

Effects of Curcumin on A Renal Ischemia/Reperfusion Injury in Rats

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ABSTRACT

This study aimed to investigate the biochemical and histopathological effects of curcumin on Renal Ischemia/Reperfusion Injury. Twenty-four female Wistar rats were used in the study and divided into control (C), sham, ischemia/reperfusion (I/R), and curcumin (CUR) groups ($n=6$). Laparotomy was performed under general anesthesia in I/R and CUR groups than the left renal pedicle was dissected it was clamped. Then, 1-h ischemia and 6-h reperfusion were applied. 500 mg/kg Curcumin was given intraperitoneally to the CUR group after ischemia application. MPO, IMA, MDA, NO, SOD, GPx, AOS, urea, and creatinine levels were measured in serum samples. MPO, MDA, NO, SOD, GPx, and AOS were also measured in tissue samples. The histopathological examination was performed. Serum and tissue AOS levels were significantly higher in the CUR group than in the I/R group ($P<0.05$). Tissue NO levels were significantly lower in the CUR group than in the I/R group (15.30 ± 5.41 and 4.8 ± 1.37 , respectively) ($P<0.05$). Histopathological scores were also significantly lower in the CUR group than in the I/R group ($P<0.05$). The results showed that curcumin prevented I/R damage by decreasing oxidative stress in serum and tissue samples in rat renal I/R model.

Keywords: Curcumin, Renal Ischemia/Reperfusion, Rats.

Ratlarda Renal İskemi/Reperfüzyon Hasarında Curcumin'in Etkileri

ÖZ

Bu araştırmada, Renal İskemi/Reperfüzyon Hasarı Modelinde Curcumin'in etkilerini biyokimyasal ve histopatolojik olarak araştırmak amaçlanmıştır. Çalışmada ratlar kontrol (C), sham, iskemi repürfüzyon (I/R) ve curcumin (CUR) grubu olmak üzere ($n=6$) toplam 24 dişi Wistar Rat kullanılmıştır. Genel anestezi altında Grup I/R ve CUR da laparotomi uygulanarak sol böbrek pedikülü diseksi edilmiş ve böbrek arteri klempe edilerek 1 saat iskemi ve 6 saat reperfüzyon gerçekleştirilmiştir. Grup CUR da 500 mg/kg Curcumin iskemi sonrası intraperitoneal olarak verilmiştir. Serum MPO, IMA, MDA, NO, SOD, GPx, AOS, üre ve kreatinin ölçümleri gerçekleştirilmiştir. Doku örneklerinde MPO, MDA, NO, SOD, GPx, AOS ölçülmüştür. Histopatolojik inceleme ile böbrek dokusunda I/R hasarı skorlanmıştır. Serum ve doku AOS Grup I/R Grup CUR ile karşılaştırıldığında istatistiksel önemi olacak şekilde Grup CUR'de yüksel bulunmuştur ($p<0.05$). Doku NO düzeyi I/R grubu ile karşılaştırıldığında Grup CUR'da istatistiksel önemi olacak şekilde düşük bulunmuştur ($15,30\pm 5,41$, $4,8\pm 1,37$) ($p<0.05$). Histopatolojik skorlamada Grup CUR, Grup I/R ile karşılaştırıldığında istatistiksel olarak anlamlı olacak şekilde düşük bulunmuştur ($p<0.05$). Sonuç olarak; bu araştırmada Curcumin'in Renal I/R hasarı modelinde serum ve doku örneklerinde oksidatif stresi azaltarak I/R hasarını önlemede olumlu etkisi olduğu ve histopatolojik inceleme ile de Curcumin'in bu olumlu etkisi desteklenmiştir.

Anahtar kelimeler: Curcumin, Renal İskemi/Reperfüzyon, Rat.

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INTRODUCTION

Acute kidney failure (AKF) is a clinical syndrome characterized by a rapid disruption of normal functions of the kidney for various reasons. This syndrome is characterized by the accumulation of nitrogenous waste materials and the deterioration of fluid and electrolyte balance as a result of a rapid decline in glomerular filtration rate (GFR) over hours or day by day (Adewusi and Afolayan, 2009; Atthe et al., 2009; He et al., 2011; Tanrıverdi and Karadağ, 2010; Uslu et al., 2009). Renal functions usually improve with an effective treatment in patients whose kidney functions were normal before the development of acute renal failure (ARF). This situation is the most important characteristic of AKF distinguishing it from chronic kidney failure (Tanrıverdi and Karadağ, 2010).

The most common cause of ARF is ischemia. Ischemic ARF occurs due to conditions such as kidney transplantation, cardiovascular surgical interventions, hemorrhage, sepsis, dehydration, and trauma (Guan et al., 2009; Kumar et al., 2009).

The pathophysiology of Ischemia/Reperfusion Injury in the kidney is quite complex. Activation of neutrophils involves the release of ROS and other inflammatory mediators such as adhesion molecules and cytokines. Studies have shown beneficial effects of some agents in combating IRH. For example, doxycycline has been shown to be effective in reducing proinflammatory cytokine levels (İhtiyar et al., 2011; Kucuk et al., 2009). Leptin has shown to reduce tumor necrosis factor alpha levels and increase nitrite levels (Erkasap et al., 2004). Levosimendan acts as an antioxidant due to nitric oxide-related mechanism (Grossini et al., 2012), and iloprost shows the same effect by suppressing lipid peroxidation (Döslüoğlu et al., 1993). Finally, ascorbic acid sweeps out all the free radicals and acts as an antioxidant (Korkmaz and Kolankaya, 2009).

Curcuma longa is a plant belonging to the Zingiberaceae family. It is commonly found in India and China. The local paramedical use of curcumin has been studied by many researchers in recent years (Pandya et al., 2000). Curcumin (CUR) has anti-inflammatory, immunomodulatory, anti-tumoral, and anti-psoriatic effects due to its antioxidant properties.

This study investigated the effect of CUR on the rat ischemia/reperfusion (I/R) injury model. All biochemical and antioxidant parameters in serum and tissue specimens were measured, and changes in electrolyte levels and histopathology samples were examined.

MATERIALS and METHODS

A total of 24 female Wistar rats weighing 250–350 g were used. In this study raised under the same environmental conditions were used. For at least one week prior to surgery, the animals were housed in standard cages in a pathogen-free environment with free access to food (until 2 h before the anesthetic procedure) and water and with a 12-hour light/dark cycle. The animals were randomly separated into four groups, each containing six rats. All the procedures were performed according to the accepted standards of the Guide for the Care and Use of Laboratory Animals. The study was started with the approval of Afyon Kocatepe University, Local Animal Experiment Community dated: 08.11.2016 with the number of 137-16.

Study design

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

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.

Curcumin group (CUR group) (n = 6): The rats in this group were injected intraperitoneally with 500 mg/kg CUR (Sigma Life Science, Lot: SLBN7214V, MO, USA) dissolved in ethanol after laparotomy, and their blood and tissue samples were collected after 6-h reperfusion following 60-min renal ischemia.

Anesthesia procedure

General anesthesia was performed intramuscularly using 8 mg/kg xylazine HCl (Alfazine, Ege-Vet, Turkey) and 80 mg/kg ketamine HCl (Alfamine, Ege-Vet, Turkey).

Renal ischemia/reperfusion procedure

Laparotomy was performed with midline incision in rats in the I/R and CUR groups. After each renal pedicle was found, renal artery occlusion was achieved using microvascular clamps (Bulldog). Fading in the kidneys after using clamps was detected as a sign of occlusion. Subsequently, the abdomen, which was temporarily closed with silk sutures, was opened after 60 min. The clamps were removed, and the color change in the kidney was

observed. The abdomen was temporally 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 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

Kidney tissue samples were homogenized in 1:10 w/v of 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA. The homogenate was centrifuged in a refrigerated centrifuge at 15,000 rpm for 15 min to obtain supernatants. Subsequently, protein levels were determined using Lowry method (Lowry et al., 1951), followed by the determination of SOD, GPx, malondialdehyde (MDA), and NO levels and antioxidant activity.

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

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 assay (Cayman Chemical Company, ELISA Kit Cat. No. 709001, USA) levels in the tissue samples and serum were determined using a commercially available ELISA kit. The absorbance of the color formed by the

reaction of MDA with thiobarbituric acid [known as Draper and Hadley method (1990) (Draper and Hadley, 1990) measured at 535 nm. The SOD activity was determined using the method reported by Sun et al. (1988) (Sun et al., 1988), which was based on the inhibitory effect of SOD on nitroblue tetrazolium reduction of superoxide anions by xanthine/xanthine oxidase system. Nitric oxide levels were determined according to the method reported by Miranda et al. (2001) (Miranda et al., 2001). 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. They were trimmed after 48 h and moved into the trays for tissue attachment. The tissues were traversed through the series of alcohol and xylene applications and blocked in paraffin. The blocks were sliced into 4- to 4- μ m sections using a microtome and placed on the slides. The sections were stained with hematoxylin–eosin (HE) technique and examined under a light microscope. The changes in the kidneys were evaluated according to the criteria given in Table 1.

Table 1. Degree of kidney histopathology
Tablo 1. Böbrek Histopatolojisinin Derecelendirilmesi

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

Statistical analysis

Data were presented as means \pm standard deviation (S.D.) values. The Kruskal–Wallis H test was used as a nonparametric test to determine changes during the biochemical and electrolyte analysis of oxidative stress. In addition, the chi-square test was used for comparing the variables obtained from the pathological analysis. The data obtained in the study was analyzed using the Statistical Package for Social Sciences (SPSS, 18.0 software, USA). Differences were considered statistically significant when $p < 0.05$.

RESULTS

The levels of MPO, IMA, MDA, NO, SOD, GPx, AOS, urea, and creatinine in the serum samples were measured in control, sham, I/R, and CUR groups in this study. Serum and tissue AOS levels were significantly higher in the CUR group than in the I/R group ($P < 0.05$). (Table 2). MPO, MDA, NO, SOD, GPx, and AOS levels were measured in the kidney tissue. Tissue NO levels were significantly lower in the CUR group than in the I/R group (15.30 ± 5.41 and 4.8 ± 1.37 , respectively) ($P < 0.05$). (Table 3). At the end of

the study, serum K⁺, Ca²⁺, Na⁺, and Cl⁻ levels were measured in the venous blood samples taken from all groups before sacrificed. Serum K⁺ levels in I/R grup were higher than in CUR Grups (8.69 ± 0.84 vs 6.93 ± 1.51) (P<0.05). The results are shown in Table 4.

Histopathological scores were also significantly lower in the CUR group than in the I/R group (83.3%=Degree 3, 0%=Degree 4, 6.7% =Degree 5 in the CUR group vs 50%, 33,3%, 16,7% in the I/R grup, respectively) (Pearson chi-square test: *P* = 0.001 (Table 5), (Fig 1a,b,c,d).

DISCUSSION

This study investigated whether the intraperitoneal application of curcumin at a dose of 500 mg/kg in rats had beneficial effects in correcting renal I/R Injury. Studies on the physiopathology of I/R injury have reported that ROS produced from the damaged tissue after reperfusion induces the release of proinflammatory cytokines by stimulating macrophages. These cytokines trigger an inflammatory response, increasing tissue damage

(Pompermayer et al., 2005). ROS which is released after neutrophil migration in ischemic tissue, protease, elastase, MPO, and proinflammatory cytokines increase tissue damage (Kettle and Winterbourn, 1997).

In the present study, both serum level and activity of MPO (an indicator of tissue neutrophil activation) in the kidney tissue increased in the control group compared with the sham and I/R groups. In contrast, MPO activity decreased in the CUR group.

IMA has been identified as a new marker of inflammatory diseases in recent years (Ellidag et al., 2013). IMA activity has been found to be high in high oxidative stress-related, inflammatory diseases (Roy et al., 2006) (Roy et al., 2006). It has been reported that the IMA level elevates within minutes after ischemia, remains high for 6–12 h, and returns to a normal level within 24 h (Anwaruddin et al., 2005).

In this study, the IMA level in the I/R group was found to be statistically significantly higher after 6 h of reperfusion compared with that in the CUR group. On the contrary, the IMA level measured at the end of the study was lower in the CUR group than in the control group.

Clinical trials have shown a major role of ROS in the pathogenesis of renal I/R Injury (Sancaktutar et al., 2014) (Sancaktutar et al., 2014). Lipid peroxidation is a complex phenomenon initiated by

the removal of a hydrogen atom from a methylene group placed between two unsaturated bonds in lipid molecules. This results in the formation of a new carbon-centered lipid free radical. Lipid peroxides or hydroperoxides are formed from this new lipid free radical in the presence of oxygen. These end products are converted into MDA, which is a relatively more stable end product and can be used as a marker for lipid peroxidation (Slater, 1988).

In this study, MDA levels in kidney tissue were significantly elevated in both I/R and CUR groups compared with the control and sham groups. However, MDA level in the CUR group was determined to be lower than that in the I/R group. However, the serum I/R level was lower in the I/R group than in the CUR group.

Tubular cells normally do not produce NO. Ischemic damage increases intracellular NOS outflow in the tubular cells. Ischemia in the tubular cells has been shown to induce peroxynitrite formation by increasing NO and superoxide production (Yaqoob et al., 1996).

The NO level in the kidney tissue was found to be statistically significantly lower in the CUR group compared with that in the I/R group. On the contrary, NO level was significantly higher in the I/R group than in the control group.

The antioxidant properties of curcumin have been shown to reduce oxidative stress and tissue destruction in the heart and brain and also I/R injury in the liver (Thiyagarajan and Sharma, 2004). Curcumin acts as an antioxidant by inhibiting the conversion of XD into XO, lipid peroxidation, and ROS in the ischemic environment. Additionally, it reduces lipid peroxidation by increasing the activity of the enzymes such as curcumin catalase, superoxide dismutase, and glutathione peroxidase (Miquel et al., 2002).

In this study, the difference in the serum SOD and GPx levels were found to be insignificant between the I/R and CUR groups. However, the SOD level in the kidney tissue was found to be significantly higher in the CUR group compared with that in the I/R group. Although GPx was found to be lower in both serum and kidney tissues in the I/R group, serum GPx level was found to be higher in the CUR group than in all other groups. AOS in the serum and kidney tissues was found to be lower in the I/R group compared with that in the CUR group.

Table 2. Measurement results of biochemical and oxidant/antioxidant parameters in serum.

Tablo 2. Serum biyokimya ve oksidan/antioksidant parametrelerin ölçüm sonuçları.

Group	MPO (ng/mL)	IMA (ng/mL)	MDA (nmol/mL)	NO (μ mol/L)	SOD (U/mL)	GPx [nmol/(min · mL)]	AOS (mmol/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control (<i>n</i> = 6)	3.43 \pm 0.94	23.73 \pm 3.91 ^a	1.56 \pm 0.34	3.76 \pm 0.34	1.21 \pm 0.16	43.50 \pm 5.46	0.3 \pm 0.02 ^b	49.42 \pm 7.14 ^a	0.31 \pm 0.09 ^a
Sham (<i>n</i> = 6)	3.87 \pm 0.43	26.97 \pm 1.16	1.86 \pm 0.39	3.49 \pm 0.41	1.35 \pm 0.35	43.83 \pm 10.3	0.26 \pm 0.02 ^{bc}	45.38 \pm 5.37 ^a	0.29 \pm 0.04 ^a
I/R (<i>n</i> = 6)	4.24 \pm 0.59	28.86 \pm 3.84 ^b	2.16 \pm 0.44	3.52 \pm 0.28	1.6 \pm 0.23	38.83 \pm 8.32	0.19 \pm 0.03 ^a	74.25 \pm 14.25 ^b	0.45 \pm 0.04 ^b
CUR (<i>n</i> = 6)	3.24 \pm 0.73	23.17 \pm 6.21 ^a	2.08 \pm 0.22	3.43 \pm 0.33	1.24 \pm 0.25	46.66 \pm 7.36	0.23 \pm 0.03 ^{ac}	96.87 \pm 12.38 ^b	0.52 \pm 0.07 ^b

The values in the same column shown with different characters are statistically significant ($P < 0.05$).

AOS: Antioxidant status; GPx, glutathione peroxidase; IMA, ischemia-modified albumin; MDA, malondialdehyde;

MPO, myeloperoxidase; NO, nitric oxide; SOD, superoxide dismutase.

Table 3. Measurement results of biochemical and a oxidant/ntioxidant parameters in the kidney tissue

Tablo 3.Böbrek dokusunda biyokimya ve oksidan/antioksidant parametrelerin ölçüm sonuçları

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 (<i>n</i> = 6)	1.06 \pm 0.28 ^a	2.62 \pm 0.49 ^a	6.88 \pm 3.58 ^a	0.81 \pm 0.07 ^{bc}	0.18 \pm \pm 0.03	11.59 \pm 1.53 ^{bc}
Sham (<i>n</i> = 6)	1.19 \pm 0.32 ^a	2.89 \pm 0.63 ^a	6.07 \pm 2.58 ^a	0.88 \pm 0.03 ^b	0.18 \pm 0.03	12.53 \pm 1.64 ^b
I/R (<i>n</i> = 6)	3.2 \pm 0.71 ^b	4.90 \pm 0.84 ^b	15.30 \pm 5.41 ^b	0.31 \pm 0.04 ^a	0.13 \pm 0.03	7.20 \pm 2.03 ^a
CUR (<i>n</i> = 6)	2.73 \pm 0.37 ^b	4.02 \pm 0.74 ^b	4.8 \pm 1.37 ^a	0.52 \pm 0.07 ^{ac}	0.16 \pm 0.03	8.61 \pm 2.33 ^{ac}

The values in the same column shown with different characters are statistically significant ($P < 0.05$).

AOS: Antioxidant status; GPx, glutathione peroxidase; IMA, ischemia-modified albumin; MDA, malondialdehyde;

MPO, myeloperoxidase; NO, nitric oxide; SOD, superoxide dismutase.

Table 4. Measurement results of serum electrolyte parameters
Tablo 4. Serum elektrolit parametrelerinin ölçüm sonuçları

Group	K ⁺	Ca ²⁺	Na ⁺	Cl ⁻
Control	5.87 ± 0.52 ^a	10.42 ± 0.66 ^a	140.83 ± 4.57	105.5 ± 3.44
Sham	5.43 ± 0.71 ^c	9.32 ± 0.4 ^b	141,33 ± 1.37 ^a	103.83 ± 3.6
I/R	8.69 ± 0.84 ^{b**}	9.95 ± 0.62 [*]	136.57 ± 2.76 ^{b*}	103 ± 4.61
CUR	6.93 ± 1.51 ^{cd}	10.06 ± 0.82 ^{bc}	139 ± 3.51	104 ± 3.78

The values in the same column shown with different characters are statistically significant ($P < 0.05$).
^{*} $P < 0.05$; ^{**} $P < 0.001$.

Table 5. Histopathological scoring results of the kidney tissues in each groups
Tablo 5. Gruplarda böbrek dokusunun histopatolojik skorları.

Group/Score	0	1	2	3	4	5
Control	100%	0%	0%	0%	0%	0%
Sham	100%	0%	0%	0%	0%	0%
I/R	0%	0%	0%	50%	33,3%	16.7%
CUR	0%	0%	0%	83.3% [*]	0% [*]	16.7%

Pearson chi-square test: ^{*} $P = 0.001$.

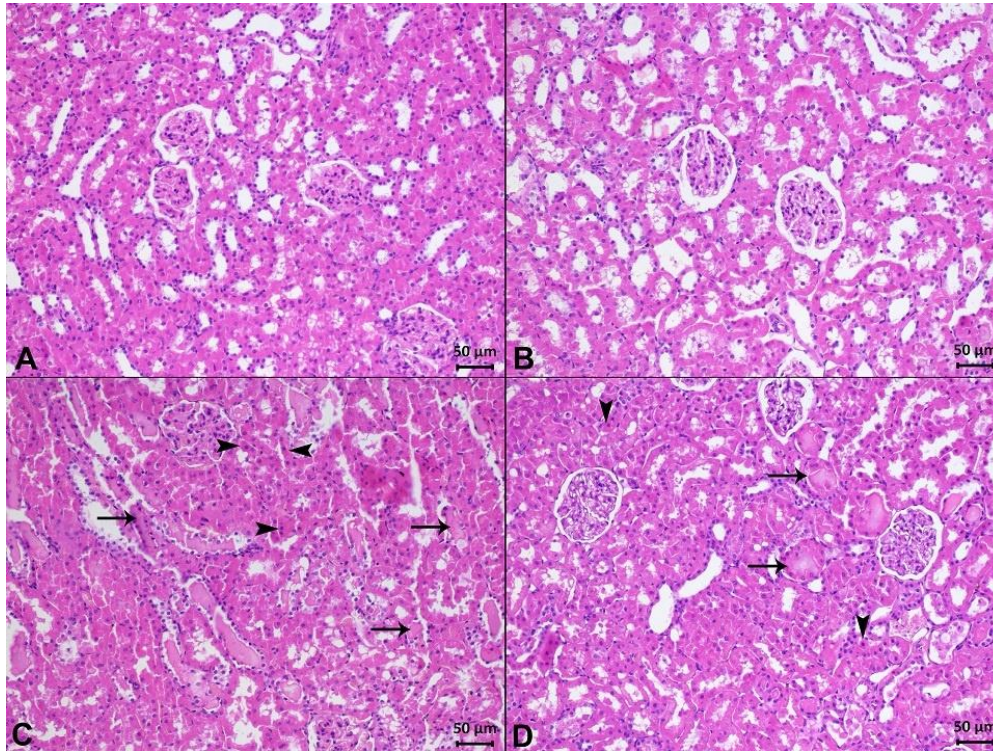


Figure 1. Histopathological view of experimental groups. **A. Control group:** histological structures were normal **B. Sham group:** histological structures are normal. **C. I/R group:** swelling in tubules in large field and severe necrosis (arrows), picnosis (arrowheads) in tubul epithelium and basal membrane separation in some epithelium. **D. CUR group:** less severe than the IR group; swelling and necrosis in the tubules (arrows), picnosis in the tubul epithelium (arrowheads) and basal membrane separation in some epithelium (HE,20x).

Şekil 1. Deney gruplarının histopatolojik görüntüsü. **A. Kontrol grubu:** histolojik yapılar normal görünümde **B. Sham grubu:** histolojik yapılar normal görünümde. **C. I/R grubu:** geniş sahalar halinde tubullerde şişme ve şiddetli nekroz (oklar), tubul epitellerinde piknoz (ok başları) ve bazı epitellerde bazal membrandan ayrılma. **D. CUR grubu:** IR grubuna göre daha az şiddette; tubullerde şişme ve nekroz (oklar), tubul epitellerinde piknoz (ok başları) ve bazı epitellerde bazal membrandan ayrılma (HE. 20x).

AKF is a clinical syndrome characterized by a rapid disruption of normal functions of the kidney for various reasons. This syndrome is characterized by the accumulation of nitrogenous waste materials and deterioration of fluid and electrolyte balance as a result of a rapid decline in GFR over hours or day by day (Adewusi and Afolayan, 2009; Atthe et al., 2009; He et al., 2011; Tanrıverdi and Karadağ, 2010; Uslu et al., 2009)

Serum urea and creatinine levels significantly increased in the I/R and CUR groups compared with those in the control group, but they remained within the normal reference levels. On the contrary, the serum K⁺ level was significantly higher in the I/R group than in the CUR group, whereas the serum Ca²⁺ level was higher in the CUR group compared with that in the I/R group. No significant difference was seen in Na⁺ and Cl⁻ levels between different groups.

Renal tubular epithelial cells may exhibit different structural and functional recovery, apoptosis, and necrosis depending on the duration and severity of ischemia (Dagher, 2004). Histopathological examination of renal tissue in the present study revealed nuclear shrinkage and severe vacuolization (moderate kidney damage) in 83.3% and complete tubular necrosis in 16.7% of the rats in the I/R group. Nuclear shrinkage and severe vacuolization (moderate kidney damage) was 50% and complete necrosis in the tubules 16.7% in the CUR group. This study showed that the intraperitoneal application of CUR at a dose of 500 mg/kg during 1-h ischemia had beneficial effects in correcting renal IRH in the rat renal ischemia/reperfusion model, considering biochemical measurements and oxidative markers. These results were also supported by histopathological results. Hence, it is believed that CUR protects the kidney against ischemia/reperfusion damage in the rat renal ischemia/reperfusion model.

Conflict of interest

Authors declared no Conflict of interest.

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