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Investigation of the Effects of Chrysin Against Amoxycillin-Clavulanic Acid-Induced Kidney Injury in Rats

Serpil AYGÖRMEZ[1,*] Elif DALKILINÇ[2] Nurhan AKARAS[3] Şaban MARAŞLI[1]

[1] *Kafkas University, Faculty of Veterinary Medicine, Department of Biochemistry, 36100, Kars, Türkiye*
[2] *Ataturk University, Faculty of Veterinary Medicine, Department of Biochemistry, 25010, Erzurum, Türkiye*
[3] *Aksaray University, Faculty of Medicine, Department of Histology and Embryology, 68100, Aksaray, Türkiye*

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ABSTRACT The amoxycillin-clavulanic acid (ACA) used in the treatment of various bacterial infections often causes drug-induced tissue damage, but the mechanism of this damage has not yet been fully elucidated. Chrysin (CHR) is a natural flavonoid with various pharmacological properties as well as antioxidant and anti-inflammatory properties. In this study, the protective effect of CHR against ACA-induced kidney damage, which is frequently used in human and animal health, was investigated. Twenty-eight female rats were divided into four groups as control, CHR, ACA and ACA+CHR. ACA (30 mg/kg) and CHR (50 mg/kg) were administered orally once a day for seven days. Renal function, oxidative stress and inflammation parameters were analyzed to determine renal tissue damage. Histopathologic analysis was also performed to detect tissue damage and structural changes. According to the data obtained from these analyses, ACA increased urea and creatinine levels in kidney tissue. ACA administration also increased malondialdehyde (MDA) and decreased glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) activities and glutathione (GSH). Nuclear factor kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α) and interleukin 1β (IL-1β) expression levels were found to increase. Administration of CHR together with ACA decreased urea, creatinine, MDA, NF-κB, TNF-α, IL-1β levels and increased GSH level and GPx, SOD, CAT activities. When the findings were evaluated together, it was determined that ACA caused renal damage by increasing renal function levels, oxidative stress and inflammation, while supportive treatment of CHR reduced renal damage by bringing these parameters closer to normal.

Keywords: *Amoxycillin-Clavulanic Acid, Chrysin, Oxidative stress, Inflammation, Kidney.*

ÖZ

Sıçanlarda Amoksisilin/Klavulanik Asit ile Oluşturulan Böbrek Hasarına Karşı Krisin'in Etkilerinin Araştırılması

Çeşitli bakteriyel enfeksiyonların tedavisinde kullanılan amoksisilin-klavulanik asit (ACA), çoğunlukla ilaç kaynaklı doku hasarına neden olmaktadır fakat bu hasarın mekanizması henüz tam olarak aydınlatılmamıştır. Krisin (CHR), antioksidan ve antiinflamatuar gibi özelliklerinin yanı sıra çeşitli farmakolojik özellikleri bulunan doğal bir flavonoiddir. Bu çalışmada, insan ve hayvan sağlığında sıklıkla kullanılan ACA'nın neden olduğu böbrek hasarına karşı CHR'nin koruyucu etkisi araştırıldı. Yirmi sekiz adet dişi sıçan kontrol, CHR, ACA ve ACA+CHR olmak üzere dört grup ayrıldı. ACA (30 mg/kg) ve CHR (50 mg/kg) yedi gün boyunca günde bir kez oral yoldan uygulandı. Böbrek dokusunda hasarı belirlemek için böbrek fonksiyonu, oksidatif stres ve inflamasyon parametreleri analiz edildi. Doku hasarı ve yapısal değişiklikleri tespit etmek için histopatolojik analiz yapıldı. Bu analizler sonucunda elde edilen verilere göre ACA, böbrek dokusunda üre ve kreatin düzeylerini artırdı. ACA uygulamasının aynı zamanda malondialdehit (MDA)'i arttırdığı, glutatyon peroksidaz (GPx), süperoksit dismutaz (SOD), katalaz (CAT) aktivitelerini ile glutatyon (GSH)'u azalttığı bulundu. Nuclear factor kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α) ve interleukin 1β (IL-1β) ekspresyon düzeylerini artırdığı tespit edildi. CHR'nin ACA ile birlikte uygulanmasının üre, kreatin, MDA, NF-κB, TNF-α, IL-1β düzeylerini azalttığı, GSH düzeyi ile GPx, SOD, CAT aktivitelerini artırdığı belirlendi. Elde edilen bulgular birlikte değerlendirildiğinde, ACA'nın böbrek fonksiyon düzeylerini, oksidatif stresi ve inflamasyonu artırarak böbrek hasarına neden olduğu, CHR'nin destekleyici tedavisinin ise bu parametreleri normale yakınlaştırarak böbrekte hasarı azalttığı tespit edildi.

Anahtar Kelimeler: *Amoksisilin/Klavulanik Asit, Krisin, Oksidatif stres, İnflamasyon, Böbrek.*

✉ *Corresponding author: serpilaygormez@hotmail.com

INTRODUCTION

Kidneys play a role in essential functions in the organism, such as detoxification of toxic substances and excretion of some drugs. Therefore, they can be exposed to many drugs or harmful substances, including antibiotics, through blood flow (Abouzed et al. 2021; Daoudi et al. 2025). Antibiotics, which are widely used in the treatment of bacterial infections, may cause kidney damage by causing the formation of free oxygen radicals due to their side effects (Badr et al. 2025; Daoudi et al. 2025). Although the exact cause of Amoxycillin-clavulanic acid (ACA)-induced tissue damage has not yet been proven, it is thought that oxidative stress plays an important role in the pathogenesis of drug-induced tissue damage and its induction is associated with tissue damage (El-Hosseiny et al. 2016; Jamshidi and Negintaji 2021). When the endogenous antioxidant system is depleted in the organism, reactive oxygen species (ROS)-mediated oxidative stress develops and tissue damage occurs, causing the onset of adverse outcomes (El-Emam et al. 2023). Interest in plant-derived compounds that can be used in the treatment of many diseases with their anti-inflammatory and antioxidant properties is increasing in research (Kankılıç et al. 2024a).

Flavonoids, which are effective in various health problems and abundant in fruits and vegetables to prevent diseases caused by oxidative stress, have been reported to be effective against multidrug resistance with their antioxidant effect (Temel et al. 2021; Öztürk et al. 2025). Chrysin (5,7-dihydroxyflavone) (CHR), found in honey, many plants and propolis, is a natural flavonoid used for therapeutic purposes (Aksu et al. 2018; Küçükler et al. 2022). It has been stated that CHR, which has no side effects, has many pharmacological effects as well as antioxidant and anti-inflammatory effects (Çelik et al. 2020; Akaras et al. 2023a). Thanks to these properties, it is effective in reducing or preventing many tissue damages (Şimşek et al. 2023; Varışlı et al. 2023).

In the present study, we aimed to investigate the effects of CHR on kidney damage caused by ACA, which is frequently used in human and animal health treatment.

MATERIAL AND METHODS

Chemicals

ACA (Amoclavin®-BID, Tablet, 1000 mg, Tekirdağ) and CHR (Sigma, Cas No: 480-40-0, 97% purity) were commercially available.

Groups and Experimental Procedures

In this study, 28 female *Sprague Dawley* rats (220-250 g) were obtained from Atatürk University Experimental Research and Application Center (ATADEM) (Erzurum/Turkey). Ethical approval was obtained from Atatürk University Animal Experiments Local Ethics Committee (Approval No: 2025/01/06, Date: 30.01.2025). Rats were housed in cages in an environment with a temperature of 24-25 ˚C and 12 h dark-light cycle. The rats were randomly divided into four groups (n=7).

Control: Saline was given orally for seven days.

Chrysin (CHR): 50 mg/kg CHR was given orally for seven days (Kankılıç et al. 2024a).

Amoxycillin-Clavulanic Acid (ACA): 30 mg/kg ACA was given orally for seven days (Mohammed et al. 2024).

Amoxycillin-Clavulanic Acid+Chrysin (ACA+CHR): Animals were given ACA (30 mg/kg) orally, followed by CHR (50 mg/kg) half an hour later. Treatment continued for seven days.

One day after the last ACA and CHR administration, blood samples were collected from the jugular vein under mild sevoflurane (Sevorane®; Queenborough, UK) anesthesia, followed by kidney tissues. Blood samples were collected in 5 mL vacuum gel tubes for urea and creatinine analyses. Serum was obtained by centrifugation at 3000 rpm for 10 minutes. One kidney tissue and serum sample was stored at -80 °C for biochemical analyses, while the other kidney tissue sample was stored in 10% formaldehyde solution for histological examination.

Kidney Function Analyses

Serum creatinine, urea levels were analyzed using commercial kits (Diasys Diagnostic Systems, Istanbul, Turkey) to evaluate renal function.

MDA and GSH Analyzes

Total protein content of kidney tissue was determined according to the method of Lowry et al. (1951). Kidney tissue, malondialdehyde (MDA) and glutathione (GSH) levels were homogenized with 1.15% potassium chloride (KCl) and the supernatant was obtained by centrifugation. The absorbance at 532 nm of the color formed by thiobarbituric acid reaction was measured to determine the level of MDA in kidney tissue (Placer et al. 1966). GSH level was analyzed according to the method of Sedlak and Lindsay (1968).

Antioxidant Enzyme Analysis

Kidney tissue was homogenized with 1.15% KCl for superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) activities and the supernatant was obtained by centrifugation. SOD activity was analyzed according to Sun et al. (1988), CAT activity according to Aebi (1984), and GPx activity according to Lawrence and Burk (1976).

RNA extraction and real-time polymerase chain reaction (RT-PCR) analysis

According to the manufacturer's guidelines, total RNA was extracted from kidey tissue using QIAzol Lysis Reagent (Qiagen, Germany). The concentration and purity of the RNA samples were evaluated using a NanoDrop® spectrophotometer (BioTek Epoch). cDNA was synthesized from 2 µg of total RNA using the Qiagen High-Capacity cDNA Kit (Thermoscientific). RT-PCR was conducted using the Power SYBR Green Master Mix PCR kit (Qiagen) on the Rotor-Gene Q 5plex HRM platform (Qiagen, Germany). The mRNA levels of NF-κB, TNF-α, and IL-1β in the kidney tissues were analyzed in triplicate using gene-specific primers (Table 1). β-actin was used as reference gene. The relative gene expression levels were determined from the Ct value and calculated using the $2^{-\Delta\Delta Ct}$ method Livak ve Schmittgen, (2001).

Histopathological Analysis

Kidney tissue obtained at the end of the experiment were kept in 10% formalin solution for 48 hours for fixative purposes. After fixation, the tissues were washed under tap water overnight and dehydrated in increasing levels of ethanol. They were then clarified with xylene and embedded in paraffin wax. From the paraffin blocks obtained, 5 µm sections were taken using a microtome. Kidney tissues were stained with hematoxylin and eosin (H&E) for light microscopy (Olympus Cx43; Japan).

The resulting slides were evaluated using a blinded method and semi-quantitative scoring was performed. The criteria included inflammatory cell infiltration, vascular congestion and glomerular atrophy. The scoring was as follows: 0 = no lesions, 1 = mild lesions (<30%), 2 = moderate lesions (30-50%) and 3 = severe lesions (>50%).

Statistical Analysis

The data obtained at the end of the study were statistically analyzed using SPSS 26.0 software. Data were presented as mean ± standard error (SEM). Tukey post hoc tests and one-way analysis of variance (ANOVA) were applied for multiple comparisons. Statistical significance was determined at $p<0.05$ level.

Table 1. Primer sequences of genes analyzed in RT-PCR. (RT-PCR: Real-time polymerase chain reaction, NF-κB: Nuclear factor kappa B, TNF-α: Tumor necrosis factor-alpha, IL-1β: İnterleukin 1β).

Gene	Accession Number	Primers	Product Size (bp)
β-actin	NM_031144.3	**F:** GGAGATTACTGCCCTGGCTCCTAGC **R:** GGCCGGACTCATCGTACTCCTGCTT	155
NF-κB	NM_001415012.1	**F:** CAGCACTCCTTATCAACCACC **R:** CTCCTGAGCGTTGACTTCTG	125
TNF-α	NM_012675.3	**F:** ATGGGCTCCCTCTCATCAGT **R:** GCTTGGTGGTTTGCTACGAC	106
IL-1β	NM_031512.2	**F:** AGCTCTCCACCTCAATGGAC **R:** TTGTTTGGGATCCACACTCTCC	187

RESULTS

Effects of ACA and CHR on Markers of Renal Function

The effects of ACA and CHR on the kidney tissue were examined and the findings are given in Figure 1. Urea (Figure 1A) and creatinine (Figure 1B) levels were evaluated to determine the markers of renal function in kidney. According to the data obtained, urea, creatinine levels in the ACA group increased compared to the control and CHR groups ($p<0.05$) and ACA+CHR treatment was found to be effective in bringing these markers closer to normal levels.

Effects of ACA and CHR on Oxidative Stress Levels in Kidney Tissue

MDA (Figure 2A), GSH (Figure 2B) levels and SOD (Figure 2C), CAT (Figure 2D) and GPx (Figure 2E) activities were evaluated to determine oxidative stress in kidney tissue. It was found that MDA level increased in ACA group compared to control and CHR groups ($p<0.05$), while GSH level and SOD, GPx, CAT activities decreased ($p<0.05$). It was determined that CHR supportive treatment with ACA decreased MDA level and increased CAT, SOD, GPx activities and GSH and strengthened the antioxidant defense system in kidney tissue.

Effects of ACA and CHR on NF-κB, TNF-α and IL-1β Levels in Kidney Tissue

The effects of ACA on the kidney of rats were examined and the findings are shown in Figure 3. TNF-α (Figure 3A), IL-1β (Figure 3B) and NF-κB (Figure 3C) levels were evaluated to determine the level of inflammation in kidney tissue. According to the data obtained, TNF-α, NF-κB, IL-1β levels increased in ACA group compared to control and CHR groups ($p<0.05$) and ACA+CHR treatment was found to be effective in bringing these markers closer to normal levels.

Histopathologic Results

Hematoxylin and eosin (H&E) staining results of kidney tissue are shown in Figure 4. No histologic changes were observed in the control and CHR treated groups. In the ACA-treated group, atrophic glomeruli and enlarged bowman spaces were observed. In the tubules, there were degenerative and pyknotic changes in epithelial cells. Vascular congestion, hemorrhage and increased inflammatory cells were also observed in interstitial areas. When the images of rats treated with CHR simultaneously with ACA were examined, there were more regular renal bodies and tubules. There was less vascular congestion and inflammatory cells in the interstitial area. According to the histopathologic score results, inflammatory cell infiltration, vascular congestion and glomerular atrophy were significantly increased in the ACA group compared to the control group, whereas they were significantly decreased in the group treated with ACA with CHR ($p<0.05$).

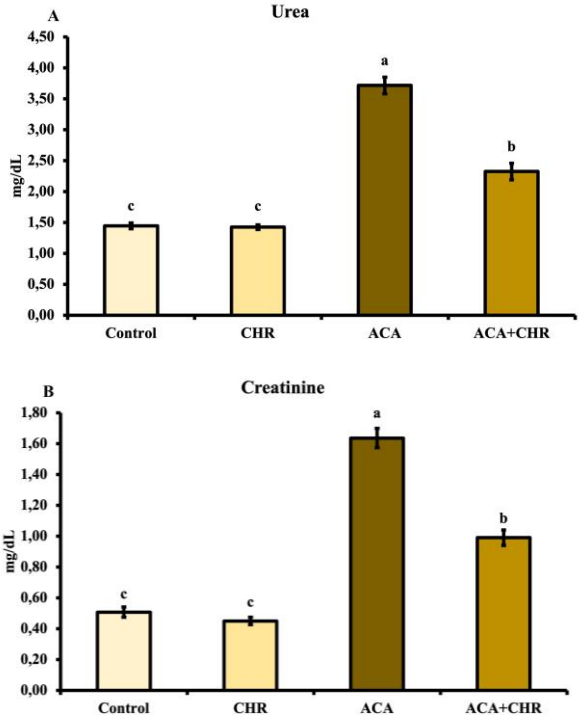

Figure 1: Effect of ACA and CHR administration on urea and creatinine levels in kidney tissue.

Each group values are given as mean ± SEM. Different letters in the columns (a-b-c) indicate the difference in the groups ($p<0.05$). (ACA: Amoxycillin-Clavulanic Acid, CHR: Chrysin).

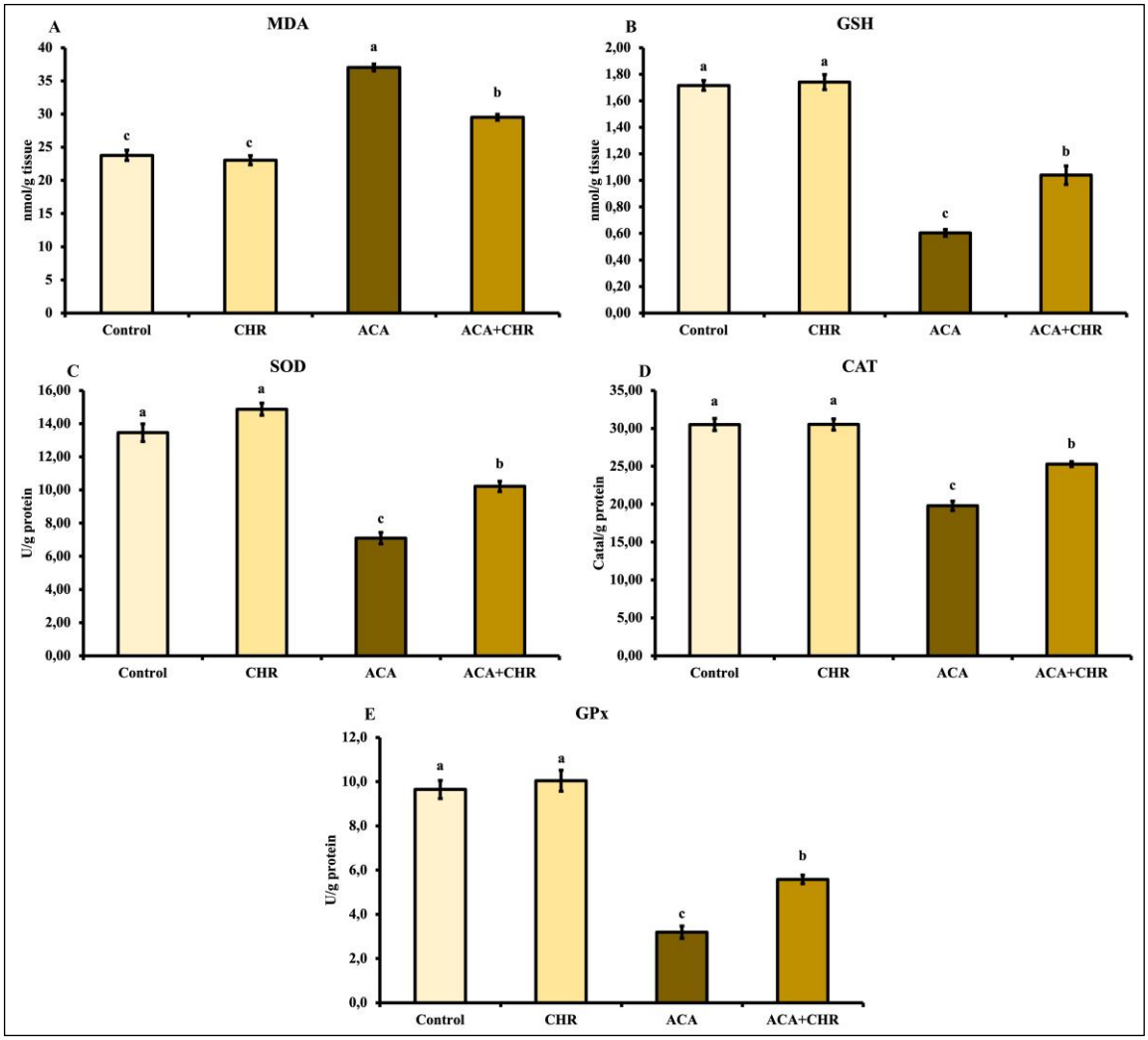

Figure 2: Effect of ACA and CHR administration on MDA and GSH levels and SOD, CAT, GPx activities in kidney tissue.

Each group values are given as mean ± SEM. Different letters in the columns (a-b-c) indicate the difference in the groups ($p<0.05$). (ACA: Amoxycillin-Clavulanic Acid, CHR: Chrysin, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, GSH: Glutathione).

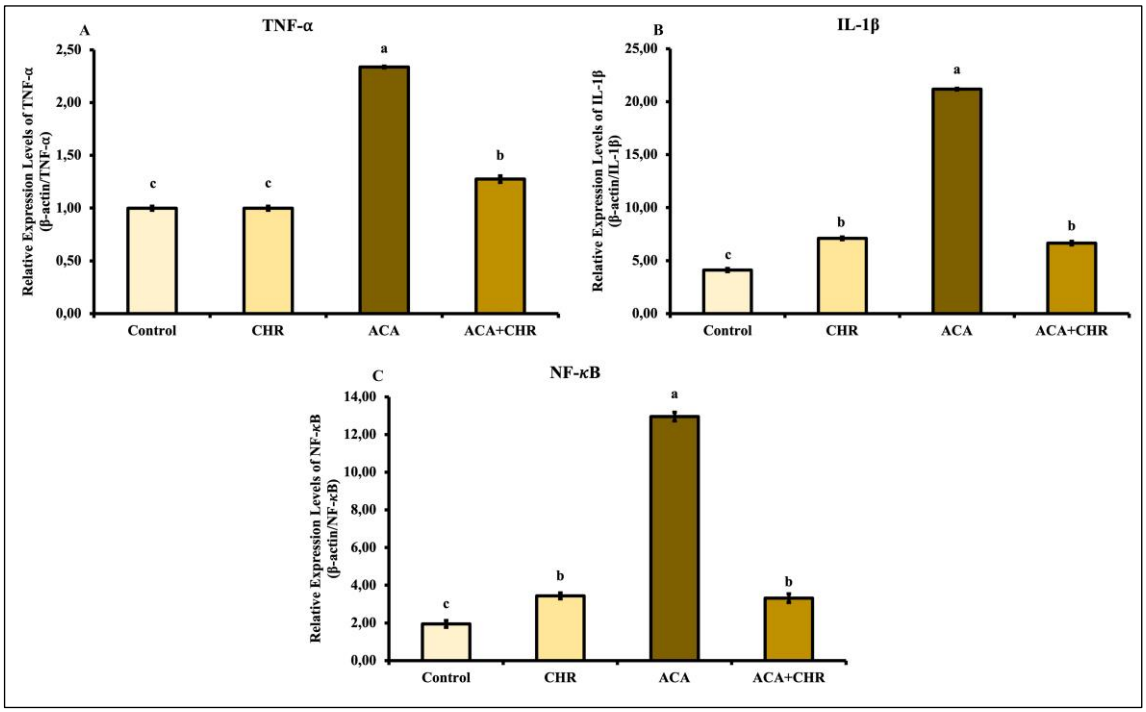

Figure 3: Effect of ACA and CHR administration on NF-κB, TNF-α and IL-1β levels in kidney tissue.

Each group values are given as mean ± SEM. Different letters in the columns (a-b-c) indicate the difference in the groups ($p<0.05$). (ACA: Amoxycillin-Clavulanic Acid, CHR: Chrysin, TNF-α: Tumor necrosis factor-alpha, IL-1β: Interleukin 1β, NF-κB: Nuclear factor kappa B).

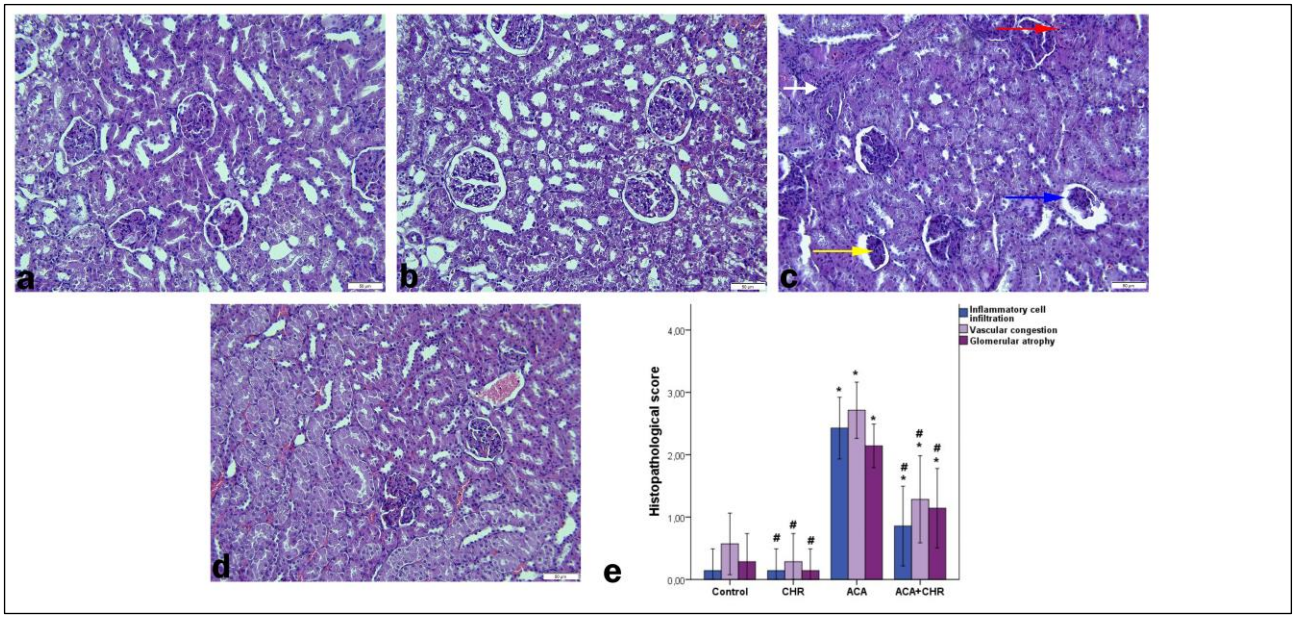

Figure 4. Histopathologic changes in kidney tissues of ACA and CHR treated rats (H&E staining, 200x).

Control (a) and CHR (b) group showing normal architecture of renal tissues, ACA (c) group showing atrophic glomeruli (yellow arrow), enlarged Bowman's spaces (blue arrow), inflammatory cell infiltration (white arrow), vascular congestion (red arrow), ACA+CHR (d) group showing histologic structure similar to control group and histopathologic score (e) graph.

DISCUSSION AND CONCLUSION

ACA, a broad-spectrum antibiotic, is used in the treatment of bacterial infections and is known to cause tissue damage. This damage is thought to be related to the formation of oxidative stress as a result of increased ROS production and activation of damage pathways such as inflammation triggered by oxidative stress. Therefore, in the present study, we investigated the effects of CHR, which has no side effects, on ACA-induced kidney damage.

Kidney function has an important role in ensuring the overall homeostasis of the organism (Bencheikh et al. 2021). Urea and creatinine, which are important indicators of the structural integrity and function of the kidneys, are the end products of metabolism and are eliminated from the body through the kidneys (Shehata et al. 2022, Gur and Kandemir 2023). In this study, it was found that ACA administration to rats increased urea and creatinine levels in renal tissue and caused damage to the renal tissue as a result. This suggests that the change in pharmacokinetic effect of ACA, along with the changes in urea and creatinine function parameters, may cause damage. In previous studies, it was stated that antibiotic administration caused damage by increasing creatinine and urea levels in kidney tissue as a result of decreased glomerular filtration rate (Babaeenezhad et al. 2024, Abukhalil et al. 2025). Coadministration of CHR, which has no side effects, with ACA was found to reduce the increased creatinine and urea levels in kidney tissue. These results suggest that CHR improves renal function directly or indirectly through its antioxidant effect. Studies show that CHR reduces kidney damage as a result of increasing antioxidant activities of kidney tissue against different toxic agents (Kucukler et al. 2021, Şimşek et al. 2023).

Oxidative stress occurs as a result of excessive ROS production in cells and tissues (Jamshidi and Negintaji 2021, Küçükler et al. 2024a). MDA, the most important indicator of oxidative stress, is the end product of polyunsaturated fatty acids (Aydin et al. 2009, Akarsu et al. 2023). SOD, which converts superoxide into hydrogen peroxide (H_2O_2), CAT, which catalyzes oxygen and water, and GPx, which neutralizes it, are antioxidant enzymes of oxidative stress (Akaras et al. 2023b, Gur et al. 2023). GSH neutralizes free radicals (Cakmak et al. 2023, Küçükler et al. 2024b). Oxidative stress increases as a result of decreased antioxidant activity in the organism and increased lipid peroxidation (Kankılıç et al. 2024b, Şimşek et al. 2024, Tuncer et al. 2023). In the present study, it was determined that MDA increased in ACA-induced damage in kidney tissue in rats, oxidative stress developed as a result of decreased GSH and SOD, CAT, GPx enzyme activities, and as a result, tissue damage was caused. The results suggest that ACA causes oxidative stress by producing free radicals and ROS at the cellular level. In different studies on the subject, it has been stated that antibiotics cause kidney damage and oxidative stress due to ROS production produced by drug metabolites (Elgendy et al. 2024, Ekinci Akdemir et al. 2025). Co-administration of CHR with ACA was found to regulate oxidative stress markers in kidney tissue. CHR, a natural flavonoid, is thought to protect kidney tissue by improving antioxidant status and reducing free radical damage. Studies have shown that CHR has the ability to reduce lipid peroxidation and increase antioxidant activities against different toxic agents and prevent kidney tissue damage (Şimşek et al. 2023, Kankılıç et al. 2024a).

Inflammation is significantly triggered by oxidative stress (Yıldız et al. 2022, Yilmaz et al. 2024a). Activation of NF-κB stimulates proinflammatory cytokines (Ileriturk et al. 2022, Yardim et al. 2022, Yilmaz et al. 2024b). Molecules involved in the initiation of acute phase reactions are proinflammatory cytokines such as IL-1β, TNF-α (Akaras et al. 2024, Akarsu et al. 2024). In the present study, it was found that ACA administration increased the mRNA expression levels of NF-κB, TNF-α and IL-1β cytokines, which play an important role in inflammation in the kidney tissue of rats, and as a result, caused damage to the kidney tissue. The results suggest that ACA causes oxidative stress and inflammation by increasing NF-κB and other inflammation parameters. It has been reported that antibiotic administration increases proinflammatory

cytokines in kidney tissue, resulting in kidney damage and dysfunction (Hassanein et al. 2021, Wu et al. 2021, Ayusso et al. 2024). In the present study, co-administration of CHR, which has no side effects, with ACA was found to alleviate inflammatory damage by showing anti-inflammatory effect by reducing the increased IL-1β, NF-κB, TNF-α mRNA expression levels in kidney. These results support that CHR has anti-inflammatory effects by reducing proinflammatory cytokine expression. Studies have reported the therapeutic potential of CHR in reducing inflammation and alleviating histopathological changes (Şimşek et al. 2023, Kankılıç et al. 2024a).

In conclusion, ACA interfered with the damage pathways in renal tissue and caused alterations in markers of oxidative stress, renal function, inflammation, and tissue structural and functional changes. CHR, a natural flavonoid, showed anti-inflammatory and antioxidant effects on ACA-induced kidney injury. These effects suggest that CHR may be considered as a potential therapeutic agent due to its ability to protect the structural integrity of kidney tissue.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: SA, ED, NA, ŞM
Supervision / Consultancy: SA, ED, NA, ŞM
Data Collection and / or Processing: SA, ED, NA
Analysis and / or Interpretation: SA, ED, NA, ŞM
Writing the Article: SA, ED, NA
Critical Review: SA, ED, NA, ŞM

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