

Allopurinol mitigates distant organ damage following bilateral renal ischemia-reperfusion injury in male rats

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Title: Allopurinol Mitigates Distant Organ Damage Following Bilateral Renal Ischemia-Reperfusion Injury in Male Rats

Running title: Allopurinol and distant organ injury

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Abstract

Introduction: Ischemia/reperfusion injury (I/R) is common in various clinical situations. A growing body of clinical evidence suggests that renal I/R can trigger dysfunction in distant organs, underscoring the need for interventions that address both local and systemic effects. This study aimed to investigate the effect of allopurinol on distant organ damages induced by renal I/R.

Methods: Twelve Sprague-Dawley rats were randomly divided into groups: I) Sham, II) I/R, and III) I/R + Allopurinol. Renal I/R was induced by bilateral clamping of the renal pedicles for 60 minutes, followed by 24 h of reperfusion. A single dose of allopurinol (100 mg/kg, gavage) was administered 1h before I/R. At the end of 24 hours, electrocardiograms and mean blood pressure were recorded. Functional biomarkers, pathological findings, and oxidative stress levels were assessed in the tissue samples of the experimental groups.

Result: Renal I/R injury significantly increased the functional biomarkers of the kidneys (Cr and BUN), heart (LDH and cardiac troponin I), and liver enzymes (ALT, AST, and, APT). Pathological changes were significant in both the site of ischemia and the distant organs. Total oxidative status increased, while total antioxidant capacity decreased in the lung, liver, and heart tissues of the I/R group. Allopurinol pretreatment significantly restored the plasma levels of functional biomarkers and oxidative stress in the lung, heart, and liver tissues. Allopurinol pretreatment improved the pathological damage in the kidneys and distant organs.

Conclusion: Allopurinol, a xanthine oxidase inhibitor, was able to reverse the structural damage and functional disorders of the kidney and remote organs caused by renal ischemia by modulating the oxidative stress pathway.

Keywords: Ischemic-Reperfusion, Liver, Heart, Lung, Distant Organ, Oxidative Stress, Allopurinol

Introduction

Acute Kidney Injury (AKI) is a rapid deterioration of kidney function, which can result from various causes, including renal ischemia-reperfusion (I/R) injury. Renal ischemia refers to the cellular damage that occurs when blood supply is restored to the kidneys after a period of ischemia (lack of blood flow)(1, 2). AKI is associated with significant morbidity and mortality, especially in critically ill patients. It may lead to complications such as fluid overload, electrolyte imbalances, uremia, and a higher risk of chronic kidney disease(3). The effects of renal I/R injury can extend beyond the kidneys, leading to distant organ effects due to a complex interplay of molecular and cellular responses(4). Clinical observations and studies on animals indicate that humans and animals respond to local tissue damage with disruptions in systemic homeostasis, which can trigger remote injury of the heart, lung, gut, brain, liver, and other organs(5-8)

Acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) can occur due to the systemic release of inflammatory cytokines. These mediators can increase vascular permeability, leading to pulmonary edema(9).

The production of reactive oxygen species (ROS), elevated levels of various cytokines, and activation of immune cells play a crucial role in the development of these distant organ disorders(10, 11). In the rat model of renal ischemic reperfusion, cellular injury in the heart and brain tissues is associated with an increase in lipid peroxidation and a decrease in antioxidant markers in both brain and heart

tissues(12). Bagheri et al. demonstrated that a compound derived from rice bran oil, gamma-oryzanol, protects the brain, heart, and liver tissues from damage caused by oxidative stress induced by renal ischemia-reperfusion injury(13).

Xanthine oxidase (XO) is produced in various organs, including the liver, gut, lungs, kidneys, heart, brain, and plasma. This is an endogenous source of ROS and plays a significant role in ischemia, heart failure, vascular injury, and inflammation. In the previous study, we indicated that the level of XO was elevated in the post-ischemic kidney(14). Allopurinol and its active metabolite, oxypurinol, are effective inhibitors of XO used in the clinical management of gout and conditions linked to hyperuricemia. Additionally, allopurinol exhibits renoprotective properties, which are supported by its ability to reduce the formation of ROS associated with apoptosis. Soliman et al. reported that reduced kidney and liver functions after renal I/R were associated with increased XO levels and decreased peroxisome proliferator-activated receptor gamma (PPAR- γ) activity. Administration of allopurinol increased the PPAR- γ expression and improved both liver and kidney functions. Additionally, allopurinol reduced inflammation and nitrosative stress, as indicated by decreases in tumor necrosis factor-alpha (TNF- α), inducible nitric oxide synthase (iNOS), nitric oxide (NO), and peroxynitrite formation(15). Therefore, the main aim of our study was to investigate the effect of allopurinol on distant organ damage induced by renal ischemia-reperfusion through oxidative stress pathways.

2-Materials and Methods

2-1-Animals

Twenty-one adult male Sprague-Dawley rats (8 week), weighing 200–240 g, were acquired from the Animal Center of the Shiraz University of Medical Sciences. All animals were kept in a single cage under standard conditions (12-hr light/dark cycles; 22 ± 2 °C) and had unrestricted access to water and standard laboratory food. Animal Ethics Committee of Shiraz University of Medical Sciences confirmed all experimental protocols of this study with the ethics code of IR.SUMS.AEC.1402.074.

2-2-Experimental design

The rats were randomly divided into three groups (n=7 per group). Sham group, I/R group, and

I/R+Allopurinol (100 mg/kg, gavage) group. In this study, our control group is the same as the sham group. The sham group consists of healthy animals. The concept of the sham group is that the surgical procedure was performed on them without clamping the renal artery and vein. By doing this, we can confirm that the results observed in the ischemia group are due to renal ischemia, not the stress of surgery.

2-3-surgical procedures

At first, rats were anesthetized with an intraperitoneal injection of ketamine (50 mg/Kg) and xylazine (10 mg/Kg). Then, anesthetized rats were placed on a surgical heating table to control the body core temperature at a range of $37 \pm 1^{\circ}\text{C}$ throughout the surgery. The abdomen was opened via a midline incision. In the I/R group, bilateral renal pedicles were clamped with nontraumatic vascular clamps for 60 min. After 60 min, the pedicle clamps were removed and the kidney reperfed. In the I/R+Allopurinol group, the rats were pretreated with allopurinol (100 mg/kg, gavage) 1 hour before the induction of ischemia. The 100 mg dose was chosen based on previous studies (16) and pilot experiments, which showed no toxic effects at this level. Our prior study demonstrated that allopurinol reduced oxidative stress without significant side effects. Therefore, an allopurinol-alone (allopurinol-only) group was not included. Once the procedure was finished, the muscular and skin layers of the abdominal wall were sutured.

After 24 h of reperfusion, rats were weighed and re-anesthetized. Animals were placed on a wooden plate because in recording the electrocardiogram, the surface on which the animal is placed should not be a conductor of electric current which could interfere with our ECG recording. Then, a filled cannula containing normal saline (Samen, Mashhad, Iran) with 15 IU/mL heparin (Alborzdarouco, Tehran, Iran) was inserted into the right femoral artery and linked to a pressure transducer. Arterial blood pressure (BP) and heart rate (HR) were continuously recorded for 10 min using a PowerLab/8SP data acquisition system (AD Instruments, BellaVista, NSW, Australia) after 15 min of equilibration (diagram 1).

Blood samples were taken from the tail vein. The plasma was separated by centrifugation at 5000 rpm for 15 min and then stored at -80°C for measuring the functional parameters. Next, the kidneys, heart, liver, and lung tissues were harvested and weighed. The animal's chest is opened to remove heart and lung tissue. This is how the animal euthanasia. The right kidney and parts of the liver, heart, and lung tissues were quickly frozen in liquid nitrogen and saved at 80°C for the oxidative stress analysis. The left kidney and other parts of these organs were placed in a buffer of 10% formalin for histopathological assessments.

After the removal of the right lung of each rat, the wet weight of the lung was determined. Then, they were incubated in an oven set to 90°C for 72 h, after which their weight was measured as dry weight. The lung weight wet-to-dry ratio (wet/dry) was determined as an index for pulmonary edema. The kidneys, heart, and liver tissues were then removed and weighed to calculate the kidney, heart, and liver index according to a previously reported method(10).

Kidney Index = Kidney weight /Body weight $\times$ 100

Liver Index = Liver weight /Body weight $\times$ 100

Heart Index = Heart weight /Body weight $\times$ 100

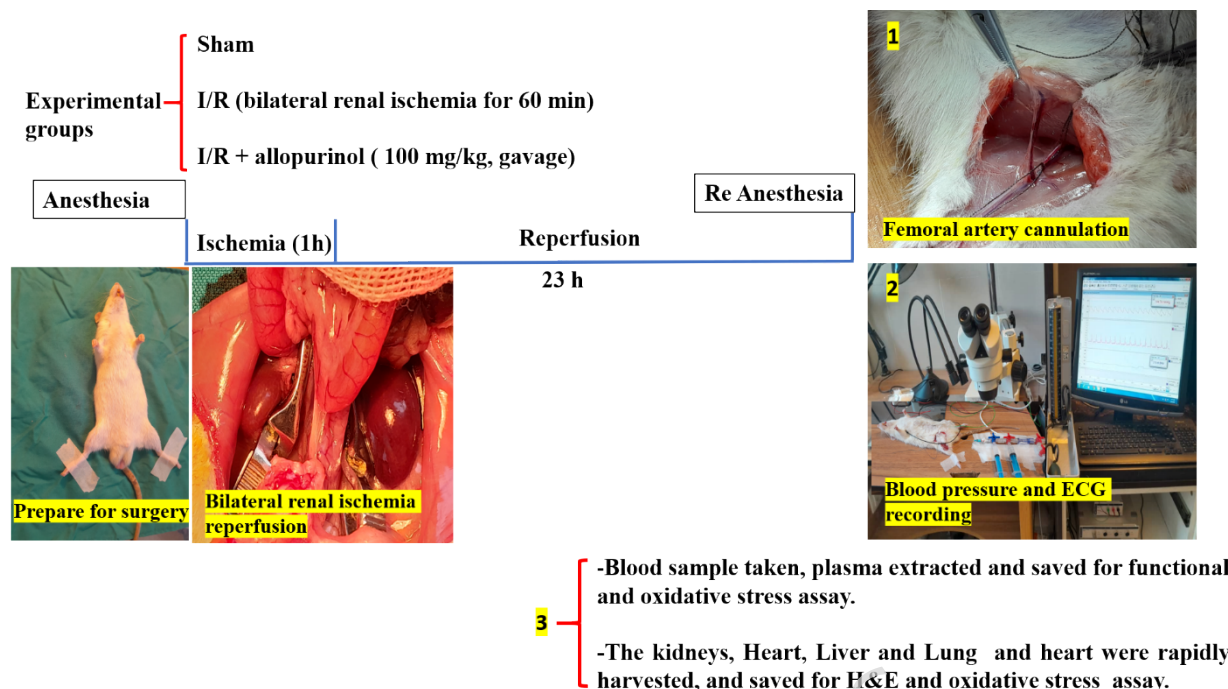


Diagram 1: Experimental design and surgical procedures

2-4-Functional biomarkers

The renal function was assessed by determining the plasma level of creatinine (Cr) and blood urea nitrogen (BUN) using Technicon® RA-1000 auto-analyzer (Technicon Corporation, Tarry-town, USA) in Namazi Hospital Laboratory, Shiraz, Iran. Cardiac troponin I (cTn-I), as a specific index of myocardium injury, was detected by an enzyme-linked fluorescent assay (ELFA) kit (VIDAS®, bioMérieux, North Carolina, USA). The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and Alkaline phosphatase (ALP) activity as a liver enzyme were measured spectrophotometrically (autoanalyser; Prestige, Biolis 24I, Japan).

2-5-Assessing the total antioxidant capacity (TAC)

The level of TAC was measured using available commercial kits (Kiazist, Hamedan, Iran) according to the manufacturer's recommendations. In the TAC assay, antioxidants reduce cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}), resulting in a color change due to the chromogen. The absorbance of this color is measured at a wavelength of 490 nm using a microplate reader

(Epoch 2TM). The assay results were expressed as nmol Trolox Equiv/mg protein.

2-6-Assessing the total oxidant status (TOS)

The total amount of oxidants in the sample is indicated as a TOS variable, which can include reactive oxygen species (ROS). TOS assays in the renal tissue supernatant fractions were done using commercially available kits (Kiazist, Hamedan, Iran) following the manufacturer's instructions. Ferrous ions are oxidized to ferric ions by the oxidant molecules present in the samples. The absorbance of color is measured at a wavelength of 560 nm using a microplate reader (Epoch 2TM). H₂O₂ was used as a standard, and the assay results were expressed as $\mu\text{mol H}_2\text{O}_2$ Equiv / mg protein.

2-7-Oxidative stress index

Protein concentration was assessed using Bradford's method (17). Oxidative stress index (OSI) was defined as the percent ratio of TOS to TAC level. OSI values were calculated using the following formula (21): OSI (arbitrary unit) = TOS (nmol H₂O₂ Equiv/mg protein.)/TAC (nmolTroloxEquiv/mg protein).

2-8- Electrocardiographic (ECG) Assessment

An electrocardiographic assessment was performed on the long Lead II of ECG recording obtained with the PowerLab/8SP data acquisition system (AD Instruments, Bella Vista, NSW, Australia) for 10 min. The rats were positioned on a wooden board to minimize interference from extraneous electrical signals. For Lead II, the negative electrode (right hand) and the positive electrode (left hand) were attached to the forelimbs of the animals, with the left limb corresponding to the positive ECG lead.

ECG interpretation focused on the durations, intervals, and amplitudes of the P wave, QRS complex, and T wave, performed with ECG analysis software LabChart Reader 8.1.30. To correct the QT interval, we applied Bazett's formula ($QT_c = QT/(RR/f)^{1/2}$) is used to estimate the heart-rate-corrected QT interval (QT_c) from the QT interval and the heart rate. f represents the normalization factor based on the RR interval from the sham group.

2-9- Histopathological assessment of kidney, heart, liver, and lungs

Tissue samples from the kidneys, heart, liver, and lungs were preserved in a 10% formalin solution (composed of 37% formaldehyde; Merck Chemicals GmbH, Darmstadt, Germany). Histological examination of the tissues was conducted on formalin-fixed, paraffin-embedded sections stained with hematoxylin and eosin (H&E) and analyzed by light microscopy. Ten randomly selected non-overlapping fields per slide were assessed by a blinded expert pathologist. The percentage of tissue damage in each field was graded on a 0–5 scale: 0 = no injury; 1 = <20%; 2 = 20–40%; 3 = 40–60%; 4 = 60–80%; 5 = >80%. The total histopathological score for each animal was obtained by summing the scores from the ten fields. Analysis of the total histopathological score was performed per animal within each experimental group.

2-10-Statistical analysis

Statistical analyses were done with the help of the GraphPad Prism software package ver. 9. (GraphPad Software Inc. La Jolla, California, USA). For multiple comparisons between the groups, one-way ANOVA was used, followed by Tukey's post-hoc test. The data are presented as means $\pm$ SEM; $P < 0.05$ was considered statistically significant.

3-Results

3-1- The effect of allopurinol on the renal functional disturbance induced by renal bilateral ischemic reperfusion

Creatinine (Cr) and blood urea nitrogen (BUN) were measured as golden standard to assay the kidney function. Renal I/R led to marked elevations in kidney-specific markers such as creatinine (Cr) and blood urea nitrogen (BUN), indicative of impaired renal function (Fig. 1A and B). Treatment with Allopurinol significantly improved BUN and Cr following ischemia-reperfusion (Fig. 1C). In agreement, BW/KW was decreased significantly following ischemia-reperfusion and this was better by allopurinol treatment to the same levels as indicated in the sham group (Fig. 1C).

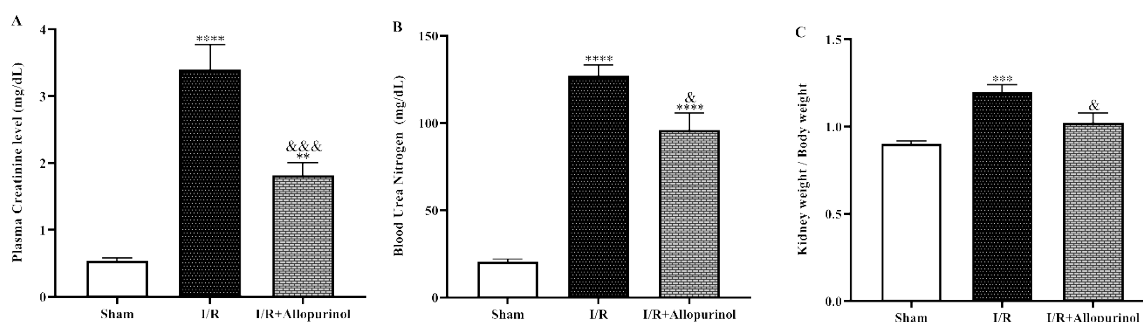


Figure 1: The effect of Allopurinol on the plasma creatinine (A), blood urea nitrogen (B), and body weight/kidney weight (C). Allopurinol (100 mg/kg, gavage) improved renal function of rats with renal I/R. Result are shown as mean $\pm$ SEM (n=8 per group). One-way ANOVA, followed by Tukey's Post-test. *** $P < 0.001$, and **** $P < 0.0001$. Compare each group vs. sham group. & $P < 0.05$ and &&& $P < 0.001$. Compare I/R+Allopurinol group vs. I/R group. I/R; ischemic reperfusion

In this study, we also evaluated the plasma functional biomarkers related to the lung, liver, and heart. The lung wet/dry ratio slightly (not significant) increased at 24 hours after renal ischemic reperfusion. Similarly, elevated levels of LDH and cardiac troponin I pointed to myocardial stress, while heightened liver enzymes (AST, ALT, and ALP) suggested hepatocellular damage—a pattern consistent with widespread organ dysfunction following I/R (Table1). These results suggest that renal ischemia/reperfusion not only triggered the kidney functional injury but also impaired the function of distant organs.

Plasma concentrations of liver enzymes (AST, ALT, and ALP) and LDH were reduced in the group receiving allopurinol (I/R+Allopurinol) compared to the I/R group, and plasma levels of cardiac function index (cTnI) were also reduced in the I/R+Allopurinol (Table1).

Table 1: The effect of allopurinol on the functional biomarkers of the lung, liver, and heart in the experimental groups

Organs Variables		Sham	I/R	I/R+Allopurinol
Lung injury markers	Lung wet/dry ratio	5.478 $\pm$ 0.142	6.323 $\pm$ 0.238	6.811 $\pm$ 0.689
	AST Activity (U.L ⁻¹)	189.3 $\pm$ 20.75	637.3 $\pm$ 69.70***	379.7 $\pm$ 27.55*&&

Liver injury markers	ALT Activity (U.L⁻¹)	30.17 ± 2.61	24.33 ± 2.498	13.71 ± 2.427**
	ALP Activity (U.L⁻¹)	267.7 ± 13.81	480.1 ± 43.52***	430.3 ± 31.12**
	LW/BW	3.07 ± 0.08	3.44 ± 0.07	3.80 ± 0.38
Cardiac injury markers	LDH (U/l)	1742 ± 464.5	5664 ± 767.9***	2341 ± 492.2 &&
	Troponin I (ng/ml)	0.070 ± 0.022	0.396 ± 0.073***	0.088 ± 0.018&&
	HW/BW	0.30 ± 0.007	0.31 ± 0.007	0.32 ± 0.023

Results are shown with the mean ± SEM (n=8 for each group). One-way ANOVA, followed by Tukey's Post-test. *P<0.05, **P<0.01, ***P<0.001; sham group vs. other groups.

&&P<0.01 I/R + Allopurinol group vs. I/R group.

I/R; ischemic reperfusion

3-2- The effects of allopurinol on histopathological changes induced by bilateral renal ischemic reperfusion

Renal ischemia/reperfusion-induced histopathological changes in kidney and distant organs. The histological examination of the kidney showed vascular congestion with an expanded Bowman's space. The renal tubules exhibit intraluminal proteinaceous casts and demonstrate signs of tubular injury in the cortex and medulla area (Fig. 2B and E). Vascular congestion was evident in the lung tissue of the I/R group (Fig. 3B). The heart and liver tissues were also examined for histological findings. The liver of rats subjected to renal I/R indicated dilated congested central vein and disturbed architecture (Fig. 4B). The crosstalk between the heart and renal was confirmed in the pathological finding of the heart. Animals received allopurinol indicated amelioration in renal, lung, and liver as well as heart histopathological derangements induced by renal I/R (Fig. 5B).

Total pathological changes of the kidney and distant organs quantified and reported in the Fig.6. Statistical analysis of histopathological changes shows that renal ischemic reperfusion significantly increased tissues injury, while allopurinol improved structural damages in the kidney as well as lung, liver, and heart (Fig. 6A and D)

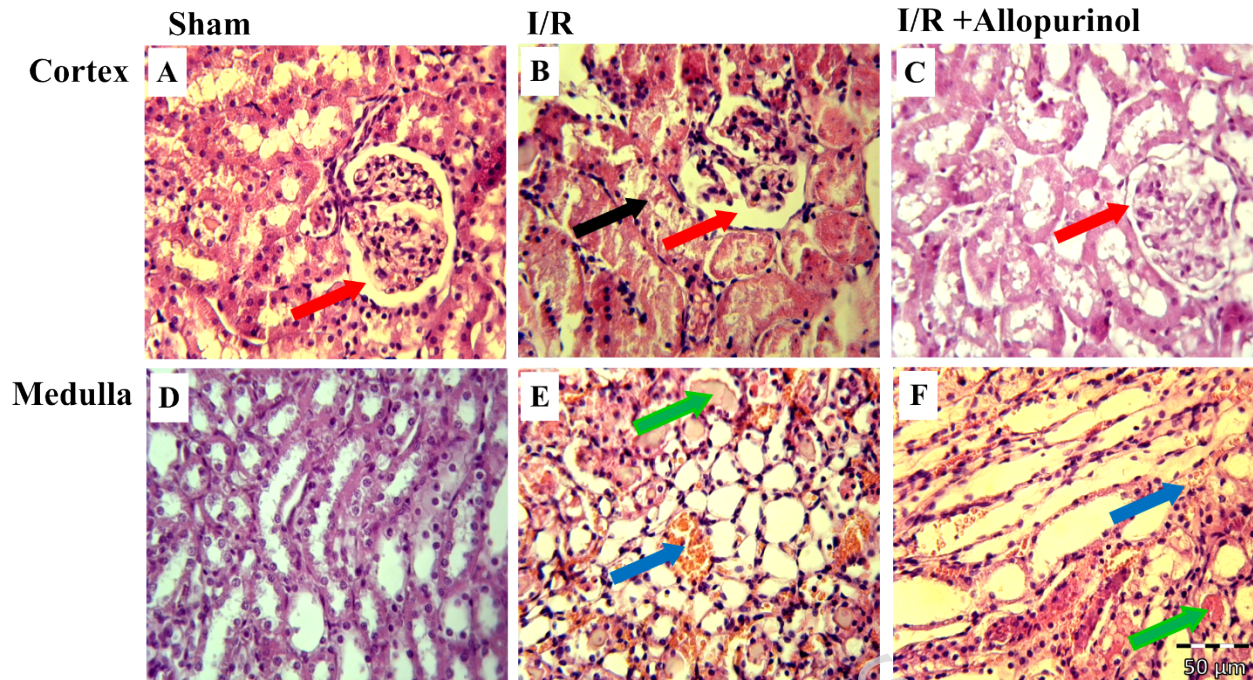


Figure 2: The effect of Allopurinol on the renal histology of rats with renal I/R. Allopurinol (100 mg/kg, gavage) improved the kidney pathological changes of rats with renal I/R. Expanded Bowman's space (red arrow), tubular injury (black arrow), intratubular cast (green arrow), and vascular congestion (blue arrow). (Hematoxylin & Eosin staining, 400X, scale bar = 50 μm).

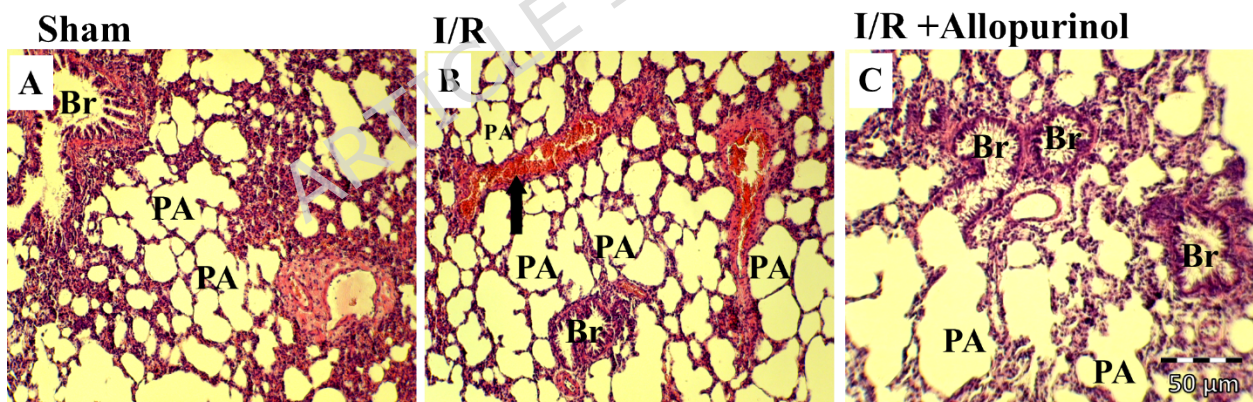


Figure 3: The effect of Allopurinol on the lung histology of rats with renal I/R. Allopurinol (100 mg/kg, gavage) improved the lung pathological changes of rats with renal I/R. Vascular congestion (black arrow). PA; patent alveoli, Bronchioles (Br). (Hematoxylin & Eosin staining, 400X, scale bar = 50 μm).

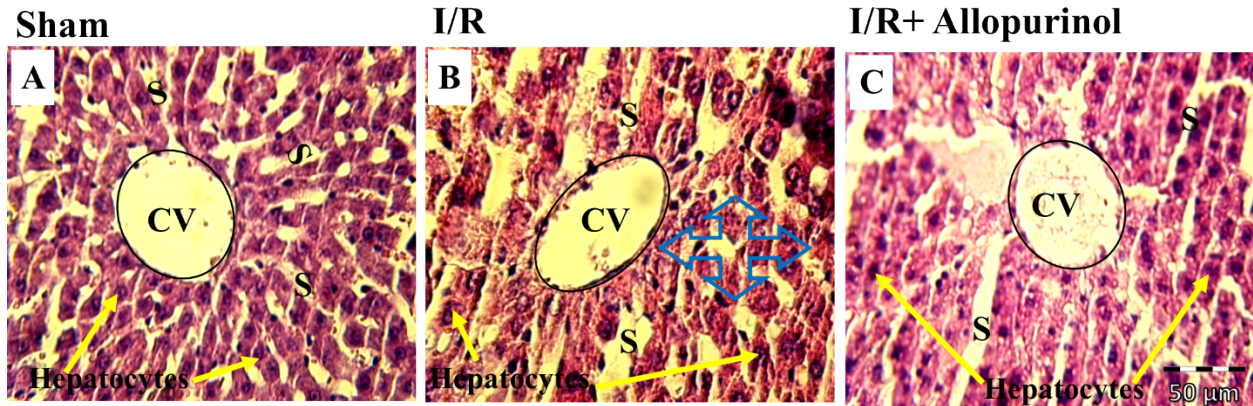


Figure 4: The effect of Allopurinol on the liver histology of rats with renal I/R.

Allopurinol (100 mg/kg, gavage) improved the liver pathological changes of rats with renal I/R. Disturbed architecture (Quad arrow) and Hepatocytes (double yellow arrows). CV; Central vein, S; Sinusoids. (Hemotoxin & Eosin staining, 400X, scale bar = 50 μm).

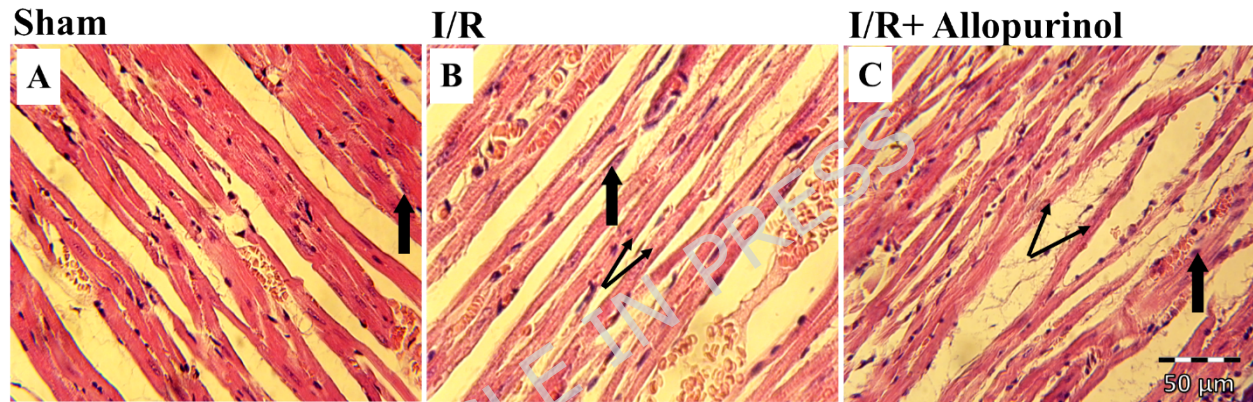


Figure 5: The effect of Allopurinol on the heart histology of rats with renal I/R.

Allopurinol (100 mg/kg, gavage) improved the heart pathological changes of rats with renal I/R. Branched fibers (double black arrows) and central nucleus (black arrow). (Hemotoxin & Eosin staining, 400X, scale bar=50 μm).

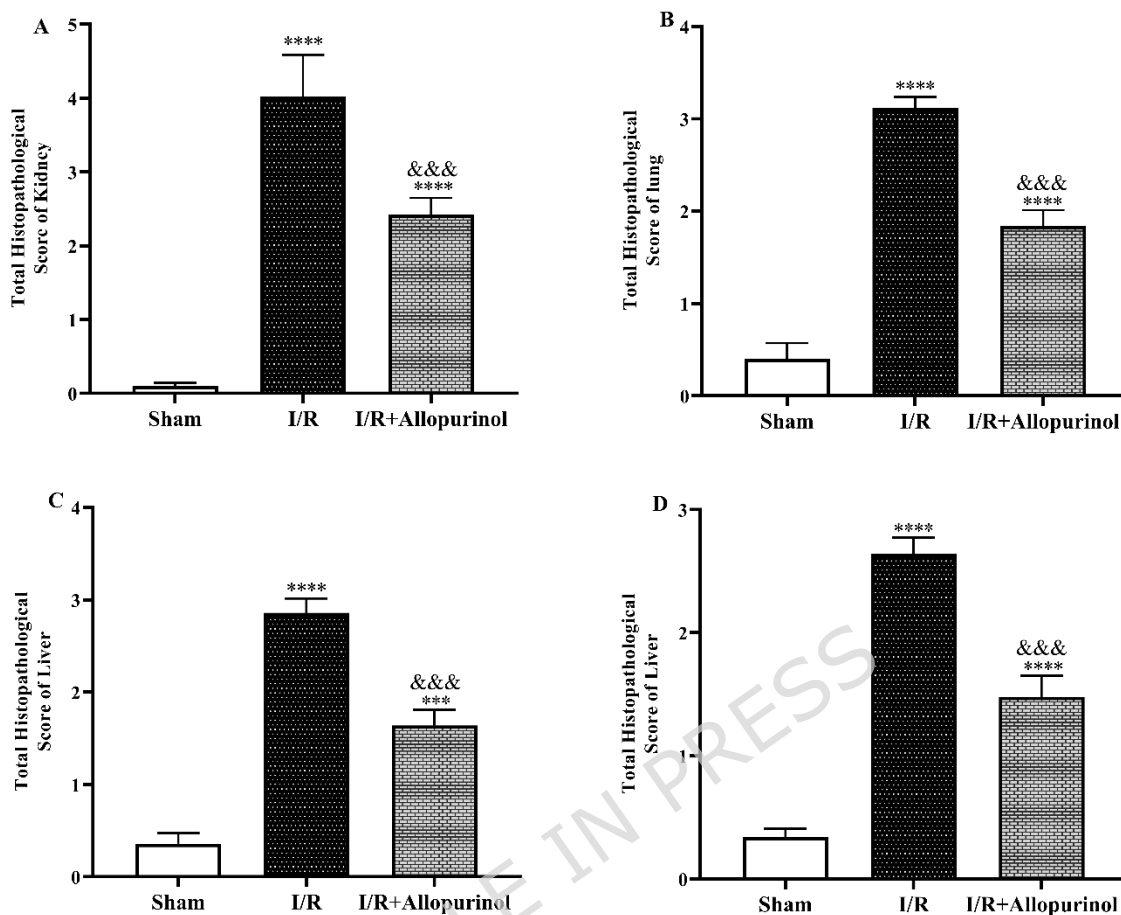


Figure 6: The effect of Allopurinol on total histopathological scores of the kidney(A), lung (B), liver(C), and heart(D). Allopurinol (100 mg/kg, gavage) improved structural damages of rats with renal I/R. Result are shown as mean $\pm$ SEM (n=8 per group). One-way ANOVA, followed by Tukey's Post-test. *** $P < 0.001$, and **** $P < 0.0001$. Compare each group vs. sham group. &&& $P < 0.001$. Compare I/R+Allopurinol group vs. I/R group. I/R; ischemic reperfusion

3-3- The effects of allopurinol on oxidative stress induced by bilateral renal ischemic reperfusion

The Total Oxidant Status (TOS) and Total Antioxidant Capacity (TAC) were measured to assess oxidative stress in tissue samples. The levels of TOS increased, while TAC decreased (not significantly) in the lung tissue following renal ischemic reperfusion injury. However, the TOS level significantly increased in both heart and liver tissues after renal ischemic reperfusion injury compared to the sham group ($p < 0.05$ for both). In contrast, levels of TAC were significantly reduced in the heart ($p < 0.01$) and liver ($p < 0.001$) tissues of the I/R group compared to the sham group. In addition, 24 h after

bilateral renal ischemia, the oxidative stress (OSI) index slightly increased in the heart and liver tissues (Table 2).

Administration of allopurinol slightly improved the oxidative stress. In the lung and liver tissues, the levels of TAC were found to compensatory increase in response to TOS levels in the I/R + Allopurinol group. Our results revealed a significant decrease in TOS and a significant increase in TAC in the heart tissue of the I/R + Allopurinol group compared to the I/R group (Table 2).

In addition, the OSI ratio reduced in the heart and liver tissue of the I/R + Allopurinol group. However, this variable did not change in the lung tissues in the experimental groups (Table 2).

Table 2: The effects of allopurinol on oxidative stress induced by bilateral renal ischemic reperfusion in the experimental group

Variables organs		Sham	I/R	I/R+Allopurinol
Total Oxidant Status (TOS) nmol/mg protein in tissue	Lung	5.491 ± 1.345	7.180 ± 0.6373	10.33 ± 0.815
	Liver	6.606 ± 0.80	10.71 ± 1.070 *	12.89 ± 1.100 **
	Heart	4.375 ± 0.809	7.400 ± 0.757 *	3.094 ± 0.436 &&
Total Antioxidant Capacity (TAC) nmol/mg protein in tissue	Lung	3.386 ± 0.328	2.576 ± 0.093	3.731 ± 0.548
	Liver	4.353 ± 0.130	3.523 ± 0.070 ***	3.606 ± 0.092 ***
	Heart	3.746 ± 0.314	2.317 ± 0.099 **	3.396 ± 0.352 &
Oxidative Stress Index (OSI) Arbitrary unit	Lung	2.838 ± 0.743	2.624 ± 0.065	2.622 ± 0.246
	Liver	1.743 ± 0.309	2.898 ± 0.454	2.381 ± 0.594
	Heart	1.844 ± 0.176	2.194 ± 0.202	1.348 ± 0.160 &

Results are shown with the mean ± SEM (n=8 for each group). One-way ANOVA, followed by Tukey's Post-test. *P<0.05, **P<0.01, ***P<0.001; sham group vs. other groups. &P<0.05 and &&P<0.01; I/R + Allopurinol group vs. I/R group. I/R; ischemic reperfusion

3-4- Electrocardiographic analysis

Chart view of ECG, HR, and BP shown in Fig.7. The increase in arterial blood pressure and QRS interval following renal ischemia were significant in the I/R group. However, R and T Amplitude depressed in the I/R group when compare with sham group (Table 3).

Table 3: The effects of allopurinol on electrocardiographic analysis in the experimental group

Groups Variables	Sham	I/R	I/R + Allopurinol
MBP (mmHg)	96.94±0.93	126.6±7.33*	96.90±7.70&
HR (BPM)	252.0± 23.76	255.6±16.96	189.4± 5.007&
RR interval (ms)	254.± 27.26	240.0±17.96	312.0±11.77
PR Interval (ms)	53.55 ± 2.055	50.12±2.227	55.59±1.428
P Duration (ms)	18.41± 2.90	20.34± 2.09	16.76± 1.07
QRS Interval (ms)	19.99±1.67	21.59± 0.74	15.45±1.31&
QT Interval (ms)	66.11± 3.08	71.75± 7.55	67.14± 8.17
J-T Interval (ms)	45.88± 3.978	48.87± 8.650	56.99 ± 7.827
P Amplitude (mV)	0.076±0.005	0.039± 0.03	0.031± 0.02
S Amplitude (mV)	-0.12±0.07	-0.03± 0.04	-0.03± 0.035
R Amplitude (mV)	-1.30± 0.44	-0.23± 0.06*	-0.17± 0.04*
ST Height (mV)	-0.59± 0.41	-0.02± 0.02	0.02± 0.02
T Amplitude (mV)	0.24± 0.009	0.11± 0.04*	0.20± 0.03
QTc interval (ms)	66.31± 3.45	73.71± 6.25	76.90± 5.00

Results are shown with the mean ± SEM (n=8 for each group). One-way ANOVA, followed by Tukey's Post-test. *P<0.05; sham group vs. other groups. &P<0.05; I/R + Allopurinol group vs. I/R group. I/R; ischemic reperfusion.

MBP; mean arterial blood pressure, **HR;** heart rate, **RR interval;** the time elapsed between two successive R-waves of the QRS signal on the electrocardiogram, **PR interval;** represents the time between atrial depolarization and ventricular depolarization, **QT interval;** is the time from the start of the Q wave to the end of the T wave.

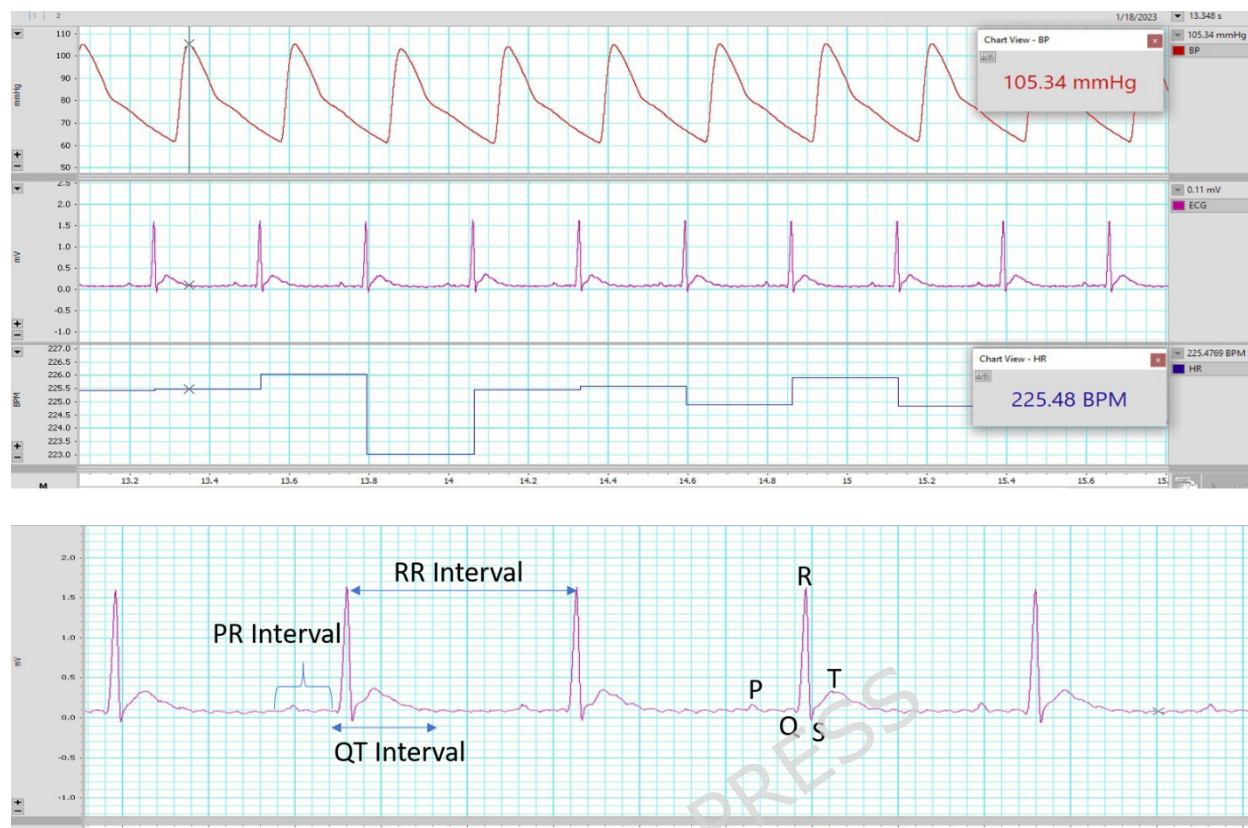


Figure 7: Chart view of electrocardiogram, heart rate, and blood pressure. **RR interval;** the time elapsed between two successive R-waves of the QRS signal on the electrocardiogram, **PR interval;** represents the time between atrial depolarization and ventricular depolarization, **QT interval;** is the time from the start of the Q wave to the end of the T wave.

4-Discussion

The current study indicated that allopurinol improved structural and functional changes in the remote organs (lungs, heart, and liver) induced by renal ischemia-reperfusion by suppressing oxidative stress. Remote organ disorders are a common complication of acute kidney injury (AKI)(18). During ischemia, renal tubular cells release endogenous agents, including pro-inflammatory cytokines, chemokines, free radicals, and apoptotic factors. Upon reperfusion, these dangerous ligands diffuse to surrounding tissues by circulation, exacerbating damage through increased oxidative stress, inflammation, and apoptosis.(19, 20).

A key player in this response is xanthine oxidase (XO), an enzyme that generates reactive oxygen species (ROS) during ischemic episodes. Elevated levels of ROS contribute to inflammation, apoptosis, and further tissue injury,

amplifying the detrimental effects of I/R. A previous study(14) showed that XO activity as well as total oxidative stress significantly increased 24 h of reperfusion in the plasma and post-ischemic kidney, and administration of allopurinol (inhibitor of XO) protects the kidney against I/R injury. The current study reports the protective effects of allopurinol on the heart, lungs, and liver injury following renal I/R injury.

Our results showed that functional biomarkers in the kidney (Cr and BUN), liver (AST and ALP), and heart (LDH and Troponin), as well as lung wet/dry ratio, were elevated after 24-hour reperfusion. In addition to functional deficits, determined structural changes were observed in these organs.

Renal ischemic injury lasting one hour has been reported to induce liver injury(21). Ashour et al. reported that one hour of renal ischemia followed 24 hours of reperfusion developed liver injuries as indicated by raised serum transaminase concentration and pathological changes(22) Previous studies done by our laboratory and other researchers have thoroughly documented the structural and functional abnormalities of the heart and lungs that occur following renal ischemia(10, 23, 24).

An imbalance between the antioxidant and oxidant systems is regarded as one of the important mediators of renal ischemia/reperfusion (I/R)-induced remote organ injury(25, 26). Our results indicated a significant increase in total oxidative status (TOS) within the lung, liver, and heart tissues following renal ischemia. Researchers have shown that plasma levels of oxidative stress markers increase while antioxidant enzyme activity decreases following renal I/R (27, 28). Systemic oxidative stress that occurs in the post-ischemic kidney can readily propagate to other organs through the circulation (12). Seifi et al. reported that renal ischemia/reperfusion caused a significant elevation in liver biomarkers (AST and ALT) along with a disarrangement in the liver architecture. These changes have been associated with an increase in MDA levels and a decrease in SOD activity in the plasma and liver tissue samples from the ischemia/reperfusion group(29). In our study, an increase in TOS level was accompanied by a decrease in antioxidant capacity in liver tissue

from the bilateral renal ischemia group. The administration of allopurinol improves structural disarrangement and functional disorders in the liver. These effects of allopurinol are accompanied by a decrease in TOS levels and an increase in TAC levels.

One may speculate that xanthine oxidase is a significant source of ROS during the ischemia and reperfusion phases of the renal I/R procedure, affecting both the kidney and distant organs(30). Thus, the inhibition of xanthine oxidase with specific inhibitors, such as allopurinol, decreases the production of superoxide anions, as indicated by TOS, thereby reducing oxidative stress, as reflected by the rise in TAC levels in liver tissue.

In line with our previous report (23), this study has also demonstrated the crosstalk between the heart and kidney. Fox et al. demonstrated that changes in cardiac metabolites were associated with imbalanced oxidative stress and impaired oxidative phosphorylation, along with evidence of alternative energy production 24 hours after renal ischemic reperfusion. Oxidative stress and protein oxidation were indicated by a significant reduction in glutathione levels and lysine degradation (8). In our study, the total antioxidative capacity decreased, while oxidative status increased in the heart tissue following postoperative ischemia. The protective impact of allopurinol pre-treatment has been indicated in both clinical and experimental models of cardiac surgery and cardiac ischemia/reperfusion(31, 32).

The current study indicated that mean systemic blood pressure increased 24 hours after reperfusion. Changes in systemic blood pressure are associated with fluctuations in glomerular filtration rate and alterations in the expression of water and electrolyte transporters in the renal epithelial cells(33, 34). Lima et al. reported changes in the renin-angiotensin-aldosterone system and sodium (Na^+) transporter-induced hypertension following renal ischemia and reperfusion(35). In the previous study, our findings showed that glomerular filtration rate significantly decreased after postoperative kidney(14). Decreased glomerular filtration increases the

concentration of sodium and water in the blood vessels, which significantly affects systemic blood pressure.

From a translational perspective, the observed changes in cardiac injury biomarkers and systemic oxidative status align with clinical considerations for patients at risk of AKI-induced cardiac and pulmonary complications(36). Regarding hyperuricemia, human data link elevated uric acid with cardiovascular risk and oxidative stress; integrating uric acid measurements in future translational work could clarify whether XO inhibition-mediated benefits extend to patients with hyperuricemia(37). Future studies should assess whether the biomarker changes observed in this model provide incremental predictive value beyond standard ECG metrics and uric acid-related risk factors.

The lungs and kidneys work together as interdependent organs to maintain the body's homeostasis(38). In addition, lung tissue is vulnerable to damage from harmful factors present in the bloodstream, because of its extensive glomerular capillary bed (39). Ambruso et al. conducted a lung metabolomics analysis and assessed lung energy production following ischemic acute kidney injury in mice. They reported an increase in oxidative stress, characterized by a reduction in the antioxidant's glutathione and thioredoxin, as well as a decrease in some metabolites involved in glutathione synthesis(26).

In our study, vascular congestion and increased alveolar wall thickness were evident in the pathological results of the I/R group. Moreover, the lung wet/dry ratio increased after 24 of reperfusion. This parameter indicates an increase in pulmonary vascular permeability and fluid leakage into the alveolar spaces. In intestinal ischemia-reperfusion I/R-induced ALI, the level of protein in bronchoalveolar lavage fluid increased, indicating a rise in pulmonary permeability. Meanwhile, the partial pressure of oxygen significantly decreased. The pathological changes are characterized by inflammation in the alveoli and interstitial spaces, and the presence of alveolar hemorrhage(6). Our findings demonstrate that allopurinol mitigates

renal I/R-related injury in distant organs (heart, lung, liver) in this animal (preclinical) model, translating these effects to humans requires careful interpretation. Clinically, oxidative stress and systemic inflammation are recognized contributors to multi-organ dysfunction after AKI, and XO inhibition has been explored as a strategy to limit I/R-mediated injury in cardiac surgery and critical care contexts. Our data showing reduced oxidative stress markers and restored antioxidant capacity in distant organs are concordant with the notion that limiting XO-derived ROS can attenuate organ crosstalk-driven injury. However, causality and effectiveness in humans remain to be established, given species differences, dosing regimens, and comorbidity profiles.

This study has several important limitations that temper the generalizability of the findings. First, the use of a specific animal model of renal I/R may not fully replicate human AKI pathophysiology or the complexity of multi-factor clinical settings. Second, sample size and the potential for inter-individual variability may limit power for subgroup analyses and the detection of smaller effects. Finally, the absence of direct clinical data limits translational claims; prospective human studies are needed to evaluate safety, efficacy, and the relevance of these pathways in AKI-associated distant-organ injury.

5-Conclusion

Our findings demonstrate that pre-treatment allopurinol, through its action as a xanthine oxidase inhibitor, mitigates structural and functional impairments in both the kidneys and distant organs following renal I/R injury, likely via modulation of oxidative stress pathways. These results align with emerging evidence highlighting the therapeutic potential of antioxidants in combating multi-organ dysfunction post-I/R. Allopurinol was administered as a pre-treatment 1 hour prior to ischemia/reperfusion exposure. However, further studies are warranted to explore the long-term effects of allopurinol. The findings reflect pre-treatment effects in an experimental context and should not be extrapolated to clinical therapeutic use without further evidence.

6-Ethics declarations: This study was reviewed and approved by Shiraz University of Medical Sciences, with the code of [IR.SUMS.AEC.1402.074]. This study was carried out in accordance with either the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities Council Directive 2010/63/EU or the National Institutes of Health – Office of Laboratory Animal Welfare policies and principles. This study complies with the ARRIVE guidelines.

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8-Authorship contribution statement: Z.K. and V.Y. were responsible for designing and overseeing the project P.Gh conducted the animal surgeries and collected samples. Z.K. and P.Gh analyzed the data, managed the database, performed statistical analyses, and prepared the manuscript. All authors participated in interpreting the data and reviewed and revised the final version of the manuscript

9-Data Availability: Data can be provided upon request.

10-Declaration of competing interest: The authors confirm that they do not have any known financial conflicts of interest

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