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Protective Effect of Pyrroloquinoline Quinone (PQQ) against Renal Ischemia-Reperfusion Injury in Rat

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ABSTRACT

Ischemia/reperfusion can cause tissue damage and affect organ function. Ischemia-reperfusion injury (IRI) occurs in a variety of clinical manifestations such as stroke, trauma, and surgery. The present study investigated the role of pyrroloquinoline quinone (PQQ) on the renal damage after IRI. Rats were assigned to four groups randomly (n = 8): Control group, Sham group, Renal IRI (45 minutes ischemia and subsequently 24 hours reperfusion) and renal IRI + PQQ (10 mg/kg /IP). Serum level of urea, creatinine, TNF-α and IL-6 were measured by a biochemical analyzer using commercial kits. In kidney tissue samples total antioxidant capacity (TAC), malondialdehyde (MDA), and myeloperoxidase (MPO) were measured using commercial kits. The results showed that IRI injury increased serum urea, creatinine levels, TNF-α and IL-6 along with MDA, MPO levels in the renal tissue, and decreased renal TAC levels. A decrease in serum urea, creatinine levels as well as MDA, MPO levels of the IR + PQQ group were observed in renal tissues. In addition, TAC levels in the kidney tissues of PQQ-treated animals were improved in comparison with the IRI group. In conclusion, pyrroloquinoline quinone treatment ameliorated renal IR injury by anti-oxidative stress and anti-inflammatory properties. Consequently, it could be promising as a potential therapeutic agent for renal ischemia-reperfusion injury.

Introduction

Tissue damage can result from ischemia in any organ. In contrast, the return of blood to ischemic tissue is attended by a second reperfusion period recognized as ischemia/reperfusion (IR) injury (IRI).[1] Renal ischemia is common in individuals who undergo cardiovascular surgery, trauma, shock, burns, main artery surgery, or transplantation.[2] Acute kidney injury and renal insufficiency are frequently caused by IR damage.[3] Acute kidney damage is a serious medical condition that affects some critically ill patients, particularly those in critical care units. Furthermore, in the case of renal transplantation, this lesion might result in acute or chronic adverse outcome, impaired function, and/or kidney failure.[4] As a result, despite numerous pathways postulated to explain the pathogenesis of IR injury, finding the most effective and protective technique to reduce the deleterious consequences of IR in transplanted kidneys is critical.

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The majority of researches have been performed on the role of inflammatory cytokine and chemokine sequences, neutrophil and macrophage infiltration, stress oxidative mechanisms and lipid peroxidation.[5]

Pyrroloquinoline quinone (PQQ) (4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-f] quinolone-2,7,9-tricarboxylic acid) is a redox active o-quinone that can be reversibly reduced to pyrroloquinoline quinol (4,5-dihydroxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid, PQQH2) through a semiquinone intermediate.[6] Several treatments, including anti-inflammatory and anti-oxidant free radical scavengers, have been found to have promising benefits in preventing of ischemia/reperfusion damage in tissues.[2] PQQ is found in plants, microorganisms, animals, foods. Antioxidative stress properties of It has become a research hot topic in latest years. PQQ has been confirmed to have beneficial effects as an antioxidant.[6] It seems protection of PQQ is mostly associated with its antioxidant properties.[7] The PQQ, as a reactive oxygen species (ROS) scavenger in oxidative stress, may continually neutralize ROS *in vitro* to generate non-reactive products, therefore, protects DNA and protein against oxidative stress impairment. As a ROS scavenger, PQQ may directly neutralize ROS, preserve mitochondrial function and avert apoptosis.[8] The current study was designed to investigate the potential protective benefits of PQQ against IR-induced renal damage in rats.

Materials and Methods

Animals

Three-month-old male Wistar rats (n = 48, 200–250 g) were obtained from Urmia University Animal House. The animals were kept in temperature of (23 ± 3) °C, steady air humidity, and a natural period of light/darkness as well as standard rodent laboratory food and tap water *ad libitum*. The procedure was directed according to the guiding principles of the ethics committees for animal experimentation, Urmia University, Iran (Code NO: IR-UU-AED-32/PD/3). All methods were performed in accordance with the relevant guidelines and regulations.

Groups

Rats were assigned randomly into four groups (n = 8): Control group (animals which were given the soybean oil as vehicle), sham group (animals were subjected to the surgical procedure with no clamp on renal artery), IRI group (induction of IR damage in both kidneys, clamped with clips for 45 minutes and subsequently reperfused for 24 hours), IR + PQQ (10 mg/kg) group was subjected to renal arterial closure and IR procedures; but just 30 minutes before ischemia initiation, PQQ (Sigma-Aldrich, USA; 10 mg/kg) was administered intraperitoneally.[8-11]

Surgical Technique for Inducing Ischemia-Reperfusion

Animals were anesthetized by intra-peritoneal administration of ketamine-xylazine (ketamine 5%, 90 mg/kg and xylazine 2%, 5 mg/kg) the abdomen wall was incised and the hilum of kidney was located and the renal artery was carefully constricted for a 45-min interval using conventional clips bilaterally.[11,12] When the arteries on both sides were clamped, the kidneys underwent into an ischemia state. Pale kidneys demonstrated the pedicles were properly blocked. Afterward, in order to stop the ischemic stage, the clamps were removed. After that, the abdomen was closed in with a 3-0 nylon. The kidney had undergone a 24-hour reperfusion phase after blood was returned to them next the ischemic period.[14,15]

Blood and Kidney Samplings

Following a 24-hour reperfusion interval, the animals were anesthetized. Ketamine (90 mg/kg) and xylazine (5 mg/kg), were used to anesthetize the animals intraperitoneally. The samples were taken for biochemical and histopathological investigation. The serum was obtained by centrifuging blood samples taken from the rats' hearts. After that, the samples were kept at -20 °C for biochemical analysis. Afterwards, one of the separated kidneys was maintained at -80 °C to determine biochemical factors in the tissues; while, the other was placed in a 10% buffered formalin for histopathologic evaluation.[12]

Serum Levels of Urea and Creatinine (Cr) Measurement

Urea and Cr levels were measured as physiological function indicators of the kidney in the stored serum samples using a biochemical analyzer (BT-1500, Biotechnica instruments, Italy) with commercial kits (Pars Azmoon, Tehran, Iran).

Lipid Peroxidation, Total Antioxidant Capacity (TAC), and Myeloperoxidase (MPO) Activity in Tissues

Quantification of malondialdehyde (MDA) is

commonly used as an indicator for lipid peroxidation, and is evaluated by its interaction with thiobarbituric acid according to the method of Ohkawa *et al*., (1979). Spectrophotometer (DANA-3200; Garni Medical Engineering Co., Tehran, Iran) by means of commercial assay kits (Navand Salamat, Urmia. Iran) was used to assess values of TAC and MDA in the kidney tissues according to the manufacturer's directions. The TAC and MDA values in homogenates of the kidney were assessed using ferric-reducing ability of plasma and the thiobarbituric acid reactive substance methods, respectively. The MPO activity in the renal tissues was assessed similar to a protocol defined formerly.[16]

Inflammatory Factors in Serum

Tumor necrosis factor TNF-α and IL-6 levels were measured in renal tissue homogenate by the commercial ELISA kits (Peprotech, London, UK) according to the manufacturer's instructions.[15]

Histopathologic Assessment

Renal tissue specimens were fixed in 10% formaldehyde for 24 h, used for light microscopic investigations. The tissues were initially immersed in paraffin and cut into 5 µm slices after they had been fixed. Then, hematoxylin and eosin (H&E) were used to stain the slices. Following that, a common light microscope (Olympus CX 22, Tokyo, Japan) was used to examine them. Microscopic examination of tissue sections was carried out by a pathologist.[10]

Statistical Analysis

Statistical analysis was performed by SPSS 22.0 (Inc., Chicago, IL, USA). After confirming the normality of data by the Kolmogorov-Smirnov test, using an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. All data were expressed as means ±standard deviation. Values of $p < 0.05$ were set as significant.

Results

Serum Level of Urea and Cr

As shown in Figure 1A, the concentration of urea in the serum of IRI group was significantly higher than the control group ($p < 0.05$). In addition, when compared to the IRI group, urea was significantly lower in groups treated with IRI+ PQQ (10 mg/kg). According to Figure 1B, the serum Cr concentration in the IRI group was significantly higher than in the control group ($p < 0.05$).

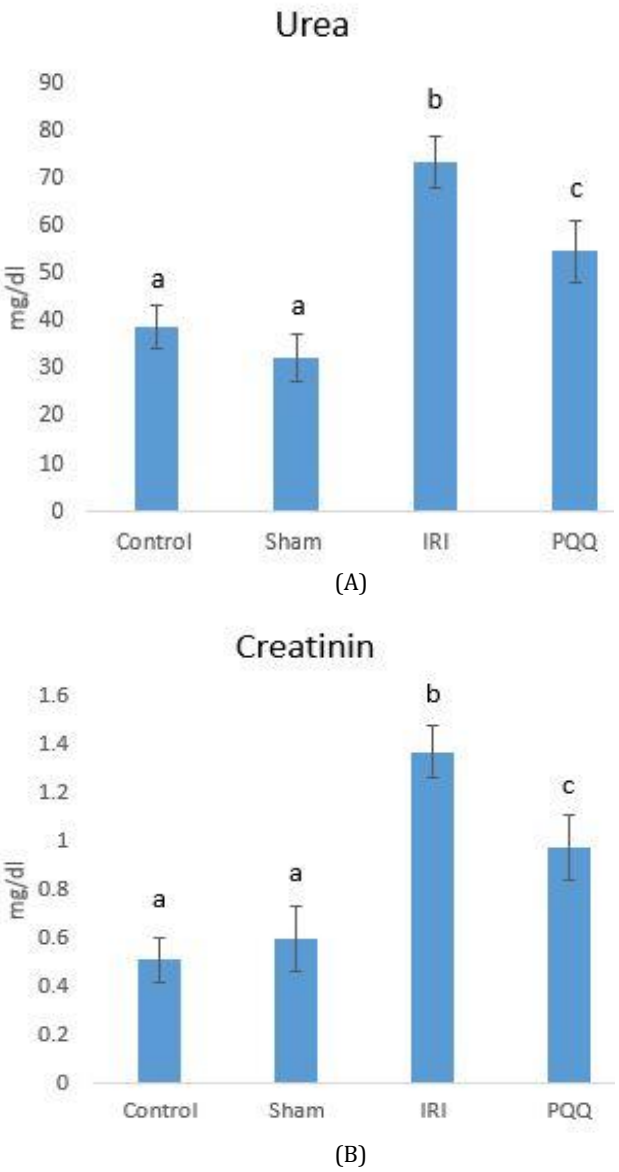

Figure 1. Serum (A) urea and (B) creatinine concentrations. a, b, c: Different superscript letters indicate significant difference ($p < 0.05$). IRI: ischemia reperfusion injury, PQQ: pyrroloquinoline quinone.

In comparison with the IRI group, rats administered with PQQ showed a significant decrease in serum Cr concentration ($p < 0.05$).

Lipid Peroxidation and Oxidative Stress Indicators

The tissue level of MDA in the IR group rats was considerably higher than in the control group ($P < 0.05$), as shown in Figure 2A. When compared to the IRI group, MDA levels were significantly lower after treatment with PQQ (10 mg/kg) ($p < 0.05$).

The renal tissue levels of TAC were significantly lower in the IRI group compared to the control group ($p < 0.05$); while, the TAC levels of renal tissues were

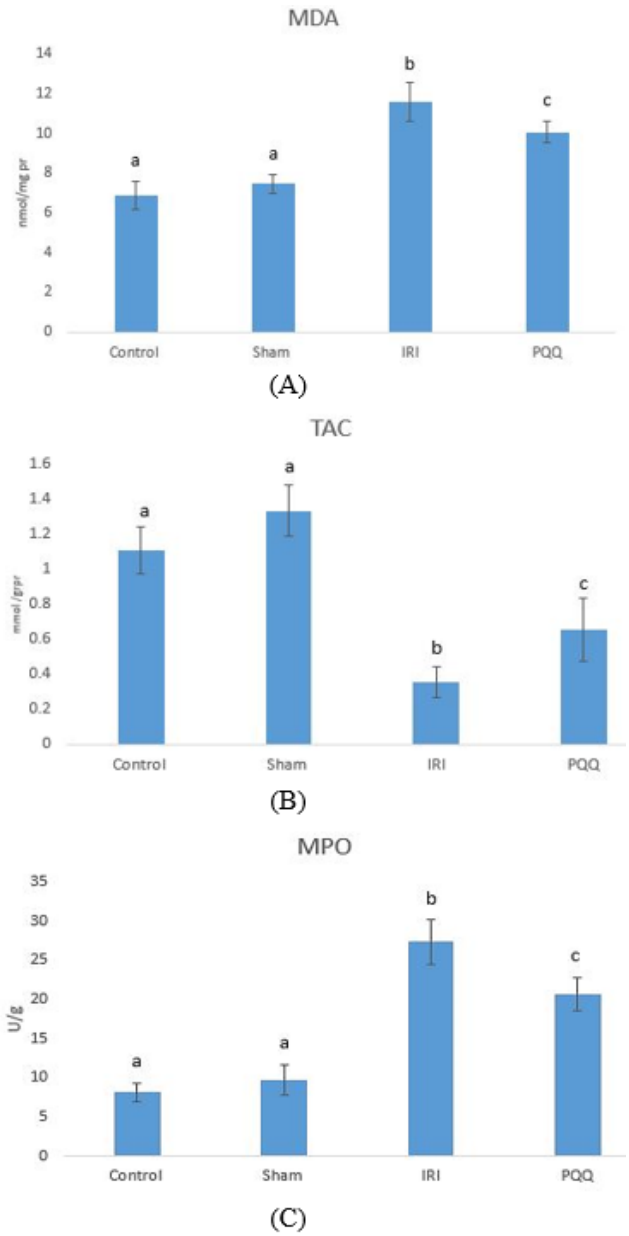

Figure 2. (A) MDA, (B) TAC and (C) MPO levels (B) of kidney tissue. a, b, c: Different superscript letters indicate significant difference (p < 0.05). MDA: malondialdehyde, TAC: total antioxidant capacity, MPO: myeloperoxidase

significantly higher in of the IRI+ PQQ group in comparison with the IRI group (p < 0.05, Figure 2B).
In the IR group, the MPO level of kidney tissue after IRI was considerably higher than in the control group, as shown in Figure 2C (p < 0.05). Comparing the IRI+ PQQ (10 mg/kg) group to the IR group, the results showed that MPO levels were significantly lower in the PQQ-treated groups (p < 0.05).

Inflammatory Markers

Following ischemia-reperfusion damage, serum TNF-α levels in the IRI group were significantly higher

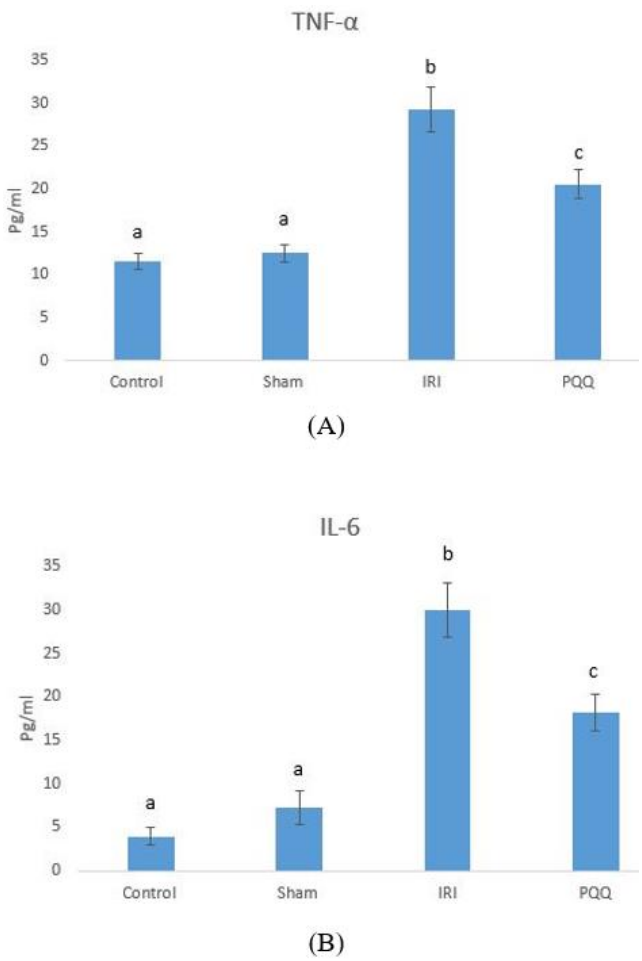

Figure 3. (A) Serum levels of TNF-α, and (B) serum levels of IL-6. a, b, c: Different superscript letters indicate significant difference (p < 0.05). TNF-α: tumor necrosis factor alpha, IL-6: interleukin-6.

than in the control group, as shown in Figure 3A (p < 0.05). The PQQ decreased the serum level of TNF-α in the treatment group compared to the IRI, as shown in Figure 3B. Values of serum IL-6 in the IR group was considerably greater than the control group (p < 0.05). Treatment with PQQ (10 mg/kg) caused a significant decrease in the serum level of IL-6 (p < .001).

Renal Histopathologic Assessment

Histopathological examinations of slides from the control and sham groups revealed an intact Bowman's capsule, proximal and distal convoluted tubes, and no evidence of inflammation (Figures 4A and 4B). Tubular damage, cell swilling and necrosis were observed in the IRI group. Moreover, inflammatory cell infiltrations were seen in the IRI group as a result of an inflammation reaction in the kidney (Figures 4C and 4D). In IRI + PQQ group (10 mg/kg) a lesser amount of inflammation, necrosis and tissue disarrangement were seen in comparison with IRI group (Figure 4E).

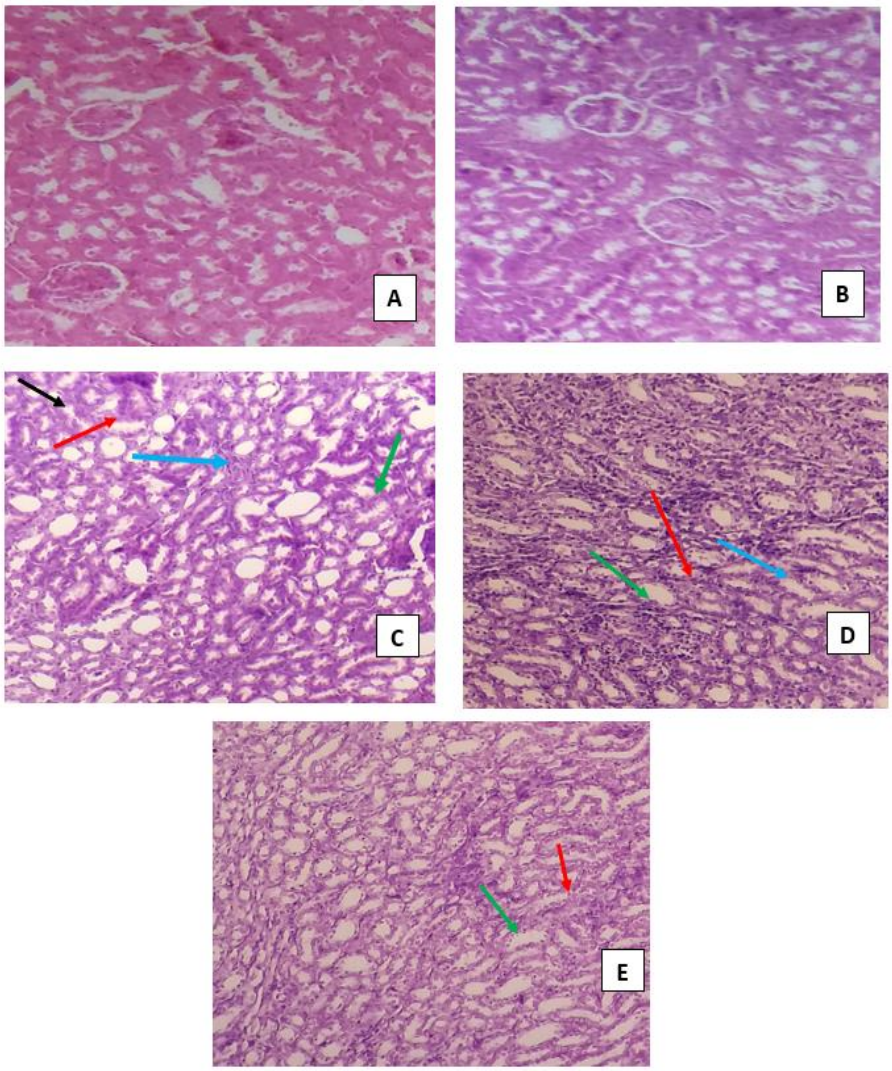

Figure 4. Histologic micrographs of the kidney samples in (A) control group, (B) sham group, (C) IRI group: Green, red and black arrows showed cell swilling and hydropic degeneration**.** Blue arrow showed nuclear pyknose and tubular necrosis. (D) IRI group: Green arrow inflammatory cell infiltration, Red arrow showed nuclear pyknose, Blue arrow showed tubular necrosis, (E) IRI + pyrroloquinoline quinone (10 mg/kg) group: Green arrow showed nuclear pyknose, Red arrow showed hydropic degeneration (H&E, 10×).

Discussion

The IRI is one of the major issues in kidney surgery.[18,19] This appears as a result of the tissue's hypoxia during and after surgery. It may cause acute kidney injury or possibly acute or chronic reject of the transplanted kidney in some situations.[20] Renal ischemia causes a cascade of complicated biochemical reactions that end in cellular death and injury.[21] Reperfusion after renal ischemia, on the other hand, causes additional tissue damage due to acute inflammation. One of the reasons of tissue injury is the production of reactive oxygen species during this phase. The most essential mechanisms thought to be involved in IRI are oxidative stress, lipid peroxidation, and inflammation. As a result, identifying therapies that inhibit these processes could be a beneficial strategy for preventing or reducing ischemia/reperfusion damage.[4,22] Protective properties of PQQ in rat models of renal ischemia-reperfusion damage were demonstrated in the present study. Protective characteristics of PQQ have been studied extensively using various animal models concluding the beneficial mechanisms of PQQ in inhibition of stress oxidative and inflammatory cascades.[6]

PQQ was given at a dose of 10 mg/kg, 30 minutes before ischemia, and successfully reduced renal function impairment, improved levels of indicators of oxidative stress and inflammatory cytokines, moreover histopathologic damages after renal IRI damage. Many studies have found that indicators of kidney function (such as urea and Cr levels) were increased with renal

IRI injury. Urea and Cr levels could be elevated as a result of glomerular filtration rate reduction and tubular disorders.[21] In the current study, administration of PQQ resulted in a significant reduction in serum concentrations of urea and Cr in IRI group. The TNF-α and IL-6 serum levels of IRI group were found to be higher in this study, which was consistent with prior studies.[22] Both of these inflammatory cytokines were effectively reduced in serum when PQQ was used. The PQQ suppressed TNF-α in a rat model of cardiotoxicity as well as IR injury during torsion/detorsion in ipsilateral and contralateral testes.[25,26] The TNF-α and IL-6 release in response to tissue damage can activate the NF-κβ inflammatory pathways.[27] Because inflammation is a key element in the progress of renal IRI injury, anti-inflammatory drugs that target TNF-α and IL-6 cytokines or the NF-κβ pathway may help reduce kidney IRI.[29,30] Formerly, NF-κβ overproduction in renal IRI has been documented.[30]

Several studies have shown that ischemic reperfusion injury is induced by a number of processes including the oxidative stress response, lipid peroxidation and overproduction of ROS.[31] Free oxygen radicals cause lipid oxidation, and MDA is one of the end products of enzymatic lipid peroxidation widely used as an index for oxidative stress.[32] In numerous investigations, MDA was reported to be associated with the development of renal ischemia reperfusion damage.[33,34] We also found an increased MDA level as a result of IR damage, which was consistent with mentioned studies. The elevated kidney quantity of MDA in the IRI group was dramatically reduced after PQQ treatment, according to the results of this investigation. The MPO and its oxidation products are important pathogenic causes in various kidney diseases, contribute to the progression of various kidney injuries.[35] According to findings of a study, neutrophils activation and infiltration play an important role in the harmful cascade that takes place within ischemic reperfusion.[36] After 24 hours, the kidney MPO level was significantly increased due to IRI in the current investigation. The findings of the present study demonstrated that PQQ (10 mg/kg) could reduce the increased level of MPO activity.

In conclusion, the PQQ exhibited a renal protective effect on a rat model of IRI, presumably by reducing inflammatory and oxidative markers. Further investigations are required to evaluate the precise mechanism of protective effect of PQQ on kidney.

Conflict of Interest

The authors declare that they have no competing interests.

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