneration of renal tubules, tubular cell necrosis, increased Bowman's space, formation of protein casts within the tubular lumen, cellular cell scattering into the tubular lumen, reduced number of RBCs in glomerula, and glomerular injury. In the hydralazine-treated rats, the improvement was not found in pathologic outcomes. The imbalance between expression and activity of eNOS and iNOS (decreased eNOS activity and increased iNOS expression) is responsible for the pathophysiology of I-R injury.⁴⁶ Previous studies have not shown the improvement effects of hydralazine on the histological changes.⁴⁷ Authors believe that the time of using hydralazine and also duration of treatment exert a very important role in pathological improvement.

Conclusion

Regarding the results of the present study, post-treatment of hydralazine had therapeutic effects on AKI caused by I-R and improved ion reabsorption and excretion, hemodynamic parameters, antioxidant defense, and reduced MDA.

IRB Ethical Approval Number

The study Ethics Committee of Arak University of Medical Sciences approved this research under registration number IR.ARAKMU.REC. 1396.287.

Informed Consent

This research was not on humans. All ethical codes developed by the Monitoring Committee for Laboratory Animals at Arak University of Medical Sciences were complied with in the experiments conducted in the present study.

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