Original Article
Investigation of the effects of boron on a renal ischemia/reperfusion injury in rats

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Abstract: Purpose: This study was intended to investigate the effects of boron on a rat renal ischemia/reperfusion (I/R) damage model on biochemical and histopathological grounds. Methods: The control, sham, I/R, and Boron groups in the study included a total of 24 female Wistar rats with weights of 250-350 grams. Groups I/R and B underwent laparotomy under the general anesthesia and the researchers dissected left kidney pedicles of all experimental animals. A kidney artery clamp was applied for 1 hour to generate ischemia, which was followed by 6 hours of reperfusion. Group C also received 10 mg/kg boron intra peritoneally after I/R. Once blood samples were taken from the rats, they were sacrificed and tissue samples were taken by means of nephrectomy. The study analyzed the serum samples seeking Myeloperoxidase (MPO), Ischemic Modified Albumin (IMA), Malondialdehyde (MDA), Nitric Oxide (NO), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Antioxidant Activity (AOS), Urea, and Creatinine. The researchers also measured MPO, MDA, NO, SOD, GPx, and AOS in the tissue samples. Results: The IMA level in the serum samples was significantly lower in group B than group I/R (P<0.05). The MDA level in the serum samples was significantly higher in group I/R than in groups C and S (P<0.05). The AOS level in the serum samples was significantly lower in groups B and I/R than in groups C and S (P<0.05). The histopathologic scoring was significantly lower in group B than in group I/R (P<0.05). Conclusion: Boron in the renal I/R injury model makes a protective effect against I/R damage by decreasing oxidative stress in serum and tissue samples. This result was supported by histopathological examination.

Keywords: Boron, ischemia/reperfusion injury, kidney, rat

Introduction

Ischemia is an inadequate blood supply to tissues that is caused by the obstruction of arterial blood flow. Reperfusion is a new bleeding in a previously ischemic tissue or organ. Ischemia/Reperfusion damage (IRH) is tissue damage that is made by the supply of blood flow to the ischemic tissue. Basic initiator pathophysiologic factors cause not only reperfusion but also inflammation [1]. The inflammation in the tissue causes an increase of reactive oxygen species (ROS), endothelial dysfunction, changes in the medullary microcirculation, and tubular damage [2]. Depending on the duration and severity of ischemia, renal tubular epithelial cells may go through various changes (e.g., structural and functional full recovery, apoptosis, necrosis) [2].

The atomic number of boron element is 5. This element is indicated in the periodic table by the letter "B", and is placed first in group 3A. It does not exist in the elemental form in nature [3]. Elemental boron was discovered in 1808 by Thenard, Gay-Lussac, and Davy. However, the use of boron compounds dates back to 4000 years ago [4].

Boron is an important element for humans and animals. It plays a role in macro-mineral metabolism, endocrine function (has an effect on calcitonin, estrogen, insulin, and thyroid hormones), metabolism of D vitamins, vision, bone metabolism, and immune function. Many studies that have been conducted since 1981 have determined its effects on energy substrates (e.g., glucose) and nitrogen-containing substances (e.g., amino acid) as well as nitrogen [5-7].

Table 1. Categorization of kidney histopathology

Degree	Damage	Pathological definition
0	Absent	Normal tubule
1	Mild	Mild swelling, loss of brush border edge
2	Moderate	Massive swelling, middle vacuolization
3	Middle	Shrinkage in the nucleus, severe vacuolization
4	Severe	Necrotic, apoptotic cells, basal membrane rupture
5	Necrosis	Complete necrosis of the tubule

Antioxidant properties of boron were demonstrated in animal studies [8, 9]. Boron increases the amount of low glutathione in cells, which leads to a reduction in oxidative stress and oxidative damage [8, 10]. The lipid peroxidation levels became higher in blood and tissues of rats that had arsenic in their drinking water; however, the addition of boric acid reduced lipid peroxidation [11]. In another study, boron increased the glutathione levels in the blood, kidney, liver, and brain tissues, and caused a faster superoxide dismutase and catalase activity in the rats that were exposed to the toxicity of malathion, an organophosphate that is frequently used as an agricultural pesticide and produces oxidative stress in the body. Thanks to these impacts, boron can reduce oxidative stress and prevent tissue damage [12].

The aim of this study was to investigate the effects of boron on a Renal Ischemia/Reperfusion injury model in rats based on the measurement of biochemical and antioxidant parameters, changes in electrolyte levels, and histopathological examination for serum and tissue samples.

Materials and methods

This study used a total of 24 female Wistar rats (weights: 250-350 g). The rats had been raised under the same environmental conditions. For at least one week prior to surgery, the animals were kept in standard cages in a pathogen-free environment with free access to food (until 2 h before the anesthetic procedure) and water within a 12-hour light/dark cycle. The animals were randomly divided into four groups, each containing six rats.

This study was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and ARRIVE. It was approved by the Ethical Committee of Afyon Kocatepe University, Local Animal Experiment Commi-

ttee (date: 08.11.2016; number of 136-16).

Study design

Control group (C group) (n = 6): The rats in this group were injected intraperitoneally with 2 mL physiological saline and sacrificed 6 h later.

Sham group (S group) (n = 6): The rats in this group were injected intraperitoneally with 2 mL physiological saline 1 h before laparotomy, and their blood and tissue samples were collected 6 h after laparotomy.

Ischemia/reperfusion group (I/R group) (n = 6): The rats in this group were injected intraperitoneally with 2 mL physiological saline 1 h before laparotomy, and their blood and tissue samples were collected after 6-h reperfusion following 60-min renal ischemia.

Boron group (B group) (n = 6): The rats in this group underwent laparotomy and 60 minutes of renal ischemia that were followed by 10 mg/kg Bor + 1 m Serum Physiologic intraperitoneal injection. Their blood and tissue samples were taken after 6 hours of reperfusion.

Anesthesia procedure

The rats received general anesthesia intramuscularly by 8 mg/kg xylazine HCl (Alfazine, Ege-Vet, Turkey) and 80 mg/kg ketamine HCl (Alfamine, Ege-Vet, Turkey).

Renal ischemia/reperfusion procedure

The researchers performed laparotomy through midline incision in rats in the I/R and CUR groups, and used microvascular clamps to complete renal artery occlusion after finding each of the renal pedicles (Bulldog). The fading in the kidneys that occurred after using clamps was regarded as a sign of occlusion. Subsequently, the researchers opened the abdomen that was temporarily closed with silk sutures after a 60-min period. There was a change of color in the kidney after removing the clamps. The abdomen was temporarily closed by using 5 mL intra-abdominal Ringer lactate and sewing up the laparotomy line with 4.0 silk sutures. The rats were allowed to awaken and anesthetized after 6-h reperfusion once their

Table 2. Measurement of biochemical and antioxidant parameters in the serum

Group	MPO (ng/ml)	IMA (ng/ml)	MDA (nmol/ml)	NO (µmol/L)	SOD (U/ml)	GPx (nmol/min/ml)	AOS (mmol/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control (n = 6)	2.98±0.58[a]	22.55±1.92[a]	1.66±0.35[a]	3.49±0.29	1.25±0.28	48.16±7.44[b]	0.28±0.03[b]	62.01±9.24[a,c]	0.32±0.08
Sham (n = 6)	3.71±0.45[a,c]	24.6±3.27[a,c]	1.89±0.26[a]	4.55±1.47	1.28±0.27	47.33±6.59[b]	0.24±0.03[b]	39.85±3.97[a]	0.31±0.04
I/R (n = 6)	4.18±0.33[b,c]	31.43±6.1[b,c]	2.46±0.5[b,c]	3.63±0.37	1.62±0.48	37.16±5.70[a]	0.13±0.02[a]	77.65±10.45[b,c]	0.41±0.13
Boron (n = 6)	4.16±0.84[b,c]	25.76±7.50[a]	1.99±0.6[a,c]	4.08±1.14	1.39±0.37	39.16±5.94[a]	0.15±0.03[a]	88.69±20.34[b]	0.52±0.16

Different letters in the same column indicate statistically significant differences (P<0.05). MPO: Myeloperoxidase; IMA: Ischemia modified albumin; MDA: Malondialdehyde; NO: Nitric oxide; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; AOS: Antioxidant status.

intracardiac blood and kidney tissue samples were taken. Finally, the rats were sacrificed by exsanguination from the abdominal aorta.

Preparation of kidney tissue samples and protein determination

A procedure of weighing and homogenizing the kidney tissue samples in a buffer with 10 times more phosphate (pH 7.4, 1/10 g/mL) provided the crude protein extracts (homogenate). The homogenate was centrifuged in a refrigerated centrifuge at 15,000 rpm for 15 min to obtain supernatants. The study determined the protein levels using Lowry method [13, 14], and spared these levels for the measurement of SOD, GPx, Malondialdehyde (MDA), and NO levels in addition to antioxidant activity.

Determination of serum ischemic modified albumin levels based on myeloperoxidase (MPO), GPx, MDA, SOD, NO, and antioxidant status in renal tissue samples and serum

The researchers used an ELISA kit that is available in the market to measure Myeloperoxidase (MPO) [Sunred Rat MPO Enzyme-Linked Immunosorbent Assay (ELISA) Kit, Cat. No. 201-11-0575, China], GPx (Cayman Chemical Company, ELISA Kit Cat No. 703102, USA) and antioxidant activity (Cayman Chemical Company, ELISA Kit Cat. No. 709001, USA) levels in the tissue samples and serum. The MDA was measured using the Draper and Hadley method, which is based on the principle of measuring at 535 nm the absorbance of the color that was created by MDA thiobarbituric acid [14]. The SOD activity was determined using the method by Sun et al. [15]; this method was based on SOD's inhibition of nitroblue tetrazolium' reduction by superoxide anions that were created by xanthine/xanthine oxidase system. Nitric oxide levels were determined according to the method by Miranda et al. [16]. The samples were

deproteinized by diluting them at a ratio of 1/3 with 10% TCA (Trichloroacetic acid) before measurement.

The ischemia-modified albumin (IMA) level in the serum was determined using the commercial dual-antibody sandwich ELISA kit (Sunred Rat Myeloperoxidase MPO ELISA Kit Cat. No. 201-11-1672, China).

Histopathological examination

The kidneys of the rats that underwent necropsy were fixed in buffered neutral 10% formaldehyde solution. The researchers trimmed the rats after 48 h and moved them into the trays for tissue attachment. The tissues were traversed during a series of alcohol and xylene applications and blocked in paraffin. Then, the researchers sliced the blocks into 4- to 4-µm sections using a microtome and placed on the slides. The sections were stained with hematoxylin-eosin (HE) technique and examined under a light microscope. **Table 1** presents the criteria that were followed for the evaluation of the changes in the kidneys.

Statistical analysis

Data were presented as mean ± standard deviation (SD) values. The Kruskal-Wallis H test acted as a non-parametric test for the determination of the changes during the biochemical and electrolyte analysis of oxidative stress. In addition, the study used chi-square test for comparing the variables that were generated by the pathological analysis. The study data were analyzed using the Statistical Package for Social Sciences (SPSS, 18.0 software, USA). The significance threshold was P<0.05.

Results

The study measured the levels of MPO, IMA, MDA, NO, SOD, GPx, AOS, urea, and creatinine

Table 3. Measurement of biochemical and antioxidant parameters in kidney tissue

Group	MPO (ng/ml)	MDA (nmol/mg protein)	NO (nmol/mg protein)	SOD (U/mg protein)	GPx (nmol/min/ml)	AOS (mmol/mg protein)
Control (n = 6)	1.23±0.58[a]	2.44±0.78[a]	7.15±2.47[a]	0.77±0.09[b]	0.19±0.05[b,c]	12.69±1.65[b,c]
Sham (n = 6)	1.47±0.53[a]	2.96±0.97[a,c]	7.33±1.43[a]	0.76±0.07[b]	0.2±0.02[b,c]	13±1.24[b,c]
I/R (n = 6)	3±0.98[b]	4.86±1.28[b]	13.52±3.45[b]	0.40±0.05[a]	0.13±0.04[a]	7.99±1.75[a]
Boron (n = 6)	2.92±1.13[b]	3.95±0.62[b,c]	6.24±1.34[a]	0.67±0.09[b]	0.15±0.01[a,c]	11.28±1.97[a,c]

Different letters in the same column indicate statistically significant differences (P<0.05). MDA: Malondialdehyde; NO: Nitric oxide; SOD: Superoxide dismutase; Gpx: Glutathione peroxidase; AOS: Antioxidant status.

Table 4. Measurement of electrolyte parameters in serum

Group	K⁺	Ca⁺⁺	Na⁺	Cl⁻
Control	6.45±0.2[a]	10.91±0.82[a]	138.33±2.94	101.5±3.2
Sham	4.33±0.09[b]	9.55±0.31[b]	140.33±1.89[a]	102.5±2.88
I/R	7.08±0.39[b,c,*]	10.07±0.78[b,c,*]	135±4.53[a,b]	99.71±2.49
Boron	6.64±1.37[b,d]	9.93±1.08[b,d]	134.4±4.21[a,c]	100±2.16

Different letters in the same column indicate statistically significant differences (P<0.05). *: P<0.05.

Table 5. Pathological scoring of kidney tissue

Group/Score	0	1	2	3	4	5
Control Group	100%	0%	0%	0%	0%	0%
Sham Group	100%	0%	0%	0%	0%	0%
I/R Group	0%	0%	0%	0%	66.7%	33.3%
Boron Group	0%	0%	0%	16.7%*	66.6%	16.7%*

(Pearson chi-square test *P: 0.002).

in the serum samples in control, sham, I/R, and Boron groups (**Table 2**).

It was determined that the serum IMA level was significantly lower in the Boron group than in the I/R group (25.76±7.5 vs 31.43±6.1) (P< 0.05). Also, serum MDA level was significantly lower in the Boron group than in the I/R group (1.99±0.6 vs 2.46±0.5) (P<0.05). Serum Urea level was significantly higher in the Boron group than in the I/R group (88.69±20.34 vs 77.65±10.45) (P<0.05) (**Table 2**).

Tissue MDA levels had significantly lower values in the Boron group than the I/R (3.95± 0.62 vs 4.86±1.28) (P<0.05). Tissue NO level was lower in the boron group compared with the I/R, Control and sham groups, and the difference between them was statistically significant (Boron Group: 6.24±1.34 vs IR Gr: 13.52±3.45) (P<0.05). Tissue GPx level Boron group was significantly higher when compared with I/R group (0.15±0.01 vs 0.13±0.04) (P<

0.05). The tissue AOS level was higher in the Boron group than in the I/R, and the difference was significant (11.28±1.97 vs 7.99± 1.75) (P<0.05) (**Table 3**). For serum electrolyte levels, K⁺ level was significantly lower in the boron group than in the I/R group (6.64±1.37 vs 7.08±0.39) (P<0.05). Similarly, serum Ca⁺⁺ level was significantly lower in the Boron group (P<0.05) (**Table 4**). For the scoring of histopathological findings, the outcomes were Grade 0, 1, 2, 3: 0% in both groups, and Grade 3: 0% vs I/R: 16.77% in the boron group. This high value in the boron group is statistically significant (P: 0.002). The other findings were 16.7% for the boron group and 33.3% in the I/R group in Grade 5. This low result in the boron group is statistically significant (P: 0.002) (**Table 5**).
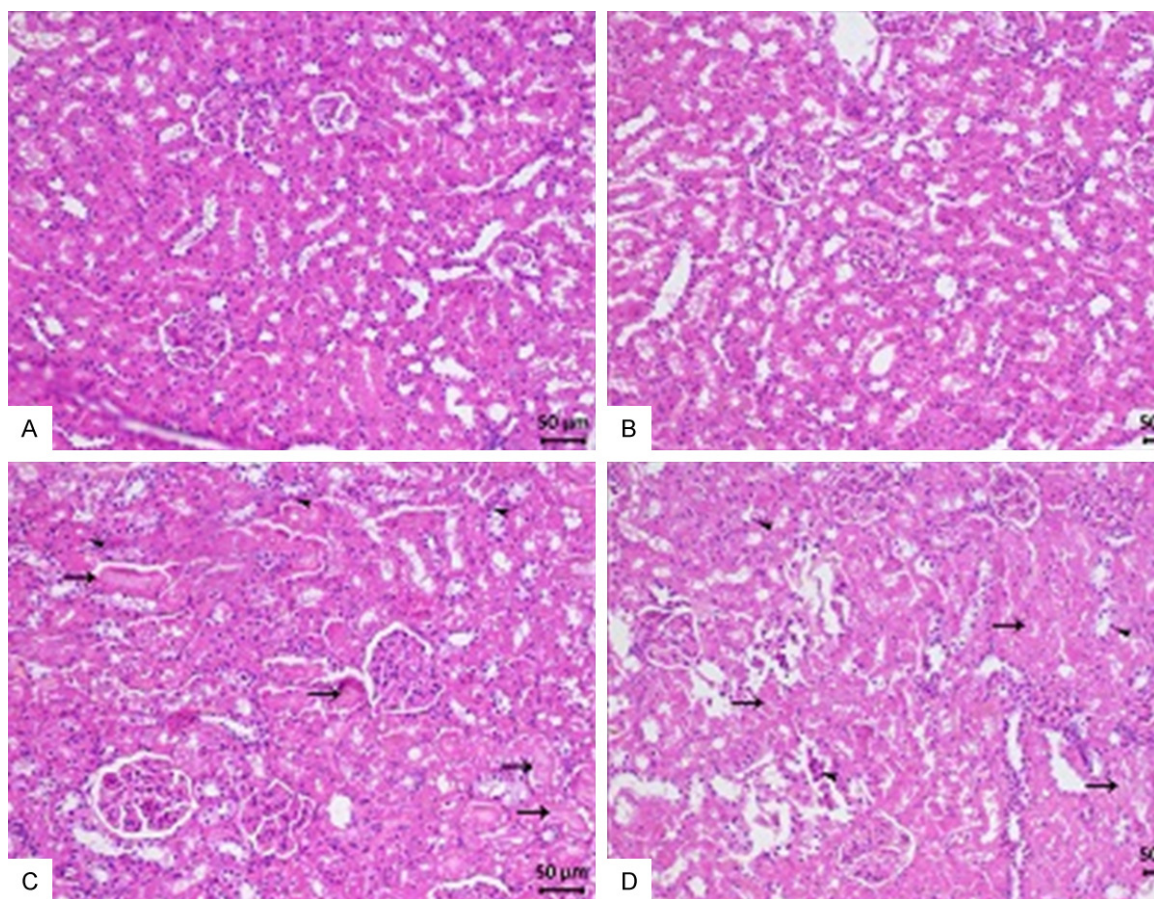
Figure 1 shows the histopathological view of experimental groups. The histological structure is normal in control and Sham groups. In the IR group there was swelling and severe necrosis in tubules as well as picnotic nuclei in intact epithelium, and basal membrane separation in some epithelium (**Figure 1**). Treatment group provided similar findings to IR group, which included swelling in tubules in large field and severe necrosis, picnotic nuclei in intact epithelium, and basal membrane separation in some epithelium (**Figure 1**).

Discussion

This study shows that intraperitoneal administration to rats of 10 mg/kg boron, which past studies proved to have beneficial antioxidant properties, is effective in correcting renal IRH.

Figure 1. (A-D) Histopathological view of experimental groups. (A) Control group; histological structures have a normal appearance (B). Sham group; histological structures are normal. (C) IR group; swelling and severe necrosis (arrows) in tubules in large field, picnotic nuclei (arrowheads) in intact epithelium and basal membrane separation in some epithelium. (D) Treatment group; similar to IR group, with IR group having swelling in tubules in large field and severe necrosis (arrows), picnotic nuclei (arrowheads) in intact epithelium and basal membrane separation in some epithelium. HE. Bar, actual length = 50 μm.

Studies on the physiopathology of I/R injury reported that the ROS, produced from damaged tissue after reperfusion, induces proinflammatory cytokine release by stimulating macrophages; these cytokines trigger inflammatory responses, which increase tissue damage [17-19]. SOR, protease, elastase, MPO, and proinflammatory cytokines are released by neutrophil migration in ischemic tissue, and they cause tissue damage as well [20].

In our study, MPO (a marker of tissue neutrophil activation) increased both at the serum level and in kidney tissue activity in the I/R group compared with the control group. In the boron group, MPO activity decreased in the tissue samples in comparison with the I/R group.

Recent studies identified IMA as a new determinant of inflammatory diseases [21]. IMA activity is high in many oxidative stress-related and inflammatory diseases [22]. The IMA level is elevated within minutes after ischemia, remaining high for 6-12 hours, and decreasing to normal values within 24 hours [23]. The IMA level was significantly higher in the I/R group than in the boron group after 6 hours of reperfusion. Clinical studies found that ROS play a major role in the pathogenesis of renal IRH [24]. Lipid peroxidation is a complex phenomenon that is initiated by the emergence of a hydrogen atom from the group of methylene between two unsaturated bonds in lipid molecules. Accordingly, a new carbon-centered lipid free radical emerges as a result of this activity. This new lipid free radical in the presence of oxygen creates lipid peroxides or hydroperoxides. These two products transform into MDA, which is a relatively more stable product and

can be used as a marker for lipid peroxidation [25].

There are studies of the effects of boron on some antioxidant or oxidant parameters in various tissues (e.g., liver, kidney, brain, and heart) [8, 9, 12]. These studies emphasize that boron significantly reduces MDA levels in the liver, kidney, and brain tissues where oxidative stress is induced due to malathion. Similarly, boric acid and boron (100 mg/kg)-supplemented diets in rats lowered MDA levels in the liver and heart. A previous study [8, 11] reported that MDA levels in liver, kidney, heart, and brain tissues significantly decreased in male and female rats. In these experimental animals, arsenic induced oxidative stress. In this study, the MDA level in the serum and kidney tissue level significantly increased in both I/R and boron groups when compared with the control group. Additionally, the MDA serum and kidney tissue levels in the boron group were significantly lower than the I/R group.

Typically, tubule cells do not produce NO. However, ischemic damage induces iNOS outflow in these cells. Ischemia in tubule cells leads to the formation of peroxynitrite by increasing NO and superoxide production [26]. The measured NO value in the kidney tissue was found to be significantly lower in the boron than in the I/R group. On the other hand, the serum NO level was not found to be significant in boron group compared to the I/R group.

SOD and catalase are the main antioxidant cellular defense system enzymes that are responsible for the elimination and detoxification of free oxygen radicals. SOD converts superoxide to hydrogen peroxide, and thus, contributes to the main defense system by eliminating the toxic effects of superoxide radicals. Catalase also protects the tissue from highly reactive hydroxyl reductants by converting hydrogen peroxide into water and oxygen [8]. In this study, the serum SOD levels were lower in the boron group than in the I/R group although the difference was not significant. At the tissue level, the SOD level in the boron group was significantly higher than the I/R group. The serum and tissue GPx levels were significantly lower in the I/R and boron groups compared with the control group. However, this decrease was less prominent in the boron group. GPx levels in kidney tissue were significantly higher in the boron group compared to the I/R group.

AOS was found to be low in serum in the I/R and Boron groups compared with the control group level. On the other hand, the tissue AOS level was significantly higher in the boron group than the I/R group.

Acute renal failure is a clinical syndrome that is characterized by the rapid degradation of normal functions of the kidney for various reasons. Nitrogenous waste accumulates as a result of the rapid decrease of glomerular filtration rate (GFR) over hours or days, leading to an impaired fluid and electrolyte balance [27-31].

The serum urea and creatinine levels significantly increased in the I/R and boron groups compared to the control group levels. The increase in the I/R group is statistically significant compared to the boron group level. The serum Ca^{++} level showed a statistically significant increase in the I/R group considering the boron group level. The serum Na^+ and Cl^- levels showed no significant change between the groups.

Depending on the duration and severity of ischemia, renal tubular epithelial cells may experience different changes including structural and functional full recovery, apoptosis, and necrosis [32]. Histopathological examination of the renal tissue in the boron group in our study showed a 16.7% shrinkage of the nucleus, dense vacuolization (moderate renal damage), and full necrosis that developed in 16.7% of the tubules. In addition, 66.6% of the tubules had serious damage. In the I/R group, however, 66.7% of the tubules had serious damage, and 33.3% had full necrosis.

This study performed the first application of boron in the rat renal ischemia/reperfusion damage model. The biochemical measurements and oxidative markers were performed 1 hour after ischemia and 6 hours after reperfusion. In the kidneys that were treated with boron, the protection from ischemia/reperfusion was significant compared to the I/R group. Additionally, histopathological findings showed the boron-treated kidneys to be better protected than the I/R group kidneys. However, the antioxidant property was not enough to protect the kidney.

Disclosure of conflict of interest

None.

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