

Investigation of the protective role of fisetin against doxorubicin-induced liver injury in rats

Ömür Gülsüm Deniz 

Department of Histology and Embryology, Faculty of Medicine, Bolu Abant İzzet Baysal University, Bolu, Türkiye

ABSTRACT

Objectives: The aim of the present research was to histopathologically investigate the potentially advantageous effects of the flavonoid fisetin on liver damage induced by the chemotherapeutic drug doxorubicin (DOX) in rats.

Methods: Thirty-five Wistar albino female rats were randomized in five as Control, dimethyl sulfoxide (DMSO, solvent), fisetin (50 mg/kg/day; 7 days i.p.), DOX (single dose, 10 mg/kg; i.p.) and DOX+fisetin (10 mg/kg DOX+50 mg/kg/day fisetin for 7 days). Livers were harvested, fixed in 10% formalin, and processed for histopathology by hematoxylin-eosin staining. Central to these analyses of damage parameters for inflammation, sinusoidal dilatation, and hepatocyte injury were examined histopathologically.

Results: The DOX group had severe hepatocyte degeneration, inflammation, and sinusoidal dilatation. In contrast, the DOX+fisetin group expressed mild sinusoidal dilatation and insignificant inflammatory change. In this context, statistical significance was found between the DOX group and the DOX+fisetin group in terms of hepatocyte degeneration and inflammation ($P<0.01$), and sinusoidal dilatation ($P=0.038$). However, no significant differences were observed in between the Control, fisetin, and DMSO groups ($P=1.000$).

Conclusions: Fisetin conferred substantial histological protection from DOX-induced liver injury in rats. The amelioration may have resulted from the fisetin's antioxidant, anti-inflammatory, and perhaps membrane-stabilizing effects, thus proving its potential as a hepatoprotective agent.

Keywords: Doxorubicin, fisetin, histopathology, liver, rat

Doxorubicin (DOX), one of the first two anthracyclines to be recovered from *Streptomyces peucetius*, was first discovered during the 1960s [1]. It is a typical chemotherapeutic antibiotic that is effective in the therapy of various cancers, for example, breast cancer, leukemia, bronchogenic carcinoma, stomach carcinoma, sarcomas, thyroid carcinomas, and hematologic malignancies [2]. Although DOX has powerful antitumor activities, it is signifi-

cant toxicity in non-malignant cells and therefore restricting its clinical utility [3]. The drug is metabolized by hepatic microsomal enzymes and cytoplasmic reductases, leading to the accumulation of toxic and immunogenic intermediates that contribute to liver injury [4]. Emerging research demonstrates that DOX-caused toxicity is due to several mechanisms that are largely diverse according to the cell type. In tumor cells, DOX mainly triggers DNA damage that leads to the suppres-

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Corresponding author: Ömür Gülsüm Deniz, PhD., Assist. Prof., Phone: +90 374 254 10 00 ext. 6519, E-mail: omur.deniz@ibu.edu.tr, omur.denizomu@gmail.com

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sion of proliferation [5], in non-malignant cells, it promotes oxidative stress and disrupts mitochondrial function through the accumulation of reactive oxygen species (ROS) [6]. Additionally, DOX has been shown to exacerbate oxidative stress by suppressing specific transcription factors [7]. This disruption of redox homeostasis by DOX has been documented in several organs, including the heart [8], kidneys [9], and liver [10, 11].

The liver bears the major role in detoxification through its ability to convert lipophiles into more aqueous compounds that can be easily removed from the body via urine [12]. Drug-induced hepatotoxicity or drug-induced liver injury (DILI) is an acute or chronic reaction to a natural or synthetic chemical [13]. Histopathological evaluation of liver tissue from animal models treated with DOX has revealed significant structural alterations. Studies have documented various hepatic abnormalities, including bile duct hyperplasia, sinusoidal dilation, and central vein congestion [14]. Other findings include hepatocyte vacuolation, nuclear condensation, degeneration of hepatic cords [15], cellular edema, focal necrosis, and disrupted hepatic trabecular organization [16]. Further observations encompass biliary duct proliferation, parenchymal necrosis [17], increased intercellular spaces, mitochondrial vacuolization and swelling [18], as well as lymphocyte infiltration [19]. These different findings reflect the extensive hepatic damage caused by DOX, with both degenerative and inflammatory liver histoarchitectural changes.

Fisetin (3,3',4',7-tetrahydroxyflavone) is a bioactive flavonoid found in numerous fruits and vegetables, such as strawberries, apples, grapes, onions, and cucumbers [20]. Fisetin demonstrates a wide range of pharmacological properties, including potent antioxidant [21], anti-inflammatory, anti-mitotic, and anticancer effects. Naturally found flavonoid fisetin has the potential to scavenge free radicals, control the signal pathways of cell proliferation [22], as well as suppress inflammatory mediators. Remarkably, the anticancer activity of fisetin was noticed to result through various mechanisms involving cell cycle arrest, apoptosis induction [23], as well as inhibition of the signal transduction pathway involving metastasis the spread of cancer cells from the place where they first formed to another part of the body [24, 25]. Fisetin demonstrates a favorable safety profile based

on preclinical toxicity assessments. Acute toxicity studies employing animal models reported no adverse effects, with no observed changes in body weight or clinical signs of toxicity such as gastrointestinal disturbances (e.g., diarrhea), neurological symptoms (e.g., restlessness or coma), respiratory dysfunction, as well as motor abnormality. These findings suggest a wide therapeutic window for fisetin administration [26]. Histopathological studies revealed no detrimental effects in the heart, lungs, kidneys, liver, stomach, intestines, spleen, or reproductive organs [27-30].

DOX, although highly effective in fighting cancer, is a major cause of liver damage that is produced because of oxidative stress and inflammation. While a few protective drug studies have undertaken work on finding ways to restore tissue damage following the use of drug therapy. Despite rising interest in the therapeutic prospects of natural flavonoids, relatively little investigation has specifically addressed the histological effect of fisetin when used in the context of DOX elicited liver injury. Most current studies concentrate on the biochemical and antioxidant activities of the fisetin, with insufficient attention to its ability to preserve tissue arrangement at the tissue level. Also, DOX is widely known to generate intense hepatotoxicity through mechanisms involving oxidative stress, inflammation, and mitochondrial injury. However, the histological impact of potential protective compounds like fisetin remains a largely untapped area of research. Bridging this gap holds significant promise for understanding the structural and functional recovery of hepatic tissue following chemotherapeutic therapy.

While there is a growing recognition of natural flavonoids as powerful therapeutic agents, the research on the role of fisetin as the main agent in the restoration of liver tissue structure after the injury caused by DOX is poorly defined. Different scientific papers emphasize fisetin's antioxidant and anti-inflammatory effects, but few address its capacity to preserve histological integrity and reserve liver tissue architecture intactness after DOX-induced stress. The study aims to fill that gap by investigating the potential of fisetin to maintain the integrity of liver tissue structure in rats that have been given DOX. Thus, the current study aims to investigate the histologic protective activity of fisetin against DOX-induced liver injury in rats. In this context, it was concentrated on histopathological changes to reveal more about the role of fisetin

in mitigating liver damage caused by chemotherapy, which is an important part of the process of enhancing the patient's condition.

METHODS

Experimental Design

All experimental procedures carried out in our study were approved by the Bolu Abant İzzet Baysal University (BAİBÜ) Animal Experiments Local Ethics Committee with the decision numbered 2025/24 and dated 16/04/2025. Following the ethics committee approval, 35 adults female *Wistar albino* rats weighing 200-250 grams were obtained from the BAİBÜ Experimental Animals Application and Research Center. The animals were maintained in an environment with a stable temperature ($24\pm 2^{\circ}\text{C}$) and humidity ($55\pm 15\%$) at the BAİBÜ Experimental Animal Application and Research Center until the commencement of the investigation and throughout its duration. The rats were granted unrestricted access to regular pellet food and tap water prior to the experiment. In our study, 35 female rats were used. The subjects were randomly divided into 5 groups, and the experimental procedure was started. The groups and the procedures to be applied are given below in order:

-Control group: 1 ml/kg saline was given intraperitoneally (i.p.) for 7 days.

-Fisetin group: 50 mg/kg fisetin dissolved in 0.1% Dimethyl sulfoxide (DMSO) [31] was injected i.p. for 7 days [32].

-DOX group: 10 mg/kg single dose of DOX [33] was given as i.p., and rats were sacrificed after 7 days.

-DOX+Fisetin group: 10 mg/kg was given as a single dose of DOX i.p. and 50 mg/kg fisetin was injected as i.p. for 7 days.

-DMSO (solvent) group: 1 ml/kg 0.1% DMSO was administered i.p. for 7 days.

Histopathological Evaluation

At the end of the experimental period, the rats were anesthetized by injecting a 5/1 mixture of ketamine (50 mg/kg)/xylazine (10 mg/kg) i.p. and their liver tissues were removed. The tissues were fixed in 10% buffered neutral formalin solution for two weeks for histopathological evaluation and then embedded in paraffin blocks with routine histological follow-up

(consisting of alcohol, xylene and paraffin series). From the obtained paraffin blocks, 4 μm thick sections were taken using a systematic random sampling method for histological procedures using a Thermo Shandon Finesse ME microtome device (Thermo Fisher Scientific, Cheshire, UK) and stained with Hematoxylin-eosin staining. Histopathological evaluation was performed using a Carl Zeiss AxioCam ERc5 model digital camera-attached microscope from the sections, and 5 different areas representing the relevant area were determined for each section taken from each animal by systematic random sampling in the relevant liver tissue, and parameters such as inflammation, hepatocyte damage, and sinusoidal dilatation were taken into consideration and evaluated under a light microscope. These morphological parameters were scored semi quantitatively between 0 and 3. If there was no change, the score was 0, if there was mild damage, the score was 1, if there was moderate damage, the score was 2, and if there was severe damage, the score was 3 [34]. All sampled were coded for the sake of all analyses would be done blindly.

Statistical Analysis

SPSS version 21.0 analysis program was used in the statistical analysis of the numerical data belonging to the groups obtained from our study. The conformity of the data to the normal distribution assumption was evaluated with the Shapiro-Wilk test. In comparing continuous variables specified by measurement, data conforming to normal distribution were evaluated using One-Way ANOVA and Bonferroni tests as post-hoc tests. In the statistical evaluations, the difference was accepted to be statistically significant when $P < 0.05$.

RESULTS

In the study, histopathological analysis was performed on sections stained with hematoxylin-eosin. When the groups were evaluated, it was seen that there was no histological difference between the control, fisetin and DMSO groups. In the relevant groups, the liver tissue contained euchromatic (Normal, uncondensed chromatin pattern), centrally located, and polygonal hepatocytes with regular cell borders around the central vein located in the center of the lobule, which had a

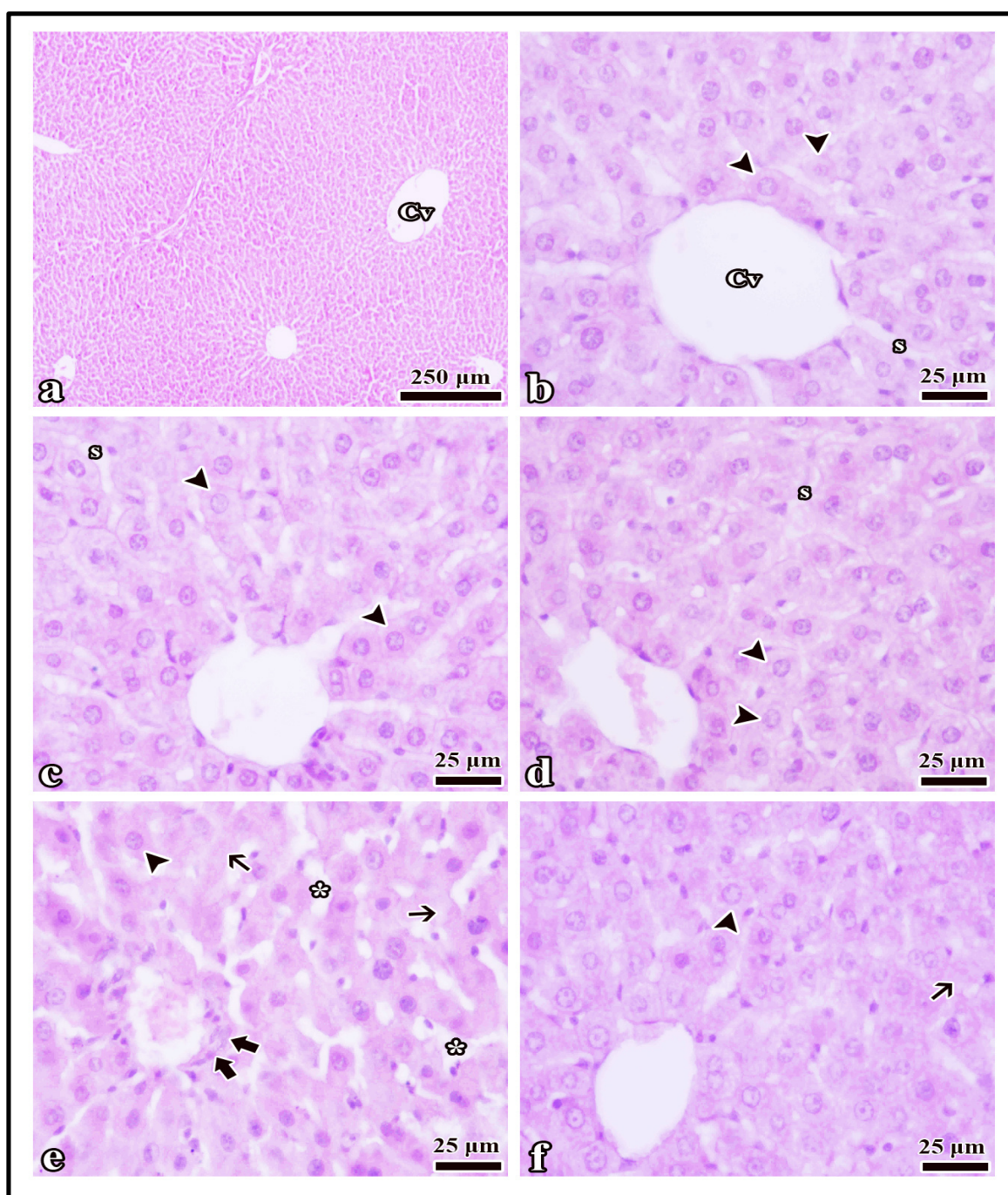


Fig. 1. The micrograph shows histological images of all groups. (a) Panoramic view of the liver. (b) Control group, (c) Fisetin group, (d) DMSO group, (e) DOX group, (f) DOX+fisetin group. Arrowhead; healthy hepatocyte, thin arrow; damaged hepatocyte, thick arrow; inflammation, (*); dilated sinusoid, Cv; central vein, s; sinusoid. (b), (c), and (d) were in normal morphology. (f) indicated that fisetin may minimize the detrimental effects of DOX (e). Hematoxylin-eosin staining $\times 40$.

small amount of stroma showing normal morphology cell cords extending radially from there and sinusoids (Vascular channels between hepatocyte cords) between these cords. The portal area, which includes the hepatic artery, portal vein, and bile duct, called the

portal triad at the corners of the hepatic lobule, appeared healthy. In the DOX group, sinusoidal dilatation, inflammation, and degeneration-related shape changes in hepatocytes in different parts of the parenchyma were remarkable. In addition, a morpho-

logical appearance similar to the control group was observed in the DOX+fisetin group, except for mild sinusoidal dilatation. No infiltration or congestion findings were observed in the relevant group (Fig. 1). When all these findings were evaluated in light of this; no statistically significant difference was observed between the control, fisetin and DMSO groups ($P=1.000$), while a statistical significance was found between these groups and the DOX and DOX+Fisetin groups ($P<0.01$). In addition, the significance between DOX and DOX+Fisetin groups was found to be at $P<0.01$ (Hepatocyte damage, inflammation) (Figs. 2a and c, respectively) and $P=0.038$ (sinusoidal dilatation) (Fig. 2b) levels.

DISCUSSION

Liver is the main organ that detoxes the detoxification of chemicals, excretion of waste in bile and performing various vital functions like synthesis, storage and redistribution of carbohydrates, lipids and proteins [35]. Like many chemotherapeutic agents, DOX is metabolized in the liver and after that it is excreted in bile. In case the liver is not functioning properly, the elimination of DOX becomes slower and delayed, and it starts to accumulate in the body which in turn leads to adverse effects on the other tissues by increased systemic toxicity. DOX is extensively metabolized by the liver to its major metabolite, doxorubicinol, along with

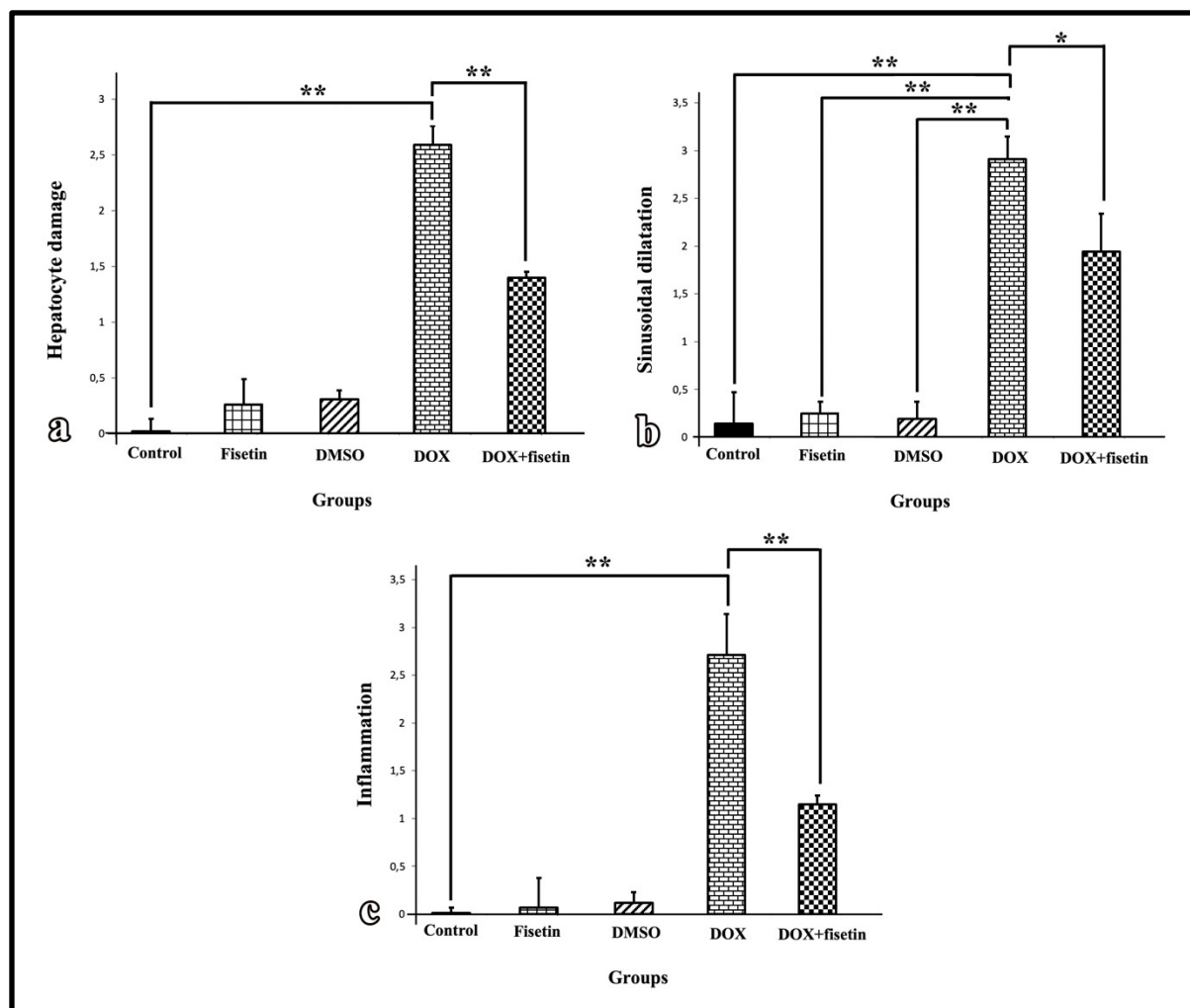


Fig. 2. Graph indicates the hepatocyte damage (a), sinusoidal dilatation (b) and inflammation (c) parameters belonging to all groups. Statistically significant differences at $P<0.01$ are shown by (**), while significant differences at $P<0.05$ are demonstrated by (*). The data obtained in the relevant graph show that DOX caused adverse effects on the liver, while fisetin minimized these effects.

several cytotoxic aglycone metabolites [36, 37]. For these drugs to be metabolized, they must first pass through the hepatic sinusoidal endothelium to enter the space of Disse and gain access to the hepatocytes [38]. Hence, understanding the mechanisms of hepatic drug clearance and how DOX induces liver toxicity is critical for developing new therapeutic drugs that can reduce these toxic effects. This underscores the urgent need for therapeutic agents that can mitigate such liver damage without compromising DOX's antitumor efficacy.

Multiple studies have documented the histological consequences of DOX-induced liver toxicity. They indicate that DOX cause expanded perisinusoidal spaces, edematous, containing material of damaged hepatocyte cytoplasm, and connective tissue proliferation and apoptosis [10, 11, 39]. Similarly, a study on male rats by El-Sayyad *et al.* [40], the most notable histological deficiencies in rats treated with doxorubicin at a dose of 1 mg/kg body weight on alternate days for 20 days included dissolution of hepatic cords appearing as hollow vacuoles organized by lines of necrotic hepatocytes. Focal inflammatory cells or necrotic tissues were observed in hepatic tissues. A higher tendency for liver fibrosis, characterized by multiple foci of focal cellular granulomatous lesions, was also a feature of DOX-treated rats. Our current findings corroborate these results in which DOX-induced hepatic toxicity was evidenced in the current histopathological investigation by the DOX group exhibited a liver that was severely damaged along with the signs of sinusoidal dilatation, hepatocyte arrangement that was disrupted, cellular degeneration, and inflammation. Another histopathology analysis of liver showed disintegration of histological architecture, there was excessive degeneration and dissolution of hepatic cords, focal inflammation and necrotic areas and increased apoptosis, vacuolations of hepatocytes with round to oval and elliptical shaped nuclei and sinusoidal dilatation, hemorrhage and congested portal vein and mononuclear cells infiltration in rats given intraperitoneally 10 mg/kg DOX [41]. Mansouri *et al.* [17] showed that the animals receiving DOX revealed mild to moderate pathological changes in the liver tissue such as degeneration of hepatocyte cords, dilatation of sinusoids, vacuolation of hepatocytes and condensation of nuclei which confirmed by our data.

Furthermore, DOX administration altered hepatic biochemical composition, resulted in a significant reduction in the levels of glycogen and nucleic acids and a significant rise in the quantity of lipids and proteins in the liver [42]. Other models, such as DOX-induced nonalcoholic steatohepatitis, revealed similar patterns of periportal necrosis and fatty degeneration, reinforcing the hepatotoxicity of DOX [43].

In the present study, histopathological evaluation of the hematoxylin-eosin-stained liver sections observed that there were no statistically significant differences among the control, fisetin, and DMSO groups ($P>0.05$). Related groups were all free of structural liver anomalies and they had normal liver histology that comprised of euchromatic hepatocytes with a central nucleus, sinusoidal structures without any damage, and healthy portal triads. Significant differences were observed between these groups and both the DOX and DOX+fisetin groups ($P<0.01$). Fisetin demonstrates notable hepatoprotective properties by preserving liver tissue structure and supporting normal hepatic function. Its antioxidant and anti-inflammatory actions contribute to reducing cellular stress and preventing liver damage under various pathological conditions.

Fisetin has been shown to exert multiple beneficial effects on liver metabolism and structure. One study reported that fisetin disrupts hepatic mitochondrial energy metabolism by inhibiting oxidative phosphorylation, reducing the mitochondrial NADH/NAD⁺ redox ratio, suppressing ketogenesis, and enhancing oleate oxidation indicating a shift toward a more oxidized mitochondrial state and a predominantly prooxidant effect on liver mitochondria [44]. Additional studies have demonstrated its protective role in alcoholic liver disease by accelerating ethanol clearance and reducing oxidative stress [45]. Furthermore, dietary fisetin has been found to prevent hepatic steatosis through the modulation of lipid metabolism genes and miR-378 expression in mice [46]. A review showed that fisetin's had protective mechanisms in organ systems treated with chemotherapy, including modulation of oxidative stress and anti-inflammatory effects. While liver-specific data is limited, it validates the fisetin's ability to reduce DOX-induced toxicity [47]. These findings support the potential of fisetin as a multifunctional compound with both regulatory and protective roles in liver health and consistent with our findings that

show that fisetin alone group rats exhibited no structural liver anomalies, maintaining normal hepatic architecture with euchromatic hepatocytes featuring central nuclei, intact sinusoidal structures, and healthy portal triads, thereby reinforcing fisetin's hepatoprotective potential under toxic conditions.

Our study found that coadministration of fisetin with DOX significantly improved liver histology compared to the DOX-only group of ($P < 0.05$). Liver architecture improved significantly in the DOX+fisetin group. This preserved lobular structure, sinusoidal dilatation and inflammation were reduced. Liver architecture improved significantly compared to the control group, suggesting significant hepatoprotection with fisetin. This hepatoprotective efficacy is likely due to fisetin's well-documented antioxidant and membrane-stabilizing properties that counteract DOX-induced oxidative injury. In methotrexate (MTX)-induced hepatotoxicity, fisetin administration effectively reversed all these changes, which points toward its hepatoprotective potential. Histopathology also indicated that fisetin diminished MTX-induced hepatocyte necrosis, fibrosis, and inflammatory infiltration. By inhibiting oxidative stress, inflammation, and apoptotic pathways, fisetin can emerge as a promising drug that leads to counteract MTX-induced hepatotoxicity [31]. Previous studies warrant these findings, providing evidence that administration of fisetin significantly reduces hepatic inflammation, preserves hepatocyte morphology, and stabilizes cell membranes in chemical-induced liver injury models [32, 36, 48]. These findings establish that fisetin enhances MTX-induced liver injury in rats through multimodal modulation of biochemical and histological damages markers [31]. Similarly, in thioacetamide-induced hepatic fibrosis, fisetin reduced the progress of histologic hepatic fibroplasia and diminished hepatic expression of α -smooth muscle actin (α -SMA) and cyclin D1 [49]. These consistent findings across various hepatotoxicity models reinforce fisetin's protective role in maintaining hepatic tissue integrity.

Fisetin administration exhibited remarkable hepatoprotective advantages by considerably lowering immunohistochemical and biochemical changes well-documented. DOX-induced oxidative stress in the liver triggers molecular events that contribute to hepatotoxicity. Hepatic enzymes metabolize DOX, gen-

erating ROS and reactive metabolites [50]. These harmful byproducts disrupt critical cellular processes, including DNA replication, transcription, and protein synthesis. The resulting DNA damage and impaired gene expression compromise hepatocyte regeneration, reducing the liver's ability to repair itself and maintain normal function [8]. Fisetin scavenges ROS, inhibits lipid peroxidation, and modulates cellular signaling pathways involved in inflammation and apoptosis [36]. Khairani and Ilyas's [50] review highlights the protective role of flavonoids, including that of fisetin, inhibiting DOX-induced oxidative liver injury. Flavonoids possess hepatoprotection through inhibiting oxidative stress through free radical formation and lipid peroxidation prevention. Flavonoids also safeguard mitochondrial integrity and reduce hepatocyte apoptosis. According to such mechanisms, flavonoids are candidate therapeutic drugs that can inhibit DOX-induced liver injury. Fisetin has been shown in Li *et al.* [51] study alleviate liver damage, reduce cell apoptosis, and minimize oxidative stress caused by hepatic ischemia-reperfusion injury, primarily through activation of the Nrf2/HO-1 signaling pathway. Fisetin protects against acetaminophen (APAP)-induced liver damage in mice and attenuates APAP-induced cytotoxicity in L-02 cells by promoting autophagy and inhibiting inflammasome activation [52]. A study investigating the protective effects of fisetin against subacute and chronic co-exposure to arsenic and fluoride in mice revealed that oxidative stress and arsenic burden in essential organs, including the liver, kidneys, and heart, were dose-dependently reduced by treatment with fisetin. These findings led to the conclusion that antioxidant activity and arsenic burden reduction by fisetin are responsible for its protective effect [53].

Strengths and Limitations

The present study has several strengths, including the use of a randomized controlled animal model, blinded histopathological evaluation, and comprehensive semi-quantitative scoring, which enhance the validity of our findings. Furthermore, the originality of investigating fisetin in DOX-induced hepatotoxicity highlights its translational potential. Nevertheless, some limitations should be acknowledged. It primarily focuses on histopathological evaluation. For future research, we recommend integrating corresponding bio-

chemical or molecular markers (e.g., serum liver enzymes, oxidative stress biomarkers, and cytokine levels). Additionally, the duration of fisetin treatment, dose-response relationships, and long-term outcomes were not assessed.

CONCLUSION

Our findings show that fisetin preserved hepatic lobular architecture, reduced sinusoidal dilatation, and prevented inflammation and hepatocyte degeneration caused by DOX administration. These protective actions appear to be exerted via the potent antioxidant, anti-inflammatory, and membrane stabilizing activities of fisetin. These findings indicate the potential of fisetin as a promising adjunctive therapeutic agent in the treatment of chemotherapy-induced hepatotoxicity. Nevertheless, more research remains to be conducted to identify its molecular targets and its long-term safety profile in human models.

In conclusion, this study suggests that fisetin is a promising natural compound with the ability to preserve liver histoarchitecture and reduce DOX-induced hepatic injury. Given its favorable safety profile and multi-targeted mechanisms of action, fisetin may offer a novel adjunctive therapeutic strategy for preventing chemotherapy-associated liver toxicity. These promising results support further exploration of fisetin as a potential adjunctive therapeutic agent to minimize chemotherapy associated with liver injury.

Ethics Approval and Consent to Participate

This study was approved by the Bolu Abant İzzet Baysal University animal experiments local ethics committee under decision no 2025/24 and date 16/04/2025. All experimental procedures were conducted in compliance with the U.K. Animals (Scientific Procedures) Act, 1986, the EU Directive 2010/63/EU for animal experimentation, and the National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on re-

quest from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: ÖGD; Study Design: ÖGD; Supervision: ÖGD; Funding: ÖGD; Materials: ÖGD; Data Collection and/or Processing: ÖGD; Statistical Analysis and/or Data Interpretation: ÖGD; Literature Review: ÖGD; Manuscript Preparation: ÖGD; and Critical Review: ÖGD.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

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Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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