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***Experimental Research***

# Chrysin May Alleviate Renal Ischemia Reperfusion Injury by Suppressing Autophagy

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**ABSTRACT**

**Objective:** Chrysin (Chr) is a flavonoid with many biological activities such as antioxidant, anti-inflammatory and antiautophagic. The effects of Chr on autophagy, oxidative stress and inflammation was investigated in rats with kidney ischemia reperfusion (I/R) injury.

**Material and Method:** Fifty male Sprague-Dawley rats were divided into 5 groups: Sham, I/R, Chr25mg/kg+I/R, Chr50mg/kg+I/R and Chr100mg/kg+I/R. The rats except sham group were give different doses of Chr and underwent bilateral kidney ischemia for 45 minutes on the 14th day then reperfusion for 24 hours. BUN, creatinine, oxidative stress parameters and cytokines levels were measured biochemically. Beclin-1 and LC-3II levels in left kidney tissues were determined immunohistochemically.

**Results:** The low and medium doses of Chr decreased BUN, SOD, GSH and TAS, but also increased MDA and TOS levels. Histopathological findings showed a significant decrease in the Chr25mg/kg+I/R and Chr50mg/kg+I/R groups. LC-3II and Beclin-1 immunoreactivity were higher in I/R and Chr100mg/kg+I/R when compared to the other groups.

**Conclusion:** Administration of low and medium doses of Chr was protective against damage via suppressing autophagy. In addition, low and moderate doses of Chr administration before ischemia also protected the tissues against oxidative damage and inflammation, while high-doses of Chr caused damage similar to I/R in the kidney tissue.

***Keywords:*** *Chrysin, Kidney, Ischemia/Reperfusion, Oxidative Stress, Autophagy, Inflammation.*

**ÖZ**

**Chrysin, Otofajiyi Baskılayarak Renal Iskemi Reperfüzyon Hasarını Hafifletebilir**

**Amaç:** Chrysin (Chr), antioksidan, antiinflamatuar ve antiotofajik gibi birçok biyolojik aktiviteye sahip bir flavonoiddir. Böbrek iskemi reperfüzyon (I/R) hasarı olan sıçanlarda Chr'nin otofaji, oksidatif stres ve inflamasyon üzerindeki etkileri araştırıldı.

**Gereç ve Yöntem:** Elli erkek Sprague-Dawley sıçan 5 gruba ayrıldı: Sham, I/R, Chr25mg/kg+I/R, Chr50mg/kg+I/R ve Chr100mg/kg+I/R. Sham grubu dışındaki sıçanlara 14 gün boyunca farklı dozlarda Chr verildi ve 14. günde 45 dakika süreyle iki taraflı böbrek iskemisi ve ardından 24 saat süreyle reperfüzyon uygulandı. BUN, kreatinin, oksidatif stres parametreleri ve sitokin düzeyleri biyokimyasal olarak ölçüldü. Sol böbrek dokularında Beclin-1 ve LC-3II düzeyleri immünohistokimyasal olarak belirlendi.

**Bulgular:** Düşük ve orta dozda Chr BUN, SOD, GSH ve TAS'ı azaltırken aynı zamanda MDA ve TOS düzeylerini de artırdı. Histopatolojik bulgular Chr25mg/kg+I/R ve Chr50mg/kg+I/R gruplarında anlamlı azalma gösterdi. LC-3II ve Beclin-1 immünreaktivitesi I/R ve Chr100mg/kg+I/R'de diğer gruplarla karşılaştırıldığında daha yüksekti.

**Sonuç:** Düşük ve orta dozda Chr uygulamasının otofajiyi baskılayarak hasara karşı koruyucu olduğu görüldü. Ayrıca iskemi öncesi düşük ve orta dozda Chr uygulamasının da dokuları oksidatif hasar ve inflamasyona karşı koruduğu, yüksek dozda Chr uygulamasının ise böbrek dokusunda I/R benzeri hasara neden olduğu görüldü.

***Anahtar Sözcükler:*** *Chrysin, Böbrek, Iskemi/Reperfüzyon, Oksidatif Stres, Otofaji, Inflamasyon.*

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Kidney ischemia-reperfusion (I/R) injury is the main cause of acute kidney injury, affecting an average of 13.3 million people worldwide each year. I/R damage to the kidneys may occur as a result of surgical interventions such as partial nephrectomy, transplantation and aortic clamping, and clinical conditions such as shock and sudden cardiac arrest. (1).

It is known that autophagy allows cells to survive by removing damaged proteins and organelles resulting from I/R injury. After kidney I/R injury, autophagy is directly related to the survival of especially podocytes, mesenchymal cells and kidney tubular cells, which are the natural cells of the kidney tissue. Therefore, it has recently become a mechanism that has been studied in

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more detail for the development of new methods in the treatment of kidney diseases (2). Beclin-1 is the main protein involved in autophagosome formation, which is the initial stage of autophagy, and mediates the transport of other autophagic proteins to the preautophagosomal membrane during the formation of autophagosomes (3). Microtubule associated protein 1 light chain 3II (LC-3II) is another important protein involved in the regulation of autophagy. It is located in the autophagosomal membrane structure and plays an important role in the elongation and closure of the autophagosome vesicle (4).

I/R injury is a pathological condition that starts with oxygen deficiency and continues with inflammatory responses via neutrophils and superoxide radicals (SORs) (5). Ischemia forces the kidney tissue to shift to anaerobic metabolism to survive. However, paradoxically, this induces inflammation and cell death and causes oxidative damage in the kidney tissue (6).

The autophagy process can be regulated by different cytokines. Increasing values of IL-1, tumor necrosis factor-α (TNF-α), IL-17, and IL-6 appear to induce autophagy, while IL-13, IL-33, IL-10, and IL-4 block the process. TNF-α plays a role in initiating the autophagy process in many cells such as osteoclasts, epithelial cells, skeletal muscle and smooth muscle cells. It has also been reported that TNF-α increases the expression level of LC3II and Beclin-1 in human atherosclerotic vascular smooth muscle cells (7). It has been shown that IL-1α and IL-1β, which start to be released in the early stages of inflammation induce autophagy by trigging autophagosome formation (8). Although cytokines can regulate the autophagy process, cytokine production, secretion and degredation can also be regulated by autophagy (7). Disruptions in autophagy pathways have been associated with increased expression levels of IL-1 α, IL-1β and IL-18 (9, 10). Detailed investigation of these interactions between autophagy and cytokines will also enable the development of new treatment methods in autoimmune diseases, infections, treatment of tumors and traumas.

Chr is a natural flavonoid found in honey, passion fruit, propolis and mushrooms with many biological activities such as antioxidant (11), anti-inflammatory (12), antiapoptotic (13) and antiautophagic (14). It has been reported that Chr prevents tubular cell apoptosis and inflammation in kidney I/R injury (15). However, no study has been found in the literature examining the effects of Chr on autophagy in kidney I/R injury.

This study was aimed to evaluate the effects of different doses of Chr taken orally prior to kidney I/R injury on oxidative damage and inflammation in kidney tissue. In addition, it was aimed to elucidate whether autophagy is induced or suppressed by Chr administration, and whether it is beneficial or harmful on kidney tissues after I/R.

## MATERIAL AND METHOD

**Animals:** The ethical approval for this study was received from the Animal Experiments Local Ethics Committee of Faculty of Medicine, Inonu University (Protocol no: 2019/A-49). Fifty male Sprague-Dawley rats with an average weight of 240-280 g obtained from Experimental Animal Production and Research Center, Inonu University were used. All rats were weighed and divided into 5 groups (n =10), with no significant difference in weights between the groups.

**Sham:** Chr solvent (olive oil) of 1ml was given daily by gavage for 14 days (16). At the end of the 14th day, the rats in this group underwent sham kidney I/R surgery.

**I/R:** Chr solvent of 1 ml was given daily by gavage for 14 days (16). At the end of the 14th day, bilateral kidney I/R model was implemented to the rats. After reperfusion, the rats were sacrificed and blood samples, right and left kidneys were collected.

**Chr25 mg/kg+I/R, Chr50mg/kg+I/R and Chr100 mg/k +I/R:** Daily 1 ml of Chr (BLDpharm, Cas no: 480-40-0) dissolved in olive oil was given by gavage for 14 days (16-18). At the end of the 14th day, bilateral kidney I/R model was implemented on the rats. After reperfusion, the rats were sacrificed and blood samples, right and left kidneys were collected.

**Application of Bilateral Kidney I/R Model and Collection of Samples**

Firstly, all rats were anesthetized (70 mg/kg ketamine (Richter Pharma AG, Australia) and 8 mg/kg xylazine (Bioveta PLC, Czech Republic)). The bilateral kidney I/R model was used. A microvascular clamp that was attached to the kidney arteries was subjected to ischemia for 45 minutes. Then the incision area was sutured and reperfusion was applied for 24 hours (19). After reperfusion, rats were sacrificed, and blood samples collected from rats were separated to serum and stored at -80 ºC for biochemical analyzes (measurement of BUN and creatinine values). Right kidney tissues collected from rats and stored at -80 ºC for biochemical analysis, while left kidney tissues were placed in 10% formaldehyde solution to be used in histological analysis.

Right kidneys stored at -80 ºC for biochemical analyzes were removed one day before the analysis and were allowed to thaw overnight at +4 ºC. Tissues were weighed and placed in glass tubes. For each tissue, 10 times the weight of wet tissue in cold Tris-HCl buffer (pH=7.4) was added to the tubes. The samples were homogenized for 3 minutes at 16000 rpm (20). The homogenate was vortexed and taken into eppendorf tubes. The malondialdehyde (MDA) measurements were made according to the method of Esterbauer and Cheeseman, which is the lipid peroxidation determination method (21). A part of the homogenate was centri-

fuged at 2200 g using a cooled centrifuge at +4 ºC for 1 hour. The determination of SOD enzyme activity from the supernatants separated after centrifugation was performed according to the method defined by Sun et al. (22), and the determination of reduced GSH was made according to the method of Beutler et al. (23). Total antioxidant status (TAS) (Cat no: EK21122A, Rel Assay Diagnostics, Turkey), total oxidant status (TOS) (Cat no: EK21135O, Rel Assay Diagnostics, Turkey), in renal tissues, blood urea nitrogen (BUN)(Cat no: 201-11-1733, SunRed Biotechnology, China) and creatinine (Cat no: 201-11-0308, SunRed Biotechnology, China) values in serum samples were determined in accordance with the protocol of commercially purchased Elisa kits and using an immino plate reader with Biotek HT Snynergy Gen 5 software.
The cytokine levels in the sera obtained from the blood samples of the experimental groups were also measured in accordance with the protocol of the commercially purchased Elisa kit (Qiagen Multi-Analyte Elisarray Kit, Germany).

**Histopathological and immunohistochemical analysis**

Collected left kidneys were fixed in formaldehyde (%10). Following the tissue follow-up procedures, the sections taken from the paraffin blocks (4-5 µm thick) were stained with the hematoxylin-eosin (H-E) staining method to determine the morphological structure. Cortical and medullary areas in left kidney sections were evaluated for tubular degeneration (tubular necrosis and dilatation), infiltration and congestion. Randomly selected 10 areas were examined and according to the degree of histological changes scored as; 0: no change, 1: mild change, 2: moderate change, 3: severe change (24).
Beclin-1 and LC-3II levels in left kidney tissues were evaluated by immunohistochemical analysis. Brown staining was observed in tubule epithelial cells due to immunoreactivity to Beclin-1 and LC-3II applications.
Stainings were scored semiquantitatively according to the prevalence of immunoreactivity (0: 0-25%, 1:26-50%, 2:51-75%, 3:76-100%) and severity (0: none, +1: mild, +2: moderate, +3: severe). It was calculated as; total staining score= prevalence x severity (25).
Leica DFC-280 research microscope (Leica Micros Imaging Solutions Ltd., Cambridge, UK) with Leica Q Win Image Analysis System was used for all histological analyzes.

**Statistical analysis**

Statistical analyzes for biochemical evaluations were performed using the IBM SPSS Statistics 25.0 program. The conformity of the data to the normal distribution was done with the Shapiro-Wilk test. The comparison of groups performed by Kruskal Wallis test. Mann-Whitney-U test and Bonferroni correction was used in multiple comparisons. p <0.05 values were considered significant. Data are given as median (min-max).

## RESULTS

**Effect of Chr on BUN and Creatinine Levels**

Serum BUN levels were significantly decreased in rats treated with Chr compared to the I/R group. Low, medium and high doses of Chr showed similar effects on serum BUN levels (Figure 1A).
No significant difference among the groups were observed for serum creatinine levels (Figure 1B).

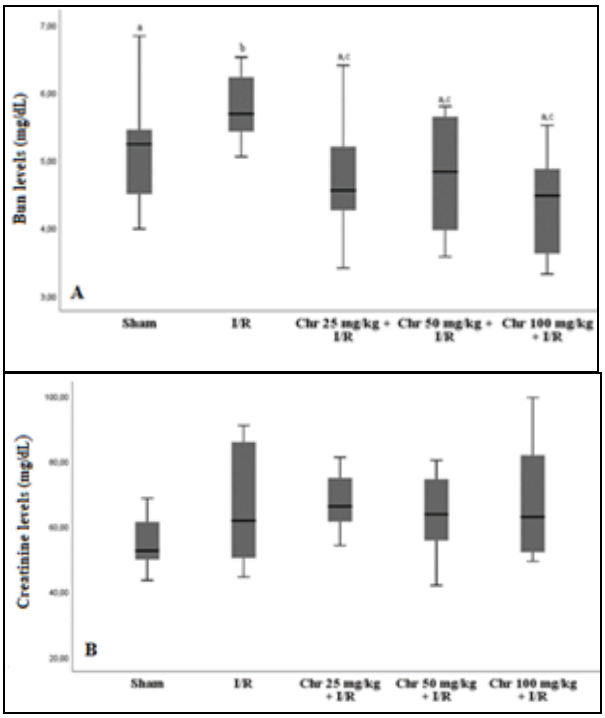


***Figure 1 (A, B).*** *Serum BUN and Creatinine levels among groups. (The Shapiro-Wilk test was used to evaluate normality (p =0.002 for BUN, p =0.0001 for Creatinine). The Kruskal Wallis Test was used to evaluate the data (p =0.001 for BUN, p =0.247 for Creatinine), and the Mann Whitney U test with Bonferroni correction was used for the Kruskal Wallis test for multiple comparisons. Values are given as median (min.-max). Differences between groups were indicated by letters. p<0.05 values were considered significant.)*

**Effect of Chr on oxidative stress in the kidney tissues**

The SOD enzyme activities and GSH levels in the I/R and high-dose Chr treated group were significantly lower compared to the sham, Chr25 mg/kg+I/R and Chr50 mg/kg+I/R groups (p <0.05). (Fig. 2A and 2B).

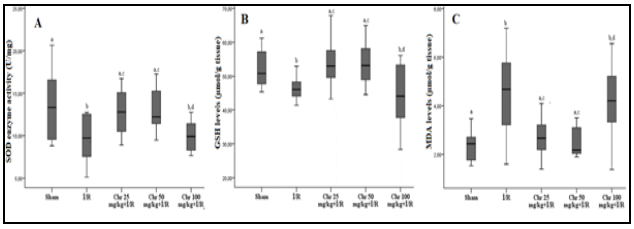


***Figure 2 (A, B, C).*** *SOD, GSH and MDA levels in the kidneys among groups (The Shapiro-Wilk test was used to evaluate normality (p =0.01 for SOD, p =0.0001 for GSH, p = 0.003 for MDA). The Kruskal Wallis Test was used to evaluate the data (p =0.007 for SOD, p =0.043 for GSH, p =0.057 for MDA), and the Mann Whitney U test with Bonferroni correction was used for the Kruskal Wallis test for*

*multiple comparisons. Values are given as median (min.-max). Differences between groups were indicated by letters. p <0.05 values were considered significant.)*

Likewise, the TAS level was found to be higher in the Chr25mg/kg+I/R and Chr50mg/kg+I/R groups (p <0.05). However, TOS levels were significantly low in the Chr25mg/kg+I/R and Chr50mg/kg+I/R groups (p<0.05) (Figure 3A and 3B).

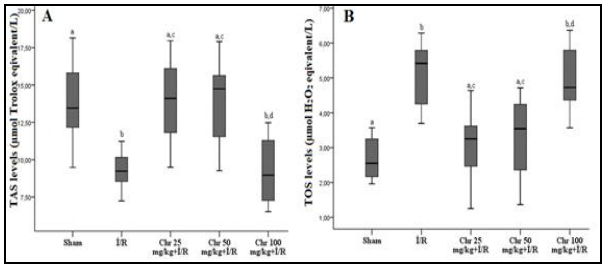


***Figure 3 (A, B).*** *TAS and TOS levels in the kidneys among groups. (The Shapiro-Wilk test was used to evaluate normality (p =0.02 for TAS, p =0.045 for TOS). The Kruskal Wallis Test was used to evaluate the data (p =0.001 for TAS, p =0.0001 for TOS), and the Mann Whitney U test with Bonferroni correction was used for the Kruskal Wallis test for multiple comparisons. Values are given as median (min.-max). Differences between groups were indicated by letters. p <0.05 values were considered significant.)*

MDA level was analyzed as an indicator of lipid peroxidation in renal tissue. MDA levels of ischemic renal tissue were significantly higher in the I/R group and the high-dose Chr-treated group. Levels of MDA in the low- and medium-dose Chr-treated groups were lower than I/R and high-dose group (p <0.05) (Figure 2C).

### Effect of Chr on Cytokine Levels in the Kidney Tissues

TNF-α, IL-1α, IL-1β, IL-6, IL-4 and IL-10 levels were also determined in the kidney tissue as indicators of inflammation. The TNF-α, IL-1α, IL-1β and IL-6 levels in the Chr25mg/kg+I/R and Chr50mg/kg+I/R groups were significantly decreased compared to the I/R and Chr100mg/kg+I/R groups (p <0.05). On the contrary, IL-10 and IL-4 levels were higher in groups Chr25mg/kg+I/R and Chr50mg/kg+I/R (p <0.05) (Figure 4).

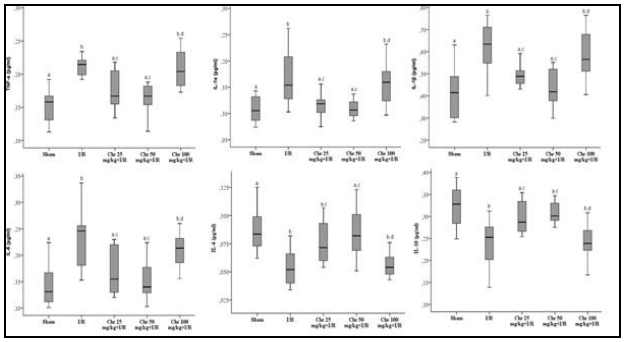

***Figure 4.*** *Kidney cytokines levels. (The Shapiro-Wilk test was used to evaluate normality. The Kruskal Wallis Test was used to evaluate the data, and the Mann Whitney U test with Bonferroni correction was used for the Kruskal Wallis test for multiple comparisons. Values are given as median (min.-max). Differences between groups were indicated by letters. p <0.05 values were considered significant.)*

### Histological Findings

### Histopathological Findings

In the sham group, the left kidney tissues had normal histological appearances, except for mild changes (tubular dilatation and congestion) observed in some samples (Figure 5A). In the I/R group, necrotic changes and dilatation were observed in tubules in both cortical and medullar areas. Moreover, another finding in this group was inflammatory cell infiltration and congestion (Figure 5B, 5C). In terms of these changes, the difference between the I/R and sham groups was found to be significant (p <0.0001).

Histopathological changes were observed to be significantly decreased in the Chr25mg/kg+I/R and Chr50mg/kg+I/R groups compared to the I/R group (p<0.05) (Figure 5D, 5E).

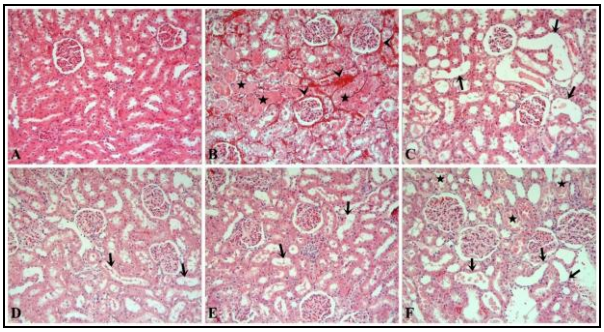


***Figure 5.*** *Histopathological Findings. (**Sham group (A);** kidney cortical tissue with normal histological appearance. **I/R group (B);** necrotic tubules (star) and congestion (arrowhead), **(C);** dilated tubules (arrow). **Chr25mg/kg+I/R (D) and Chr50mg/kg+I/R (E) groups**, significant decrease in histopathological changes except tubular dilatation. group. HE, 200x.)*

In the Chr100mg/kg+I/R group the congestion, tubular damage and infiltration was similar to I/R group (Figure 5D) (Table 1).

***Table 1.*** *Histopathological findings.*

| Groups | Tubular damage | Infiltration | Congestion |
|---|---|---|---|
| **Sham** | 0 (0-1) | 0 (0-0) | 0 (0-1) |
| **I/R** | 2 (0-3)[a] | 0 (0-3)[a] | 2 (0-3)[a] |
| **Chr25mg/kg+IR** | 1 (0-3)[b] | 0 (0-2)[b] | 0.5 (0-3)[b] |
| **Chr50mg/kg+I/R** | 1 (0-3)[b] | 0 (0-2)[b] | 0 (0-3)[b] |
| **Chr100mg/kg+I/R** | 2 (0-3)[a] | 0 (0-3)[a] | 0 (0-3)[b] |

*(The Kruskal Wallis Test was used to evaluate the data, and the Mann- Whitney U test and Bonferroni correction was used for the Kruskal Wallis test for multiple comparisons. Values are given as median (min.-max). Differences between groups were indicated by letters. [a,b] p <0.05 values were considered significant.) [a] Higher than sham group (p <0.0001). [b]Lower than I/R group (p <0.05).*

### Immunohistochemical analyzes

Beclin-1 immunoreactivity was prominently observed in the cytoplasm of tubule epithelial cells (Figure 6).

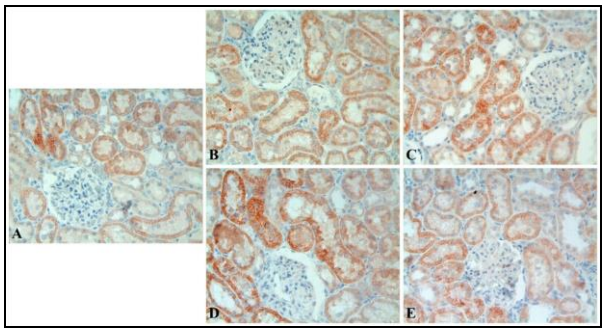


***Figure 6.*** *Beclin-1 Immunoreactivity. (Beclin-1 immunoreactivity observed in tubule epithelial cells, 400x.* ***A;*** *sham group,* ***B;*** *I/R group,* ***C;*** *Chr25 g/kg+I/R group,* ***D;*** *Chr50mg/kg+I/R group,* ***E;*** *Chr100mg/kg+I/R group.)*

In Chr25mg/kg+I/R and Chr50mg/kg+I/R groups, immunoreactivity was found statistically lower than in the I/R group ($p < 0.05$). Beclin-1 immunoreactivity observed in the I/R and Chr100mg/kg+I/R group was found significantly higher than the Chr25mg/kg+I/R and Chr50mg/kg+I/R groups ($p < 0.05$) (Table 2).
LC-3II immunoreactivity was observed in the cytoplasm of tubule epithelial cells (Figure 7).

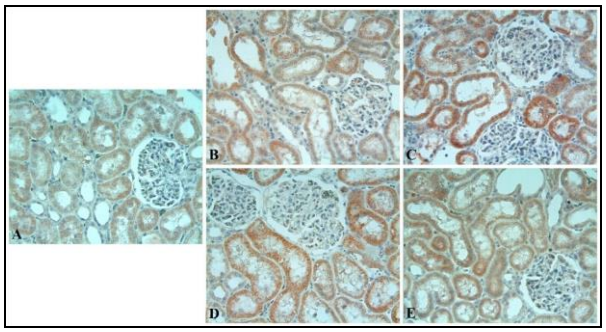


***Figure 7.*** *LC-3II Immunoreactivity. (Brownish LC-3II immunoreactivity in tubule epithelial cells, 400x.* ***A;*** *Sham group,* ***B;*** *I/R group,* ***C;*** *Chr25mg/kg+I/R group,* ***D;*** *Chr5 mg/kg+I/R group,* ***E;*** *Chr100kg/kg+I/R group.)*

LC-3II immunoreactivity observed in the Chr25mg/kg+I/R and Chr50mg/kg+I/R groups was lower than the I/R and Chr100mg/kg+I/R groups ($p < 0.05$) (Table 2).

***Table 2.*** *Immunohistochemical findings.*

| Groups | Beclin-1 | LC-3II |
|---|---|---|
| **Sham** | 8 (4-12) [a] | 4 (2-12) [a] |
| **I/R** | 12 (4-12) [b] | 8 (4-12) [b] |
| **Chr25mg/kg+I/R** | 8 (3-12) [a] | 4 (2-12) [a] |
| **Chr50mg/kg+I/R** | 8 (2-12) [a] | 4 (2-12) [a] |
| **Chr100mg/kg+I/R** | 12 (4-12) [b] | 6 (2-12 ) [b] |

*(The Kruskal Wallis Test was used to evaluate the data, and the Mann- Whitney U test and Bonferroni correction was used for the Kruskal Wallis test for multiple comparisons. Values are given as median (min.-max). Differences between groups were indicated by letters. [a,b] $p < 0.05$ values were considered significant.) [a] Lower compared to the I/R and Chr100mg/kg+I/R groups ($p < 0.0001$). [b] Higher compared to the Sham and Chr25mg/kg+I/R and Chr50mg/kg+I/R groups ($p < 0.05$).*

## DISCUSSION

In this study, the effects of different doses of Chr, which has antioxidant properties, on oxidative damage, inflammatory damage, and autophagy in kidney tissue in rats with kidney I/R damage were investigated. As a result of our study, although the serum BUN level was found to be quite high in the I/R group, it was observed that the creatinine level was similar in all groups. The lower BUN levels in the groups pretreated with low and medium dose Chr indicate that Chr has a protective effect on kidney functions. Although Xu et al. (26) showed that high-dose (100 mg/kg) Chr administration significantly reduced BUN and creatinine levels, high-dose Chr pretreatment increased BUN levels in our study. While they administered Chr once daily for three days after ischemia, in our study, Chr pretreatment was administered for 14 days before ischemia. Therefore, high-dose Chr may have had a toxic effect due to the longer application period.
It is known that one of the main causes of kidney I/R damage is the increase in the production of free oxygen radicals in the kidney tissue. Increasing oxygen radicals cause lipid peroxidation and thereby tissue damage by disrupting the cell structure. In the present study, it was observed that TAS, GSH and SOD enzyme activities were increased, while TOS and MDA levels were decreased in the groups that received low and medium dose Chr pretreatment. This indicates that Chr pretreatment protects the tissues against oxidative damage by activating antioxidant systems in kidney tissues after I/R. Similar with our results, low and medium doses of Chr treatment have been shown to have a protective effect by significantly reducing oxidative damage in nephrotoxicity and paracetamol-induced kidney damage (14). It was also stated that the Chr dose administered to rats treated with moderate dose Chr for 14 days after ovarian I/R significantly increased the antioxidant system parameters and reduced oxidative damage (27).
ROS, which causes oxidative damage, contributes to inflammation by activating the NF-κB pathway. Stimulated NF-κB travels to the nucleus and triggers the production of cytokines such as TNF-α and IL-1β (28). It has been reported that following cerebral ischemia, Chr suppresses the release of proinflammatory cytokines such as IL-6, IL-1β and TNF-α in the brain tissue (29). In addition, it was stated in a study that Chr administration before kidney I/R injury also reduced IL-6, IL-1β and TNF-α levels in the kidney tissue after I/R (26). The anti-inflammatory effects of Chr against the damage caused by paracetamol in the kidney tissue were also investigated, and it was reported that IL-33, IL-1β and TNF-α levels were significantly decreased in the Chr treated group (14). In agreement with these studies, in our study, it was observed that low and moderate doses of Chr administration before kidney I/R reduced TNF-α, IL-1 α, IL-1β, IL-6. However, it was observed that Chr given in high-doses had a toxic effect on the kidney tissue and caused an image like I/R damage. In a different study showed that IL-10 also

plays a protective role against ischemic and cisplatin-induced kidney damage (30). Studies have shown that decreasing IL-4 levels increase I/R damage in tissues (31, 32). Similarly, in our study, low and medium doses of Chr treatment increased IL-4 and IL-10 levels.

There is also a different argument that permanent autophagy activation can trigger cell death pathways and increase kidney damage. It is a common view that Chr protects tissues from damage through up- or down-regulation of autophagy. The researchers reported that the LC-3II level, in the kidney tissues of rats given 25 and 50 mg/kg Chr was higher than the rats in the cyclophosphamide group. Thus, it has been shown that Chr administration prevents tissue damage in the kidney by inducing autophagy (33). Lee et al. (34) on the other hand, stated that Chr protects kidney tissues against damage by reducing the induction of autophagic genes such as Beclin-1, LC-3II, Atg3 and Atg7 in diabetic kidneys. Additionally, it was shown that Chr treatment could ameliorate testicular damage in cadmium-induced testicular toxicity by reducing the expressions of Beclin-1 and LC-3II, which are key genes of autophagy (35). In a study, it was determined that high-dose paracetamol treatment induced autophagic tissue damage by increasing the LC-3II level in kidney tissue. When the protective effects of Chr against paracetamol toxicity in kidney tissue were examined, it was seen that it significantly improved this damage (14). Similarly, in our study, we have also observed decreased Beclin-1 and LC-3II levels with low and medium dose Chr pretreatment. It was determined that these decreases suppressed autophagy in the kidney tissue. In addition, necrotic changes in the cortical and medullary tubules and inflammatory cells in the interstitial tissue seen in the high-dose Chr group and reduction of infiltration in low and moderate dose groups, suggest that Chr administration protected tissues from damage through the blocking of autophagy.

Our results showed that low and moderate dose of Chr suppresses autophagy in kidney I/R injury and decreased autophagy ensures survival especially of tubular epithelial cells in the kidney tissue during I/R. However, a high-dose of Chr caused an increase in cellular damage by significantly inducing autophagy. Also, low and moderate doses of Chr were found to be an effective method to protect cells against inccreased kidney functions and increased oxidative and inflammatory damage as a result of I/R. However, it was determined that high-dose Chr administration caused damage similar to I/R damage in the kidney tissue. All our results clearly demonstrate the antioxidant, anti-inflammatory and antiautophagic effects of Chr in renal I/R injury. However, our findings need confirmation by additional studies for Chr to be considered clinically useful as a therapeutic agent in kidney injury.

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