EXPERIMENTAL STUDY

Protective effect of oxymatrine against renal ischemia/reperfusion injury in rats

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ABSTRACT

BACKGROUND: Renal ischemia/reperfusion (I/R) injury is a common cause of acute kidney injury. The pathologic mechanisms underlying renal I/R injury are complicated, involving reactive oxygen species, necrosis, cell apoptosis, and inflammation, but the exact mechanisms remain unclear.

OBJECTIVES: This study aimed to investigate the effect of oxymatrine (OMT) on renal I/R injury and its underlying mechanism.

METHODS: Thirty male Sprague–Dawley rats were randomly allocated to three groups (n = 10): the sham-control group, the renal I/R-untreated (I/R-untreated) group, and the I/R-OMT group. Renal I/R injury were induced by clamping the left renal artery for 45 min followed by 24 h of reperfusion. At 10 min before reperfusion, the rats in the I/R-OMT-treated group rats received an intravenous injection of 40 mg/kg OMT. Renal function and histological changes were compared and the relevant parameters of oxidative stress and inflammation were detected.

RESULTS: Oxymatrine pretreatment significantly decreased the level of renal dysfunction, attenuated the renal histological changes, the levels of reactive oxygen species production in renal tissue upon I/R. Additionally, OMT pretreatment could further activate the serum antioxidant enzyme activities.

CONCLUSION: The beneficial effects of OMT were likely mediated by the inhibition of lipid peroxidation and the increase in endogenous antioxidant activity. The results of this study indicate that oxymatrine may represent a potent anti-oxidant drug to protect the kidney against I/R injury *(Fig. 5, Ref. 29)*. Text in PDF *www.elis.sk*.

KEY WORDS: oxymatrine, renal ischemia/reperfusion, oxidative stress, rat.

Introduction

Renal ischemia/reperfusion (I/R) injury is a common cause of acute kidney injury that occurs in a variety of clinical entities, such as renal resection, hydronephrosis, kidney transplantation, renal artery angioplasty, accidental or iatrogenic trauma, and shock (1–4). The pathologic mechanisms underlying renal I/R injury are complicated and involve oxidative stress, necrosis, apoptosis, calcium ion overloading, and inflammatory reaction (5, 6).

In I/R, tissues are subjected to the destructive proinflammatory effects of cytokine release and the production of oxygen free radicals by neutrophils (7). Therefore, oxidative stress plays an important role in renal I/R injury (8, 9). The production of reactive oxygen species (ROS) in the reperfusion period is considered a key reason for uncontrolled oxidative stress, and the increased

amount of ROS can also drive the inflammatory cascade (6). Additionally, cell apoptosis has been considered one of the most serious consequences of renal I/R injury in previous studies, and it determines the outcome of renal damage (10, 11). The balance between pro- and anti-apoptotic mediators prevents and regulates cell death (12). Therefore, oxidative stress and apoptosis are extremely important in renal I/R injury, and targeting its processes is the ideal therapeutic approach.

Oxymatrine (OMT), a natural quinolizidine alkaloid, is a major bioactive component extracted from the root of traditional Chinese herbal medicine *Sophora japonica* (sophora flavescesn Ait) (13). OMT has been proved to have such effects as anti-inflammatory, anti-apoptosis, anti-tumor, anti-viral, anti-hypersensitive, and inhibition of histamine release (14). OMT has also been shown to exert a protective effect on ischemia or I/R damage (15, 16).

This experiment aimed to investigate the cytoprotective effects of OMT using a rat model of renal I/R injury and to further identify the underlying mechanisms of OMT.

Materials and methods

All surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee of Abant Izzet Baysal University (Bolu, Turkey) (Number/ID of the approval (s):2015.08.10.905). The procedures were conducted according

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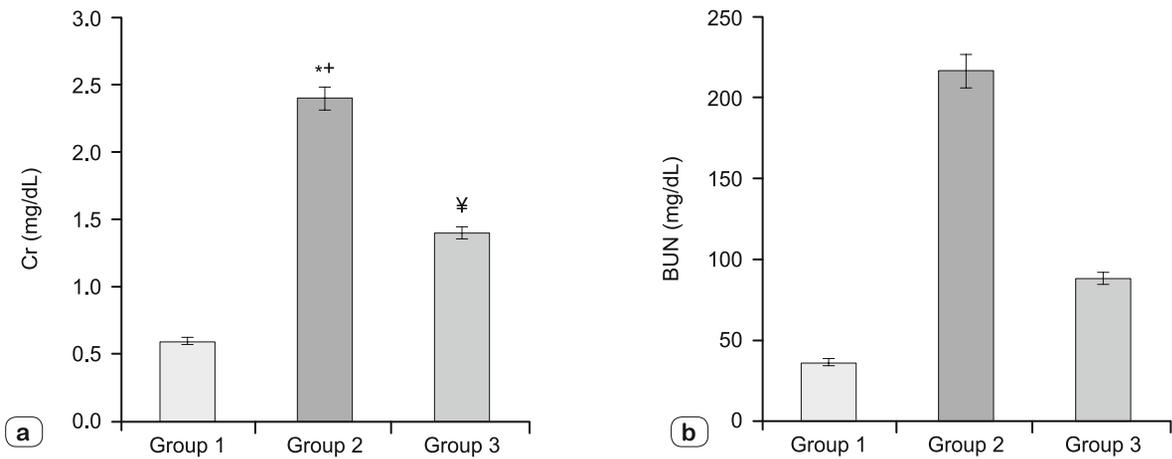
Fig. 1. The effects of oxymatrine (OMT) treatment on alterations of renal function following renal I/R-induced injury. Serum creatinine (a) and urea (a) were measured to assess the reno-protective effect of OMT treatment against renal injury in groups. Data were represented as mean ± SEM (n = 10). Group 1: sham-operated control; Group 2: I-R/untreated; Group 3: I-R/oxymatrine treated. IR: Ischemia/reperfusion. * p < 0.0001 (Group 2 vs 1); † p < 0.0001 (Group 2 vs 3), ¥ p < 0.0001 (Group 3 vs 1).
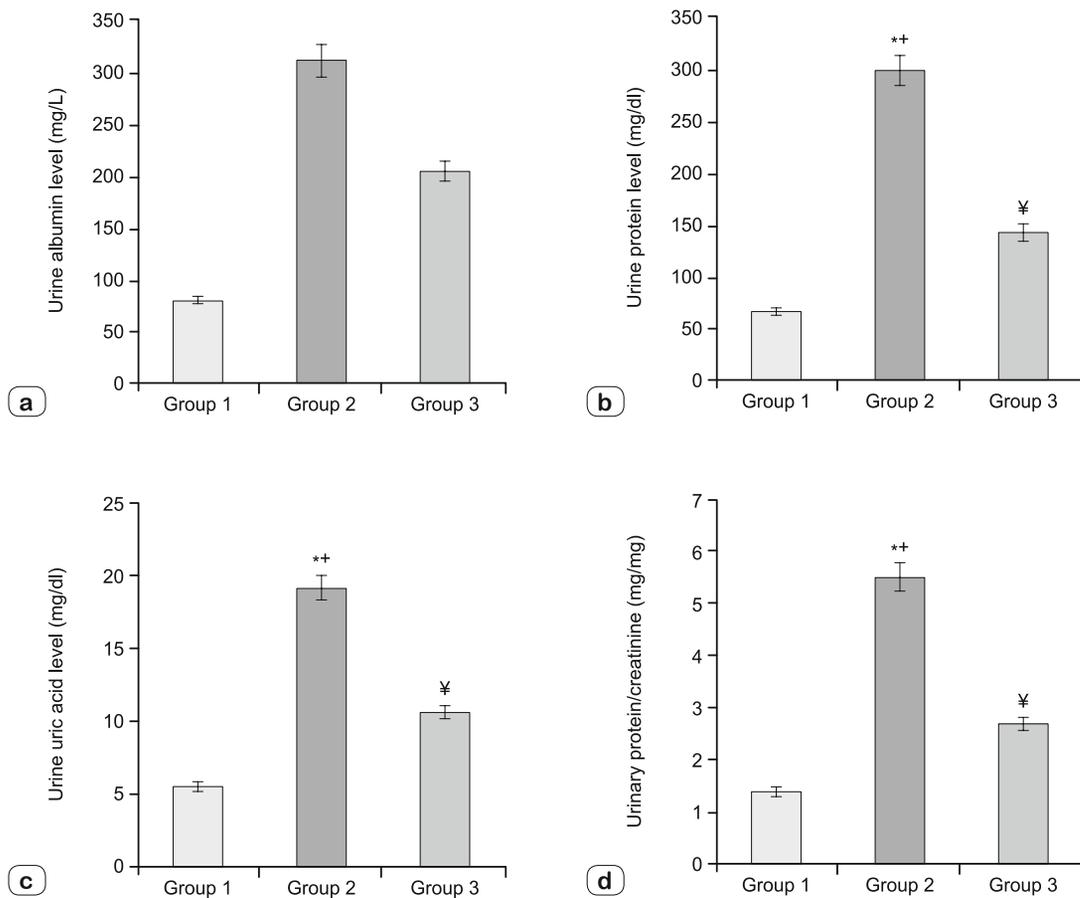

Fig. 2. The levels of urinary albumin (a), protein (b) and uric acid (c) were decreased by OMT treatment in the kidneys exposed to renal ischemia/reperfusion. The ratio of urinary protein/creatinine was significantly increased in the kidneys subjected to IR injury but decreased by OMT treatment (d). Data were represented as mean ± SEM (n = 10). Group 1: sham-operated control; Group 2: I-R/untreated; Group 3: I-R/oxymatrine treated. IR: Ischemia/reperfusion. *p < 0.0001 (Group 2 vs 1); †p < 0.0001 (Group 2 vs 3), ¥ p < 0.0001 (Group 3 vs 1).

to routine animal care guidelines, and all experimental procedures complied with the Guide for the Care and Use of Laboratory Animals (1996). The animals were all kept in captivity under the same environmental and nutritional conditions. The rats were kept in sawdust-lined cages (47 cm x 34 cm x 18 cm; four animals per cage) in one room at a constant temperature (22 ± 2 °C), with light from 7:00 h to 19:00 h and water and food ad libitum.

Renal I/R injury

Briefly, the rats were anesthetized by an intraperitoneal injection of xylazine (10 mg/kg) and ketamine hydrochloride (100 mg/kg, Ketalar, Eczacıbasi, Turkey) and placed on a homeothermic table to maintain a core body temperature of 37 °C. Following catheterization of the right femoral vein, fluid replacement was performed with 3 mL·kg⁻¹·h⁻¹ lactated Ringer's solution using an infusion pump. Then, a midline laparotomy was performed: the right kidney was removed and the left kidney pedicle was shut by an artery clamp for 45 min. After 45 min of left renal ischemia, occlusion clips were removed and the incision was closed in two layers. The same procedure was performed in the sham operation group without the unilateral clamping process.

The 30 rats were randomly divided into three groups, namely, the sham operation group (Group 1), I/R-untreated group (Group 2), and I/R-OMT-treated group (Group 3), with 10 animals in each group. The sham operation group underwent only the separation of the right renal pedicle but not occlusion. In the I/R-untreated and I/R-OMT-treated groups, the left kidney vessels were clamped for 45 min, followed by 24 h of reperfusion. At 10 min before reperfusion, the rats in the I/R-OMT-treated group rats received an intravenous injection of 40 mg/kg OMT (Sigma–Aldrich-O0891, St. Louis, MO, USA).

We collected 24-h urine samples using metabolic cages. The animals were anesthetized again, blood samples were collected from the abdominal aorta, left kidneys were removed for later analysis, and then the animals were sacrificed.

Preservation of kidneys

The left kidney was removed under fully maintained anesthesia. After removal, the kidney was fixed in 10% phosphate-buffered formalin or immediately frozen, and stored at – 80 °C for different determinations.

Assessment of renal function

Serum and urinary supernatants were stored at –80 °C until laboratory analysis. Samples were thawed before study. Serum BUN, creatinine, and urinary uric acid, creatinine, protein, albumin and the ratio of urinary protein/creatinine analyses were performed with autoanalyzers (Architect c 8000, Abbot Laboratories, USA).

Histopathological evaluation

The kidney was fixed in 10% neutral-buffered formalin, paraffin embedded, and sectioned at 4 mm thickness according to the standard procedure. The sections were deparaffinized and hydrated gradually, and then examined by hematoxylin and eosin staining. Morphologic assessment was performed by an expe-

rienced renal pathologist who was unaware of the treatment. A grading scale of 0–4, as outlined by Jablonski et al (17), was used for the histopathologic assessment of the I/R-induced damage of the proximal tubules.

Measurements of serum antioxidant enzyme activities and lipid peroxidation

Kidney tissues weighing 100 mg each were placed in labeled glass tubes. Samples were washed with phosphate-buffered saline once and stored at –80 °C until the day of biochemical analysis. Samples were thawed before study. Each tissue was homogenized once in 1 ml phosphate-buffered saline. Samples were frozen and thawed twice to facilitate cell membrane disintegration. The homogenate was centrifuged at 4 °C at 5,000 g for 5 min, and the supernatant was removed. Catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-PX) activities were measured using commercially available enzyme-linked immunosorbent assay kits according to manufacturer instructions (Cusabio Biotech, Wuhan, PRC). Malondialdehyde (MDA) concentrations were measured using commercially available colorimetric assay kits according to manufacturer instructions (Cayman Chemical Company, Ann Arbor, U.S.A.). BCA protein assay was used for the quantitation of tissue total protein (Thermo Fisher Scientific Inc. Rockford, USA).

Statistical analysis

All data were analyzed by SPSS 17.0 software. The results are expressed as mean ± SD. The Kruskal–Wallis test was used for comparison of the groups. In two group comparisons, the Mann–Whitney U test was performed. p values below 0.05 were considered statistically significant.

Results

The serum BUN and creatinine levels increased in the I/R group compared with those in the sham operation group ($p < 0.0001$ and $p < 0.0001$, respectively). However, in rats treated with OMT, a reduction in the serum levels of BUN and creatinine was observed unlike in the I/R-untreated group ($p < 0.0001$) (Fig. 1 a, b). The levels of urinary albumin, protein, and uric acid increased in the kidneys exposed to I/R ($p < 0.0001$) but decreased in the OMT treatment ($p < 0.0001$). The ratio of urinary protein/creatinine also increased by I/R injury ($p < 0.0001$) but decreased by OMT treatment ($p < 0.0001$) (Fig. 2 a, b, c, d).

The value of MDA decreased in the I/R-OMT-treated group ($p < 0.0001$) compared with that in the I/R-untreated group. Moreover, the SOD, CAT, and GSH-Px levels increased in the I/R-OMT-treated group compared with those in the I/R-untreated group ($p < 0.0001$, $p < 0.0001$, and $p < 0.0001$ respectively) (Fig. 3 a,b,c,d).

The renal injury score (RIS) increased in the experimental groups compared with that in the sham operation ($p < 0.0001$ and $p < 0.0001$, respectively). However, quantitative analysis showed a significantly decreased RIS in the I/R-OMT-treated group compared with that in the I/R-untreated group (Fig. 4). Normative histological changes were found in the sham operation group (Fig. 5
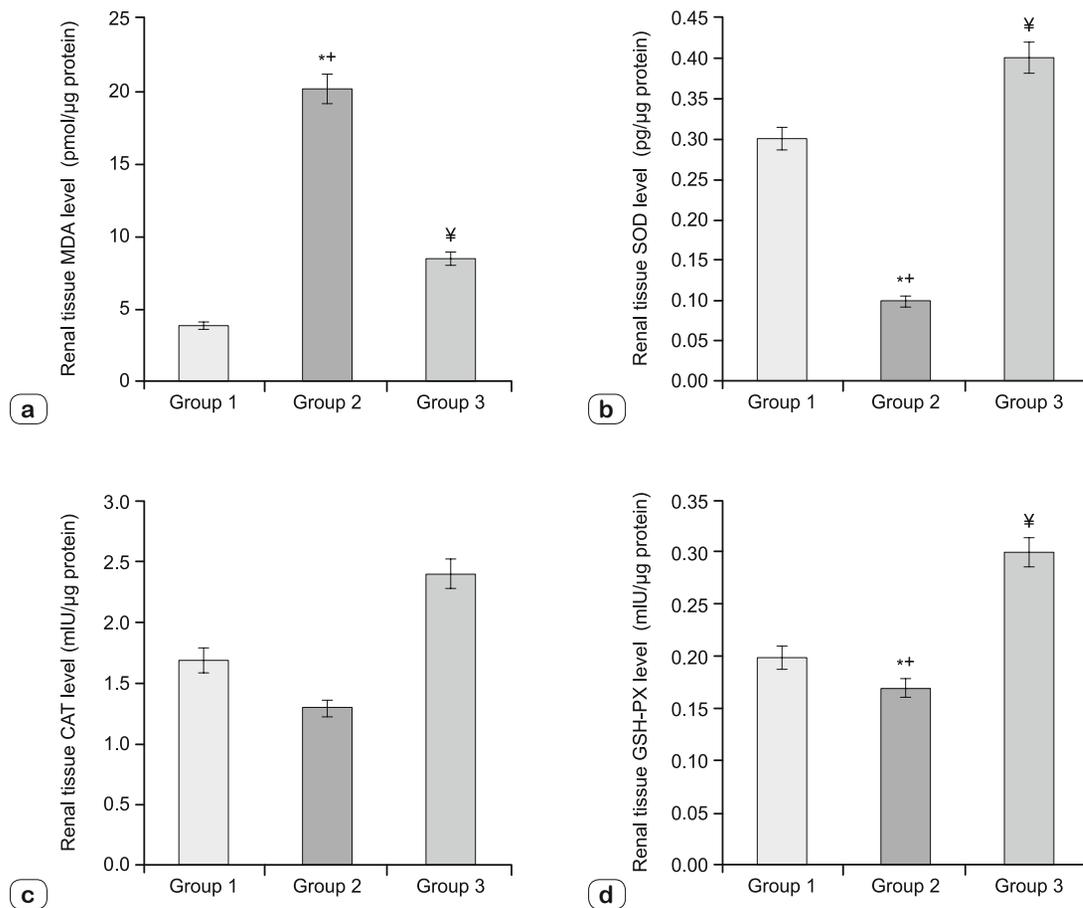
Fig. 3. Malondialdehyde (MDA) was significantly increased in the kidneys subjected to IR injury but decreased by OMT treatment (a). The levels of SOD, CAT and GSH-Px in the renal tissue exposed to renal ischemia/reperfusion were increased by OMT treatment (b, c, d). Data were presented as mean ± SEM (n = 10). Group 1: sham-operated control; Group 2: I-R/untreated; Group 3: I-R/oxymatrine treated. IR: Ischaemia/reperfusion. SOD: superoxide dismutase. CAT: Catalase. GSH-Px: glutathione peroxidase. * p < 0.0001 (Group 2 vs 1); † p < 0.0001 (Group 2 vs 3), ¥ p < 0.0001 (Group 3 vs 1).

A). Compared with those in the sham operation group, the rats in the I/R-untreated group showed a significant renal histopathologic changes, which included tubular dilation, cloudy swelling and hydropic degeneration in the tubular epithelial cells, vascular congestion and tubular necrosis (Fig. 5 B). In the I/R-OMT-treated group, minimal hydropic changes in the tubular epithelial cells *and* regeneration of tubular epithelium were observed (Fig. 5 C).

Discussion

OMT is a natural alkaloid, and its separation and determination have currently matured (18, 19). Several studies have shown that OMT has the following pharmacological effects: immune reaction inhibition, anti-viral effect, hepatocyte protection, and anti-hepatic fibrosis effect (14–16, 20, 21). Additionally, OMT has been reported to be effective because of its anti-apoptotic and anti-inflammatory activities; it can also decrease NF-kappaB expression in myocardial, liver, intestinal, and cerebral I/R injury in animal models (14–16, 22, 23). In this study, the obtained results

showed that the renal I/R injury induced pathological changes in the renal tissue, such as widespread degeneration of tubular architecture, tubular dilation, tubular cell swelling, cellular vacuolization, inflammatory cell infiltration, severe tubular necrosis, and luminal congestion. The plasma urea and creatinine levels significantly increased in the I/R group compared with those in the control group. The levels of urinary albumin, protein, uric acid, and protein/creatinine were also increased by I/R injury. We found decreased levels of plasma creatinine and BUN and ameliorated pathological changes in the OMT-treated group unlike in the I/R-untreated group. Results from these studies suggested that OMT intravenous injection prior to renal reperfusion significantly attenuated the improvement in the renal dysfunction caused by severe ischemia (45 min) and reperfusion in the animals.

The anti-inflammatory and antioxidative effects of OMT, as well as its roles in immunological regulation, have attracted attention in the past few years (14, 22, 24). OMT has been confirmed to act on nonspecific inflammatory reactions (25). OMT exerts its anti-inflammatory effect independent of the pituitary–adrenal
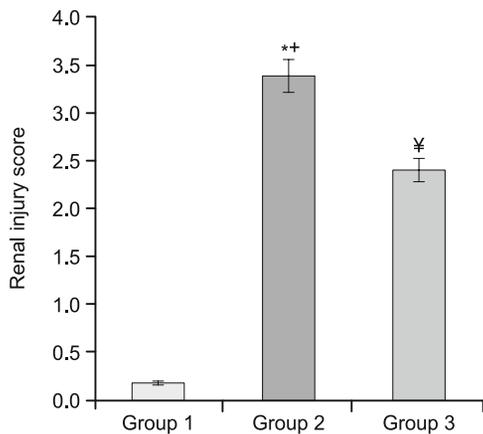
Fig. 4. Jablonski scores for histological appearance of acute tubular necrosis from sham-operated control, I-R/untreated and I-R/oxymatrine treated groups. Renal injury score was significantly increased in the kidneys subjected to IR injury but decreased by OMT treatment. Data were presented as mean ± SEM. Group 1: sham-operated control; Group 2: I-R/untreated; Group 3: I-R/oxymatrine treated. IR: Ischemia/reperfusion. * p < 0.0001 (Group 2 vs 1); † p < 0.0001 (Group 2 vs 3), ¥ p < 0.0001 (Group 3 vs 1).

of cellular membranes.

In the renal ischemia, free radicals generated as a result of lipid peroxidation in the kidney cell membrane cause tissue damage. The best indication of a structural oxidative injury of the cell membrane is the tissue MDA value (28). The biological activity of free radicals is inhibited by antioxidant enzymes SOD and CAT (22). SOD catalyzes the reduction of superoxide anions to H_2O_2, which is then converted to H_2O and O_2 by CAT. Moreover, GSH is widely accepted as an important constituent of intracellular protective mechanisms against various noxious stimuli, including oxidative injury (29). In a study on intestinal I/R in rats, Zhao et al (16) showed that OMT significantly attenuated intestinal I/R injury and inhibited cell apoptosis by impeding the production of lipid peroxides and serum levels of TNF-α and by downregulating the expression of phosphorylated p38 mitogen-activated protein kinase, Fas, and FasL. In another experimental study, Hong et al (22) explored the possible role of OMT against myocardial ischemic damage and several related signaling pathways. They suggested that one of the beneficial effects of OMT was likely mediated by the inhibition of lipid peroxidation and the increase in endogenous antioxidant activity.

As the results of the present study support the notion that depletion of renal GSH, SOD, and CAT and increase in MDA content are some of the major factors causing lipid peroxidation and subsequent tissue damage because of I/R, the replenishment of renal GSH, SOD, and CAT contents by OMT treatment may be considered as the antioxidant function of OMT. Our recent study also confirms that OMT exerts antioxidant and minimal histopathologic changes and regeneration of tubular epithelium properties in renal tissue by scavenging ROS and increasing the activities of GSH, superoxide dismutase, and catalase.

The current study demonstrated that renal tubular damage and inflammatory cell infiltration caused by renal I/R injury were markedly alleviated in rats following OMT treatment. The beneficial effects of OMT were likely mediated by the inhibition of lipid peroxidation (MDA production) and the increase in endogenous antioxidant activity (CAT, SOD, and GSH-PX). This finding indicates that OMT exhibits renoprotective effects against renal I/R injury by inhibiting oxidative stress.

system: it acts directly on inflammatory cells (25). OMT is a two-way immunoregulant. That is, it can stimulate the proliferation of lymphocytes at low concentrations but inhibits this proliferation at high concentrations (26). Jiang et al (15) suggested that the protective function of OMT against I/R also depends on its anti-inflammatory activity. The release of destructive proinflammatory cytokines during I/R is one of the main mechanisms of liver damage, and evidence shows that OMT can inhibit cytokines, such as TNF-a, IL-1, IL-6, and IL-8,14–16, thus supporting the use of oxymatrine to protect the liver against I/R. Zhu et al (27) demonstrated that OMT could significantly induce the expression of IL-10 and downregulate the secretion of TNF-a and IL-8 from multinuclear leukocytes in a lung I/R model in rabbit. Thus, OMT can reduce damage in microvascular endothelial cells, suppress the increasing vascular wall permeability, increase the anti-inflammatory and anti-oxidative capacity of tissues, and increase the stability
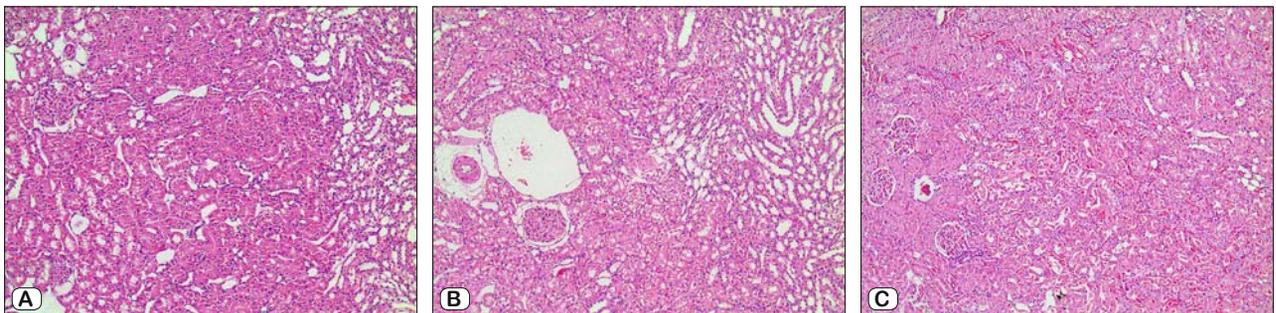

Fig. 5. Representative kidney sections stained with H&E at the end of 24 hours' reperfusion. A) Sham-operated animals: normal histological characteristic of glomeruli and tubules. B) Rats subjected to renal I-R injury: marked necrosis with tubular dilation, swelling, and luminal congestion. C) Rats subjected to renal I-R injury, pretreated with Oxymatrine: minimal hydropic changes in the tubular epithelial cells and regeneration of tubular epithelium. (H&E, x100). I-R: Ischaemia/reperfusion.

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