HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Evaluation of the Protective Effect of Cranberry in Gentamicin-Induced Nephrotoxicity in Rats

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Received: 17.10.2025

Accepted: 09.12.2025

How to cite this article: KARAPINAR M, GÜNCÜM E, KURTDEDE E, BİŞKİN TÜRKMEN M, ŞAHİN Y, BAYDAN E. (2025). Evaluation of the Protective Effect of Cranberry in Gentamicin-Induced Nephrotoxicity in Rats. Harran Üniversitesi Veteriner Fakültesi Dergisi, 14(2): 206-216. DOI:10.31196/huvfd.1804391.

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Available on-line at: https://dergipark.org.tr/tr/pub/huvfd

Abstract: Gentamicin (GTC) is a widely used aminoglycoside antibiotic, but its therapeutic application is limited by nephrotoxic and hepatotoxic effects primarily driven by oxidative stress. This study investigated the potential protective role of cranberry (CRB) (*Vaccinium macrocarpon*), known for its antioxidant and anti-inflammatory properties, against GTC-induced nephrotoxicity in rats. Hepatic biochemical and histopathological parameters were also evaluated as supportive markers of systemic toxicity. Thirty-two rats were divided into four groups: Control, CRB, GTC + CRB, and GTC. GTC (80 mg/kg, s.c.) and CRB (200 mg/kg, p.o.) were administered for seven consecutive days. Biochemical analyses showed significant increases in serum BUN, urea, creatinine, ALT, and AST levels in the GTC group, confirming renal and hepatic impairment. Co-treatment with CRB reduced these elevations and improved antioxidant parameters, reflected by decreased MDA and oxidative stress index levels and increased total and native thiols ($p < 0.001$). Gene expression analyses demonstrated that GTC upregulated pro-apoptotic genes (Bax, caspase-3, -9) and downregulated the anti-apoptotic Bcl-2, whereas CRB co-administration reversed these alterations. Histopathological evaluations supported the biochemical findings, revealing severe tubular and hepatocellular necrosis, inflammatory infiltration, and hemorrhage in the GTC group, which were markedly alleviated by CRB supplementation. These results suggest that CRB may exert a partial renoprotective effect against GTC-induced oxidative and apoptotic damage, possibly through its antioxidant and cytoprotective properties.

Keywords: *Antioxidant, Cranberry, Gentamicin, Nephrotoxicity, Oxidative stress*

Introduction

Aminoglycosides have been employed over the years for the treatment of infectious diseases affecting both humans and animals (Şahin et al., 2022). They act as bactericidal antibiotics with wide antimicrobial coverage, primarily through inhibition of protein synthesis, and share close similarities in their chemical composition, spectrum of activity, pharmacological properties, and toxicity. These drugs have a narrow therapeutic window and tend to cause similar adverse effects at effective doses. Gentamicin (GTC), a member of the aminoglycoside group, exhibits strong antibacterial activity against Gram-negative pathogens responsible for serious infections in both humans and animals, and is widely used for their treatment (Elgazzar et al., 2022; Khalili et al., 2021). Despite GTC's effectiveness as an antibacterial drug, its use is limited by its potential to cause kidney damage (nephrotoxicity) as a side effect (Ijaz et al., 2023). At the cellular level, GTC tends to concentrate within the proximal tubular segments, causing the development of tubular necrosis and congestion in the glomeruli that impair overall kidney function. It also promotes oxidative stress and activates inflammatory cascades, both of which are key drivers of its nephrotoxic effects (Abouzed et al., 2021).

Recently, various agents with anti-inflammatory, antioxidant, and nephroprotective properties have been investigated. Among them, cranberry (CRB) (*Vaccinium macrocarpon*) has been widely investigated due to its rich chemical profile (De Souza Gouveia Moreira et al., 2024). Fruits of this species contain high levels of phenolic compounds, primarily anthocyanins, flavonols, phenolic acids, and proanthocyanidins, all of which contribute to their notable antioxidant, anti-inflammatory, and antimicrobial effects. CRBs are also abundant in other bioactive phytochemicals including flavones, flavonoids, and organic acids, which contribute to their nutritional importance and therapeutic potential (Balawejder et al., 2023; Nemzer et al., 2022). The characteristic red pigmentation of CRB arises from anthocyanins and their aglycone forms (anthocyanidins), compounds known for their pronounced antioxidant and anti-inflammatory activity (Boira et al., 2025). Moreover, evidence from clinical research suggests that the consumption of CRB, like other polyphenol-rich fruits, has a positive influence on lipid metabolism and helps maintain normal blood pressure. It also protects neural and endothelial tissues from oxidative stress and consequently reduces the likelihood of cardiovascular disease (Xue et al., 2023). However, despite CRB's known therapeutic potential, its role in protecting against GTC-induced renal toxicity has not yet been clearly elucidated. In the present study, multiple indicators of kidney injury, including serum BUN, urea, and creatinine levels; oxidative stress parameters (TAS, TOS, OSI, MDA, total and native thiols); apoptotic markers (Bax, Bcl-2, caspase-3, -9, and Cyt-c); and renal histopathological changes were evaluated. Based on these evaluations, the study aimed to clarify the renoprotective potential of CRB in a rat model of GTC-induced nephrotoxicity.

Materials and Methods

Drugs and Chemicals

Cranberry (*Vaccinium macrocarpon*) capsules were obtained from Orzax products (Cranberry 30 Capsules, Turkey). Gentamicin, commercially available as Gentasol 10%, was provided by Netfarma (Istanbul, Turkey). Ketamine (Ketasol 10%) was supplied by Richter Pharma (Austria). Xylazine (Xylazinbio 2%) was purchased from Bioveta (Czech Republic).

Cranberry Extract Characteristics and Dose Selection

The cranberry extract used in this study contained 514 mg of cranberry extract, providing 36 mg of proanthocyanidins (PACs) per two capsules, as specified by the manufacturer (Orzax, Turkey). Cranberry was administered at a dose of 200 mg/kg by oral gavage. This dose was selected based on previous studies demonstrating the nephroprotective effects of cranberry species at similar dose ranges in experimental models of nephrotoxicity and diabetic nephropathy (Shareef et al., 2024).

Experimental Protocol

Thirty-two male Sprague–Dawley rats were selected for the study. All animals were certified healthy, aged between 8 and 12 weeks, and possessed a body mass ranging from 180 to 220 g. The experimental procedures involving animals adhered strictly to the established guidelines for the care and utilization of laboratory animals. Formal approval was obtained from the Kırıkkale University Animal Experimentation Ethics Committee (Decision Number: 2024/08-29). Following a 14-day quarantine, the 32 rats were randomly allocated into four groups, each containing 8 animals. Subcutaneous (SC) and oral (PO) administrations were performed once daily throughout the experimental period, as detailed for each group below.

Group 1 (Control): 1 mL isotonic saline SC + distilled water PO daily (Şahin et al., 2022)

Group 2 (GTC): 80 mg/kg gentamicin SC + distilled water PO daily (Şahin et al., 2022)

Group 3 (GTC + CRB): 80 mg/kg gentamicin SC + 200 mg/kg cranberry PO daily (Şahin et al., 2022; Shareef et al., 2024)

Group 4 (CRB): 1 mL isotonic saline SC + 200 mg/kg cranberry PO daily (Şahin et al., 2022; Shareef et al., 2024)

The entire treatment course lasted 7 consecutive days, with all doses administered daily. On the eighth day, 24 hours after the last administration, the animals were anesthetized intraperitoneally with ketamine and xylazine, and blood samples were subsequently collected. After blood collection, animals were sacrificed, and kidney and liver tissues were taken. Plasma was retrieved from the collected blood samples by centrifuging at 3000 rpm over a 10-minute period. Following extraction, the plasma was immediately frozen and maintained at a temperature of −20 °C until the analytical phase.

Biochemical Analysis

Kidney samples collected from sacrificed rats were labelled, placed in sample containers, and stored at −80 °C

until analysis. For biochemical assays, each tissue sample was homogenized in ice-cold phosphate-buffered saline (PBS, pH 7.4) at a proportion of 140 mg tissue per 1 mL buffer (1:10, w/v). The serum levels of urea, blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were calculated using an automated clinical chemistry analyzer (BS-120, Mindray, China).

The total antioxidant status (TAS), measured in the plasma and renal tissue supernatants, was assessed using commercial kits supplied by Rel Assay Diagnostics (Turkey) and analyzed spectrophotometrically with a UV-1202 device (Shimadzu, Japan). Total oxidant status (TOS) was calculated spectrophotometrically with the same system (Erel, 2005), and the oxidative stress index (OSI) was computed as the ratio of TOS to TAS. Malondialdehyde (MDA) concentrations in plasma and renal tissue supernatants were assessed through the thiobarbituric acid reactive substances (TBARS) technique described by Ohkawa et al. (1979), employing commercial kits (Rel Assay Diagnostics, Turkey), and readings were taken at 532 nm with a UV-1202 spectrophotometer (Shimadzu, Japan) (Ohkawa et al., 1979). Renal apoptosis-related markers, including B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (Bax), cytochrome c (Cyt-c), and caspases-3 and -9, were quantified using rat-specific ELISA kits (Reed Biotech, China) according to the manufacturers' protocols and read on an ELISA microplate reader (Tecan, Austria).

Histopathological Examination

Fixation of the collected liver and kidney tissue samples was achieved by placing them in a 10% neutral buffered formalin solution. The samples were maintained in the fixative for 48 to 72 hours following necropsy. After fixation, a routine histological tissue processing protocol was applied. To facilitate embedding, the tissue samples were initially dehydrated using an ascending ethanol series (50%, 70%, 80%, 96%, and 100%). Subsequently, they were cleared using xylene before being placed into paraffin blocks. The paraffin-embedded tissues were cut into 4-µm-thick sections. Prepared slides were examined and photographed using an Olympus BX51 light microscope equipped with a camera attachment (Japan). For the liver, histopathological evaluation included assessment of hepatocyte morphology, sinusoidal architecture, integrity of portal areas, vascular alterations, inflammatory cell infiltration, and degenerative or necrotic changes. The evaluation focused on key hepatic lesions such as vacuolar degeneration, hepatocellular necrosis, and portal inflammation. These parameters were semi-quantitatively scored according to the four-grade scale described by Klopfleisch (2013) as follows: 0 (absent), 1 (mild), 2 (moderate), and 3 (severe) (Klopfleisch, 2013). Kidney tissues were examined using the same histological procedures. Evaluated parameters included the integrity of glomerular structures, Bowman's capsule morphology, degenerative and necrotic changes in proximal and distal tubules, tubular dilatation, epithelial cell alterations, and interstitial inflammatory infiltration. Vascular structures and tubulointerstitial regions were also analyzed for potential pathological findings.

Statistical Analysis

The results are expressed as the mean ± standard error (SE). Parametric variables were analyzed by one-way ANOVA followed by Duncan's post hoc test ($p < 0.05$), while nonparametric variables were evaluated using the Kruskal–Wallis test with pairwise comparisons by the Mann–Whitney U test and Bonferroni correction ($p < 0.0083$). Statistical evaluations were conducted using SPSS version 18.0 software (PASW Statistics, Chicago, IL, USA).

Results

The integrity of renal function is routinely assessed using serum biochemical parameters, including urea, BUN, and creatinine. In a distinct role, ALT and AST serve as biomarkers reflecting hepatic cellular integrity (Gounden et al., 2024; McGill, 2016). In this study, serum concentrations of urea, BUN, creatinine, ALT, and AST were evaluated, and the obtained data are presented in Table 1. Treatment with gentamicin (GTC) led to a significant increase in the serum concentrations of urea, BUN, and creatinine when compared to the control group ($p < 0.001$), as detailed in the table, indicating marked renal impairment. Moreover, AST and ALT serum activities were markedly elevated in the GTC group, reflecting hepatocellular injury. Co-treatment with cranberry (CRB) significantly attenuated these changes; the GTC + CRB group showed lower urea, BUN, creatinine, AST, and ALT levels in comparison with the GTC group (although still higher than control, the improvement was significant; $p < 0.001$). CRB alone did not cause any adverse alterations and the measured parameters remained similar to the control group.

In the present study, the levels of TAS, TOS, and OSI were measured to assess the oxidative stress status. The findings are demonstrated in Table 2. Compared with the control group, GTC administration caused a slight but non-significant decrease in TAS, while significantly elevating TOS and OSI ($p < 0.001$), indicating increased oxidative stress. Co-treatment with CRB maintained TAS at a level comparable to the control and significantly lowered OSI compared to the GTC group ($p < 0.0083$), although TOS remained above the values detected in the control group. CRB alone produced the highest TAS values and kept both TOS and OSI close to the control group.

Total and native thiols are key components of the antioxidant defense system, reflecting the redox balance of biological tissues, while MDA is a key biomarker reflecting lipid peroxidation and oxidative stress. In this study, total thiol, native thiol, total/native thiol ratio, and MDA levels were evaluated, and the findings are presented in Table 3. GTC administration significantly decreased total and native thiol levels compared to the control group ($p < 0.001$) and markedly increased MDA, indicating enhanced lipid peroxidation. The total/native thiol ratio also displayed a significant decline in the GTC group. Co-treatment with CRB significantly improved total and native thiol levels compared with GTC alone ($p < 0.001$). Total thiol levels were restored to and slightly exceeded control values, whereas native thiol and the total/native thiol ratio remained lower than in the

Table 1. Serum urea, blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels in the groups (mean ± SE).

Group	Urea (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)	AST (mg/dl)	ALT (mg/dl)
Control	24.04 ± 1.11^{b}	11.23 ± 1.37^{b}	0.93 ± 0.09^{b}	140.52 ± 28.86^{b}	63.16 ± 1.38^{b}
GTC	33.58 ± 4.39^{a}	15.69 ± 5.02^{a}	1.25 ± 0.10^{a}	255.15 ± 22.60^{a}	143.71 ± 15.50^{a}
GTC+CRB	31.85 ± 1.38^{a}	15.07 ± 0.59^{a}	1.22 ± 0.08^{a}	243.15 ± 11.58^{a}	137.79 ± 6.04^{a}
CRB	17.44 ± 2.44^{b}	8.15 ± 1.14^{b}	0.80 ± 0.04^{b}	159.73 ± 9.95^{b}	68.10 ± 9.95^{b}

GTC (gentamicin), GTC+CRB (gentamicin + cranberry), CRB (cranberry). Different superscript letters within a column indicate statistically significant differences between groups ($p < 0.001$).

Table 2. Total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) levels in the groups (mean ± SE).

Group	TAS (mmol/L)	TOS (µmol H_2O_2 eq/L)	OSI (AU)
Control	1.12 ± 0.08^{b}	9.15 ± 0.34^{b}	0.84 ± 0.66^{bc}
GTC	1.01 ± 0.05^{b}	15.18 ± 0.43^{a}	1.56 ± 0.16^{a}
GTC+CRB	1.12 ± 0.05^{b}	14.14 ± 0.80^{a}	1.26 ± 0.03^{ad}
CRB	1.43 ± 0.03^{a}	8.55 ± 0.44^{b}	0.79 ± 0.19^{cd}

GTC (gentamicin), GTC+CRB (gentamicin + cranberry), CRB (cranberry). AU (arbitrary unit). Different superscript letters within a column indicate statistically significant differences between groups ($p < 0.001$ for TAS and TOS; $p < 0.0083$ for OSI).

Table 3. Total thiol, native thiol, total/native thiol ratio, and malondialdehyde (MDA) levels in the experimental groups (mean ± SE).

Group	Total thiol (µmol/L)	Native thiol (µmol/L)	Thiol/Native thiol ratio	MDA (nmol/mL)
Control	53.80 ± 1.40^{b}	133.86 ± 2.97^{a}	40.02 ± 1.42^{a}	12.48 ± 0.68^{c}
GTC	43.41 ± 1.35^{c}	86.00 ± 2.44^{c}	21.29 ± 1.17^{c}	20.50 ± 0.68^{a}
GTC+CRB	59.75 ± 2.04^{a}	103.33 ± 4.32^{b}	21.78 ± 1.94^{c}	17.30 ± 0.79^{b}
CRB	55.74 ± 2.0^{ab}	110.00 ± 1.88^{b}	27.12 ± 1.61^{b}	13.37 ± 0.47^{c}

GTC (gentamicin), GTC+CRB (gentamicin + cranberry), CRB (cranberry). Different superscript letters in the same column indicate statistically significant differences between groups ($p < 0.001$).

Table 4. Bax, Bcl-2, and Bax/Bcl-2 ratio levels in the experimental groups (mean ± SE).

Group	Bax (ng/mL)	Bcl-2 (ng/mL)	Bax/Bcl-2 (AU)
Control	6.42 ± 0.09^{d}	4.63 ± 0.17^{a}	1.40 ± 0.06^{c}
GTC	9.31 ± 0.08^{a}	3.08 ± 0.11^{b}	3.05 ± 0.10^{a}
GTC+CRB	8.50 ± 0.11^{b}	4.14 ± 0.18^{a}	2.08 ± 0.11^{b}
CRB	6.77 ± 0.09^{c}	4.32 ± 0.30^{a}	1.62 ± 0.11^{c}

GTC (gentamicin), GTC+CRB (gentamicin + cranberry), CRB (cranberry) bax (Bcl-2 associated X protein), bcl-2 (B-cell lymphoma 2). AU (arbitrary unit). Different superscript letters within a column indicate statistically significant differences between groups ($p < 0.001$).

Table 5. Cytochrome c (Cyt-c), Caspase-3, and Caspase-9 levels in the experimental groups (mean ± SE).

Group	Cyt-c (ng/mL)	Caspase-3 (ng/mL)	Caspase-9 (ng/mL)
Control	9.46 ± 0.21^{c}	15.26 ± 0.17^{c}	7.64 ± 0.18^{c}
GTC	11.91 ± 0.36^{a}	18.16 ± 0.10^{a}	10.73 ± 0.17^{a}
GTC+CRB	10.97 ± 0.27^{b}	16.11 ± 0.30^{b}	8.68 ± 0.17^{b}
CRB	9.48 ± 0.14^{c}	15.12 ± 0.18^{c}	7.61 ± 0.22^{c}

GTC (gentamicin), GTC+CRB (gentamicin + cranberry), CRB (cranberry). Different superscript letters in the same column indicate statistically significant differences between groups ($p < 0.001$).

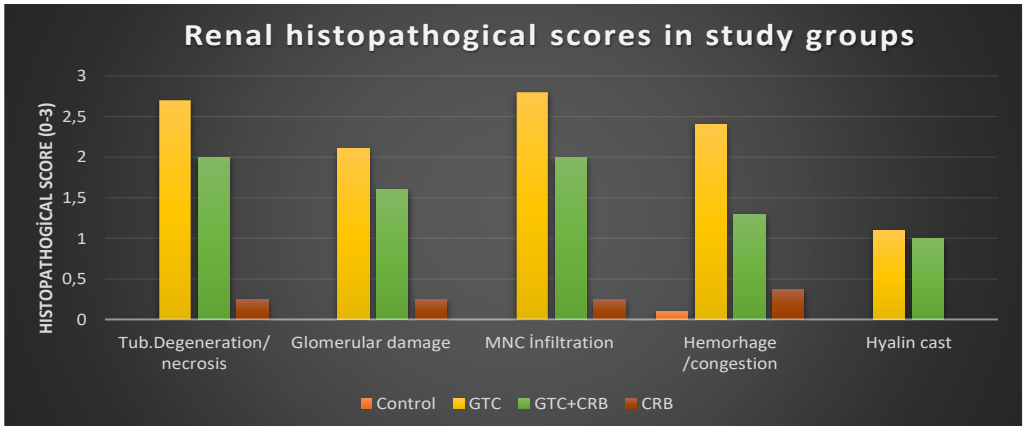
control group. MDA levels were significantly reduced in the GTC + CRB group compared to the GTC group ($p < 0.001$), indicating partial protection against oxidative damage. CRB alone maintained thiol parameters close to control values and significantly lowered MDA levels compared with GTC.

A fine balance between pro- and anti-apoptotic proteins is essential in regulating programmed cell death. Bax contributes to apoptosis by increasing mitochondrial membrane permeability, while Bcl-2 counteracts this effect, maintaining cellular survival. In the present study, Bax, Bcl-2, and the Bax/Bcl-2 ratio were evaluated as indicators of apoptotic activity, and the findings are presented in Table 4. As shown in the table GTC administration markedly increased renal Bax expression compared to the control group ($p < 0.001$) and significantly diminished Bcl-2 values, leading in a marked rise in the Bax/Bcl-2 ratio, an indicator of enhanced pro-apoptotic signaling. Co-treatment with CRB significantly decreased Bax levels and partially restored Bcl-2 expression compared to the GTC group ($p < 0.001$), thereby lowering Bax/Bcl-2 ratio, although not fully reaching control values. CRB alone maintained Bax and Bcl-2 levels close to those of the control group, with a Bax/Bcl-2 ratio similar to control.

Cyt-c, and caspases are key indicators of mitochondrial-mediated apoptosis. In our study, Cyt-c, caspase-3, and caspase-9 levels were determined to assess apoptotic activation, and the results are shown in Table 5. GTC administration significantly elevated Cyt-c, caspase-3, and caspase-9 concentrations compared with the control group ($p < 0.001$), indicating intrinsic apoptotic pathway activation. Co-treatment with CRB significantly reduced these elevations relative to the GTC group ($p < 0.001$) but did not fully restore the levels to control values. CRB alone maintained Cyt-c, caspase-3, and caspase-9 levels at values similar to those recorded in the control group.

Histopathological examination was conducted to evaluate structural alterations and tissue damage in renal tissues. The representative findings are summarized in Figure 1, and Figure 2 illustrates representative histological changes observed in the study groups. Kidneys from the control group demonstrated normal architecture with well-preserved glomeruli and tubules and only minimal degenerative changes. GTC administration caused marked tubular epithelial degeneration and necrosis, glomerular damage, mononuclear cell infiltration, hemorrhage/congestion, and hyaline cast formation, reflecting severe structural injury. In contrast, co-treatment with CRB markedly ameliorated these pathological alterations, with less tubular and glomerular damage and milder inflammatory cell infiltration and cast formation compared with the GTC group, although complete structural restoration was not achieved. CRB alone preserved nearly normal renal histology, showing only minor changes comparable to the control group. A detailed examination of histopathological findings that overall architecture of renal parenchyma was preserved. The proximal and distal tubules were regular, the glomeruli were of normal size and structure, and there was no evidence of inflammation (Figure 2A). In the CRB group, renal morphology was largely similar to control group, showing intact glomeruli and Bowman's capsules, with only mild tubular dilation, minimal vacuolar degeneration in tubular epithelium and congestion (Figure 2B). In the GTC group, cortical disorganization was evident, accompanied by extensive tubular necrosis, intraluminal hyaline casts, interstitial edema, and intense mononuclear cell infiltration (Figures 2C). These features represent classic signs of GTC-induced nephrotoxicity. Co-treatment with CRB considerably alleviated these lesions, resulting in less tubular necrosis and reduced inflammatory infiltration, while vascular congestion was also diminished compared with the GTC group (Figures 2D). Collectively, these histopathological observations confirm that CRB administration provided partial morphological protection against GTC-induced renal injury.

Liver histopathological findings are summarized in Figure 3 and Figure 4 illustrates the microscopic features of hepatic tissues from the experimental groups. The control group showed normal hepatic histology, with no vacuolar degeneration, necrosis, portal inflammation, or hemorrhage/congestion. GTC administration caused marked hepatic injury recognized by extensive vacuolar degeneration, hepatocellular necrosis, prominent portal inflammatory cell infiltration, and areas of hemorrhage/congestion. In contrast, co-treatment with CRB



Group	Tubular degeneration / necrosis	Glomerular damage	MNC infiltration	Hemorrhage / congestion	Hyaline cast
Control	0	0	0	0.1	0
GTC	2.7	2.1	2.8	2.4	1.1
GTC+CRB	2.0	1.6	2.0	1.3	1.0
CRB	0.25	0.25	0.25	0.375	0

Figure 1. Renal histopathological scores in study groups. Scores are presented as mean values (0 = none, 1 = mild, 2 = moderate, 3 = severe) for tubular degeneration/necrosis, glomerular damage, mononuclear cell (MNC) infiltration, hemorrhage/congestion, and hyaline cast formation. GTC (gentamicin), GTC+CRB (gentamicin + cranberry), CRB (cranberry).

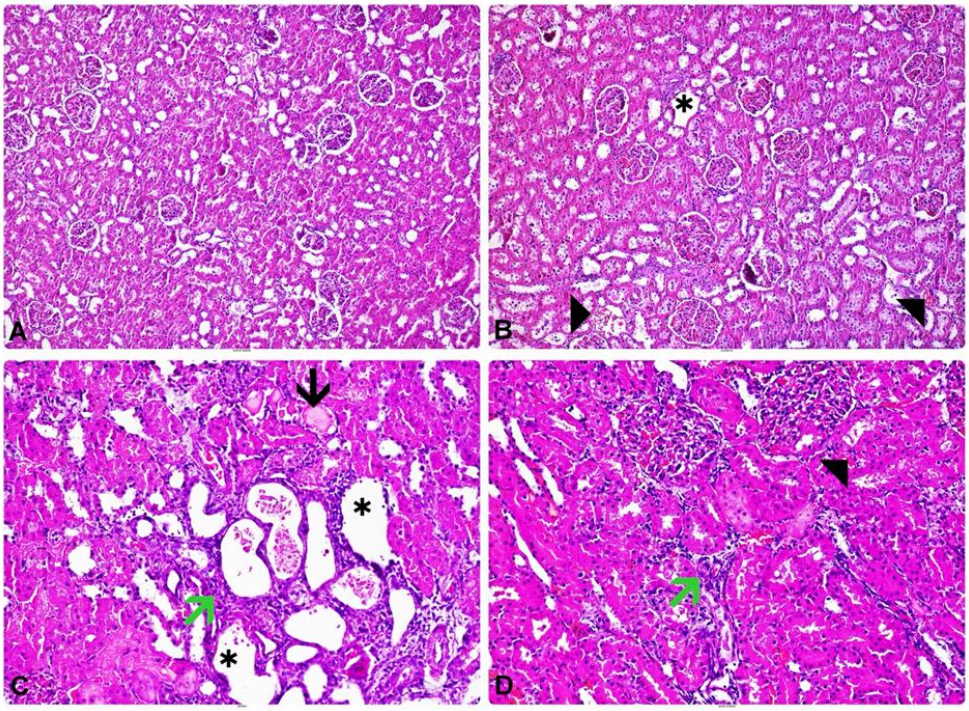
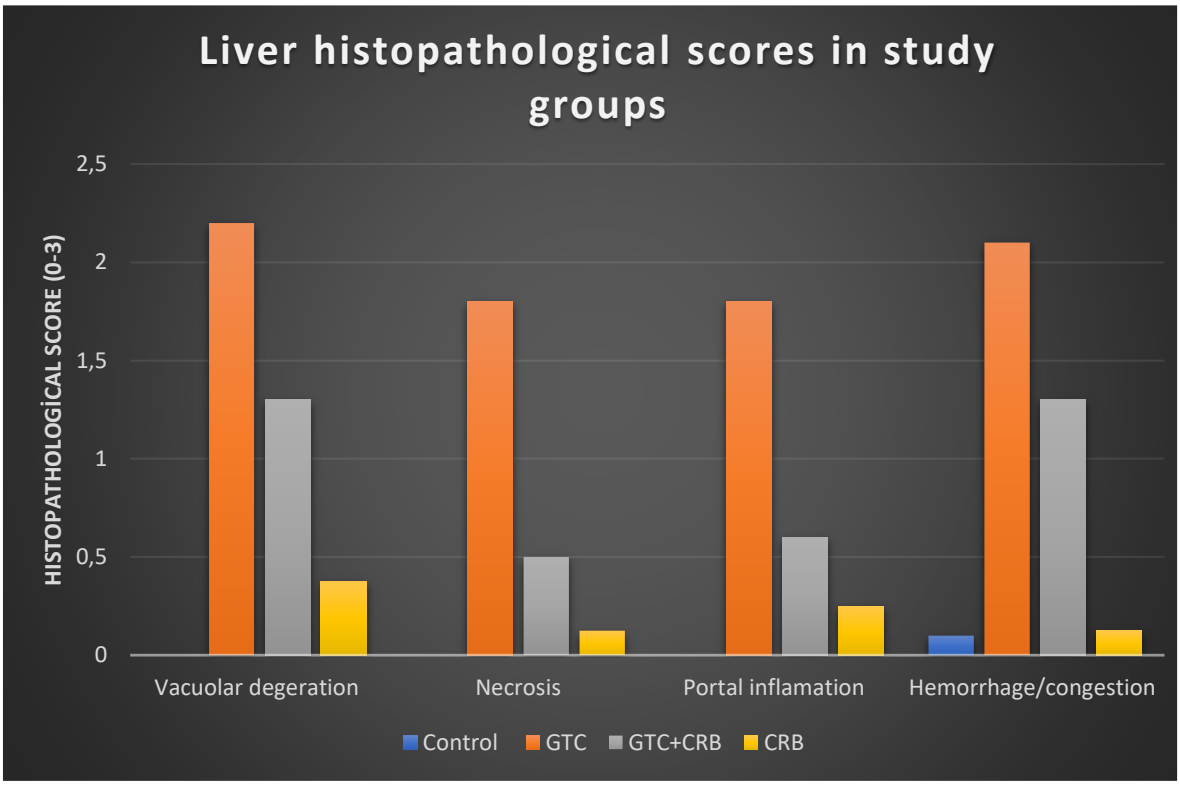


Figure 2: Microscopic images of hematoxylin-eosin (HE) stained sections of rat kidney tissues from experimental groups

(A) Control group showing normal renal histology with well-preserved tubules and glomeruli (×100). (B) CRB group showing mild congestion (arrowheads, ▲) and dilated tubules (asterisks, *) (×100). (C) GTC group showing severe mononuclear cell infiltration in the interstitium (green arrow, →), tubular casts (black arrow, →), and extensively necrotic and dilated tubules (asterisks) (×200). (D) GTC + CRB group showing mild to moderate mononuclear cell infiltration (green arrow, →) and mild congestion (arrowhead, ▲) (×200).



Group	Vacuolar degeneration	Necrosis	Portal inflammation	Hemorrhage / congestion
Control	0	0	0	0.1
GTC	2.2	1.8	1.8	2.1
GTC+CRB	1.3	0.5	0.6	1.3
CRB	0.375	0.125	0.25	0.125

Figure 3. Liver histopathological scores in study groups. Scores are presented as mean values (0 = none, 1 = mild, 2 = moderate, 3 = severe) for vacuolar degeneration, necrosis, portal inflammation, hemorrhage/congestion. GTC (gentamicin), GTC+CRB (gentamicin + cranberry), CRB (cranberry).

markedly ameliorated these lesions, showing reduced vacuolar degeneration and necrosis, milder portal inflammation, and less pronounced vascular congestion compared with the GTC group, although complete structural restoration was not achieved. CRB alone maintained nearly normal hepatic morphology, with only slight changes comparable to the control group. When the histopathological data were examined, it was found that liver sections from the control group displayed normal hepatic architecture, with well-preserved remark cords, intact lobular organization, and sinusoids of normal appearance (Figure 4A). In the CRB group, the overall architecture was similar to the control, though occasional mild vascular congestion in the portal vein and limited vacuolar degeneration around the central vein were observed (Figures 4B). GTC administration caused prominent histopathological alterations, including moderate to severe vacuolar degeneration, multifocal hepatocellular necrosis, and pronounced sinusoidal and vascular congestion. Distinct inflammatory cell aggregates were also observed in the portal and pericentral regions, indicating marked hepatic inflammation (Figures 4C). In contrast, co-treatment with CRB substantially alleviated these alterations. The extent of vacuolar degeneration and inflammatory infiltration was markedly reduced, with only mild periportal congestion and preserved lobular structure in most sections (Figure 4D). These observations indicate that CRB co-administration provides partial histological protection against GTC-induced hepatic injury.

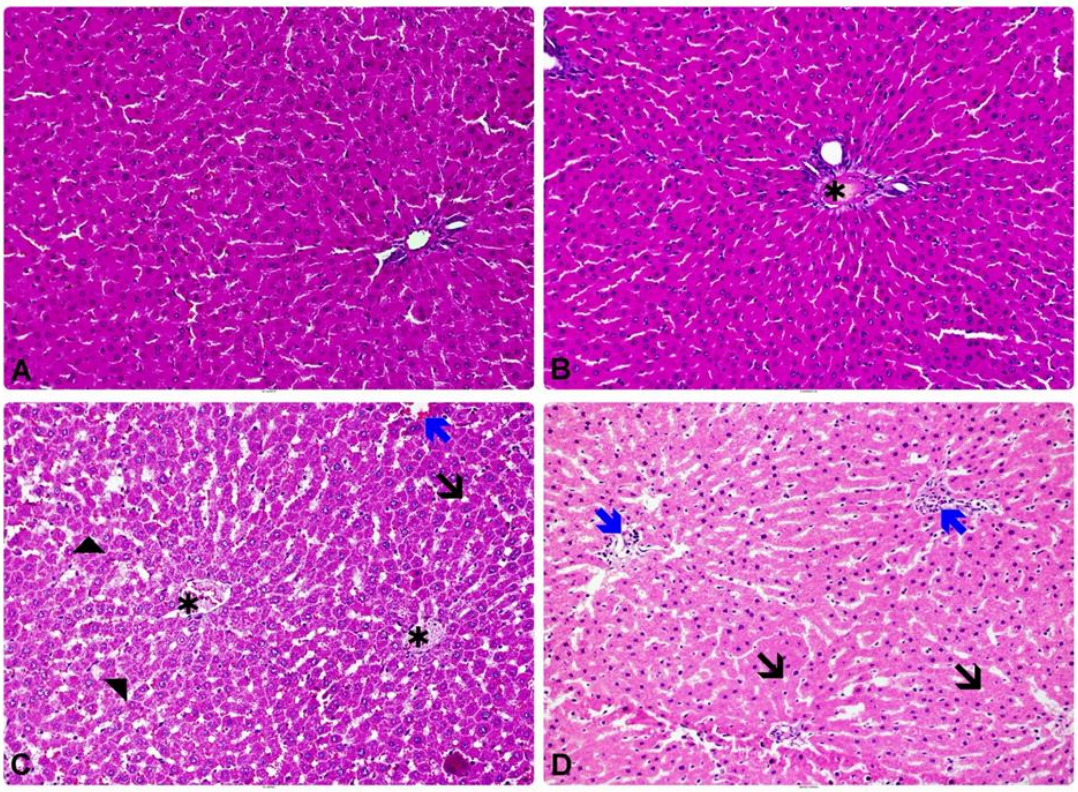


Figure 4: Microscopic images of hematoxylin–eosin (HE)–stained liver sections from the experimental groups.

(A) Control group: regular hepatic cords with normal sinusoidal and portal areas (×100). (B) CRB group: congestion in the portal vein (asterisk, *) (×100). (C) GTC group: mild-to-moderate vacuolar degeneration (black arrow, →), necrotic areas (arrowheads), and widespread congestion in the central vein (asterisk, *) and sinusoids (blue arrow, →), (×200). (D) GTC + CRB group: mild mononuclear cell infiltration in the portal area (blue arrows) and very mild vacuolar degeneration (black arrows, →), (×200).

Discussion

Gentamicin (GTC), an aminoglycoside antibiotic effective against Gram-negative bacterial strains, is also associated with nephrotoxic effect that restrict its clinical use. The present study was designed to assess whether cranberry (CRB) (*Vaccinium macrocarpon*) could mitigate these toxic effects through its antioxidant and anti-apoptotic mechanisms. In the present study, GTC administration caused marked biochemical and structural alterations in renal tissue, confirming its nephrotoxic potential. In this context, remarkable elevations in serum urea, BUN, creatinine, ALT, and AST were observed following GTC administration. Co-treatment with CRB, however, improved these parameters, reflecting partial protection of tissue integrity and function. These findings support the well-known nephrotoxic potential of GTC and demonstrate that CRB supplementation exerts a partial protective effect. Similar results have been reported in previous studies. For example, Shareef et al. (2024) demonstrated that CRB extract significantly improved renal function markers in mice with streptozotocin-induced nephropathy. Treatment with CRB led to marked reductions in serum BUN, creatinine, and uric acid, along with increased antioxidant capacity (Shareef et al., 2024). In another study, Alkhazragy and Alshawi (2023) demonstrated that CRB extract significantly reduced serum creatinine and improved urea levels in mice with cisplatin-induced nephrotoxicity and increased renal GSH (glutathione) content, supporting its role in improving oxidative status and preserving renal function (Alkhazragy and Alshawi, 2023). These findings are consistent with the current study, where CRB co-administration attenuated GTC-induced elevations in renal function markers, suggesting that CRB provides functional protection through antioxidant-mediated mechanisms. Collectively, these results indicate that the antioxidant capacity of CRB contributes to mitigating GTC-induced kidney injury. However, as emphasized in previous reports, the protective effect can vary depending on dose, route, and duration of administration, which should be considered in future experimental designs.

The present research evaluated oxidative balance using TAS, TOS, and OSI determination. GTC administration markedly increased TOS and OSI levels while slightly reducing TAS, confirming its strong pro-oxidant effect. Co-treatment with CRB normalized OSI and maintained TAS values close to the control group, showing that CRB supplementation mitigated GTC-induced oxidative stress. A similar pattern was also reported by Celik and Irak (2018), who demonstrated that GTC administration significantly reduced TAS while markedly increasing TOS and OSI levels in renal tissue. Co-treatment with a plant-derived extract (date extract) restored TAS and significantly decreased TOS and OSI, supporting the idea that antioxidant-rich phytochemicals can counteract GTC-induced oxidative imbalance. These findings parallel the results of the current study, in which CRB co-administration reduced OSI and

partially normalized oxidative status, suggesting that CRB exerts its renoprotective effect primarily by limiting excessive oxidant production (Celik and Irak, 2018).

Evaluation of total and native thiols together with malondialdehyde (MDA) levels provided further insight into the oxidative status of renal tissues. GTC administration led to a marked depletion of total and native thiols and a significant rise in MDA ($p < 0.001$), indicating elevated lipid peroxidation, whereas CRB supplementation reversed these alterations to a considerable extent. The partial restoration of thiol levels and reduction in MDA suggest that CRB helps maintain redox balance and limits oxidative damage. Similar findings were also reported by Edeogu et al. (2020), who demonstrated that gentamicin administration markedly increased renal MDA levels while reducing GSH and antioxidant enzyme activities in rats. Co-treatment with Moringa oleifera seed oil significantly restored antioxidant status and reduced lipid peroxidation, supporting the role of plant-derived antioxidants in mitigating gentamicin-induced oxidative injury. These results parallel our observations that CRB supplementation decreased MDA and partially restored thiol homeostasis (Edeogu et al., 2020).

In the present study, the apoptotic response was evaluated through the Bax and Bcl-2 proteins expression, which regulate the balance between cell death and survival. GTC administration significantly enhanced Bax expression while significantly reducing Bcl-2 levels ($p < 0.001$), causing a pronounced elevation in the Bax/Bcl-2 ratio and indicating enhanced pro-apoptotic signaling. Co-treatment with CRB effectively decreased Bax expression and partially restored Bcl-2 levels, reflecting a modulatory, anti-apoptotic influence. These findings suggest that CRB has a protective role, anti-apoptotic activity against GTC-induced renal injury by modulating the Bax/Bcl-2 balance. Similar anti-apoptotic properties have been described for other plant-derived compounds. Ijaz et al. (2023) reported that administration of the flavonoid glabridin in a GTC-induced nephrotoxicity model increased Bcl-2 levels and reduced Bax expression (Ijaz et al., 2023). Likewise, Abukhalil et al. (2025) showed that galangin attenuated GTC-induced apoptosis by downregulating Bax and caspase-3 while enhancing Bcl-2 expression. These findings parallel the results of the present study and support the conclusion that CRB may mitigate GTC-induced renal apoptosis by modulating pro- and anti-apoptotic regulatory proteins (Abukhalil et al., 2025).

Cyt-c, caspase-3, and caspase-9 levels were evaluated to further elucidate the mitochondrial apoptotic mechanism. GTC administration markedly increased all three markers ($p < 0.001$), indicating activation of the intrinsic apoptosis pathway, whereas CRB co-treatment significantly reduced these elevations, suggesting partial inhibition of apoptosis. CRB alone maintained Cyt-c, caspase-3, and caspase-9 levels similar to the control group values, reflecting its safety and lack of pro-apoptotic effects. Recent studies have similarly reported that natural antioxidants mitigate GTC-induced mitochondrial apoptosis. Laorodphun et al. (2022) also demonstrated that gentamicin-induced nephrotoxicity is strongly mediated by oxidative stress and activation of mitochondrial apoptosis, reflected by increased cytochrome-c and cleaved caspase-3 expression. Pre-treatment with curcumin effectively reversed these alterations, suppressing caspase activation and restoring redox balance. Likewise, Kang et al. (2022) demonstrated that oxymatrine significantly reduced renal caspase-9 and caspase-3 activities, thereby alleviating GTC-induced oxidative and nitrative stress.These findings support the current results showing that CRB reduces Cyt-c and caspase-3/9 levels, suggesting attenuation of gentamicin-triggered intrinsic apoptotic signaling.

Histopathological evaluations supported the biochemical outcomes by clearly demonstrating gentamicin-induced nephrotoxicity. GTC caused characteristic renal structural injury, including tubular degeneration and necrosis, inflammatory cell infiltration, hyaline cast formation, and vascular congestion. CRB co-administration markedly attenuated these lesions, reflected by reduced tubular and glomerular damage and milder inflammation, although full restoration of the architecture was not achieved. CRB alone preserved normal renal morphology, confirming the absence of structural toxicity. These histopathological observations are consistent with recent studies reporting structural protection against GTC-induced renal injury. Ashour et al. (2025) demonstrated that GTC administration caused pronounced cortical and medullary injury, including glomerular shrinkage, tubular necrosis with loss of brush borders, interstitial edema, and epithelial apoptosis. Pretreatment with nicardipine markedly preserved renal architecture, reducing tubular and glomerular damage. These findings parallel the present findings, where CRB co-administration substantially ameliorated tubular necrosis, inflammatory infiltration, and vascular congestion, suggesting that CRB exerts nephroprotective effects through preservation of renal tissue morphology (Ashour et al., 2025).

Histopathological evaluation of hepatic tissues revealed findings parallel to those observed in the kidney. GTC administration resulted in pronounced hepatic damage, including vacuolar degeneration, multifocal hepatocellular necrosis, portal inflammatory infiltration, and vascular congestion, all indicating severe hepatotoxicity. Co-treatment with CRB notably ameliorated these structural alterations, as reflected by reduced vacuolar degeneration, milder inflammation, and preservation of lobular architecture, although full histological recovery was not achieved. CRB alone maintained nearly normal hepatic morphology, demonstrating its safety and absence of hepatotoxic effects. These observations confirm that CRB provides partial protection against GTC-induced hepatic injury, likely through its antioxidant and anti-inflammatory mechanisms that limit cellular degeneration and inflammatory infiltration. Recent evidence supports these findings. Althobaiti et al. (2024) reported that gentamicin administration caused marked hepatic injury characterized by portal vein congestion, micro- and macrovesicular steatosis, and hepatocellular pyknosis in mice. Co-treatment with Artemisia annua extract substantially ameliorated these lesions and preserved hepatic architecture. These observations align with the present study, in which CRB co-

administration reduced vacuolar degeneration, inflammation, and congestion, suggesting that phytochemical-rich agents may mitigate gentamicin-induced hepatotoxicity through antioxidant and anti-inflammatory mechanisms (Althobaiti et al., 2024).

Conclusion

In this study, CRB's protective effect (*Vaccinium macrocarpon*) against GTC-induced nephrotoxicity was investigated in rats. Biochemical analyses revealed that CRB supplementation ameliorated the GTC-induced increases in serum BUN, urea, and creatinine levels, reduced oxidative stress index (OSI), and enhanced antioxidant defense by elevating total and native thiol levels. CRB also attenuated lipid peroxidation and partially restored redox homeostasis. Histopathological and apoptotic evaluations supported these biochemical findings, showing reduced tubular degeneration, decreased inflammatory cell infiltration, and partial normalization of Bax/Bcl-2 and caspase signaling. Although CRB could not completely prevent renal injury, it significantly mitigated GTC-induced oxidative and apoptotic damage. These findings suggest that CRB exerts a partial renoprotective effect mainly through its antioxidant, anti-inflammatory, and anti-apoptotic mechanisms. Further investigations are warranted to clarify dose-dependent effects, molecular pathways, and long-term efficacy of CRB in preventing aminoglycoside-induced renal toxicity.

Conflict of Interest Statement

The authors report no conflict of interest concerning the publication of this study.

Acknowledgment

This study was supported by the Scientific Research Projects Coordination Unit of Kırıkkale University (Project no: 2024/145).

Ethical Approval

This study was approved by the Kırıkkale University Animal Experiments Local Ethics Committee (23.10.2024, 2024/08, 29 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

Funding

This study was supported by the Scientific Research Projects Coordination Unit of Kırıkkale University, (Project Number: 2024/145).

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Author Contributions

Motivation / Concept: EG, YŞ.
Design: EG, YŞ
Control/Supervision: EG, YŞ, EB.
Data Collection and / or Processing: MK, MBT, EK, YŞ.
Analysis and / or Interpretation: MK,EG, MBT, EK, YŞ, EB
Literature Review: MK, EG.
Writing the Article: MK, EG.
Critical Review: EG, EK, MBT, YŞ, EB.

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