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Role of Antioxidant and Anti-inflammatory Parameters in the Renoprotective Effects of Valsartan against Renal Ischemia-Reperfusion Injury in Male Rats

Parvin Ramian[1];Ph.D, Izadpanah Gheitasi[2*];Ph.D, Delaram Eslimi Esfahani[1*];Ph.D, Hossein Sadeghi[2,3];Ph.D, Elmira Beirami[1];Ph.D

[1]Department of Animal Sciences, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
[2]Department of Physiology, Faculty of Medicine, Yasuj University of Medical Sciences, Yasuj, Iran
[3]Department of Pharmacology, Faculty of Medicine, Yasuj University of Medical Sciences, Yasuj, Iran

Abstract

Renal ischemia–reperfusion (I/R) injury, a major cause of acute kidney injury, is characterized by inflammation, oxidative stress, and functional impairment. Valsartan, an angiotensin II receptor blocker, reduces blood pressure and exhibits anti-inflammatory and antioxidant properties. This study evaluated the renoprotective effects of valsartan through antioxidant and anti-inflammatory parameters in a male rat model of renal I/R. Eighteen male Wistar rats (250–300 g) were randomly assigned to three groups: sham control, I/R control, and I/R treated with valsartan. Valsartan (60 mg/kg) was administered orally by gavage for one week before and immediately after I/R induction. After 24 hours, urine, blood, and kidney tissue samples were collected to assess biochemical and functional parameters including mean arterial pressure (MAP), inflammatory and oxidative stress markers, and histopathological alterations. In the I/R group, blood urea nitrogen (BUN), tumor necrosis factor-alpha (TNF-α), tissue malondialdehyde (MDA), fractional excretion of sodium (FENa) and potassium (FEK), urinary sodium, and plasma creatinine significantly increased, while glomerular filtration rate (GFR), ferric reducing antioxidant power (FRAP), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and MAP significantly decreased ($P<0.05$). Valsartan treatment significantly reduced urinary urea, BUN, TNF-α, and FENa, improved antioxidant enzyme activities and plasma FRAP, and increased urinary creatinine, potassium, and sodium compared with the I/R group ($P<0.05$). Histopathological examination demonstrated reduced renal tissue injury following valsartan treatment. Overall, valsartan attenuated renal I/R injury by reducing oxidative stress, inflammation, and structural damage, suggesting potential therapeutic benefits that warrant further clinical investigation.

Keywords: Valsartan; Renal ischemia-reperfusion; TNF-α; Oxidative stress.

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1. Introduction

Ischemia-reperfusion (I/R) is a complex pathological process in which blood flow to a tissue is temporarily interrupted and subsequently restored. In early ischemia, cessation of arterial flow (usually due to embolic obstruction) reduces oxygen delivery and impairs the tissue's metabolic needs, leading to hypoxia and cellular damage. Reperfusion injury may further exacerbate tissue damage due

Corresponding Author: Izadpanah Gheitasi, Department of Physiology, Faculty of Medicine, Yasuj University of Medical Sciences, Yasuj, Iran
E-mail: gheitasiizadpanah@gmail.com

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to intense inflammatory responses (1).

Ischemia-reperfusion injury (IRI) commonly occurs in clinical procedures including partial nephrectomy, kidney transplantation and cardiac surgery with cardiopulmonary bypass. Such injury can result in both structural and functional renal damage (2). This condition triggers the activation of both innate and adaptive immune responses, including complement activation, platelet aggregation, microvascular dysfunction, cellular apoptosis, and the infiltration of inflammatory cells into the tissue, ultimately culminating in acute kidney injury (AKI) (3).

In this context, oxidative stress plays a central role in I/R-induced injury. A disturbance in the balance between the production of reactive oxygen species (ROS) and the body's antioxidant defense capacity is one of the principal mechanisms underlying such pathological injury (4). Excessive production of ROS, which primarily results from electron leakage in the mitochondrial electron transport chain, can cause severe damage to DNA, lipids, and proteins, ultimately leading to cellular death (5). Under such conditions, endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) play a protective role by directly neutralizing free radicals or by enhancing the body's antioxidant defense system (4). However, under conditions of intense oxidative stress, the normal function of these antioxidant enzymes may be insufficient (5, 6). Consequently, exogenous antioxidants have been suggested as a potential therapeutic approach.

The renin-angiotensin-aldosterone system (RAAS) plays a critical role in the pathophysiology of I/R-induced renal injury. Angiotensin II (AngII), the key effector molecule of this system, contributes to vasoconstriction, increased oxidative stress, induction of apoptosis, and amplification of inflammatory responses by binding to AT1 receptors (7).

Activation of RAAS and increased angiotensin II (Ang II) are key factors in renal injury after I/R. Ang II exacerbates renal injury by inducing oxidative stress, apoptosis, vasoconstriction, and increased vascular sensitivity to sympathetic stimulation. RAAS also modulates renal inflammation through two opposing pathways: the pro-inflammatory ACE/Ang II/AT1 and ACE2/Ang (1–7)/Mas pathways. ACE inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) have shown protective effects against I/R-induced injury (8, 9).

Valsartan, an ARB approved by the FDA, is recognized for its ability to lower blood pressure, reduce oxidative stress and cellular injury, and protect renal tissue (10-12).

Although the anti-inflammatory and antioxidant properties of valsartan have been demonstrated in various renal injury models, including acute kidney injury and diabetic nephropathy (9, 13–15), its renoprotective effects in acute renal ischemia-reperfusion (I/R) injury remain less well-characterized. Most previous investigations have employed short-term pretreatment or post-injury administration protocols and focused on a limited set of biomarkers.

This study aimed to address this knowledge gap by investigating a chronic pretreatment regimen with valsartan (60 mg/kg/day orally for one week prior to ischemia, supplemented with an additional dose immediately post-reperfusion) in a male rat model of bilateral renal I/R injury. This approach not only allowed exploration of potential preconditioning effects but also enhanced translational relevance by simulating clinical scenarios involving prophylactic and perioperative administration. Furthermore, evaluations conducted at 24 hours post-reperfusion enabled a comprehensive assessment of early acute-phase alterations in functional, biochemical, oxidative stress, inflammatory, and histopathological parameters, thereby advancing our understanding of the renoprotective mechanisms of valsartan in acute I/R settings.

2. Materials and Methods

2.1. Animals, study design, and surgical procedure

In this study, adult male Wistar rats weighing 250-300 g were used. The animals were obtained from the Animal Research Center of Isfahan University of Medical Sciences,Isfahan, Iran). They were housed for

10 days under controlled conditions(22±2 ºC, 12-hour light/dark cycle) with free access to water and standard chow, to allow acclimatization to the new environment. All experimental procedures were conducted in accordance with the guidelines approved by the Ethics Committee of Kharazmi University, Tehran, Iran (Ethics code: IR.KHU.REC.1400.108).

The rats were randomly divided into three experimental groups (n=6 per group):

1. Sham control group: Rats underwent laparotomy and right total nephrectomy without clamping of the left renal artery.
2. I/R group: Following right total nephrectomy, the left renal artery was clamped for 45 minutes, followed by reperfusion.
3. Valsartan-treated I/R group: Rats received valsartan (Abidi Pharmaceutical Co., Tehran, Iran) at a dose of 60 mg/kg body weight (16). These rats underwent laparotomy, right total nephrectomy and left renal artery ischemia for 45 minutes, followed by reperfusion.

Groups 1 and 2 received equivalent volumes of normal saline via oral gavage on the same schedule as the valsartan group. In the valsartan-treated I/R group, valsartan was administered orally via gavage daily for one week prior to surgery, with an additional single dose given immediately after reperfusion (upon clamp removal). No further doses were administered thereafter, as the experiment concluded twenty-four hours post-reperfusion. This duration was chosen to focus on acute-phase changes following I/R injury.

For induction of anesthesia, sodium pentobarbital (60 mg/kg) was injected intraperitoneally (17). A midline abdominal incision was then made, and a right nephrectomy was performed in all groups. In the I/R and valsartan groups, the left renal artery was clamped for 45 minutes using a microvascular atraumatic clamp, followed by reperfusion. In the sham group, the left kidney was exposed whitout arterial clamping. After surgery, the incision was sutured, and the animals were allowed to recover from anesthesia under standard postoperative conditions before being returned to their cages.

2.2. Collection of urine, plasma, and renal Tissue samples

Twenty-four hours after reperfusion, the rats were re-anesthetized. A tracheostomy was performed, and the tracheal tube was connected to an oxygen supply. Body temperature was monitored using a rectal thermometer and maintained at 37 °C with a heating pad. The right femoral vein was catheterized for the continuous infusion of saline (3 mL/h) and for the administration of anesthesia. The right femoral artery was catheterized for blood pressure measurement using a transducer connected to a PowerLab/USP data acquisition system (AD Instruments, Australia).

A bladder catheter was inserted, and after a 30-minute stabilization period, urine was collected for 1 hour into pre-weighed tubes. Then, 5 mL of blood was drawn from the artery, centrifuged, and the plasma was separated and stored at -30 °C. Urine volume was measured and, if necessary, diluted and stored at a low temperature.

At the end of the experiment, the left kidney was removed and weighed, then divided into two parts: one part was snap-frozen in liquid nitrogen and stored at -80 °C for measurement of TNF-a and oxidative stress markers, while the other was fixed in 10% formalin for histopathological analysis.

Following terminal sample collection, rats under deep anesthesia were euthanized by intravenous injection of potassium chloride solution (2 mmol/kg), sufficient dose to induce immediate cardiac arrest, in accordance with institutional ethical guidelines

2.3. Measurement of biochemical and renal functional parameters

Urine and plasma samples were analyzed to measure creatinine (Cr) and urea levels using commercially available assay kits

(Pars Azmoon Co., Iran). The concentrations of potassium (K) and sodium (Na) ions were determined using the ion-selective electrode method. The volume of urine excreted from the left kidney was measured gravimetrically, and urine flow rate was calculated relative to kidney weight (KW) as V0 (µL/min/gKW).

Cr clearance was evaluated as an index for determining glomerular filtration rate (GFR) (18). The absolute excretion of Na (UNaV0), absolute excretion of K (UKV0), fractional excretion of Na (FENa), and fractional excretion of K (FEK) were also calculated using standard mathematical formulas.

2.4. Measurement of oxidative stress and inflammatory markers

Plasma nitric oxide (NO) levels were indirectly determined by measuring nitrite concentration using the Griess colorimetric method. Due to the instability of NO and its rapid conversion to nitrite and nitrate, nitrite concentration was measured as an indirect indicator. After plasma separation, the Griess reagent (containing sulfanilamide and N-(1-naphthyl) ethylenediamine (NED)) was added to the samples. The reaction resulted in the formation of a chromophore, which was measured spectrophotometrically at 540 nm. Plasma nitrite concentration was determined using a sodium nitrite standard curve (19).

Renal tissue malondialdehyde (MDA) levels were measured based on the modified Ohkawa method, adapted for this study. In this method, 250 µL of tissue homogenate was mixed with a solution containing 15% trichloroacetic acid (TCA), 0.25 N hydrochloric acid (HCl), and 0.375% thiobarbituric acid (TBA), and incubated in a water bath at 100 °C for 30 min. Samples were then centrifuged at 3500×g for 10 min, and absorbance was measured at 570 nm (20).

The tissue ferric reducing antioxidant power (FRAP) assay was used to assess the sample's ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions in the presence of TPTZ reagent. A blue TPTZ-Fe^{2+} complex with maximum absorbance at 570 nm was formed. Calibration was performed using $FeSO_4 \cdot 7H_2O$ standard solutions ranging from 0 to 1000 µmol/L (21).

Antioxidant enzyme activities, including glutathione peroxidase (GPx), were assessed using a commercial kit (Navand Salamat Co., Iran). SOD and CAT were measured using kits from Karmania Parsgen (Iran), following the manufacturers' instructions. The concentration of tumor necrosis factor-alpha (TNF-α) in kidney tissue was measured using a rat-specific ELISA kit (Karmania Parsgen Co., Iran) according to the supplier's protocol, and the absorbance for this inflammatory cytokine was recorded at 450 nm.

2.5. Histological examination

Following blood sampling, kidney tissue samples were collected and fixed in 10% formalin solution. The tissues were then embedded and sectioned into slices of 3-4 µm in thickness. These sections were mounted on glass slides and stained with hematoxylin and eosin (H&E). A pathologist performed a blinded evaluation of slides.

2.6. Statistical analysis

All collected data were analyzed using one-way analysis of variance (ANOVA). Tukey's post hoc test was used to determine significant differences between groups. The results were expressed as mean ± standard error of the mean (SEM), and a P value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. Effect of valsartan on biochemical, functional, and blood pressure changes

3.1.1. Plasma and urinary creatinine, urinary urea, and blood urea nitrogen

Plasma Cr levels were significantly increased in the I/R group compared to the sham group ($P<0.001$); however, treatment with valsartan did not significantly alter this parameter

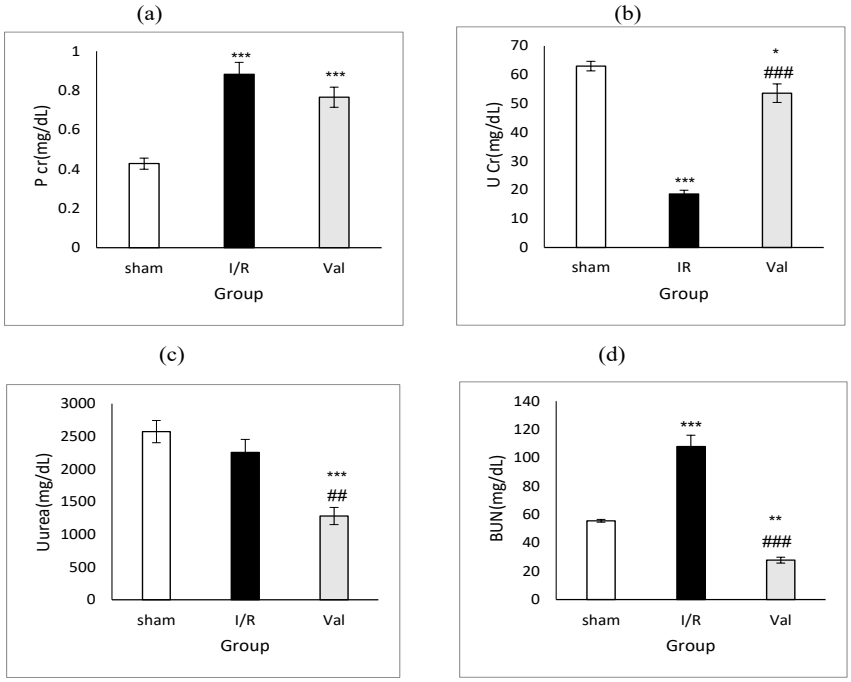

Figure 1. Effect of valsartan (Val) on plasma Cr (a), urine Cr (b), urine Urea (c) and BUN (d) following I/R injuryData are expressed as mean ± SEM. ***Significant differences with sham group (P<0.001), **Significant differences with sham group (P<0. 01), *Significant differences with sham group (P<0. 05), ### Significant differences with IR group (P<0.001), ## Significant differences with IR group (P<0.01). IR: ischemia-reperfusion, BUN: blood urea nitrogen, Cr: creatinine, p, plasma, U, urine, Cr; creatinine.

compared to the I/R group (P>0.05). The corresponding data are shown in Figure 1-a.

As shown in Figure 1-b, urinary Cr in the I/R group was significantly reduced compared with the sham group (P<0.001). Treatment with valsartan significantly increased this parameter compared with the I/R group (P<0.001), although it remained lower than that of the sham group.

As shown in Figure 1-c, urinary urea level in the I/R group did not show a significant difference compared to the sham group. However, valsartan treatment significantly reduced this parameter compared with both the I/R group (P<0.01) and the sham group (P<0.001).

According to the results presented in Figure 1-d, BUN level in the I/R group significantly increased compared with the sham group (P<0.001). Administration of valsartan resulted in a significant reduction in BUN compared with the I/R group (P<0.001).

3.1.2. kidney weight, urine flow and volume, rlectrolytes, absolute sodium excretion, absolute potassium excretion, fractional excretion of sodium, and fractional excretion of potassium

As shown in Table 1, the mean KW did not differ significantly among the studied groups (P>0.05). In the I/R group, urine volume (P<0.01) and urine flow rate (V0) (P<0.05) increased significantly compared with the sham group. There was no significant change in V0 in the valsartan group compared to the I/R group (P>0.05), wherease UV showed a significant increase (P<0.05). There was no significant difference in plasma Na and K levels among the studied groups (P>0.05). Urinary Na level in the I/R group significant-

Table 1. Effect of valsartan (Val) on some biochemical markers following I/R injury.

Parameters	Sham	IR	IR+Val
KW(gr)	0.98±0.08	1.04±0.05	1.01±0.004
UV(mL)	0.49±0.03	0.63±0.023**	0.74±0.026***#
V0(µl/min/gKW)	7.9±0.45	10.23±0.62*	11.11±0.74**
PNa(mmol/L)	147.6±2.66	144.5±1.63	143.8±1.24
PK(mmol/L)	5.33±0.39	5.04±0.38	4.61±0.15
UNa(mmol/L)	34.43±4.15	55.98±2.64***	39±1.98##
UK(mmol/L)	63.02±1.65	35.43±1.31***	66.95±2.79###
UNaV0 (µmol/min/g KW)	0.271±0.04	0.573±0.047**	0.432±0.03*
UKV0 (µmol/min/g KW)	0.497±0.038	0.362±0.025*	0.74±0.042**###
FENa(%)	0.15±0.02	0.96±0.08***	0.30±0.03###
FEK(%)	8.02±2.4	17.49±4.3**	16.58±0.20*

Renal functional and electrolyte parameters in different experimental groups. Data are presented as mean ± SEM. ***Significant differences with sham group (P < 0.001), **Significant differences with sham group (P < 0. 01), *Significant differences with sham group (P < 0. 05), ### Significant differences with IR group (P < 0.001), ## Significant differences with IR group (P < 0.01), # Significant differences with IR group (P < 0.05). IR: ischemia–reperfusion, Val: valsartan-treated group. KW: kidney weight, UV: urine volume, V_0: urine flow rate, PNa: plasma sodium concentration, PK: plasma potassium concentration,UNa: urinary sodium concentration, UK: urinary potassium concentration, $UNaV_0$: absolute sodium excretion, UKV_0: absolute potassium excretion, FENa: Fractional excretion of sodium %, FEK: Fractional excretion of potassium %.

ly increased compared with the sham group (P<0.001). Administration of valsartan significantly reduced the urinary Na concentration compared with the I/R group (P<0.01). Urinary K in the I/R group showed a significant decrease compared with the sham group (P<0.001). In the valsartan-treated group, a significant increase in urinary K was observed compared with the I/R group (P<0.001). In the I/R group, UNaV0 significantly increased compared with the sham group (P<0.01). Valsartan administration resulted in a non-significant reduction in this parameter compared to the I/R group.

In the I/R group, UKV0 significantly decreased compared to the sham group (P<0.05). Treatment with valsartan resulted in an increase in this parameter compared with the I/R group (P<0.001). The FENa% in the I/R group greatly increased compared with the sham group (P<0.001). The use of valsartan significantly reduced this parameter compared with the I/R group (P<0.001). Regarding the FEK%, the I/R group showed a significant increase compared with the sham group (P<0.001), but valsartan treatment did not cause a significant reduction in this parameter (P>0.05).

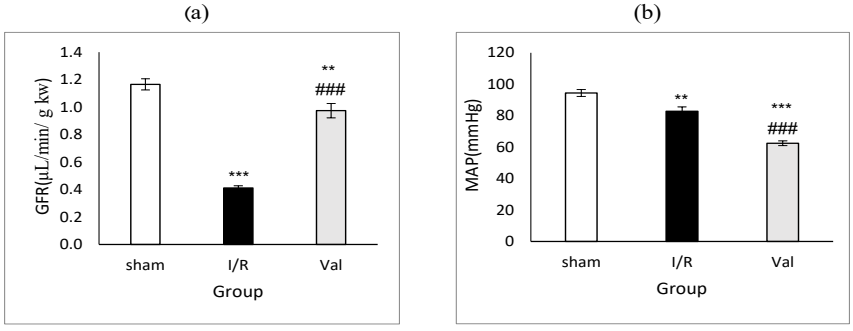

Figure 2. Effect of valsartan (Val) on GFR (a) and MAP (b) following I/R injury. Data are expressed as mean ± SEM. ***Significant differences with sham group (P<0.001), **Significant differences with sham group (P<0.01), ###Significant differences with IR group (P<0.001). IR: ischemia-reperfusion, GFR: glomerular filtration rate and MAP: mean arterial pressure.

3.1.3. glomerular filtration rate and mean arterial pressure

As shown in Figure 2-a, GFR was significantly reduced in the I/R group compared with the sham group (P<0.001). Valsartan administration improved this parameter compared with the I/R group (P<0.001).

According to Figure 2-b, the MAP in the I/R group showed a significant decrease compared with the sham group (P<0.01). Valsartan treatment caused a further reduction in MAP compared with the I/R group (P<0.001).

3.2. Effect of valsartan on changes in oxidative stress and inflammatory markers

According to Figure 3-a, the concentration of the inflammatory cytokine tumor necrosis factor alpha (TNF-α) showed a significant increase in the I/R group (P<0.01). Treatment with valsartan significantly reduced TNF-α levels (P<0.01).

Plasma nitric oxide (NO) levels in the I/R group were significantly increased compared with the sham group (P<0.01). Valsartan treatment resulted in a non-significant decrease in plasma NO compared with the I/R group (P>0.05),as shown in Figure 3-b.

Renal tissue ferric reducing antioxidant power (FRAP levels in the I/R group showed a significant decrease compared with the sham group (P<0.01). Valsartan caused a non-significant increase in tissue FRAP compared with the I/R group (P>0.05) In plasma,

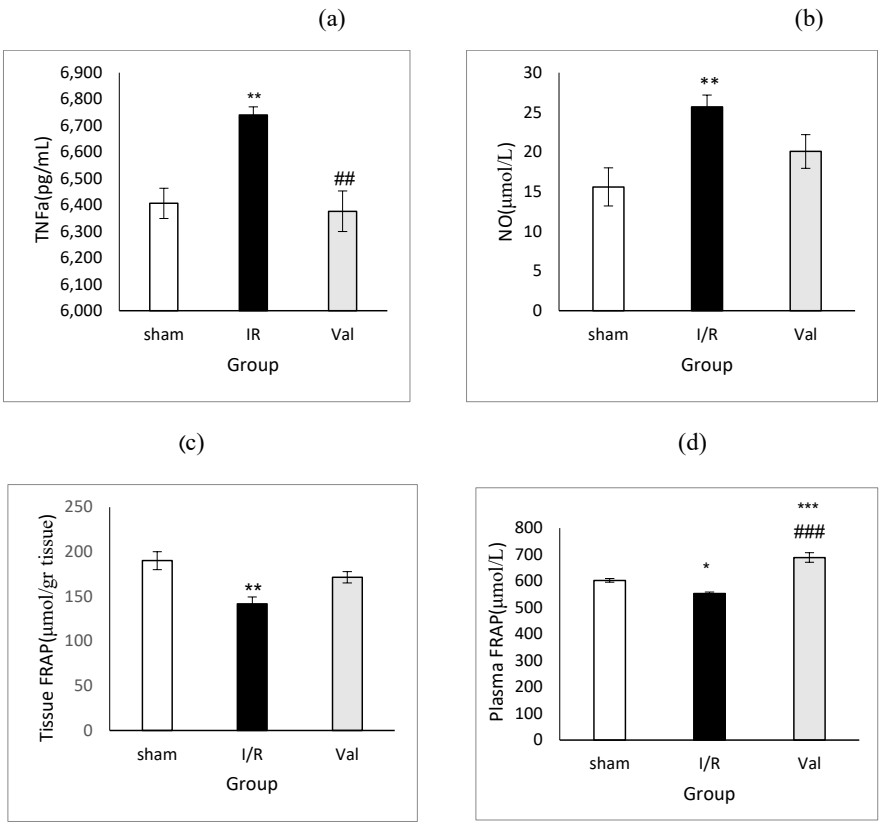

Figure 3. Effect of valsartan (Val) on inflammatory, nitrosative and total antioxidant capacity indices including: TNF-α (a), Plasma NO (b), Tissue FRAP (c), and Plasma FRAP (d) following I/R injury. Data are expressed as mean±SEM.***Significant differences with sham group (P<0.001), **Significant differences with sham group (P<0.01), *Significant differences with sham group (P<0.05), ### Significant differences with IR group (P<0.001), ## Significant differences with IR group (P<0.01), IR: ischemia-reperfusion, TNFα: tumor necrosis factor alpha, NO: nitric oxide, FRAP: ferric reducing antioxidant power.

FRAP levels significantly decreased in the I/R group(P<0.05), while treatment with valsartan showed a significant increase compared with the I/R group (P<0.001), as illustrated in Figures 3-c and 3-d.

According to Figures 4-a and 4-b, the activity of the antioxidant enzyme SOD in plasma significantly decreased in the I/R group compared with the sham group (P<0.001). In the treatment group, a significant increase in SOD levels was observed compared with the I/R group (P<0.001). Similarly, SOD levels in the kidney tissue of the I/R group were significantly lower than those in the sham group (P<0.05), and valsartan significantly increased these levels compared with the I/R group

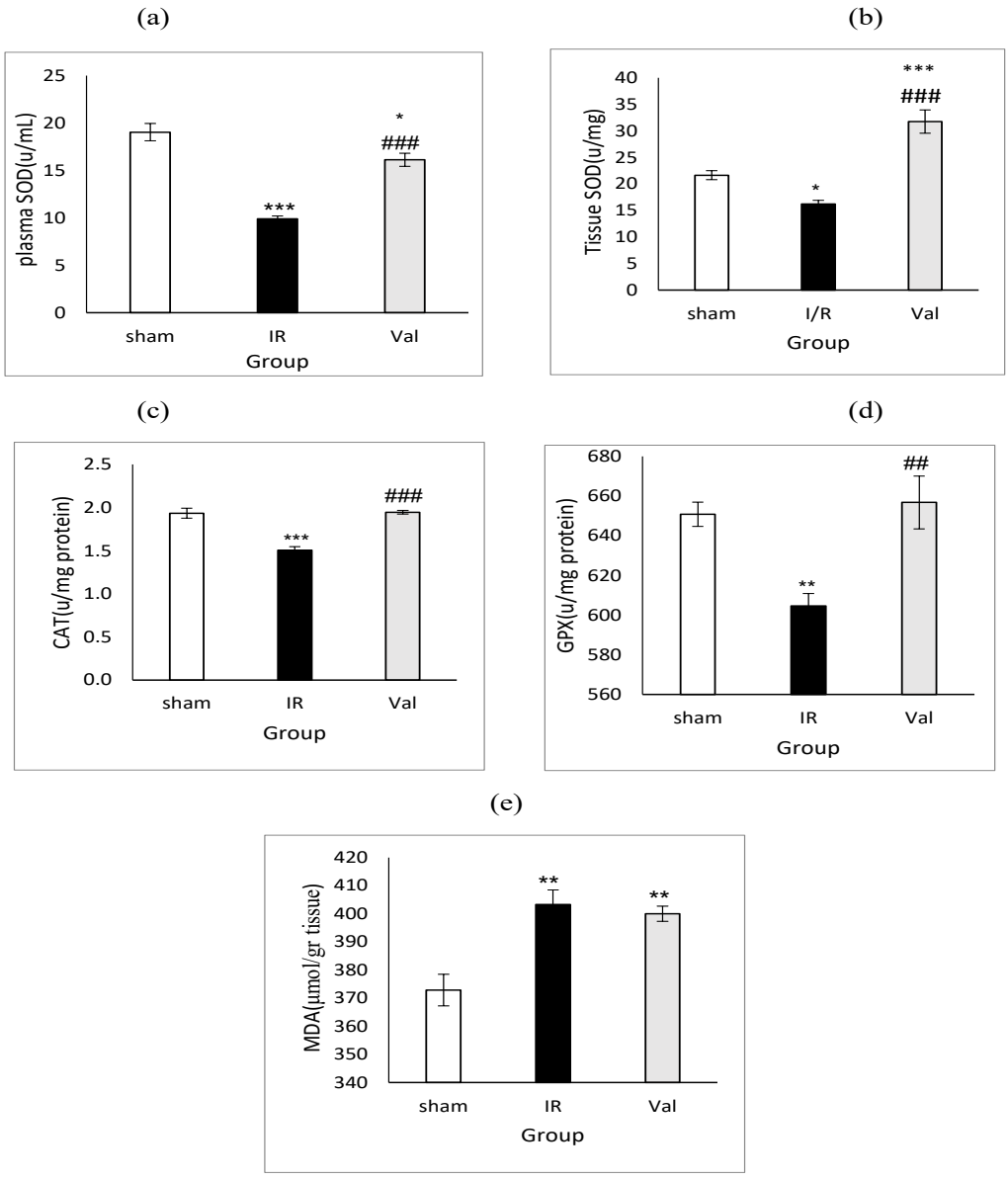

Figure 4. Effect of valsartan (Val) on antioxidant enzyme activities and lipid peroxidation, plasma SOD (a), Tissue SOD (b), Tissue CAT (c), Tissue GPX (d) and Tissue MDA (e) following I/R injury. Data are expressed as mean±SEM.***Significant differences with sham group (P<0.001), **Significant differences with sham group (P<0.01), *Significant differences with sham group (P<0.05), ### Significant differences with IR group (P<0.001), ## Significant differences with IR group (P<0.01), IR: ischemia-reperfusion, SOD: superoxide dismutase, CAT: catalase, GPX: glutathione peroxidase, MDA: malondialdehyde.

(P<0.001).

As shown in Figure 4-c, the activity of the antioxidant enzyme CAT in kidney tissue was significantly decreased in the I/R group (P<0.001). Valsartan treatment significantly increased CAT levels in kidney tissue (P<0.001).

As observed in Figure 4-d, the level of the enzyme GPx in the I/R group showed a significant decrease compared with the sham group (P<0.01). Valsartan treatment resulted in a significant improvement in the activity of this enzyme (P<0.01).

As shown in Figure 4-e, MDA levels in the I/R group were significantly increased compared with the sham group (P<0.01).valsartan treatment did not produce a statistically significant change in MDA levels.

3.3. Histological evaluation

According to the results shown in Figure 5 and Table 2, the kidney tissue sections in the sham group maintained their normal structure without any pathological alterations. In contrast, the I/R group exhibited severe tissue damage. Observed alterations included cellular vacuolization, necrosis of tubular epithelial cells, dilation of tubular lumens, detachment of epithelial cells, formation of proteinaceous casts, vascular congestion, and glomerular injuries such as Bowman's space dilation and increased mesangial cell number. The histological injury score in this group was reported as 17. In the group treated with valsartan, tissue damage was substantially reduced, and the injury score in this group was 6.

For clarification, a semi-quantitative scoring system was applied as follows: 0 = no tissue affected, 1 = mild (1–20%), 2 = moderate (21–40%), 3 = severe (41–60%), 4 = very severe (61–80%), and 5 = extensive (81–100% of tissue affected).

4. Discussion

The findings of this study indicate that valsartan, through its antioxidant and anti-inflammatory effects, can reduce the severity of ischemia-reperfusion (I/R)-induced kidney injury by improving biochemical and hemodynamic parameters and protecting renal tissue.

The I/R-induced renal injury model in male rats, involving temporary occlusion and reperfusion, leads to disturbances in renal homeostasis, increased free radical production, reduced antioxidant capacity, and activation of inflammatory pathways (3,22). These alterations in kidney function and structure provide a suitable platform to assess renoprotective interventions.

The results of this study showed that administration of valsartan significantly reduced arterial blood pressure in the renal I/R animal model. This blood pressure-lowering effect is consistent with previous findings reporting the inhibitory role of valsartan on the renin-angiotensin pathway and its protective effect on hemodynamics in diabetic rat models and *Zucker obese* rats (23).

Table 2. Histopathological scores of kidney tissue in experimental groups based on hematoxylin and eosin (H&E) staining.

Histological markers	Sham	IR	Valsartan
Glomerulus injury	0	1	0
Tubular epithelial cell necrosis	0	4	1
Tubular injury	0	5	3
Inner and outer medulla for epithelial cell necrosis	0	1	0
Proteinaceous cast	0	1	0
Medullary vessel congestion	0	5	2
Total score	0	17	6

Semi-quantitative scoring system: 0 = no tissue affected, 1 = mild (1–20%), 2 = moderate (21–40%), 3 = severe (41–60%), 4 = very severe (61–80%), 5 = extensive (81–100% of tissue affected). IR: ischemia-reperfusion.

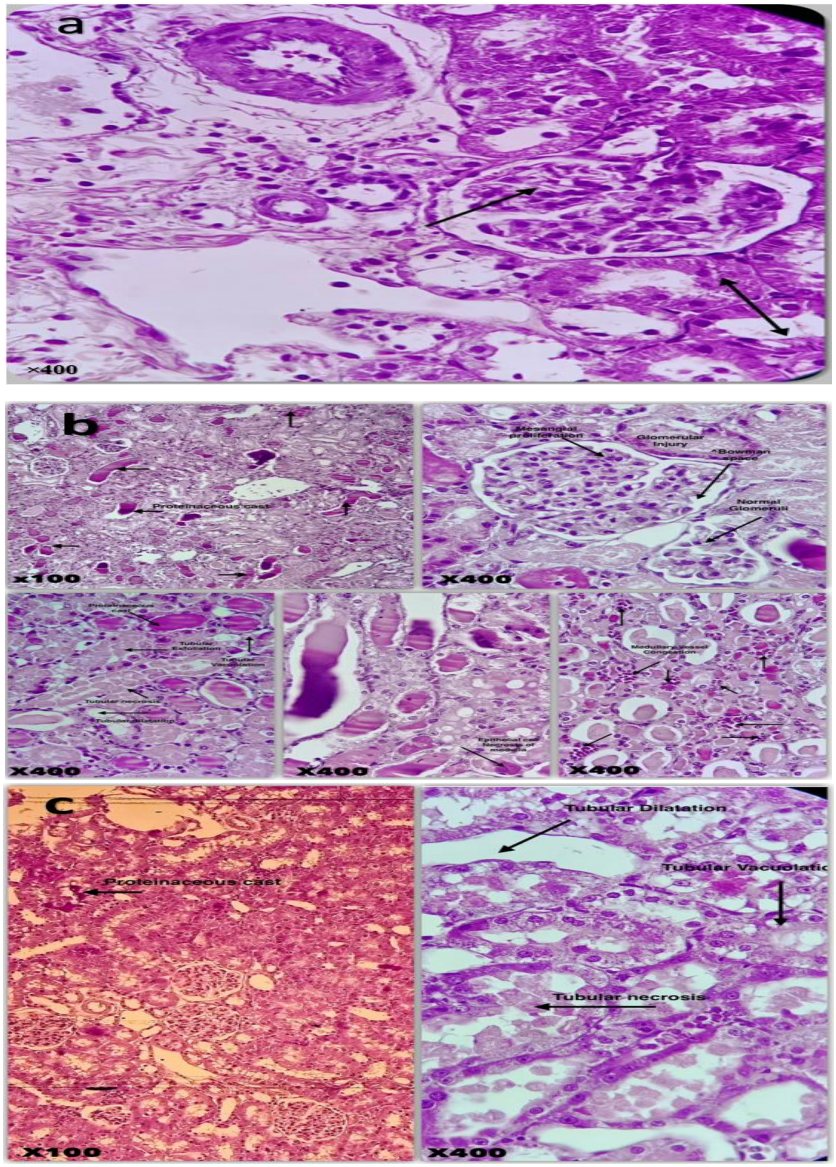

Figure 5. Microscopic images of kidney tissue in different groups, stained with hematoxylin and eosin (H&E).

The observed blood pressure reduction in the valsartan group, compared with the sham group, can be explained by the therapeutic effects of the drug. Angiotensin II receptor blockade by valsartan not only reduces blood pressure but also contributes to improved renal structure and function through the modulation of signaling pathways and reduction of inflammation, oxidative stress, and fibrosis. in line with previous observations in chronic kidney disease (CKD) models, although the present findings were obtained in an acute renal I/R setting (24).

Moreover, our findings indicate that renal I/R impaired kidney function, as shown by increased BUN and plasma Cr and decreased GFR, consistent with previous I/R studies (25). These changes reflect tissue damage caused by interrupted perfusion followed by oxidative stress and inflammation.

Valsartan administration significantly reduced BUN compared to the I/R group. However, this contrasts with Jing et al. (2017), who found no significant BUN reduction in a CKD model—could be related to differences in drug dosage (26). Although plasma Cr lev-

els were reduced in the treatment group, the difference was not statistically significant, aligning with findings from subtotal nephrectomy models (27).

The significant reduction in GFR observed in the I/R group reflects hemodynamic failure and structural damage (28). Valsartan treatment significantly improved GFR, possibly through its antioxidant properties and inhibition of the renin-angiotensin system,—mechanisms that have been shown to enhance renal function in experimental animal models (14, 15).

Regarding urinary urea, the I/R group showed a non-significant reduction compared with the sham group, consistent with earlier reports (29). Valsartan further decreased urea excretion, possibly by modulating tubular handling of urea or by reducing inflammation-induced protein catabolism. Although limited animal data are available on this effect, these findings differ from studies using ginger in I/R models, likely due to differences in pharmacological profiles, anti-inflammatory mechanisms and timing of tubular recovery (30).

In the I/R group, plasma Na and K levels were not significantly different from controls, consistent with previous studies (28, 29). Urinary K was significantly decreased in the I/R group, aligning with earlier reports (30), but valsartan significantly increased urinary K compared with the I/R group. Urinary Na was elevated in I/R group and was reduced by valsartan treatment, contrasting with findings in salt-sensitive Dahl SS rats, where valsartan increased urinary Na without affecting K excretion (31).

This difference may be because I/R reduces the expression of Na transporter proteins in renal tubular cells, such as Na^+/H^+exchanger3 (NHE3), sodium-phosphate cotransporter IIa (NaPi-IIa), and Na^+/K^+-ATPase. This reduction subsequently leads to impaired Na reabsorption in renal tubules and altered altered urinary electrolyte excretion patterns, while plasma levels of Na and other ions remain relatively unchanged (32).

In assessing absolute electrolyte excretion, the I/R group showed a significant increase in UNaV0 and a significant decrease in UKV0. Valsartan caused a non-significant reduction in UNaV0, though not statistically significant, while UKV0 significantly increased compared with the I/R group. Although direct data on the effects of valsartan on UNaV0, UKV0, FENa, and FEK in animal models are limited, previous renal I/R and renal perfusion studies provide mechanistic support for these findings (25, 28).

These changes likely result from proximal tubular injury and impaired Na^+/K-ATPase function due to I/R (33).

Additionally, previous studies have shown that I/R leads to an increase in FENa and FEK compared with the control group, which was confirmed in this study (34). Valsartan significantly reduced FENa, wherease FEK was not significantly altered by valsartan treatment. The FENa increase in I/R is due to impaired tubular sodium reabsorption and oxidative tubular damage (32).

Urine volume and flow were significantly increased in the I/R group, consistent with previous reports showing either no change or increased urine volume after I/R injury (25, 28, 30). Valsartan did not significantly alter urine volume or urine floe compared with the I/R group. The increased urine volume may be due to reduced aquaporin (AQP2) expression and decreased water reabsorption in renal tubules after IRI (30, 32).

I/R induction significantly increased TNF-α levels in renal tissue, while valsartan treatment significantly reduced this cytokine.

These findings are consistent with previous observations in HFD/STZ-induced diabetic mouse models, supporting the anti-inflammatory effects of valsartan, although the present results were obtained in an acute renal I/R setting (14).

TNF-α promotes kidney injury by activating inflammatory and survival pathways

such as NF-κB, AP-1, JNKs, and p38 MAPK, and its inhibition may reduce AKI severity (35). Ke *et al*. also showed that blocking the TNFα/RIP1/RIP3 pathway reduces I/R-induced damage (36).

Furthermore, NO levels were significantly elevated in the I/R group, likely due to iNOS induction and peroxynitrite formation, which contribute to tissue damage via oxidative stress, vascular permeability, and cell death (37).

This mechanism aligns with previous studies that have shown TNF-α, IL-1β, and IL-6 can upregulate iNOS expression (38).

Valsartan-treated rats showed a reduction in NO levels compared with the I/R group; however, this change was not statistically significant. This finding may reflect an insufficient dose or duration of treatment to effectively modulate nitric oxide production, highlighting the need for future studies to explore optimized dosing regimens and underlying mechanisms, including the iNOS pathway (37).

In this investigation, exposure to I/R significantly increased MDA levels in kidney tissue compared with the sham group, indicating enhanced lipid peroxidation and intensified oxidative stress. These finding are consistent with Previous reports in animal models (39, 40).

Previous studies have reported reduction in MDA levels following valsartan administration (14, 15). In the present study, although a relative decrease in MDA was observed in the valsartan group, it was not statistically significant. This lack of significance may result from the chemical stability and tissue retention of MDA, which is suggested to contribute to the maintenance of elevated levels despite reduced oxidative stress (41). Variations in experimental model, dosage, or treatment duration may also contribute to these differences.

I/R-induced oxidative stress significantly reduced the activity of key antioxidant enzymes (SOD, CAT, and GPx) in plasma and kidney tissue, indicating a weakened defense system against ROS, consistent with earlier studies (39, 40). Valsartan treatment notably increased SOD activity in both tissue and plasma. Similarly, it improved CAT activity, which may have been suppressed due to H_2O_2 accumulation and intracellular acidosis under I/R conditions (42, 43). GPx activity was also significantly elevated following valsartan administration. These results support the antioxidant role of valsartan, in line with prior findings in various experimental models (14, 15).

Consistent with previous studies using I/R animal models (28,29), FRAP levels were significantly reduced in I/R-injured kidneys, reflecting impaired antioxidant capacity. Although studies using FRAP to evaluate valsartan's effects are limited, some evidence suggests that the drug may enhance total antioxidant capacity, potentially through such as mitochondrial protection (44).

In the present study, valsartan significantly increased plasma FRAP. Although an increase in kidney tissue FRAP was observed, it was not statistically significant. Histopathology revealed severe I/R-induced kidney damage, including necrosis, tubular dilation, and glomerular injury, as reported in previous studies (45, 46). Valsartan markedly reduced injury severity, indicating its protective effects against I/R damage.

In addition to blocking AngII receptors, valsartan has been reported to modulate renal calcium signaling pathways, including proteins such as calmodulin, PKCα, and CaMKIV (24). These pathways have been associated with reduced inflammation, oxidative stress, and renal fibrosis, as well as improved glomerular and interstitial structures and decreased collagen deposition. Furthermore, valsartan has been reported to support renal tissue survival and repair through pathways involving proteins such as EGFR and ErbB2, thereby contributing to kidney integrity beyond blood pressure control (24).

5. Conclusion

Administration of valsartan in the renal I/R model in male rats resulted in improvements in renal function indicators, reductions in inflammatory and oxidative stress markers, such as TNF-α and MDA, and increased levels of antioxidant enzymes, including SOD, CAT, and GPx. Histological observations also showed that valsartan significantly prevented structural damage in kidney tissue.

Therefore, by acting on the angiotensin II pathway, valsartan may represent a valuable option for reducing renal injury caused by ischemia–reperfusion and may serve as a foundation for further therapeutic research in this field.

Authors contributions

A .Ramian, B. Gheitasi, C. Eslimi, D. Sadeghi and E. Beirami.

A and B wrote the manuscript.
B and D designed the study.
A and B performed the experiments.
C and E performed the analytic calculations.

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Study Limitations and Recommendations

1. This study has several limitations:
2. The short treatment duration and 24-hour reperfusion period may not have fully captured the long-term renoprotective effects of valsartan.
3. Key early renal injury biomarkers, including NGAL, KIM-1, and cystatin C, were not assessed.
4. Detailed mechanistic analyses (e.g., apoptosis, IL-6 and NF-κB expression, and Na^+/K^+-ATPase activity) were not performed.
5. To address these limitations, further studies with longer treatment and follow-up periods, along with comprehensive mechanistic investigations and additional biomarkers, are recommended.

Fundings

None to declare.

Ethical approval

The experimental protocol was approved by the institutional ethics committee (Ethics code: IR.KHU.REC.1400.108).

Conflict of Interest

The authors declare that they have no conflict of interest.

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