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Research Article

Protective Effects of Quercetin Against Cyclophosphamide-Induced Hepatic and Renal Injury: Histopathological and Immunohistochemical Evaluation of HSP70

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Abstract

Objective: *Cyclophosphamide is a widely used alkylating agent in the treatment of various malignancies and autoimmune disorders; however, its clinical application is limited by its toxic effects on several organs, particularly the liver and kidneys. This study aimed to investigate the effects of cyclophosphamide-induced toxicity on histopathological alterations and HSP70 immunoreactivity in liver and kidney tissues, as well as to evaluate the potential protective effect of quercetin.*
Methods: *A total of 28 female Wistar albino rats weighing 250–350 g were randomly allocated into four experimental groups. At the end of the experimental period, liver and kidney tissues were collected, processed using routine histological procedures, and embedded in paraffin. Histopathological changes were evaluated using hematoxylin and eosin (H&E) staining, while HSP70 immunoreactivity was assessed by immunohistochemical analysis.*
Results: *Severe histopathological alterations were observed in the group III, whereas these changes were markedly attenuated in the group IV. In addition, HSP70 immunoreactivity was markedly reduced in the liver and kidney tissues of the group III compared with the group I. In contrast, HSP70 immunoreactivity in the group IV was comparable to that observed in the group I.*
Conclusion: *These results suggest that HSP70 may serve as a potential biomarker for the assessment of cyclophosphamide-induced hepatic and renal injury. Furthermore, quercetin appears to exert a protective effect against cyclophosphamide-induced tissue damage by preserving the cellular stress response, as evidenced by the maintenance of HSP70 immunoreactivity.*

Key Words: *Cyclophosphamide, Hepatorenal Toxicity, HSP70, Quercetin*

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Introduction

Spinal Cyclophosphamide (CP) is a chemotherapeutic agent frequently used in the treatment of many malignant and autoimmune diseases. It also possesses alkylating properties. Following administration, CP is metabolized in the liver by the cytochrome P450 enzyme system to its active metabolite, 4-hydroxycyclophosphamide. This metabolite is subsequently converted into phosphoramide mustard and acrolein, which are responsible for both the therapeutic efficacy and the toxic side effects of CP (1-3).

Despite its broad clinical application, the therapeutic use of CP is limited by its dose-dependent toxicity, particularly in the liver and kidneys. CP-induced hepatotoxicity has been associated with hepatocellular injury, sinusoidal obstruction syndrome (veno-occlusive disease), and significant elevations in serum liver enzyme levels (4). High-dose and long-term CP exposure causes severe histopathological changes in the liver and kidney, including cellular degeneration, necrosis, and inflammatory cell infiltration (5). In addition, oxidative stress has been identified as one of the major mechanisms underlying CP-induced nephrotoxicity (6). Previous studies have demonstrated that enhancement of the antioxidant defense system attenuates CP-induced cytotoxicity by suppressing oxidative damage and inflammatory cytokine production, thereby protecting against tissue injury in various organs, including the lungs and kidneys (7).

Quercetin is a flavonoid belonging to the polyphenol family and occurs naturally (8). It is a potent antioxidant. Additionally, it possesses anti-inflammatory and anti-aging properties. For this reason, it has been used in numerous studies. Quercetin exerts its antioxidant activity primarily through free radical scavenging and the maintenance of cellular redox homeostasis (9). Owing to these biological properties, quercetin has been widely studied as a potential therapeutic agent for various pathological conditions, including cancer, diabetes, obesity, neurodegenerative disorders, and other oxidative stress-related diseases (10).

Heat shock proteins (HSPs) constitute a highly conserved family of molecular chaperones that play essential roles in maintaining cellular homeostasis and protecting cells against physiological and pathological stress (11). Among them, HSP70, also known as the HSPA family, is one of the most extensively studied stress-inducible proteins and is involved in numerous biological processes under both physiological and pathological conditions (12). The HSP70 family comprises 13

homologous genes encoding proteins localized in various intracellular compartments, including the cytoplasm, mitochondria, lysosomes, and nucleus (13). In addition to its cytoprotective functions, dysregulated HSP70 expression has been associated with several types of cancer, and its overexpression is frequently correlated with poor prognosis (14).

Although the protective effects of quercetin against cyclophosphamide-induced organ toxicity have been demonstrated in previous studies, limited information is available regarding its effects on HSP70 immunoreactivity in hepatic and renal tissues. Therefore, the present study was designed to investigate the effects of quercetin on HSP70 immunoreactivity and histopathological alterations in the liver and kidneys following cyclophosphamide-induced toxicity. We hypothesized that quercetin may attenuate cyclophosphamide-induced tissue injury by preserving HSP70 immunoreactivity and maintaining the cellular stress response. Our results may contribute to understanding the molecular mechanisms underlying cyclophosphamide-induced liver and kidney damage. Furthermore, they may provide additional evidence supporting the potential value of HSP70 as a biomarker for tissue damage and of quercetin as a promising therapeutic agent for preventing cyclophosphamide-induced organ toxicity.

Material and Methods

Ethical approval was obtained from the Kafkas University Ethics Committee (Ethics Council Number: KAU-HADYEK/2025-253). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Twenty-eight female *Wistar albino* rats weighing 250–350 g were used in the study. The animals were housed under standard laboratory conditions at 22 ± 2°C, with a 12-h light/12-h dark cycle, and had free access to standard laboratory chow and tap water throughout the experimental period. The rats were randomly assigned to four experimental groups (n = 7 per group):

Group I (control): No experimental procedure was performed on the rats.

Group II (quercetin): Rats were administered quercetin (50 mg/kg/day) by oral gavage for five consecutive days (15).

Group III (cyclophosphamide): Rats received cyclophosphamide (100 mg/kg/day) via intraperitoneal (i.p.) injection for five consecutive days (16).

Group IV (quercetin + cyclophosphamide): Quercetin (50 mg/kg/day, oral gavage) administration was initiated 72 h before the first cyclophosphamide injection (3 days pretreatment + 5 days concomitant) and

continued once daily throughout the experimental period. Cyclophosphamide (100 mg/kg/day, i.p.) was administered concomitantly with quercetin for five consecutive days. The 72-h pretreatment period was selected to allow quercetin to be administered before the onset of cyclophosphamide-induced oxidative injury and to establish its potential antioxidant and cytoprotective effects prior to repeated cyclophosphamide exposure. This approach was based on previous experimental evidence showing that oral quercetin pretreatment can enhance antioxidant defenses and attenuate oxidative tissue injury in rats. (15, 16).

Tissue collection

At the end of the study, the rats were euthanized following intramuscular administration of ketamine hydrochloride (75 mg/kg) and xylazine (15 mg/kg). The tissues were collected and preserved in 10% formalin for histopathological and immunohistochemical analyses. The tissues underwent routine histological processing and were subsequently embedded in paraffin blocks.

Histopathological examination

Serial sections (5 μm thick) were cut from the paraffin-embedded tissue blocks and stained with hematoxylin and eosin (H&E) for histopathological evaluation. Six randomly selected microscopic fields were examined from each tissue section (ZEISS Primostar 3 light microscope /Carl Zeiss Microscopy GmbH, Jena, Germany). Each histopathological parameter was assessed based on the overall findings observed across these six fields, and a single semi-quantitative score reflecting the overall histopathological condition of the section was assigned. In the liver, histopathological evaluation included degeneration of the central vein, disruption of the hepatic cords, cytoplasmic lipid vacuolization in hepatocytes, hepatocellular degeneration, and the presence of apoptotic cell clusters (Table 1).

Table 1. Semiquantitative histopathological scores of liver tissue.

Histopathological Parameters	Group I	Group II	Group III	Group IV
Central vein degeneration	0	0	2	0
Structural disruption of hepatic cords	0	0	3	1
Hepatocellular lipid vacuolization	0	0	3	0
Hepatocellular degeneration	0	0	3	1
Apoptotic cell clusters	0	0	2	1

Scoring criteria: 0 = no histopathological lesion, 1 = mild lesion, 2 = moderate lesion and 3 = severe lesion.

In the kidney, the evaluated parameters included dilation and disruption of Bowman's space, structural alterations in the renal tubules and blood vessels, hemorrhagic areas in the cortex and medulla, and the presence of apoptotic regions (Table 2). Each histopathological parameter was scored using a semiquantitative grading system as follows: 0 = no histopathological lesion, 1 = mild lesion, 2 = moderate lesion, and 3 = severe lesion. Histopathological evaluation and scoring were performed independently by two investigators who were blinded to the experimental groups.

Table 2. Semiquantitative histopathological scores of kidney tissue.

Histopathological Parameters	Group I	Group II	Group III	Group IV
Dilatation and disruption of Bowman's space	0	0	2	1
Structural alterations in renal tubules and blood vessels	0	0	3	0
Hemorrhagic foci in the renal cortex	0	0	2	0
Hemorrhagic foci in the renal medulla	0	0	2	0
Apoptotic areas	0	0	3	0

Scoring criteria: 0 = no histopathological lesion, 1 = mild lesion, 2 = moderate lesion and 3 = severe lesion.

Immunohistochemical analysis

Sections (5 μm thick) mounted on chrome alum-gelatin-coated slides were processed using the streptavidin–biotin–peroxidase immunohistochemical method. Throughout the procedure, phosphate-buffered saline (PBS; 0.1 M, pH 7.2) was used for all washing steps. The prepared sections were incubated in a 3% hydrogen peroxide (H_2O_2) solution for 15 min. For antigen retrieval, the sections were heated in citrate buffer in a microwave oven at maximum power for 10 min. The sections were incubated with Large Volume Ultra V Block (Thermo Scientific, LOT: PHLT811) for 10 min. Subsequently, the sections were incubated overnight at 4°C with the primary antibody against HSP70 (Santa Cruz, sc-32239, dilution, 1/100). Sections were sequentially incubated with Biotinylated Goat Anti-Polyvalent secondary antibody (Thermo Scientific, LOT: PHLT811) and Streptavidin Peroxidase solution (Thermo Scientific, LOT: PHLT811) for 15 min each at room temperature. Immunoreactivity was visualized using 3,3′-diaminobenzidine tetrahydrochloride (DAB) substrate (Thermo Scientific, LOT: HD53495), followed by counterstaining with Modified Gill III hematoxylin (Merck 105174). Histological and immunohistochemical sections were examined and photographed using a ZEISS Primostar 3

light microscope (Carl Zeiss Microscopy GmbH, Jena, Germany). Negative control sections were processed using the same protocol, except that the primary antibody was omitted and replaced with PBS to verify the specificity of the immunostaining. HSP70 immunoreactivity was evaluated in hepatocytes and bile duct epithelial cells in the liver and in the glomeruli, proximal tubules, distal tubules, and collecting ducts in the kidney. These anatomical compartments were evaluated separately to characterize the distribution and intensity of HSP70 immunoreactivity in the liver and kidney. These anatomical regions were evaluated separately for HSP70 immunoreactivity. Staining intensity was graded as follows: 0 = no staining, 1 = weak staining, 2 = moderate staining, and 3 = strong staining.

Statistical analysis

Semi-quantitative immunohistochemical scores were expressed as median (min–max)**.** Statistical comparisons among the four experimental groups were performed using the Kruskal–Wallis test**.** Statistical analyses were performed using Python (version 3.12; Python Software Foundation, Wilmington, DE, USA) with the SciPy statistical package**.** A p value < 0.05 was considered statistically significant.

Results

Histopathological results

In Group I and Group II, liver and kidney tissues exhibited normal histological structure. Based on the semiquantitative histopathological scoring system, liver tissues from the group III exhibited moderate central vein degeneration, severe structural disruption of the hepatic cords (cords of Remak), marked hepatocellular lipid vacuolization, severe hepatocellular degeneration, and moderate apoptotic cell clusters (Figure 1, Table 1).

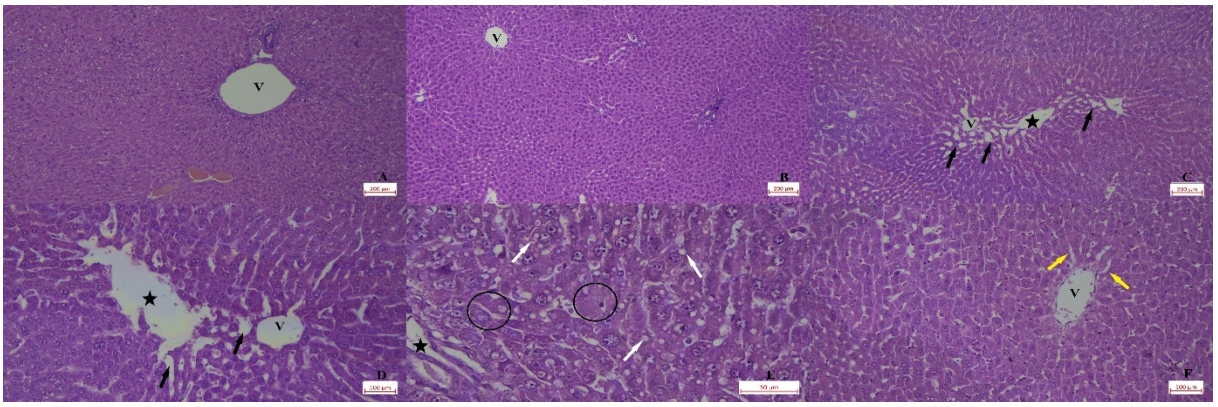


Figure 1. Representative histopathological results in liver tissue. Group I (A), Group II (B), Group III (C, D, E), and Group IV (F) groups. Central vein (V); structural disruption of hepatic cords (arrows); hepatocellular degeneration (black asterisks); apoptotic cell clusters (circles); lipid vacuoles in hepatocytes (white arrows); and nearly normal hepatic cord architecture (yellow arrows). Scale bars are shown in each image.

According to the semiquantitative histopathological scores, kidney tissues from the group III showed moderate dilation and disruption of Bowman's space, severe structural alterations in the renal tubules and blood vessels, moderate hemorrhagic foci in both the renal cortex and medulla, and severe apoptotic areas (Figure 2, Table 2).

In the group IV, degenerative changes, hemorrhagic foci, and apoptotic areas were observed in both liver and kidney tissues. Histopathological lesions were generally less severe than those observed in the group III, and the overall tissue architecture appeared better preserved (Tables 1 and 2, Figure 1 and 2).

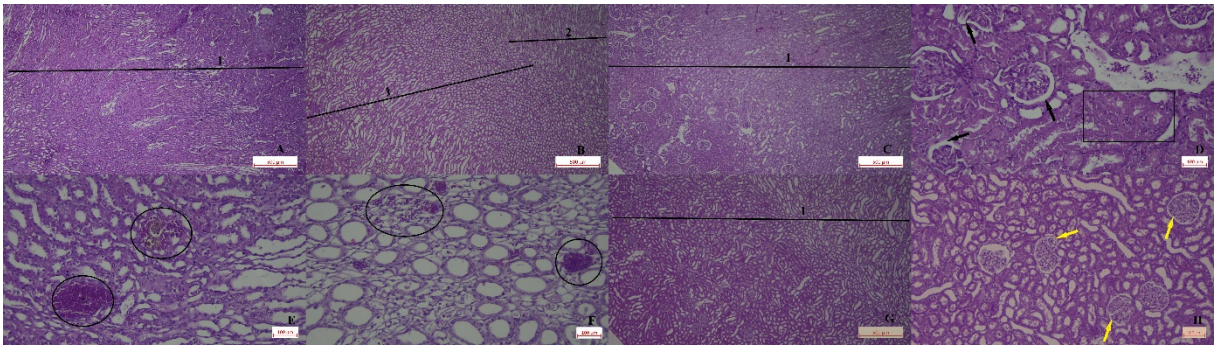


Figure 2. Representative histopathological results in kidney tissue. Group I (A), Group II (B), Group III (C–F), and Group IV (G, H) groups. Cortex (1), medulla (2), widening and disruption of Bowman's space (black arrows), tubular degeneration and apoptotic cell clusters (squares), hemorrhagic foci in the cortex and medulla (circles), and nearly normal Bowman's space (yellow arrows). Scale bars are shown in each image.

Immunohistochemical results

Representative HSP70 immunohistochemical staining patterns in liver tissue are shown in Figure 3, and the semi-quantitative immunohistochemical scores are presented in Table 3.

Strong HSP70 immunoreactivity was observed in hepatocytes and bile duct epithelial cells in the group I, group II and group IV. In contrast, the group III exhibited moderate HSP70 immunoreactivity in both hepatocytes and bile duct epithelial cells. HSP70 immunoreactivity in Group II was comparable to that observed in the control group (Group I), indicating that quercetin administration alone did not alter basal HSP70 immunoreactivity in the liver. Semi-quantitative analysis demonstrated statistically significant differences in HSP70 immunoreactivity among the experimental groups in hepatocytes (p <0.01) and bile duct epithelial cells (p <0.01).

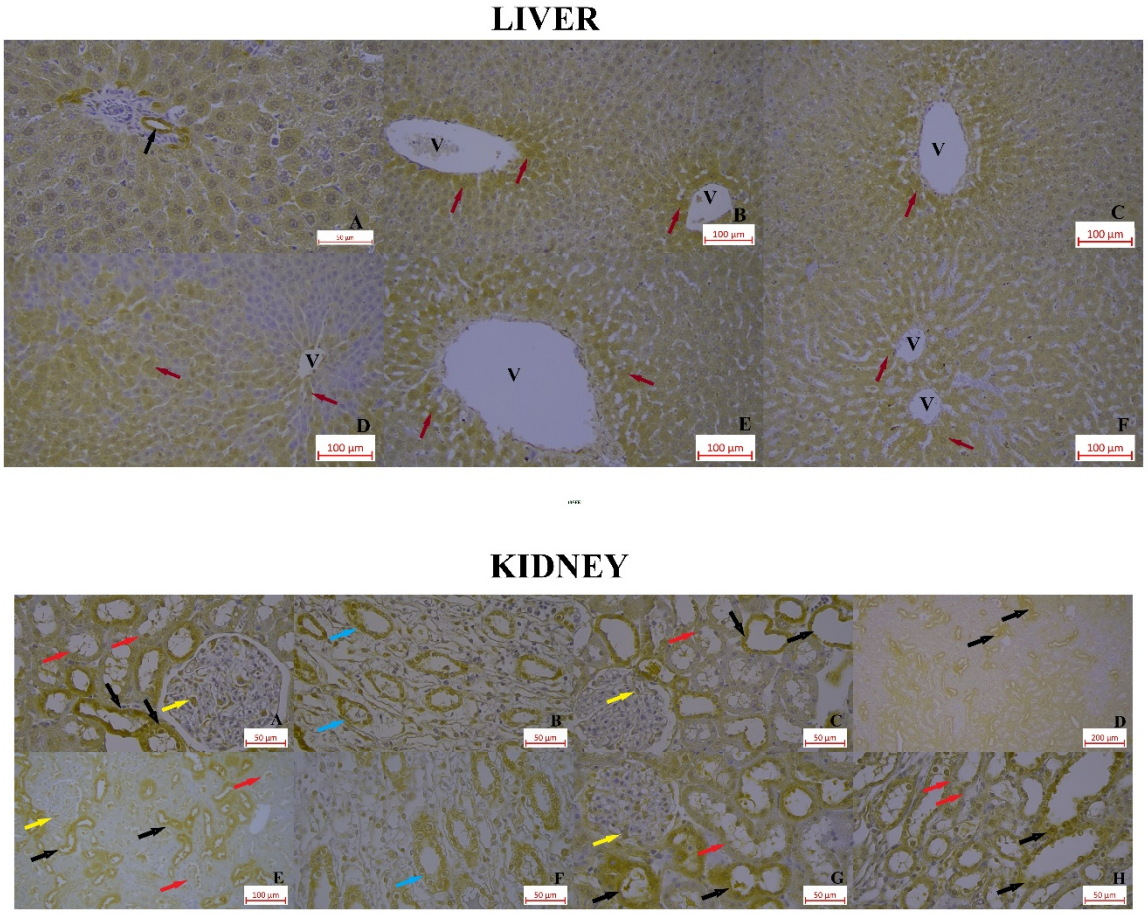


Figure 3. HSP70 immunoreactivity in liver and kidney tissues. LIVER; Group I (A, B), Group II (C), Group III (D), and Group IV (E, F) groups. Bile duct epithelium (black arrows), central vein (V), and hepatocytes (red arrows). KIDNEY; Group I (A, B), Group II (C), Group III (D–F), and Group IV (G, H) groups. Glomeruli (yellow arrows), distal tubules (black arrows), proximal tubules (red arrows), and collecting ducts (blue arrows). Scale bars are shown in each image.

Table 3. Semi-quantitative analysis of HSP70 immunoreactivity in liver tissue.

Tissue region	Group I Median (Min–Max)	Group II Median (Min–Max)	Group III Median (Min–Max)	Group IV Median (Min–Max)	*p* value
Hepatocytes	3 (2–3)	3 (2–3)	2 (2–3)	3 (3–3)	**<0.01**
Bile ducts	3 (2–3)	3 (2–3)	2 (1–2)	3 (2–3)	**<0.01**

Values are presented as median (min–max). *p* values were calculated using the Kruskal–Wallis test.

Representative HSP70 immunohistochemical staining patterns in kidney tissue are shown in Figure 4, and the semi-quantitative immunohistochemical scores are presented in Table 4. Weak HSP70 immunoreactivity was observed in the glomeruli and proximal tubules, moderate immunoreactivity in the collecting ducts, and strong immunoreactivity in the distal tubules of the group I, group II and group IV. In the group III, weak immunoreactivity was observed in the glomeruli, moderate immunoreactivity in the distal tubules and collecting ducts, whereas no HSP70 immunoreactivity was detected in the proximal tubules. HSP70 immunoreactivity in Group II was comparable to that in the control group (Group I), indicating that quercetin administration alone did not alter basal HSP70 immunoreactivity in the kidney. Semi-quantitative analysis revealed statistically significant differences among the experimental groups in the proximal tubules (p <0.01) and distal tubules (p <0.01). No statistically significant differences were detected in the glomeruli (p = 0.78) or collecting ducts (p = 0.91).

Table 4. Semi-quantitative analysis of HSP70 immunoreactivity in kidney tissue.

Tissue region	Group I Median (Min–Max)	Group II Median (Min–Max)	Group III Median (Min–Max)	Group IV Median (Min–Max)	*p* value
Glomeruli	1 (1–2)	1 (1–2)	1 (1–1)	1 (1–2)	0.78
Proximal tubules	1 (1–2)	1 (1–1)	0 (0–1)	1 (1–2)	**<0.01**
Distal tubules	3 (3–3)	3 (2–3)	2 (2–2)	3 (2–3)	**<0.01**
Collecting ducts	2 (1–2)	2 (1–2)	2 (1–2)	2 (1–2)	0.91

Values are presented as median (min–max). p values were calculated using the Kruskal–Wallis test.

Discussion

Oxidative stress is considered one of the principal mechanisms underlying cyclophosphamide-induced organ toxicity. Cyclophosphamide causes lipid peroxidation, DNA damage, and protein oxidation by increasing the production of reactive oxygen species (ROS). These oxidative alterations contribute to cellular dysfunction and tissue injury in multiple organs. In the kidney, oxidative stress primarily affects the glomerular and tubular compartments, resulting in structural damage and impaired renal function, thereby playing a pivotal role in the development of nephrotoxicity (17, 18). Similarly, oxidative stress triggers inflammatory and apoptotic signaling pathways, which are recognized as major contributors to cyclophosphamide-induced hepatotoxicity. Previous studies have demonstrated that cyclophosphamide administration increases hepatic oxidative stress markers, promotes inflammatory responses, impairs antioxidant defense mechanisms,

induces apoptosis and fibrosis, and disrupts hepatocyte integrity. In addition, elevated oxidative stress biomarkers accompanied by suppression of endogenous antioxidant systems have been consistently reported following cyclophosphamide exposure (19, 20). Consistent with these results, the group III in the present study exhibited characteristic histopathological alterations in kidney tissue, including widening of Bowman's space, tubular degeneration, cortical and medullary hemorrhagic foci, and apoptotic cell clusters, all of which support the established mechanisms of cyclophosphamide-induced nephrotoxicity. Likewise, liver tissue from the group III demonstrated apoptotic cell clusters, hepatocellular degeneration, lipid vacuolization, and structural disruption of the hepatic cords, indicating morphological evidence of oxidative stress-mediated hepatic injury.

Quercetin is a naturally occurring flavonoid polyphenol with well-documented antioxidant, anti-inflammatory, and cytoprotective properties. Previous studies have demonstrated that quercetin exerts a wide range of biological activities, including anticancer, anti-inflammatory, and antimicrobial effects. In addition, quercetin has been shown to preserve tissue integrity by modulating inflammatory responses, maintaining mitochondrial function, and suppressing apoptosis-related signaling pathways (21, 22). The potent antioxidant activity of quercetin is largely attributed to its phenolic structure, which enables the scavenging of reactive oxygen species, attenuation of oxidative stress, and preservation of cellular integrity (23). These mechanisms are considered to underlie its protective effects against oxidative stress-induced tissue injury. Consistent with these results, the group IV in the present study exhibited markedly fewer histopathological alterations in both liver and kidney tissues than the group III. Cellular degeneration, hemorrhagic foci, and apoptotic cell clusters were less pronounced, suggesting that quercetin alleviated cyclophosphamide-induced tissue injury and contributed to the preservation of cellular and tissue architecture.

HSP70 is a molecular chaperone that plays a critical role in maintaining cellular homeostasis. It facilitates the refolding of misfolded proteins, prevents oxidative damage, preserves mitochondrial function, and suppresses apoptosis-related signaling pathways. Through these cytoprotective mechanisms, HSP70 enhances cell survival under conditions of cellular stress. In the kidney, HSP70 contributes to the maintenance of mitochondrial function and promotes cell viability following tissue injury. Therefore, alterations in HSP70 expression are widely recognized as reliable indicators of the cellular stress response (24). HSP70 expression is generally considered an early adaptive response to cellular stress and is rapidly upregulated to protect cells against oxidative injury. However, when the intensity or duration of stress exceeds the cellular adaptive capacity, protein quality control mechanisms become impaired, resulting in an insufficient HSP70-mediated cytoprotective response (25, 26). Importantly, in the present study, HSP70 immunoreactivity in the quercetin-only group was comparable to that

observed in the control group in both liver and kidney tissues. This finding suggests that quercetin administration alone did not alter basal HSP70 immunoreactivity under physiological conditions. The absence of an increase in HSP70 immunoreactivity in the quercetin-only group may be related to the stress-dependent nature of HSP70 regulation. Under basal physiological conditions, HSP70 expression is maintained at a level sufficient for normal protein homeostasis, and quercetin may not induce a marked additional HSP70 response in the absence of substantial cellular stress. Rather, the cytoprotective effects of quercetin may become more evident under conditions of oxidative or cellular stress, when enhanced antioxidant and cellular defense mechanisms are required.

Among renal structures, proximal tubules are particularly susceptible to toxic injury because of their high metabolic activity and energy demand. Consequently, these cells are highly vulnerable to oxidative stress, which disrupts cellular repair mechanisms and contributes to the progression of chronic kidney injury (27, 28). In the present study, the HSP70 immunoreactivity observed in the quercetin-only group was comparable to that of the control group, whereas HSP70 immunoreactivity was preserved in the quercetin plus cyclophosphamide group despite cyclophosphamide exposure. This pattern suggests that quercetin did not stimulate HSP70 immunoreactivity under basal physiological conditions but may have contributed to the preservation of the cellular stress response under cyclophosphamide-induced oxidative stress. In particular, the strong HSP70 immunoreactivity observed in hepatocytes and renal tubular cells in the quercetin plus cyclophosphamide group may indicate that quercetin helped maintain cellular defense mechanisms during cyclophosphamide-induced tissue injury. However, because HSP70 expression was evaluated by immunohistochemistry, the present findings demonstrate an association between quercetin treatment and preservation of HSP70 immunoreactivity rather than establishing a direct molecular mechanism (25, 26). In addition, the immunohistochemical findings were consistent with the histopathological observations in the present study. The cyclophosphamide group showed reduced HSP70 immunoreactivity in both liver and kidney tissues, together with more pronounced degenerative, hemorrhagic, and apoptotic changes. In particular, the absence of HSP70 immunoreactivity in renal proximal tubular cells was accompanied by marked tubular and vascular alterations and apoptotic areas. In contrast, the quercetin plus cyclophosphamide group showed stronger HSP70 immunoreactivity and less pronounced histopathological alterations than the cyclophosphamide group. This parallel relationship between HSP70 immunoreactivity and the degree of tissue injury suggests that preservation of HSP70 may be associated with maintenance of cellular integrity during cyclophosphamide-induced injury. Nevertheless, the present findings do not establish a direct causal relationship between HSP70 preservation and the protective effect of

quercetin.

Quercetin has been extensively investigated for its antioxidant and anti-inflammatory properties and has been reported to exert protective effects against pesticide- and heavy metal-induced toxicity. In addition, quercetin reduces mycotoxin-induced damage and plays an important role in protecting cells against oxidative injury (29). The antioxidant activity of quercetin is concentration-dependent, with beneficial effects observed at lower concentrations, whereas excessive antioxidant activity may produce adverse biological effects (30). Previous studies have also demonstrated that quercetin attenuates oxidative damage induced by acrylamide, radiation, cadmium fluoride, and diabetes through the regulation of redox homeostasis and enhancement of endogenous antioxidant defense mechanisms (31-33). These findings indicate that quercetin protects neurons and other tissues against oxidative stress-mediated cellular injury by maintaining the balance between oxidants and antioxidants. In the present study, HSP70 immunoreactivity was largely preserved in the group II and group IV. This observation suggests that the attenuation of oxidative stress by quercetin may have contributed to the maintenance of protein homeostasis and cellular viability, thereby supporting HSP70 expression. In particular, the strong HSP70 immunoreactivity observed in hepatocytes and renal tubular cells indicates that quercetin may enhance cellular defense mechanisms against cyclophosphamide-induced tissue injury.

Conclusion

In conclusion, the present study demonstrated that cyclophosphamide induced marked histopathological alterations in both liver and kidney tissues, accompanied by reduced HSP70 immunoreactivity. Notably, the absence of HSP70 immunoreactivity in the proximal tubules highlights the susceptibility of this renal compartment to cyclophosphamide-induced toxicity. Quercetin administration attenuated histopathological damage and was associated with the preservation of HSP70 immunoreactivity in both liver and kidney tissues. Importantly, quercetin administration alone did not alter HSP70 immunoreactivity compared with the control group, suggesting that its effect on HSP70 may be more relevant under conditions of cellular stress than under basal physiological conditions. Collectively, these results suggest that HSP70 immunoreactivity may serve as a potential biomarker for evaluating cyclophosphamide-induced organ injury and that the protective effects of quercetin may, at least in part, be mediated through the preservation of cellular stress responses and tissue integrity. Further experimental and molecular studies are warranted to clarify the precise mechanisms underlying the interaction between quercetin and HSP70-mediated cytoprotective pathways.

Conflict of Interest

The authors report there are no competing interests to declare.

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Authorship Contributions

Concept: SYA, EKS, Design: SYA, EKS, MM, Data collection and/or processing: KY, İE, MM, Analysis and/or interpretation: SYA, EKS, Literature search: KY, İE, Writing: SYA, Approval: SYA, EKS, KY, İE, MM.

Ethical Approval

Ethical approval was obtained from the Kafkas University. Ethical Council Number KAU-HADYEK/2025-253 permission was taken. In this research the ethical rules of Helsinki Declearation was obeyed.

Statement on the Use of Artificial Intelligence

In this article, artificial intelligence (ChatGPT) was used solely for literature review and statistical analysis.

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