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Safflower seed oil extracts mitigate oxidative stress, inflammation, and apoptosis in cyclophosphamide-induced multi-organ injury

Esma OCAK* [1], Atilla OCAK [2], Arzu KÖSE [3], Betül PEKER CENGİZ [4], Adnan AYHANCİ [2]
ORCID: 0000-0002-9085-4151; 0000-0003-1149-1194; 0000-0003-0675-8958; 0000-0002-2503-7446; 0000-0003-4866-9814

[1] Eskişehir Osmangazi University, ARUM, 26040 Eskişehir, Türkiye
[2] Eskişehir Osmangazi University, Department of Biology, 26040 Eskişehir, Türkiye
[3] Republic of Türkiye Ministry of Agriculture and Forestry, Transitional Zone Agricultural Research Institute Directorate, Head of Oilseed Crops Unit, 26040 Eskişehir, Türkiye
[4] Eskişehir Yunus Emre State Hospital, Department of Pathology, 26080 Eskişehir, Türkiye

Abstract

Purpose: Cyclophosphamide (CP) is an antimitotic agent widely used in cancer treatment; however, its use may cause oxidative stress, inflammation, apoptosis, and subsequent tissue damage in multiple organs. This study aimed to evaluate the protective and therapeutic effects of *Carthamus tinctorius* L. (safflower) seed oil extracts against CP-induced liver, kidney, lung, heart, and bladder injury.

Method: Healthy Wistar Albino male rats weighing 160±200 g and aged 5-6 weeks were used in the study. The animals were divided into nine experimental groups, and different doses of CP and *Carthamus tinctorius* seed oil extracts were administered. Biochemical analyses, histopathological examinations, and antioxidant parameters were evaluated to determine the extent of tissue injury and the protective effects of the extracts.

Findings: CP administration caused significant histopathological alterations in the liver, kidney, lung, heart, and bladder tissues. CP exposure was also associated with increased oxidative stress parameters (NT, TT, SDS) and elevated apoptotic markers, including Bax and caspase-3. These findings were consistent with biochemical alterations, including changes in ALT, AST, ALP, LDH, BUN, and creatinine levels. In contrast, treatment with Carthamus tinctorius seed oil extracts markedly reduced tissue damage and enhanced antioxidant defense mechanisms.

Conclusion: The findings indicate that Carthamus tinctorius seed oil extracts may exert protective effects against CP-induced multi-organ injury by attenuating oxidative stress, inflammation, and apoptosis. Therefore, safflower seed oil may have potential as a complementary pharmacological agent for the prevention and management of cyclophosphamide-induced organ toxicity.

Keywords: *Carthamus tinctorius*, cyclophosphamide, inflammation, apoptosis, multi-organ injury

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Aspir yağı ekstraktları siklofosfamid kaynaklı çoklu organ hasarında oksidatif stres, inflamasyon ve apoptozu azaltır

Özet

Amaç: iklofosfamid (CP), kanser tedavisinde yaygın olarak kullanılan antimitotik bir ajandır; ancak kullanımı birçok organda oksidatif stres, inflamasyon ve apoptoza bağlı doku hasarına neden olabilmektedir. Bu çalışmada, *Carthamus tinctorius* L. (aspir) tohum yağı ekstraktlarının CP kaynaklı karaciğer, böbrek, akciğer, kalp ve mesane hasarı üzerindeki koruyucu ve terapötik etkilerinin değerlendirilmesi amaçlandı.

* *Corresponding author*: Tel.: +95419262680; Fax.: +902222393750; E-mail: aayhanci@ogu.edu.tr
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Metod: Çalışmada 160±200 g ağırlığında ve 5–6 haftalık sağlıklı Wistar Albino erkek sıçanlar kullanıldı. Hayvanlar dokuz deney grubuna ayrıldı ve gruplara farklı dozlarda CP ve *Carthamus tinctorius* tohum yağı ekstraktları uygulandı. Doku hasarının ve ekstraktların koruyucu etkilerinin belirlenmesi amacıyla biyokimyasal analizler, histopatolojik incelemeler ve antioksidan parametreler değerlendirildi.
Bulgular: CP uygulanan gruplarda karaciğer, böbrek, akciğer, kalp ve mesane dokularında belirgin histopatolojik değişiklikler gözlendi. CP uygulaması oksidatif stres parametrelerinde (NT, TT, SDS) ve Bax ile kaspaz-3 gibi apoptotik belirteçlerde artışa neden oldu. Bu bulgular ALT, AST, ALP, LDH, BUN ve kreatinin düzeylerindeki biyokimyasal değişikliklerle de uyumluydu. Buna karşılık, Carthamus tinctorius tohum yağı ekstraktları ile tedavi edilen gruplarda doku hasarında belirgin azalma ve antioksidan savunma mekanizmalarında iyileşme gözlendi.
Sonuç: Elde edilen bulgular, Carthamus tinctorius tohum yağı ekstraktlarının oksidatif stres, inflamasyon ve apoptozu azaltarak CP kaynaklı çoklu organ hasarına karşı koruyucu etki gösterebileceğini ortaya koymaktadır. Bu nedenle aspir tohum yağının siklofosfamid kaynaklı organ toksisitesinin önlenmesi ve yönetiminde tamamlayıcı bir farmakolojik ajan olarak potansiyel taşıdığı düşünülmektedir.

Anahtar kelimeler: *Carthamus tinctorius*, siklofosfamid, inflamasyon, apoptoz, çoklu organ hasarı

1. Introduction

Cancer is one of the most common causes of death worldwide [1]. Currently, chemotherapy and radiotherapy are the most effective and comprehensive approaches for cancer treatment [2, 3]. However, the adverse effects associated with chemotherapy and radiotherapy, including oral mucositis, gastrointestinal toxicity, hepatotoxicity, nephrotoxicity, hematopoietic system damage, cardiotoxicity, and neurotoxicity, complicate their clinical use. These side effects often reduce the quality of life in cancer patients and can lead to the termination of treatment [4]. Therefore, it is crucial to develop effective management strategies to counteract the side effects caused by chemotherapy and radiotherapy.

Cyclophosphamide (CP) is a widely used drug in clinical practice that works by inhibiting cell proliferation through altering DNA synthesis. However, the optimal clinical benefit of CP is limited due to its multi-organ toxicity CP, a cytotoxic drug, is metabolized in the liver to form the metabolites phosphoramide mustard (PAM) and acrolein (ACR). The anti-neoplastic effects of CP are associated with PAM. PAM is thought to inhibit cell division by binding to DNA, mediating CP's immunosuppressive and anti-tumor effects. On the other hand, the toxic effects of CP are believed to be related to its active metabolite, ACR. ACR interferes with the tissue antioxidant (AO) defense system, leading to a high formation of free radicals. Raw extracts, fractions enriched with bioactive compounds, and pure compounds derived from plants are natural products that are involved in the prevention and treatment of various cancers. Clinical and preclinical studies have shown that some natural products can reduce the adverse side effects of chemotherapy and radiotherapy [5, 6].

Carthamus tinctorius (CT; safflower), a branched, herbaceous, and perennial plant from the Asteraceae family, is widely found in Central Asia, Southwest Asia, and the Mediterranean regions. The genus *Carthamus* includes approximately 19 species of which only a few are used as food additives and dyes. Moreover, it has shown therapeutic efficacy in treating diseases such as anti-tumor, antioxidant, inhibition of melanin production, anti-hypertension, gynecological disorders, osteoporosis, and cerebrovascular diseases. The biological activities of safflower have been associated with various compound classes isolated from different morphological parts, including flavonoids, alkaloids, steroids, lignans, and phenolic compounds [7].

Although previous studies have investigated the biological activities and anticancer properties of *Carthamus* species, limited information is available regarding the chemical composition and comparative protective effects of *Carthamus tinctorius* (CT) genotypes (Yenice, Dinçer, and Balcı) developed by the Eskişehir Geçit Kuşağı Agricultural Research Institute against cyclophosphamide (CP)-induced multi-organ damage. The main novelty of this study lies in the comparative evaluation of these three safflower genotypes, which differ in their fatty acid compositions, and their potential protective effects on multiple organs. The experimental design was established by considering the doses previously reported for other *Carthamus* genotype [8, 9]. Therefore, this study aimed to investigate and compare the protective effects of Yenice, Dinçer, and Balcı seed oil extracts against CP-induced liver, kidney, lung, heart, and bladder damage through biochemical, histopathological, and immunohistochemical analyses.

2. Materials and methods

Yenice variety is the first registered spineless safflower variety in Türkiye. It features deep red flowers and white seeds. The weight of 1000 seeds vary between 38 and 40 grams. The oil content of the seeds in this variety is between 26% and 28%, while the protein content is around 13%.

Dinçer variety's plant height is around 90-110 cm. The flower color is orange, and the seed color is white. This variety has a moderately thorny structure, with a 1000-seed weight ranging from 45 to 49 grams, a hull percentage of 46%, a protein content of 14%, and an oil content ranging from 28 to 32%.

Balcı variety is characterized by yellow flowers and thorns, has cream-colored seeds. Plant height ranging from 55 to 70 cm. It is an early maturing, summer-type variety that is highly resistant to drought. The 1000-seed weight is between 40-48 g. Oil percentage ranging from 38 to 41%. The crude cellulose content is between 26-27%.

2.1. Fatty Acid Composition of Safflower Seeds Oil

The Yenice, Dinçer, and Balcı seeds were ground using a grinder to prepare them for extraction. 10 g of each variety were weighed and placed into the Soxhlet cartridge. Extraction was performed using petroleum ether in a Soxhlet apparatus for 6 hours. The solvent was removed at 45°C in a rotary evaporator. The obtained seed oils were separately labeled and stored in a dark environment at +4°C. The safflower seed oil extracts obtained using the Soxhlet extraction method were used for the animal experiments, with the Yenice, Dinçer, and Balcı preparations administered orally at a dose of 200 mg/kg to the corresponding experimental groups. The oils obtained from the seeds through extraction were converted into methyl esters and analyzed by GC (gas chromatography). Fatty acids were converted into methyl esters using a BF3 (boron trifluoride) catalyst dissolved in methanol, and this process was followed by GC analysis. The GC analysis was performed using an Agilent 5975 GC-FID system. Helium was used as the carrier gas (0.8 mL/min) and an Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used. The GC oven temperature was held at 60°C for 10 minutes and then programmed to increase to 220 °C at a rate of 4°C/min, where it was held constant for 10 minutes. Afterward, it was programmed to increase to 240°C at a rate of 1°C/min. The split ratio was set to 40:1. The injector temperature was set to 250 °C, and the detector (FID) was set to 300 °C for the analysis [10].

2.2. Experimental Animals

In this study, a total of 63 healthy male albino Wistar rats weighing 160–200 g and aged 5-6 weeks were used. The animals were randomly divided into nine experimental groups, with seven rats in each group (n = 7)[11]. All experimental animals were obtained from Eskişehir Osmangazi University Medical and Surgical Experimental Animals Application and Research Center (ESOGÜ-TICAM) and kept in rooms with 12:12 light/dark artificial lighting, automatically controlled temperature (22±2) and humidity (45-50%) throughout the experiment. They were given standard pellet feed and tap water as needed. All experimental procedures were approved by the Eskişehir Osmangazi University Local Ethics Committee for Animal Experiments.

2.3. Chemicals and Injections

Cyclophosphamide (CP) and all other chemicals were obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). To prepare a 150 mg/kg CP injection, 500 mg of CP was dissolved in 25 mL of double-distilled water [12]. Carthamus tinctorius (CT) was purchased from the Oilseeds Unit of the Transitional Region Agricultural Research Institute Directorate, affiliated with the Ministry of Agriculture and Forestry of the Republic of Turkey, located in Eskişehir/Türkiye. Chemical injections were administered intraperitoneally (i.p.) using sterile disposable syringes. All animals were weighed before the first injection and before euthanasia to determine their weight, and the chemicals were administered at the determined doses.

2.4. Safflower Seed Oil Extraction Method

Seeds (Yenice, Balcı and Dinçer) were dried in an oven at 40 ˚C for 4 hours using a ventilated oven to a moisture content of approximately 5%, and then ground with a Waring mixer. The dried safflower seeds were subjected to hydro-distillation for 3 hours in a Clevenger-type apparatus using 2 L of deionized water. The essential oil was collected and dried with anhydrous sodium sulfate (Na_2SO_4) [10]. The obtained extract was stored in a dark environment at 4°C. The essential oil obtained by hydrodistillation was distinct from the Soxhlet-extracted seed oil preparations used in the animal experiments. The sample was prepared for GC/MS analysis by dilution with n-hexane. In the instrument using helium as the carrier gas, 2.00 µL of sample was injected at a flow rate of 1 mL/min. The column oven program was set to 80 °C for 1 minute, then to 250 ˚C for 20 minutes with 5 °C/min increments. The ionization source was analyzed at 200 °C. At the end of the GC-MS analysis, the results were interpreted according to the library data. The components were analyzed using an LC-MS instrument. The mobile phase was prepared as H_2O + 0.1% formic acid (A) and CH_3CN + 0.1% formic acid (B). As a gradient program: 0.2 ml/min, 0-50 min, 0-40% B flow rate; 51–56 min, 100% B; Then, 100-0% B was applied within 5 minutes. Mass spectrometry was performed in negative ion mode using an electrospray ionization (ESI) source. Sample injection was done as 2 µL. Results were interpreted according to library data at the end of LC-MS analysis.

2.5. Induction and Determination of Multiple Organ Damage with Cyclophosphamide (CP)

150 mg/kg CP was injected intraperitoneally as a single dose to groups 2, 7, 8, and 9. 14 days after CP injection, bone marrow aspiration was performed to determine whether the expected damage had occurred in the experimental animals. To determine CP-induced multiple organ damage, aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), and creatinine (CR) levels were measured biochemically in serum samples using a HITACHI-917 auto analyzer with commercial kits from Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany [6]. In addition, serum thiol-disulfide (SDS) profile tests were performed with an improved clinical chemical analyzer (Roche, Cobas 501, Mannheim, Germany) to reveal the developing oxidative stress (OS) and the possible antioxidant (AO) activity of safflower extract. Serum total thiol (TT) (-SH + S-S) and natural thiol (NT) (-SH) amounts were calculated as a matching test. Thiol disulfide (S-S) amounts were determined by subtracting the NT concentration from the TT concentration. SDS/NT, SDS/TT and NT/TT ratios were measured [13].

The experimental animals were divided into nine groups, each consisting of seven rats (n = 7). Cyclophosphamide (150 mg/kg) was administered as a single intraperitoneal injection to groups 2, 7, 8, and 9. Carthamus tinctorius seed oil extracts (Yenice, Dinçer, and Balcı) were administered orally at a dose of 200 mg/kg to the corresponding treatment groups.

Experimental Groups and Injections

1. Group: Control group: Each animal received an injection of 0.5 mL of saline.
2. Group: Each animal received a single intraperitoneal injection of 150 mg/kg CP.
3. Group: Each animal received an injection of 0.5 mL of 95% ethanol.
4. Group: Each animal received 200 mg/kg CT-Yenice extract orally.
5. Group: Each animal received 200 mg/kg CT-Dinçer extract orally.
6. Group: Each animal received 200 mg/kg CT-Balcı extract orally.
7. Group: Each animal received 200 mg/kg CT-Yenice extract orally and 150 mg/kg CP intraperitoneally.
8. Group: Each animal received 200 mg/kg CT-Dinçer extract orally and 150 mg/kg CP intraperitoneally.
9. Group: Each animal received 200 mg/kg CT-Balcı extract orally and 150 mg/kg CP intraperitoneally.

2.6. Anesthesia and Surgical Procedures

All experimental studies were performed in a sterile environment and using sterile surgical instruments. Intracardiac blood was collected from animals anesthetized with ketamine/xylazine. The collected blood was centrifuged for 10 minutes at 3000 rpm using an Eppendorf Centrifuge 5804 R model device to obtain serums. Serum samples transferred to polyethylene tubes were kept in a -80 ºC deep freezer for biochemical analyses.

2.7. GC-MS Analysis

Oils obtained from seeds by extraction were converted to their methyl esters and analyzed by GC. Fatty acids were converted to methyl esters with a catalyst of BF3 (boron trifluoride) dissolved in methanol, and then analyzed by GC. GC analysis was performed with an Agilent 5975 GC-FID system. An Innowax FSC column (60m x 0.25mm, 0.25 µm film thickness) with helium (0.8 mL/min) as the carrier gas was used. The GC oven temperature was held at 60 °C for 10 minutes, then programmed to 220 °C at a rate of 4 °C/min and held at 220 °C for another 10 minutes. Subsequently, it was programmed to 240 °C at a rate of 1 °C/min. The partition ratio was set to 40:1. The injector temperature was set to 250 °C and the detector (FID) to 300 °C for analysis.

2.8. Histological and Immunohistochemical Studies

The liver, kidneys, lungs, hearts, and bladders of the autopsied rats were carefully dissected and rinsed with saline for histological and immunohistochemical examinations (apoptotic markers such as caspase-3, Bcl-2, and Bax). The organs were then placed in containers containing 10% formalin. Three hours after removal, the fixatives were changed to ensure better fixation. After routine histological tissue processing, 5-6 µm-thick sections were obtained from paraffin-embedded tissue blocks, stained with hematoxylin and eosin (H&E), examined under a light microscope, and photographed using an Olympus PM10 AD. For immunohistochemical tissue processing, the sections were deparaffinized and rehydrated. Subsequently, antigen retrieval was performed by heating in a microwave oven at 700 W for 10 minutes in citrate buffer (pH 6.0). After blocking the sections with 3 mL/L H_2O_2 and porcine serum, the sections were incubated with primary antibodies against Bcl-2 (Abcam, Cambridge, UK), Bax (Abcam), and caspase-3 (Thermo, Waltham, MA, USA) at appropriate dilutions, followed by detection using the UltraVision Quantum Detection System (Thermo Scientific)[14]. Immunohistochemical staining for Bax, Bcl-2, and caspase-3 was evaluated qualitatively under a light microscope based on staining intensity, distribution, and cellular localization. Representative microscopic images

were used to illustrate the observed staining patterns. No quantitative immunohistochemical scoring or statistical comparison of staining intensity was performed.

2.9. Statistical Assessments

Based on the frequency distribution analysis performed, it was determined that the numerical data did not follow a normal distribution. Therefore, numerical data were first analyzed using the nonparametric Kruskal–Wallis test to assess overall differences among the experimental groups. The Mann–Whitney U test was subsequently used for pairwise comparisons between groups. Results were expressed as median (minimum–maximum), and $p < 0.05$ was considered statistically significant. Statistical analyses were applied to numerical experimental data; qualitative histopathological and immunohistochemical observations were described without statistical comparisons.

3. Results

3.1. GC-MS Analysis Results

In the present study, the linoleic acid percentages in Balcı, Dinçer and Yenice varieties were determined as 71.5%, 78.1% and 79.3%, respectively. These values confirm that safflower oil is a good source of polyunsaturated fatty acids (PUFA). When examined in terms of oleic acid (C18:1, cis) content, it was determined as 17.5% in the Balcı variety and 11.7% and 11.6% in the Dinçer and Yenice varieties. In terms of saturated fatty acids, the levels of palmitic acid (C16:0) and stearic acid (C18:0) were determined. In our study, palmitic acid levels ranged from 5.6–7.3%, while the stearic acid level ranged from 2.3–2.6%. However, the trans fatty acid methyl elaidate (elaic acid, C18:1, trans) level was quite low. In addition, the arachidic acid (C20:0) level was determined as 0.4% in all varieties (Table 1). Overall, this study shows that the fatty acid composition of safflower oil is characterized by a high linoleic acid content, with linoleic acid being the predominant fatty acid in all three varieties.

Table 1. Fatty acid composition of Balcı, Dinçer and Yenice seeds.

Ingredient	**Balcı**	**Dinçer**	**Yenice**
Methyl palmitate	7,3	6,7	5,6
Methyl stearate	2,6	2,3	2,4
Methyl oleate	17,5	11,7	11,6
Methyl elaidate	0,7	0,8	0,7
Methyl linoleate	**71,5**	**78,1**	**79,3**
Methyl arachidate	0,4	0,4	0,4
Total %	100	100	100

3.2. Histological Results

In this study, the potential protective and therapeutic effects of *Carthamus tinctorius* (CT) seed oil extracts against cyclophosphamide (CP)-induced liver, kidney, lung, heart, and bladder damage were evaluated histopathologically and immunohistochemically. Histological examinations showed that CP administration caused significant tissue damage in liver and kidney tissues, including marked necrosis, degeneration, congestion, and mononuclear inflammatory cell infiltration. In the CP group, extensive areas of necrosis in the liver parenchyma, hepatocyte degeneration, and microvacuolar steatosis were observed, while glomerular structural abnormalities, tubular degeneration, and vascular congestion were detected in the kidney parenchyma ($p < 0.001$). In contrast, in the groups treated with CT seed oil extract along with CP (Yenice, Dinçer, and Balcı), liver and kidney tissue damage were lessened, the inflammatory response decreased, and tissue integrity was better preserved ($p < 0.001$). In all three groups given Yenice, Dinçer, and Balcı extracts, it was determined that CP-induced tissue damage was significantly reduced, portal area expansion was decreased, inflammatory cell infiltration was attenuated, and vascular structures were better preserved (Figure 1).

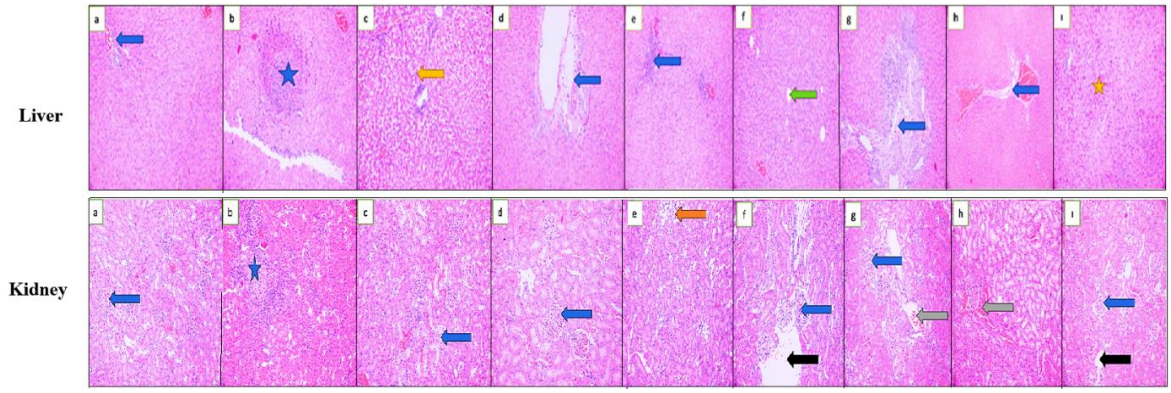

Figure 1. Representative hematoxylin and eosin (H&E)-stained sections of liver and kidney tissues from the experimental groups. (a) Control, (b) CP, (c) ethanol, (d) CT-Yenice, (e) CT-Dinçer, (f) CT-Balcı, (g) CT-Yenice + CP, (h) CT-Dinçer + CP, and (i) CT-Balcı + CP. Original magnification: ×200

In the group treated with only 150 mg/kg CP, increased proapoptotic Bax and Caspase-3 immunoreactivity and significantly decreased antiapoptotic Bcl-2 immunoreactivity were observed ($p < 0.001$); this indicates that CP increases tissue damage and consequently apoptotic activity in liver tissue. In the control group, and especially in the groups treated with only 200 mg/kg CT oil extract, strong antiapoptotic Bcl-2 immunoreactivity and mild preapoptotic Bax and Caspase-3 staining were observed ($p < 0.001$). This indicates that the liver is in a state of balanced apoptotic regulation. The results support the possibility that safflower oil, with its antioxidant and anti-apoptotic properties, may be a hepatoprotective agent (Figure 2, Table 1).

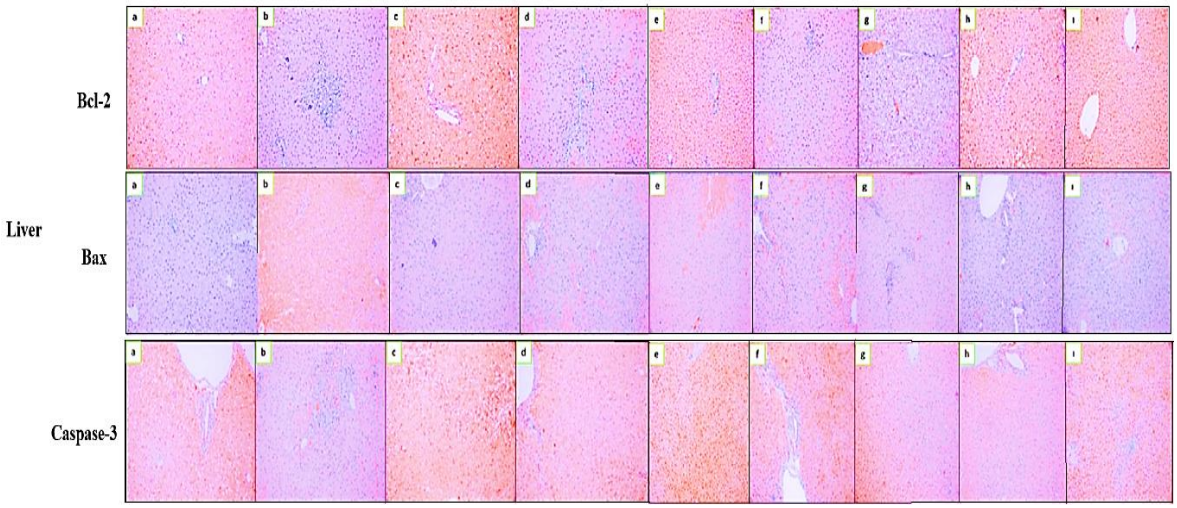

Figure 2. Representative immunohistochemical staining of Bcl-2, Bax, and caspase-3 in liver tissues from the experimental groups. (a) Control, (b) CP, (c) ethanol, (d) CT-Yenice, (e) CT-Dinçer, (f) CT-Balcı, (g) CT-Yenice + CP, (h) CT-Dinçer + CP, and (i) CT-Balcı + CP. Original magnification: ×200

The protective effects of safflower seed oil extracts against kidney damage were investigated using Bcl-2, Bax, and Caspase-3 immunohistochemical staining methods. In groups treated with only 150 mg/kg CP, increased Bax and Caspase-3 staining ($p < 0.05$) and decreased Bcl-2 expression ($p < 0.001$) in the renal parenchyma indicated the presence of apoptotic activity. Treatment with CT oil extracts (Yenice, Dinçer, and Balcı) administered in combination with CP showed equally effective anti-apoptotic effects, decreasing Bax and Caspase-3 immunopositivity while increasing Bcl-2 expression (Figure 3).

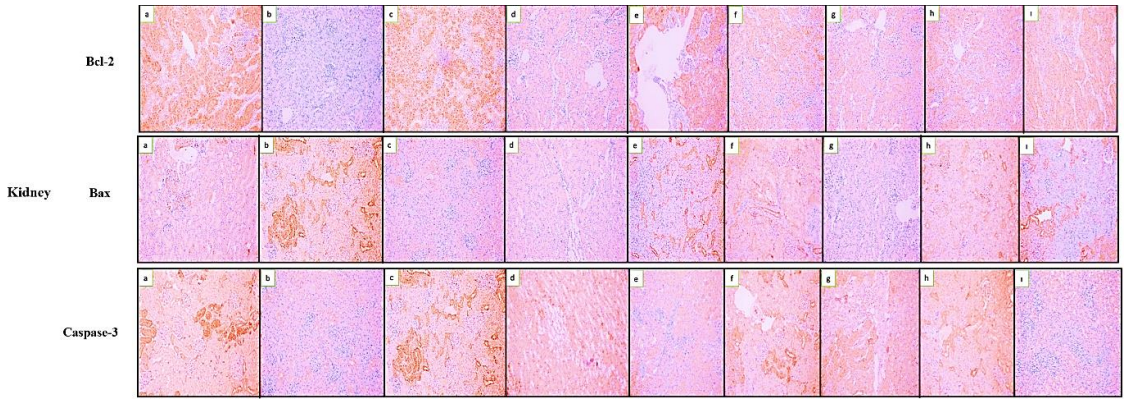

Figure 3. Representative immunohistochemical staining of Bcl-2, Bax, and caspase-3 in kidney tissues from the experimental groups. (a) Control, (b) CP, (c) ethanol, (d) CT-Yenice, (e) CT-Dinçer, (f) CT-Balcı, (g) CT-Yenice + CP, (h) CT-Dinçer + CP, and (i) CT-Balcı + CP. Original magnification: ×200

The effects of safflower seed oil extracts against CP-induced pulmonary, cardiac, and bladder toxicities were evaluated both histologically and immunohistochemically. All three CT extracts showed that they alleviated inflammatory cell infiltration and congestion in the lung parenchyma caused by 150 mg/kg CP, and exhibited significantly ameliorative effects on the heart muscle and bladder wall. The Balcı, Yenice, and Dinçer extracts provided regenerative effects, particularly by limiting inflammation and tissue damage. The Balcı extract contributed to tissue healing by reducing the effects of CP-induced edema and dysplasia on the bladder and lungs. In conclusion, it was determined that CT seeds showed preventive and ameliorative effects on tissue damage in rats treated with CP (Figures 4-6).

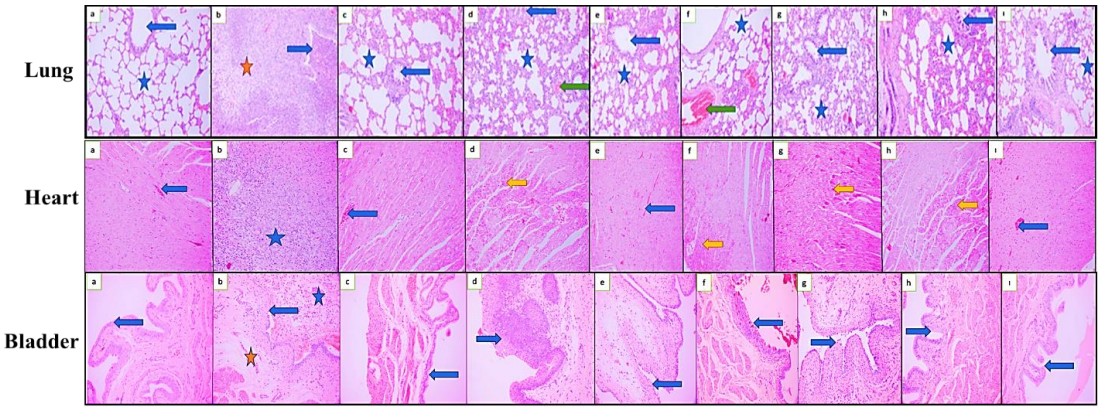

Figure 4. Representative hematoxylin and eosin (H&E)-stained sections of lung, heart, and bladder tissues from the experimental groups. (a) Control, (b) CP, (c) ethanol, (d) CT-Yenice, (e) CT-Dinçer, (f) CT-Balcı, (g) CT-Yenice + CP, (h) CT-Dinçer + CP, and (i) CT-Balcı + CP. Original magnification: ×200

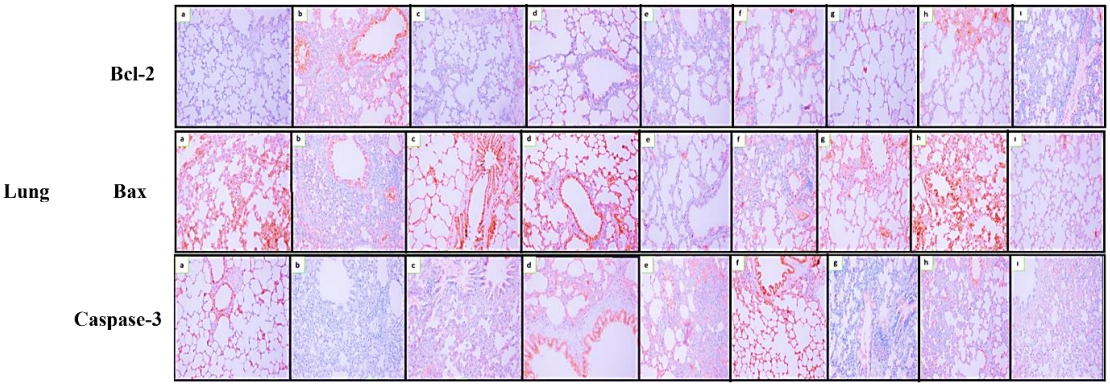

Figure 5. Representative immunohistochemical staining of Bcl-2, Bax, and caspase-3 in lung tissues from the experimental groups. (a) Control, (b) CP, (c) ethanol, (d) CT-Yenice, (e) CT-Dinçer, (f) CT-Balcı, (g) CT-Yenice + CP, (h) CT-Dinçer + CP, and (i) CT-Balcı + CP. Original magnification: ×200

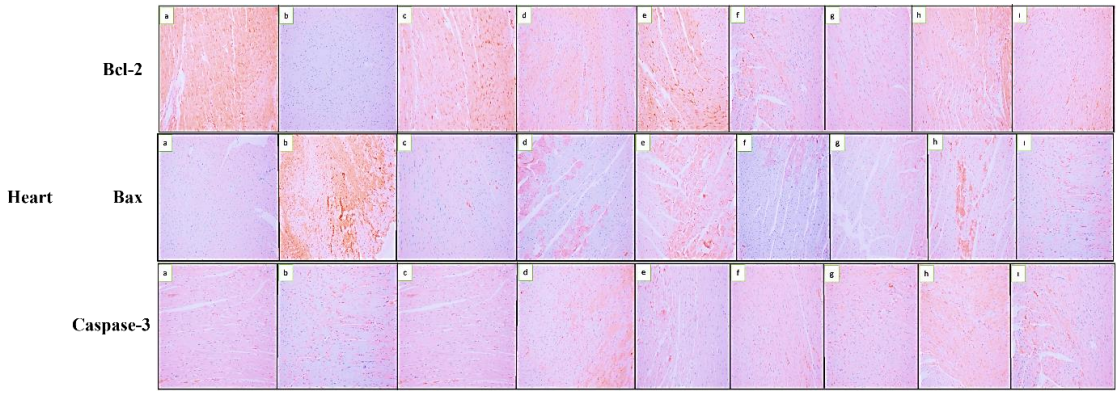

Figure 6. Representative immunohistochemical staining of Bcl-2, Bax, and caspase-3 in cardiac tissues from the experimental groups. (a) Control, (b) CP, (c) ethanol, (d) CT-Yenice, (e) CT-Dinçer, (f) CT-Balcı, (g) CT-Yenice + CP, (h) CT-Dinçer + CP, and (i) CT-Balcı + CP. Original magnification: ×200

3.3. Biochemical Results

According to biochemical data, serum AST, ALT, and ALP levels were significantly increased in the CP group, indicating liver damage. CT applications, especially in the CT-Yenice group, showed hepatoprotective effects by reducing these enzyme levels. While the increase in BUN and Cre levels in the CP group indicated impaired kidney function, CT applications partially prevented this increase, exhibiting a nephroprotective effect. The significant increase in LDH levels indicates that CP causes tissue damage, and the suppression of this increase by CT applications supports its protective effect on tissues. Overall, liver, kidney, and bladder tissue damage caused by CP was mitigated by CT genotypes, and the strongest protective effect was observed in the CT-Yenice group (Figure 8).

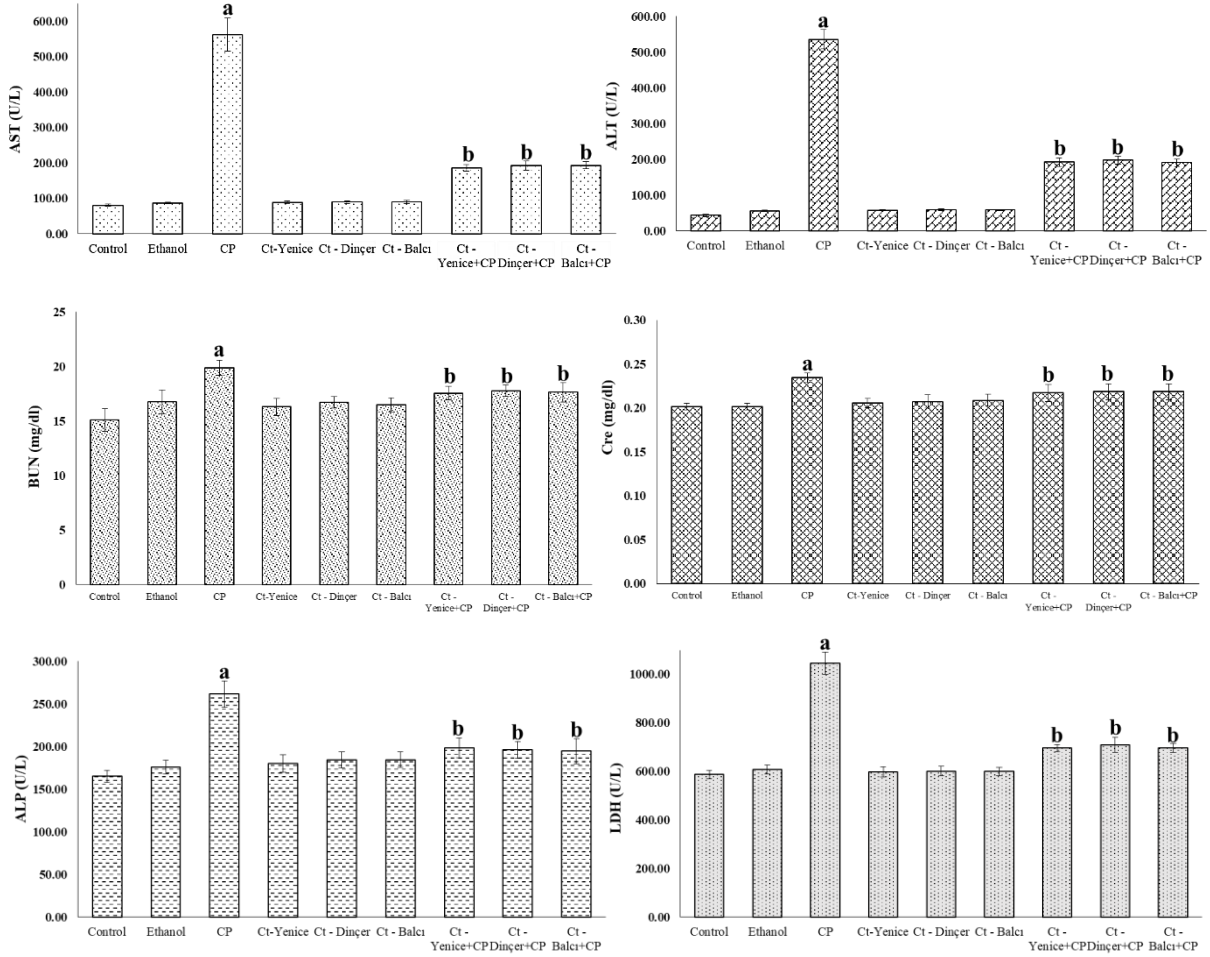

Figure 8. Effect of CP, ethanol, CT-Yenice, CT-Dinçer, and CT-Balcı on the levels of BUN, Cre, ALT, AST, ALP, and LDH. a: significant difference compared with the control group; b: significant difference compared with the CP group

On the other hand, Total Thiol (TT) and Natural Thiol (NT) levels were significantly decreased in the CP group ($p<0.05$), but CT applications, especially CT-Yenice, showed a protective effect by increasing TT and NT levels ($p<0.05$). The significant increase in serum disulfide (SDS) and the SDS/TT ratio in the CP group ($p<0.05$) indicates increased oxidative stress. CT applications, especially CT-Yenice, Dinçer, and Balcı genotypes, suppressed this increase ($p<0.05$). These results support the protective effect of CT against oxidative stress

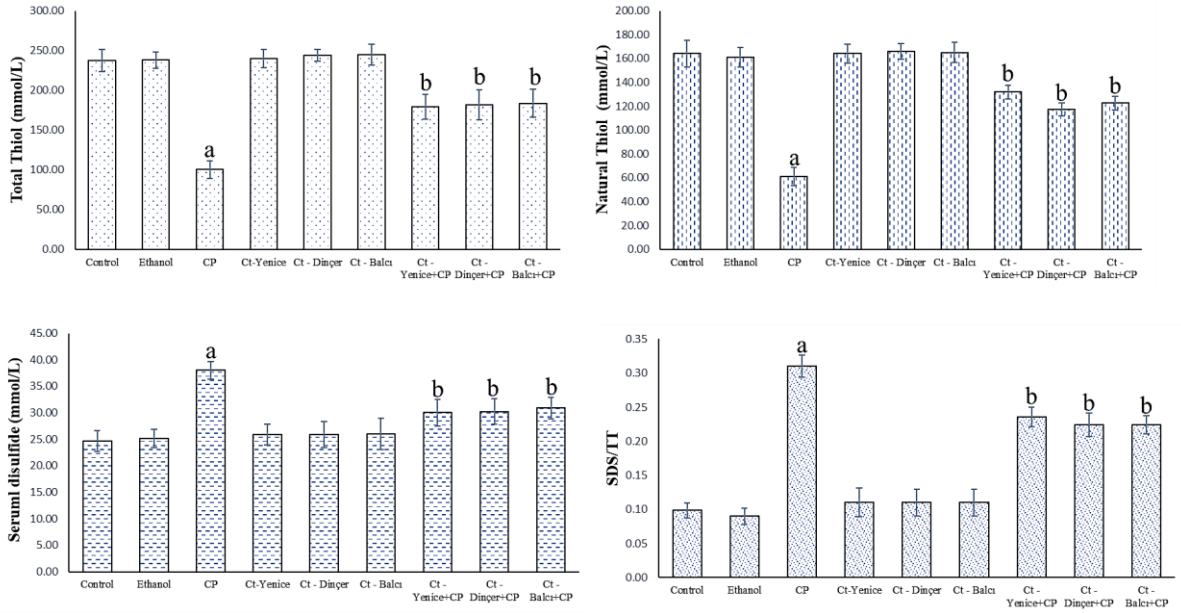

Figure 9. Showing the effect of CP, Ethanol, CT-Yenice, CT-Dinçer, and CT Balcı on the levels of TT, NT, and SDS. The significant difference compared to control = a, significant difference compared to CP=b

4. Discussion

This study evaluated the protective and therapeutic effects of *Carthamus tinctorius* (CT) seed oil extracts against cyclophosphamide (CP)-induced damage to liver, kidney, lung, heart, and bladder tissues. Our findings show that while CP administration causes significant histopathological changes, oxidative stress, and alterations in apoptosis-related markers, CT seed oil extracts significantly reduce this damage. These results suggest the potential of safflower oil as a protective agent against CP-induced multi-organ toxicity.

Histopathological examinations revealed that CP administration led to significant degenerative changes in liver and kidney tissues. Pathological findings such as hepatocyte necrosis, tubular degeneration, congestion, and inflammatory cell infiltration were particularly prominent. CP-induced cytotoxicity is associated with acrolein, a CP metabolite. Previous studies reporting that acrolein causes tissue damage via oxidative stress are consistent with our experimental findings [15]. However, in groups treated with CT extracts, these histopathological alterations were significantly reduced, and tissue architecture was considerably preserved. Previous studies reporting the protective effects of herbal antioxidants, flavonoids, and polyphenols against chemotherapy-induced toxicity support our findings [7, 16].

CP administration was associated with increased serum levels of biochemical damage markers AST, ALT, LDH, BUN, and creatinine, and consequently, multiple organ damage, primarily in the liver and kidneys. CP impairs organ function by causing lipid peroxidation, DNA damage, and apoptosis through oxidative stress [6]. However, safflower oil extract administration significantly reduced the alterations in these biochemical markers, supporting its potential protective role against CP-induced toxicity. Thiol-disulfide balance analyses also showed a shift towards oxidative stress in the CP-treated groups, while CT extract administration improved thiol-disulfide homeostasis. This is consistent with previous studies reporting the hepatoprotective effects of plant antioxidants such as silymarin and curcumin [17, 18].

In the group treated with CP alone, a significant increase in the expression of proapoptotic immunohistochemical markers Bax and Caspase-3, and a decrease in the expression of the anti-apoptotic marker Bcl-2 were detected. These findings suggest increased apoptosis-related activity following CP administration and are consistent with previous studies implicating mitochondrial apoptotic signaling in CP-induced tissue injury [19, 20]. However, in the groups treated with safflower oil extract, Bax and Caspase-3 immunoreactivity decreased, while Bcl-2 expression increased. Although these findings support a potential anti-apoptotic effect of CT extracts, direct activation

or inhibition of specific apoptotic pathways was not investigated in the present study. Studies reporting that natural antioxidants such as resveratrol and quercetin reduce CP-induced toxicity by modulating apoptotic pathways support our results [21].

An important aspect of the present study is the comparative evaluation of the three *Carthamus tinctorius* genotypes, Yenice, Dinçer, and Balcı, against CP-induced multi-organ injury. All three genotypes exhibited protective effects, although differences in the magnitude of these effects were observed. While the Yenice genotype generally showed more pronounced improvements in oxidative stress-related biochemical parameters and tissue damage, the Dinçer and Balcı genotypes also exhibited significant protective effects. These differences may be associated with variations in fatty acid composition among the genotypes**.** Gas chromatographic analysis revealed that all three genotypes (Balcı, Dinçer, and Yenice) contained high levels of linoleic acid (71.5%, 78.1%, and 79.3%, respectively). In contrast, oleic acid content was higher in Balcı (17.5%) than in Dinçer (11.7%) and Yenice (11.6%). These compositional differences highlight the comparative importance of the three genotypes and may contribute to variations in their biological activities. Linoleic acid is an important polyunsaturated fatty acid that has been associated with biological processes related to inflammation and cellular membrane function [22]. However, the present findings do not establish a direct causal relationship between individual fatty acids and the observed protective effects. Further investigations are required to clarify the contribution of specific fatty acids and other bioactive compounds to the protective activities of these genotypes.

The protective effects observed following CT seed oil extract administration may involve several complementary biological mechanisms. The improvement in thiol-disulfide homeostasis suggests attenuation of oxidative stress, while the reduced inflammatory cell infiltration observed in histopathological examinations indicates a decrease in tissue inflammatory responses. Furthermore, the observed changes in Bax, Bcl-2, and Caspase-3 immunoreactivity are consistent with the modulation of apoptosis-related processes**.** Previous studies have suggested that bioactive compounds derived from Carthamus tinctorius may exhibit antioxidant and anti-inflammatory activities [23] and influence apoptosis-related signaling pathways [24]. Nevertheless, inflammatory cytokine production, specific intracellular signaling pathways, and the direct contribution of individual bioactive compounds were not investigated in the present study. Therefore, these mechanisms should be considered plausible explanations rather than directly demonstrated molecular effects.

The findings of this study suggest that CT seed oil extract shows promise as a natural protective agent in reducing CP-induced organ toxicity. Given the widespread use of CP in chemotherapy processes, identifying natural compounds that can mitigate its side effects is of great clinical importance. Future studies should focus on identifying the specific bioactive components responsible for CT's protective effects, clarifying the underlying molecular mechanisms, and evaluating whether safflower seed oil extracts interfere with the antitumor efficacy of CP before their potential clinical application as complementary agents is considered.

5. Conclusions

In conclusion, this study demonstrates that CT seed oil extract provides effective protection against CP-induced multi-organ damage. Safflower offers a robust defense mechanism against CP toxicity by reducing oxidative stress, modulating apoptosis, and maintaining tissue integrity. These findings support the potential of safflower oil as a complementary therapy in reducing chemotherapy-induced toxicity and highlight the need for further preclinical and clinical research.

Artificial Intelligence Statement

( ) This article did not use artificial intelligence.
(x) This article does use artificial intelligence.

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Author contributions: Esma Ocak, Atila Ocak, Arzu Köse, Betul Peker Cengiz, and Adnan Ayhanci contributed to the study conception and design. Esma Ocak and Atila Ocak performed the experimental research. Esma Ocak and Atila Ocak collected and analyzed the data. Arzu Köse contributed to the preparation and characterization of the safflower seed oil extracts. Betul Peker Cengiz contributed to the histopathological evaluation of the tissue samples. Adnan Ayhanci supervised the study and contributed to the interpretation of the findings. Esma Ocak and Adnan Ayhanci were involved in drafting and revising the manuscript. All authors read and approved the final version of the manuscript.

Ethical statement: The study was approved by the Animal Experiments Local Ethics Committee of Eskişehir Osmangazi University (HADYEK) under decision number **785-2**, dated **23 June 2020**.

Data Availability Statement (Please see the Data Availability Policy section for details.)
( ) Option A (Public Repository): "The research data supporting this study are publicly available in [Name of Repository: e.g., Zenodo, Figshare, Dryad] with the permanent DOI link [URL/DOI]."
( ) Option B (Upon Request): "The raw data supporting the findings of this study are available from the corresponding author [Author Initials] upon reasonable request and within ethical boundaries."
( ) Option C (Third-Party Data): "The data used in this study were obtained from [Name of Institution/Source] and are subject to third-party restrictions regarding redistribution."
(X) Option D (No Data): "No new data were created or analyzed in this study. Data sharing is not applicable to this article."

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