Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Yavuz H, Kandemir Ö, Şimşek H, Akaras N, Kandemir FM (2026).** Investigation of the Effects of Carvacrol on Oxytetracycline-Induced Kidney Damage. *Van Vet J*, 37(1), 66-73. DOI: https://doi.org/10.36483/vanvetj.1819779

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Investigation of the Effects of Carvacrol on Oxytetracycline-Induced Kidney Damage

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Received: 07.11.2025 Accepted: 14.01.2026

ABSTRACT The main objective of this study was to assess the antioxidant capacity and the protective impact of carvacrol (CRV) against oxytetracycline (OXI)-mediated nephrotoxicity in albino rats. CRV, a naturally occurring phenolic compound found in various plant species, has significant antioxidant capacity, the ability to mitigate inflammatory processes, and tumor proliferation inhibiting properties. The study evaluated the efficacy of CRV in reducing oxidative stress-induced kidney damage in albino rats. Findings showed that CRV administration significantly reduced the kidney damage relative to the control group. Evaluation of the enzymatic antioxidant profile indicated a significant decrease in the activities of catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) enzymes and GSH levels in the OXI-treated group compared to the CRV group. Furthermore, CRV treatment decreased malondialdehyde (MDA), a key biomarker of lipid peroxidation. The protective properties of CRV were confirmed by histological examination of the kidney. CRV treatment markedly suppressed pro-inflammatory molecules, specifically diminishing the expression of interleukin-1 beta (IL-1β) and mitigating nuclear factor kappa-B (NF-κB) activation. Furthermore, CRV administration attenuated OXI-induced increases in the proapoptotic proteins Bax and Caspase-3, while increasing the transcriptional activity of the antiapoptotic gene B-cell lymphoma-2 (Bcl-2). CRV diminished OXI-induced kidney tissue damage by decreasing Kidney Injury Molecule-1 (KIM-1) and increasing Aquaporin-2 (AQP-2) levels. The study results indicate that CRV administration can alleviate tissue damage in the kidney caused by OXI toxicity.

Keywords: *Albino rat, Antioxidant activity, Carvacrol, Kidney function, Lipid peroxidation, Oxytetracycline.*

ÖZ

Oksitetrasiklin Kaynaklı Böbrek Hasarı Üzerine Karvakrolün Etkilerinin Araştırılması

Bu çalışmanın amacı, albino sıçanlarda oksitetrasiklin (OXI) kaynaklı nefrotoksisiteye karşı karvakrolün (CRV) antioksidan potansiyelini ve koruyucu rolünü araştırmaktır. Çeşitli bitki türlerinde doğal olarak bulunan fenolik bir bileşik olan CRV'nin önemli antioksidan, anti-inflamatuar ve antikanser özellikleri vardır. Çalışmada, albino sıçanlarda oksidatif stres kaynaklı böbrek hasarını azaltmada CRV'nin etkinliği değerlendirilmiştir. Bulgular, CRV uygulamasının böbrek hasarını kontrol grubuna kıyasla önemli ölçüde azalttığını göstermiştir. OXI uygulanan grupta, CRV uygulanan gruba kıyasla süperoksit dismutaz (SOD), glutatyon peroksidaz (GPx) ile katalaz (CAT) da dâhil antioksidan etkinliği olan enzimlerin aktivitelerinde ve GSH seviyelerinde önemli bir azalma gözlenmiştir. Ayrıca, CRV tedavisi, lipid peroksidasyonunun önemli bir biyobelirteci olan malondialdehit (MDA) düzeyini düşürmüştür. CRV'nin koruyucu özellikleri, böbreğin histolojik incelemesiyle doğrulanmıştır. CRV tedavisi, nükleer faktör kappa-B (NF-κB) ile interlökin-1 beta (IL-1β) gibi inflamasyon medyatörleri önemli ölçüde azalttı. Ayrıca, CRV uygulaması, proapoptotik proteinler Bax ve Kaspaz-3'te OXI kaynaklı artışları azaltırken, antiapoptotik belirteç B hücreli lenfoma-2 (Bcl-2) ekspresyonunu artırdı. CRV, Böbrek Hasarı Molekülü-1'i (KIM-1) azaltarak ve Aquaporin-2 (AQP-2) seviyelerini artırarak OXI kaynaklı böbrek doku hasarını azalttı. Çalışma sonuçları, CRV uygulamasının OXI toksisitesinden kaynaklanan böbrek doku hasarını hafifletebileceğini göstermektedir.

Anahtar Kelimeler: *Albino sıçan, Antioksidan aktivite, Böbrek fonksiyonu, Karvakrol, Lipit peroksidasyonu, Oksitetrasiklin.*

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INTRODUCTION

Oxytetracycline (OXI) is a broad-spectrum antibiotic widely used to control bacterial infections in humans and animals. Use of high doses of OXI without medical supervision has harmful effects on kidney and liver tissues (Abdel-Daim and Ghazy 2015).

OXI disrupts mitochondrial β-oxidation, consequently elevating the generation of free radicals and reactive oxygen species (ROS) (Gibson 2005). These harmful molecules, in turn, interact with cell membranes, causing lipid peroxidation and subsequent structural damage (Skakun and Vysotski 1982). Additionally, the decrease in tissue antioxidant biomarkers is associated with OXI overdose. A wide variety of antioxidants with different structures have been used against these side effects of OXI and their effects have been investigated.

Carvacrol (CRV) is found in the essential oils of aromatic plants. Besides, CRV (5-isopropyl-2-methylphenol) is a liquid monoterpenoid compound that is soluble in ethanol but insoluble in water. Its density at 20°C is approximately 0.976 g/mL, and its boiling point is between 236 and 237 °C (Mączka et al. 2023). The hydroxyl group (OH^-) in CRV's structure is what gives it its antioxidant qualities and its ability to scavenge radicals including hydrogen peroxide, nitric oxide, and superoxide radicals (Imran et al. 2022).

CRV has antimicrobial biological activity (Mączka et al. 2023). Its anti-inflammatory activity is primarily mediated through suppression of cyclooxygenase-2 (COX-2) expression and reduction of IL-1β levels (Akcılar et al. 2015).

There are also important studies on the anticancer properties of CRV (Ahmad et al. 2021; Bouhtit et al. 2021; Sampaio et al. 2021; Bansal et al. 2022; Gunes-Bayir et al. 2022; Tomsuk et al. 2024; Sathi-Devi et al. 2025; Yousef et al. 2025). CRV is documented to possess diverse biological properties, comprising anticancer, antimutagenic, inflammation-suppressing, acetylcholinesterase (AChE) inhibitory, analgesic, antioxidant, antihepatotoxic, antiparasitic, insecticidal, and antibacterial properties (Nostro and Papalia 2012).

The current research was designed to elucidate the protective efficacy of CRV against renal tissue injury induced by the commonly administered antibiotic OXI, employing a comprehensive suite of molecular, biochemical, and histopathological assessments.

MATERIAL AND METHODS

Research and Publication Ethics

This study was conducted with the permission of Konya Necmettin Erbakan University Animal Experiments Local Ethics Committee on 20.02.2025 with the number 2025-011.

Chemicals

Pioxy LA Injection Solution 100 ml (liter contains oxytetracycline dihydrate at a concentration corresponding to 200 mg of oxytetracycline base in each milliliter) by Pi Farma (Ankara, Türkiye) was purchased. CRV (CAS No: 499-75-2, with a purity of 98%) was purchased from Sigma-Aldrich.

The OXI dose was determined using the studies reported by Marza et al. (2020) and Oda et al. (2018). The study published by Şimşek et al. (2024) was used to determine the CRV dose.

Experimental design and animals

This study involved 28 albino rats of the Wistar, aged 10-12 weeks, each with a mean body weight of 235±15 g. All animals were maintained under standardized environmental conditions, including a steady temperature of 24-25 °C and a controlled 12-hour light-dark cycle (darkness commencing at 19:00 h). They had access to unlimited amounts of water and standard chow. After a week of resting in their cages, the rats were acclimated to their new surroundings, and the studies started. All animal procedures were carried out at the KONÜDAM Experimental Medicine Application and Research Center. A total of 28 rats were randomly divided into four experimental groups (n=7 per group). Literature was used to determine the dose of the active ingredients (Oda et al. 2018; Marza et al. 2020).

1. Control group (C): For seven days, 1 mL of physiological saline was administered intraperitoneally once daily.

2. Carvacrol group (CRV): For seven days, the treatment regimen involved daily oral delivery of CRV at 50 mg/kg by gavage.

3. Oxytetracycline group (OXI): OXI was delivered via a seven-day regimen of once-daily intraperitoneal injections at a fixed dose of 200 mg/kg.

4. Oxytetracycline+Carvacrol (OXI+CRV): Animals received OXI via daily intraperitoneal injection at a fixed dose of 200 mg/kg for 7 days, and at the same time 50 mg/kg of CRV was given orally by gavage once daily.

Tissue and Blood Collection

The rats were decapitated under light sevoflurane (Sevorane®; Queenborough, UK) anesthesia, which was conducted 24 hours after the final dose of CRV (on Day 8 of the protocol), and samples of kidney tissue and blood were taken. Kidney tissues were kept at -20 °C until they were subjected to molecular and biochemical investigations. Blood samples were transferred to vacuum tubes without anticoagulant and then centrifuged at 3000 rpm for 10 minutes at 4 °C. The separated serum was kept at -20 °C in a deep freezer until it was subjected to biochemical analysis.

Biochemical analysis

Kidney Function Tests

Renal function was evaluated by determining serum urea and creatinine concentrations, following the instructions provided by the commercial assay kit.

Determination of lipid peroxidation

Lipid peroxidation levels in the kidney tissues were determined via spectrophotometry. This method involves measuring the absorbance at 532 nm resulting from the chromogen formed by the reaction between MDA and thiobarbituric acid (TBA). Kidney samples were analyzed using a homogenizer. A potassium chloride (KCl) solution containing 1.15% was used to homogenize the tissues. Following homogenization, the resulting tissue suspensions underwent centrifugation at a force of 1,000 x g for 15 minutes at 4 °C. Centrifugation was followed by the use of the supernatant. MDA levels were calculated using the Placer et al. (1966) method.

Determination of Antioxidant Activity

Renal antioxidant capacity was assessed through the determination of the activities of the antioxidant enzymes CAT, GPx, and SOD using commercial ELISA kits. Supernatants were obtained using the same method used for lipid peroxidation to evaluate CAT, GPx, with SOD activities and GSH concentrations. The enzymatic activity of catalase (CAT) was measured following the established spectrophotometric procedure by Aebi (1984), SOD activity using the Sun et al. (1988) method, and GPx activity performing the method established by Lawrence and Burk (1976). GSH levels were also determined using the Sedlak and Lindsay (1968) method. The protein concentration of renal samples, needed for calculating enzyme assays, was performed using the Lowry et al. (1951) protocol.

Real Time PCR (RT-PCR)

At the conclusion of the research, mRNA expression grades of the genes were evaluated in kidney tissues using the RT-PCR technique (Table 1). RNA isolation was performed using commercially available QIAzol Lysis Reagent (Qiagen, 79306). Isolated total RNA was converted to cDNA with OneScript Plus cDNA Synthesis Kit (ABM, G236, Richmond, Canada). Then, the PCR mixture was prepared by adding BlasTaq™ 2X qPCR MasterMix (ABM, G891, Richmond, Canada) with primer sequences, and the reaction started. The procedures were carried out in appropriate temperature cycles in the Rotor-Gene Q (Qiagen) instrument according to the protocol specified by the producer.

Gene expressions obtained from the analysis were standardized with the β-Actin reference gene and assessed via the 2-ΔΔCT method (Livak and Schmittgen 2001).

Table 1: Primer sequences of genes analyzed in RT-PCR.

Gene	Sequences (5'-3')	(bp)	Accession No
NF-κB	F: AGTCCCGCCCCTTCTAAAAC R: CAATGGCCTCTGTGTAGCCC	106	NM_001276711.1
IL-1β	F: ATGGCAACTGTCCCTGAACT R: AGTGACACTGCCTTCCTGAA	197	NM_031512.2
Caspase-3	F: ACTGGAATGTCAGCTCGCAA R: GCAGTAGTCGCCTCTGAAGA	270	NM_012922.2
Bax	F: TTTCATCCAGGATCGAGCAG R: AATCATCCTCTGCAGCTCCA	154	NM_017059.2
Bcl-2	F: GACTTTGCAGAGATGTCCAG R: TCAGGTACTCAGTCATCCAC	214	NM_016993.2
KIM1	F: AGCACATTCTCCAGGAAGCC R: AGGCCAGCCCTCTAATGGTA	298	NM_173149.2
AQP2	F: AGCTGCCTTCTATGTGGCT R: GCGTTGTTGTGGAGAGCATT	120	NM_012909.2
β-Actin	F: CAGCCTTCCTTCTTGGGTATG R: AGCTCAGTAACAGTCCGCCT	360	NM_031144.3

Histopathological Examination for kidney

At the conclusion of the study, the rat kidney samples were immersed in 10% neutral-buffered formaldehyde solution for 2 days to ensure complete fixation. The tissues were submerged overnight to remove formalin. They were then dehydrated through ascending alcohols and then cleared in xylene. Following paraffin embedding, the samples were cut into 5 μm slices utilizing a microtome device. The obtained tissue sections were subjected to Hematoxylin-Eosin (H&E) staining for the observation of tissue morphology. The stained tissue sections were visualized and analyzed utilizing a binocular Olympus CX43 light microscope (Olympus Inc., Tokyo, Japan). They were subsequently captured using an EP50 digital camera.

Statistical Analysis

Statistical analysis of the dataset generated in this investigation was conducted utilizing IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Shapiro-Wilk and Kolmogorov-Smirnov normality tests and QQ plots were applied to determine the distributional properties of continuous variables. When the necessary assumptions for the parametric test were met, intergroup differences were assessed through a one-way analysis of variance (ANOVA). When significant differences were identified, subsequent pairwise comparisons were conducted using the Tukey post-hoc test. In the statistical analysis, the value ($p<0.05$) was considered significant.

RESULTS

Identification of creatinine and urea concentrations in OXI and CRV groups

To evaluate renal function, serum concentrations of urea and creatinine were quantified. These values are known as key biochemical indicators of kidney health. A significant increase in these parameters was observed in the OXI group compared to the control group ($p<0.05$). Rats that received CRV therapy had much lower concentrations of serum creatinine and urea compared to rats that only received OXI ($p<0.05$). However, CRV only slightly changed creatinine, a marker of kidney function, as seen in Figure 1.

Identification of antioxidant enzymes levels in the OXI and CRV groups

Kidney SOD, CAT and GPx activities were measured to evaluate oxidative stress. The effects of OXI and CRV on antioxidant enzyme activity are shown in Figure 2. CRV administration resulted in increased SOD, CAT, and GPx activities and increased GSH levels, while OXI treatment resulted in a reduction in these parameters. The status of lipid peroxidation, as indicated by the malondialdehyde (MDA) concentration, was measured, and an elevated level of MDA was detected in the OXI-treated group, compared to all the other experimental groups (Figure 2).

Identification of mRNA transcription levels in the OXI and CRV groups

The effects of OXI and CRV on mRNA transcription levels are shown in Figure 3. Transcriptional measures of pro-inflammatory mediators, including IL-1β and NF-κB, were assessed. OXI treatment resulted in a significant increase in NF-κB and IL-1β expression compared to the control group ($p<0.05$). However, CRV administration significantly attenuated the OXI-induced elevation in these proinflammatory markers ($p<0.05$, Figure 3).

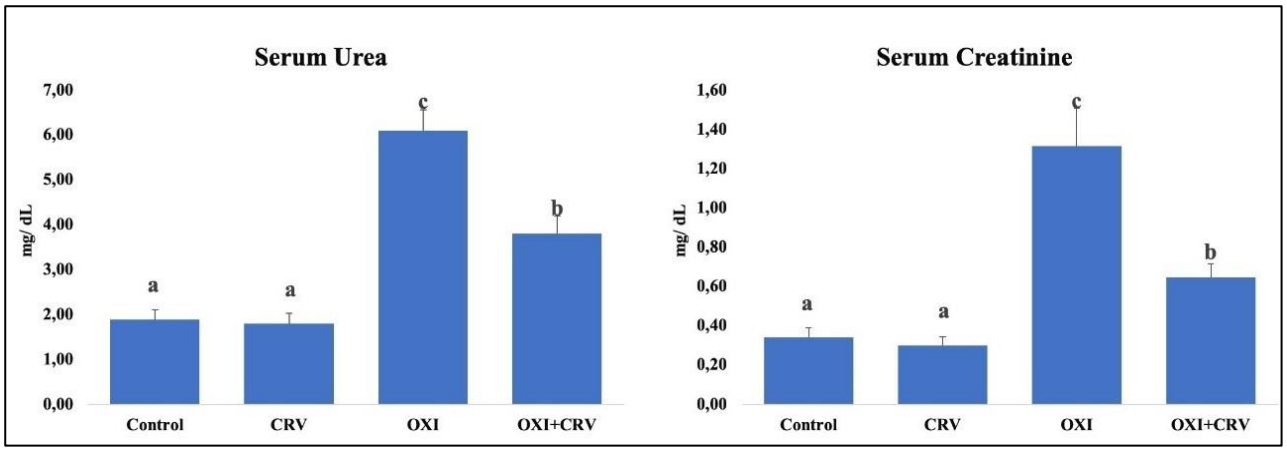

Figure 1: Effects of OXI and CRV on serum urea and serum creatinine levels. Values are given as mean±SD. Different letters indicate statistical difference: (a, b, c: p<0.05) (CRV: Carvacrol, OXI: Oxytetracycline).

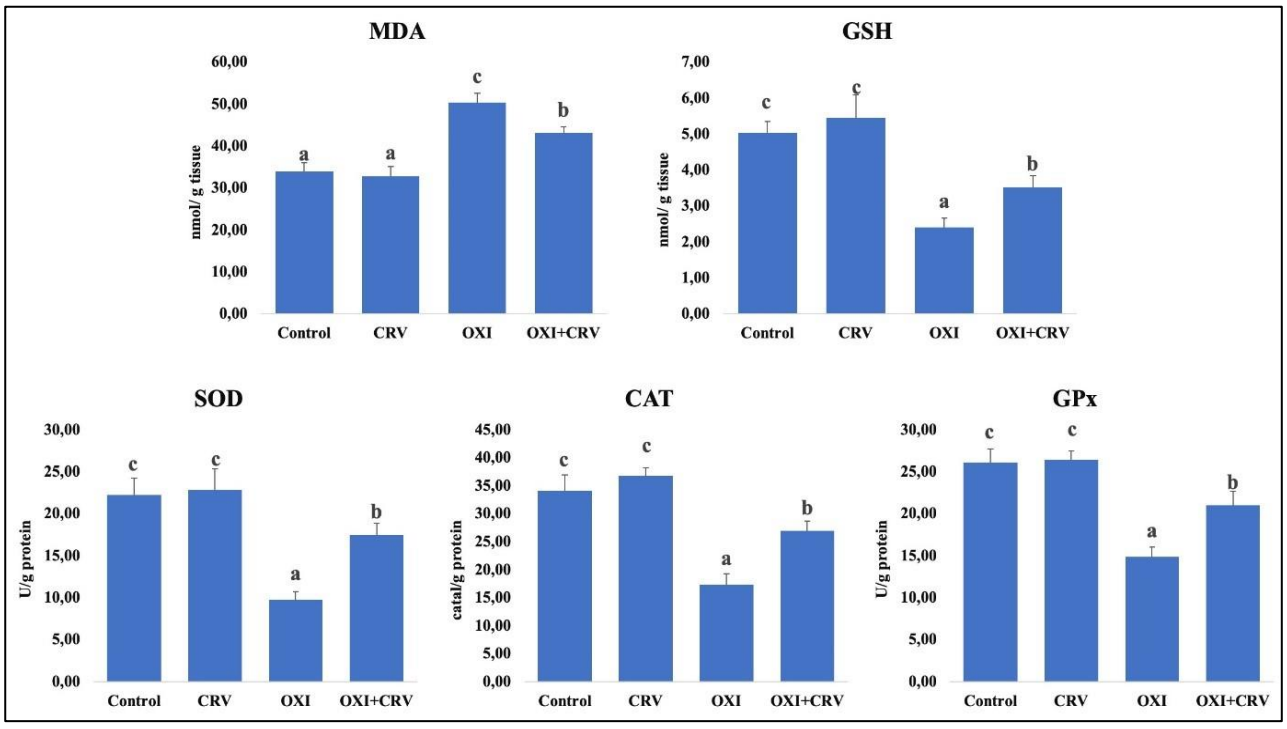

Figure 2: Effects of OXI and CRV on MDA and GSH levels and SOD, CAT, and GPx activities in rat kidney tissues. Values are given as mean±SD. Different letters indicate statistical difference: (a, b, c: p<0.05). (CRV: Carvacrol, OXI: Oxytetracycline, MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase).

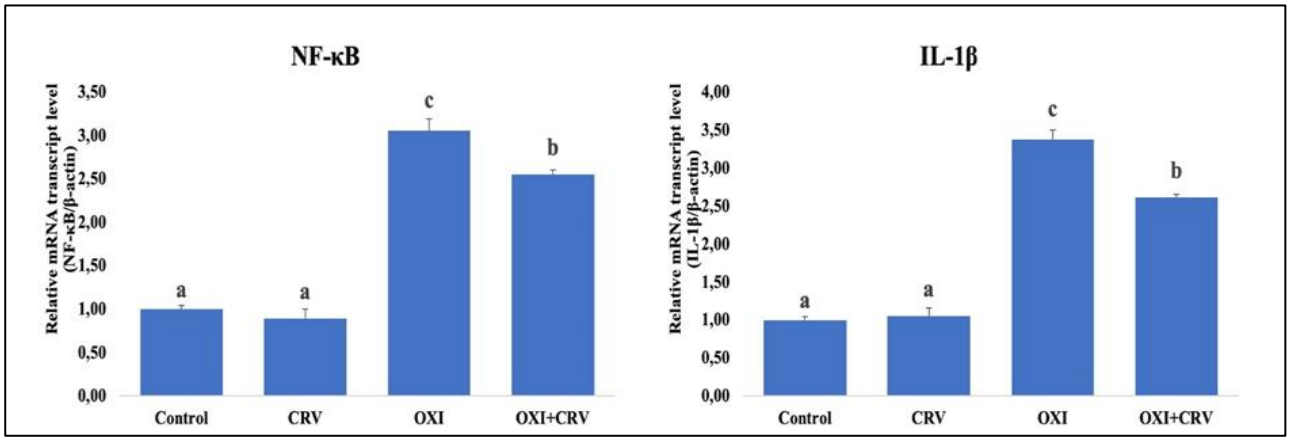

Figure 3: Effects of OXI and CRV on NF-κB and IL-1β (mRNA transcription levels) in rat kidney tissues. Values are given as mean±SD. Different letters indicate statistical difference: (a, b, c: p<0.05). (CRV: Carvacrol, OXI: Oxytetracycline, NF-κB: Nuclear factor kappa B, IL-1β: Interleukin 1β).

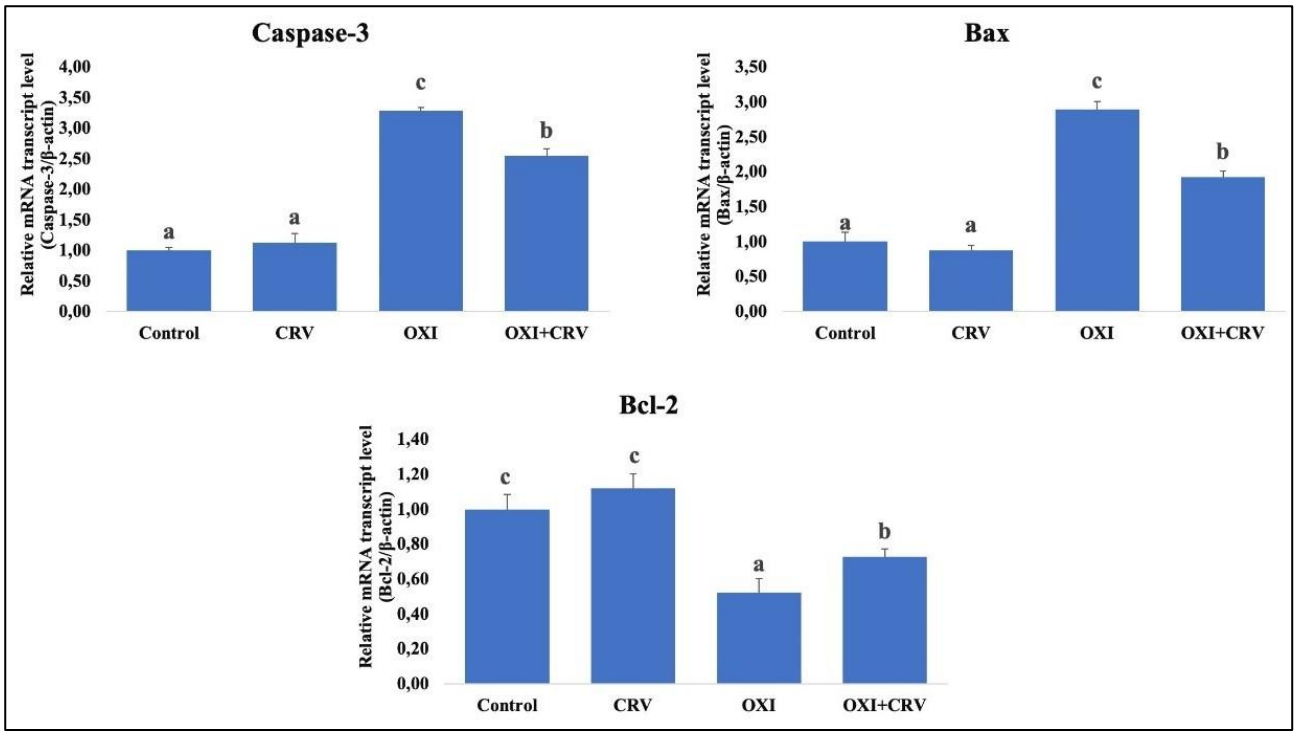

Figure 4: Effects of OXI and CRV on Caspase-3, Bax, and Bcl-2 (pro- and anti-apoptotic markers) in rat kidney tissues. Values are given as mean±SD. Different letters indicate statistical difference: (a, b, c: $p<0.05$). (CRV: Carvacrol, OXI: Oxytetracycline, Bcl-2: B-cell lymphoma-2).

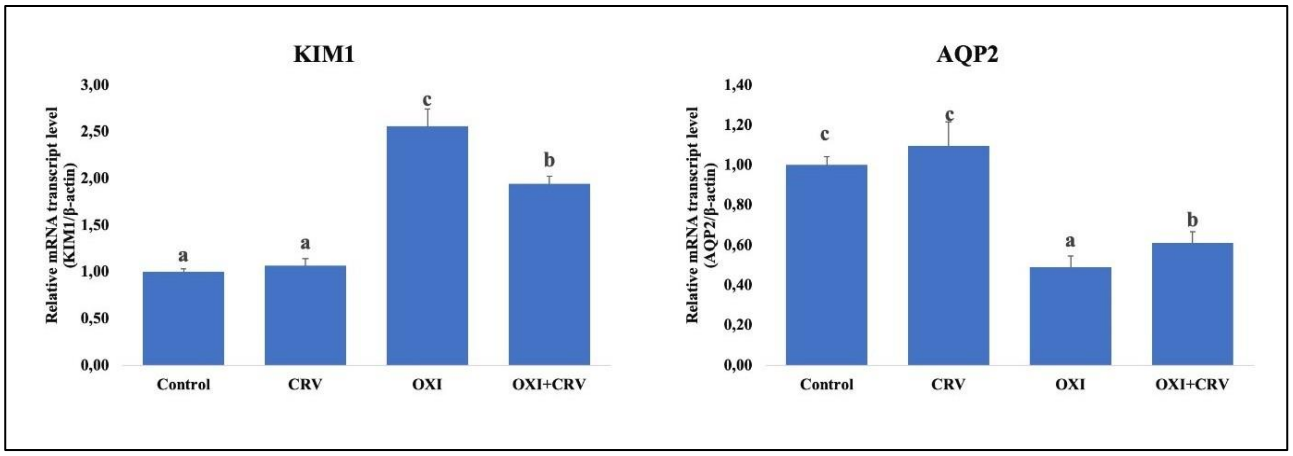

Figure 5: Effects of OXI and CRV on Kidney injury molecule-1 (KIM1) and AQP2 (mRNA transcription levels) in rat kidney tissues. Values are given as mean±SD. Different letters indicate statistical difference: (a, b, c: $p<0.05$). (CRV: Carvacrol, OXI: Oxytetracycline, KIM1: Kidney injury molecule-1, AQP2: Aquaporin-2).

Identification of apoptosis-related factors in the OXI and CRV groups

The effects of OXI and CRV on apoptosis-related factors are shown in Figure 4. As indicators of apoptosis, Caspase-3 and Bax activity were assessed. Pro- and anti-apoptotic indicators were assessed in relation to Bcl-2 levels. Animals given OXI had higher levels of Caspase-3 and Bax activity compared to the control group ($p<0.05$). Significantly reduced Bcl-2 levels were observed ($p<0.05$). Conversely, CRV administration dramatically increased Bcl-2 activity ($p<0.05$, Figure 4) and significantly decreased the higher levels of Caspase-3 and Bax ($p<0.05$) in the OXI-treated group.

Identification of nephrotoxicity biomarkers in the OXI and CRV groups

The effects of OXI and CRV on nephrotoxicity biomarkers are shown in Figure 5. Biomarkers of nephrotoxicity were found to be significantly lower in AQP-2 ($p<0.05$) and significantly higher in KIM-1 levels ($p<0.05$) in OXI-treated rats than in the control group. KIM-1 levels considerably decreased ($p<0.05$), and AQP-2 degree dramatically increased ($p<0.05$) during CRV treatment (Figure 5).

Light Microscopy Findings

Figure 6 shows the histopathological alterations in H&E-stained kidney tissue sections obtained from rats.

The results for the kidney samples in the CRV and control groups were comparable. The outcomes of the control, CRV, OXI, and OXI+CRV groups of kidney microscopic examinations are shown in Figure 6. Histological examination of kidney tissues in the control and CRV groups showed that the kidney bodies and tubular structures were of regular histological structure (Figures 6A, B). There was congestion in the glomerular capillaries and the vessels in the interstitial area in the OXI-

administered group. However, shedding and vacuolization in tubular epithelial cells, atrophy in the glomerular structure, dilatation in the Bowman's space, and inflammatory cell infiltration were noted (Figure 6 C). On the other hand, there was a significant improvement in kidney tissue in the group in which CRV was administered along with OXI. Congestion and bleeding decreased in both glomerular capillaries and interstitial vessels. Renal bodies and tubule structures were more normal (Figure 6 D).

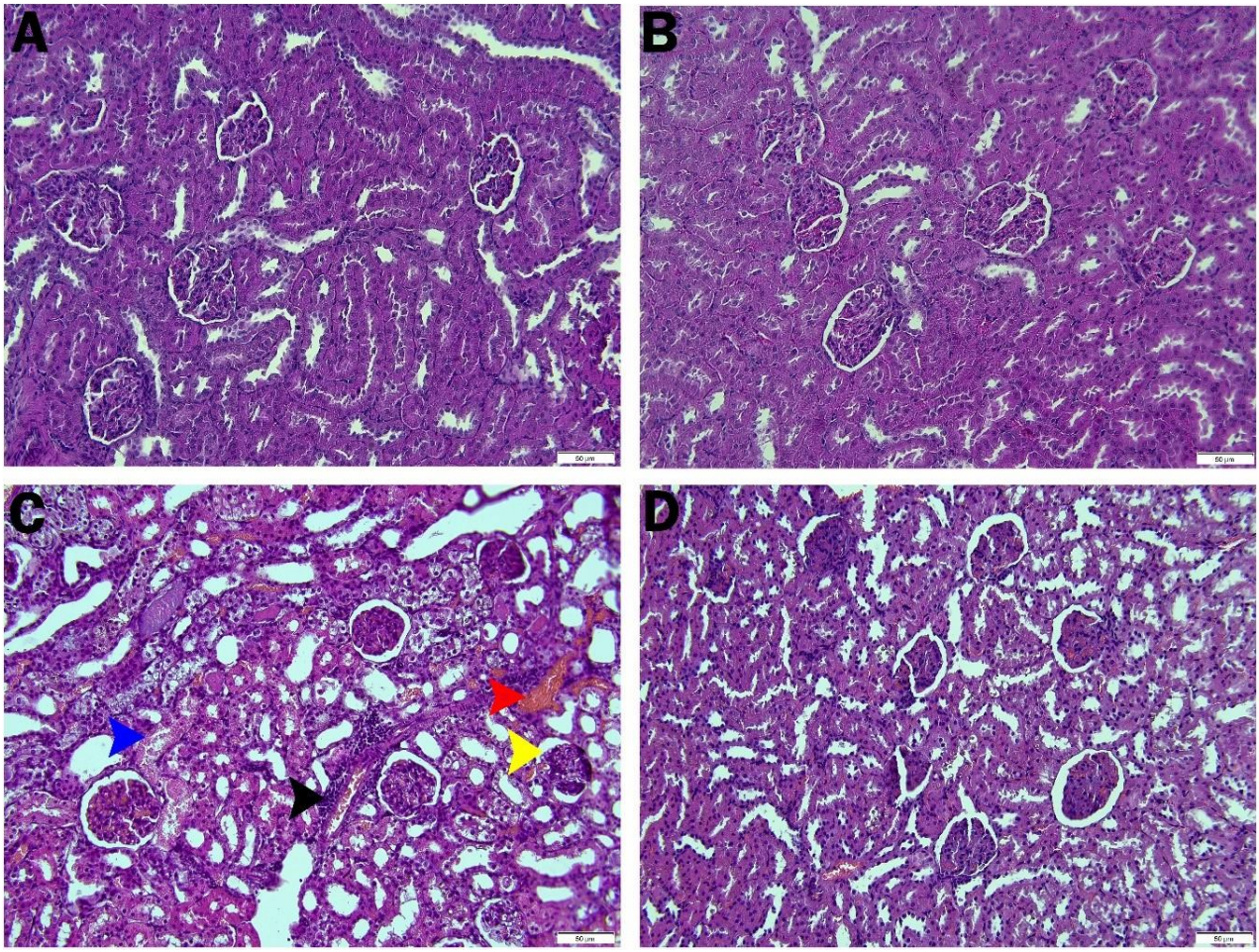

Figure 6: Photomicrographs of H&E-stained kidney sections. (H&E staining, 200x).

(A) control group, (B) CRV group, (C) OXI group, (D) OXI+CRV group. Red arrow: vascular congestion, yellow arrow: dilation in Bowman's space, black arrow: inflammatory cell infiltration, blue arrow: tubular epithelial cell loss.

DISCUSSION AND CONCLUSION

This study showed that CRV protects against kidney damage caused by OXI. The hypothesis that CRV may reduce the nephrotoxic effects of OXI was supported by its anti-inflammatory, interleukin-lowering, and antioxidant properties. OXI, a broad-spectrum antibiotic widely used in human and veterinary medicine, also has numerous side effects. High-dose OXI use without medical supervision has harmful effects on the kidneys and liver (Abdel-Daim and Ghazy 2015). The finding that OXI administration increased MDA levels and significantly inhibited the enzymatic antioxidants SOD, CAT, and GPx is consistent with EL-Akkad et al. (2022). Güvenç et al. (2019) reported in their research that CRV reduced MDA levels in kidney tissues. The results of this study revealed that OXI reduces renal function by causing histopathological changes in the glomerular capsule, accompanied by increased creatinine levels. Increased lipid peroxidation may deform membrane components, leading to changes in internal membrane properties such as enzyme activity and cell surface markers (Imbabi et al. 2024). The protective properties of CRV were confirmed by histological examination of the kidney. The OXI-treated group had higher concentrations of serum urea and creatinine than the control group. Rats that received CRV and OXI were shielded from this degradation. These findings also demonstrated the contribution of oral CRV administration to improving renal function. These results demonstrated a notable decline in urea and creatinine levels in rats treated with OXI+CRV, suggesting that CRV has an antioxidant effect as a free radical scavenger. Ahmadvand et al. (2016) reported a remarkable increase in urea and creatinine concentrations in rats treated with CRV for 12 days compared to the control group, showing a decrease in glomerular filtration in the kidneys. In the present study, high serum urea and creatinine levels resulting from OXI-induced kidney damage support the findings of previous studies (Ellero et al. 2020). Since lipids form the fundamental structure of cell membranes, increased ROS concentrations lead to the oxidative degradation of membrane lipids, a process known as lipid peroxidation (Şimşek et al. 2023). Serum MDA levels are used as a biomarker of lipid peroxidation (Yilgor and Demir 2024). ROS are neutralized by enzymatic antioxidants such as GPx, SOD, and CAT (Bhattacharyya et al. 2014). GSH, a non-enzymatic antioxidant, is a tripeptide compound that neutralizes free radicals and regenerates other antioxidants, maintaining the balance of oxidative stress in cells (Kurutas 2015).

Similar to our study, in the study conducted by Sharma et al. (2015), GSH reduction was observed in the kidneys of rats treated with OXI. Antioxidant defense systems in the body scavenge free radicals and protect against tissue damage (Akaras et al. 2023). In this study, antioxidant enzyme (SOD, CAT, and GPx) activities and non-enzymatic GSH levels decreased, while MDA levels increased in the OXI group. CRV administered in conjunction with OXI decreased these levels and also reduced the increased MDA levels. There are also some studies suggesting that CRV protects kidney health. CRV, with its antioxidant properties, protects kidney cells against free radical damage (Ram et al. 2022). Furthermore, thanks to its anti-inflammatory activities, CRV can alleviate renal inflammation and thus protect renal function (Riaz et al. 2023). Ram et al. (2022) demonstrated that CRV administration provided significant protection against experimentally induced renal injury in mice. Kidney damage has been correlated with increased levels of proinflammatory cytokines and the activation of systemic inflammation (Erdoğan et al. 2025). The pathophysiology of OXI-induced nephropathy is predicted to involve the release of proinflammatory cytokines and chemokines by macrophages, for instance, inducible nitric oxide synthase (iNOS), IL-1β, tumor necrosis factor alpha (TNF-α), macrophage migration inhibitory factor (MIF), NF-κB, and monocyte chemotactic protein-1 (MCP-1). Controlling the synthesis of proinflammatory cytokines that are useful in the inflammatory response is one of NF-κB's primary roles (Zhang et al. 2024). It has been demonstrated that oxidative stress activates the transcription factor NF-κB (Kankılıç et al. 2024). Tissue pathology is significantly influenced by inflammatory mediators including NF-κB and IL-1β (Gencer et al. 2025). Neutrophils, macrophages, monocytes, platelets, and mastocytes are inflammatory cells that are activated by these cytokines. These cells damage cells in a number of ways, such as by releasing ROS, causing lipid peroxidation in cell membranes, and oxidatively damaging proteins and DNA (Gür et al. 2022). It is known that DNA damage resulting from cancer treatment also activates NF-κB. For this reason, inhibiting NF-κB may be effective in reducing damage to the kidneys. Thus, inhibiting NF-κB may increase cellular protection against apoptosis. This study showed that the OXI-treated group resulted in higher levels of proinflammatory cytokines, especially NF-κB and IL-1β, in contrast to the control group. Additionally, higher KIM-1 and lower AQP-2 measures were detected in the OXI group in contrast with the control group. Conversely, the elevated KIM-1 levels were reduced, and the decreased AQP-2 levels increased in the OXI+CRV group compared to the control group. Ram et al. reported in 2022 that CRV administration significantly improved antioxidant proteins and kidney histological changes. They also reported that CRV lessened NF-κB (p65) phosphorylation and reduced IL-1β capacity. Consequently, CRV administration alleviates kidney fibrosis by targeting oxidative stress and inflammation (Ram et al. 2022). Additionally, Samarghandian et al. (2016) also reported renoprotective effects of CRV in rats exposed to oxidative stress. Apoptosis has an important role in maintaining homeostasis in the organism (Karaca et al. 2025). Caspases are members of the cysteine protease family and are key regulators of apoptosis, the tightly controlled cell death process crucial for the elimination of excess or unnecessary cells during development (Ozyigit et al. 2024). Execution caspases, including caspase-3, are triggered by initiator caspases and carry out most of the cellular destruction during apoptosis (Lestari et al. 2024). Doğu et al. (2022) reported that OXI not only reduces DNA damage but also reduces apoptosis. Overdosage of OXI without medical advice has harmful effects on the liver and kidney (Oda et al. 2018). Our study revealed increased apoptotic markers for Bax and Caspase-3 in the OXI group but diminished Bcl-2 levels. The opposite results were observed in the CRV+OXI group. Consequently, we concluded that OXI induces apoptosis.

In conclusion, CRV prevented OXI-induced kidney damage in rat kidney tissue. Furthermore, it inhibited lipid peroxidation by acting as an antioxidant against oxidative damage. Therefore, CRV can be considered an effective treatment method against nephrotoxicity by preventing OXI-induced oxidative stress in kidney tissues and the associated inflammation, apoptosis, and impairment of tissue integrity.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea/Concept: FMK, HŞ, HY
Supervision/Consultancy: FMK, HŞ, HY
Data Collecting and/or Processing: HY, HŞ, OK, NA
Analysis and/or Interpretation: OK, NA, HŞ, HY
Writing the Article: HY, HŞ
Critical Review: FMK, HŞ, HY

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