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ORIGINAL RESEARCH

The effect of *Daucus carota* L. var atrorubens on the treatment of experimental sepsis models

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Abstract

Background: Sepsis secondary to infections is a critical health issue with limited preventive treatments. Various studies have shown that *Daucus carota L. ssp. Atrorubens* (black carrot) possesses antibacterial, antifungal, anti-inflammatory, antiseptic and hepatoprotective effects. This study investigated the therapeutic potential of black carrot, focusing on its anti-inflammatory and antioxidant effects in an experimental sepsis model. **Methods**: Thirty two Wistar Albino rats were divided equally into four groups. Group I served as the control without any treatment. The caecal ligation and perforation (CLP) procedure was performed on Groups II, III and IV. Group II underwent only the CLP procedure. Group III received 3 mL intraperitoneal isotonic sodium chloride, and Group IV was administered 3 mL black carrot solution every 24 h for 7 days post-CLP. On day 7, all rats were sacrificed, and blood, kidney, and liver samples were collected for analysis. Data were evaluated using GraphPad Prism 6.0 software and analysed by one-way analysis of variance and Tukey multiple comparison tests. **Results**: Biochemical analysis showed similar Aspartate Transaminase (AST) and Alanine Aminotransferase (ALT) values between the control and black carrot groups. Histopathological examination revealed minimal polymorphonuclear leukocyte infiltration in the CLP + black carrot group, and normal-appearing arteria hepaticus interlobularis, vena interlobularis and ductus biliferi, similar to the control group. Despite no significant decrease in serum urea levels, histopathological findings indicated a nephroprotective effect. The CLP + black carrot group showed reduced tubular dilatation and brush border loss and appeared similar to the control group. **Conclusions**: Overall, black carrot showedhepatoprotective, nephroprotective, anti-inflammatory and antioxidant effects, as supported by both biochemical and histological data. These findings suggest that black carrot may offer therapeutic benefits in managing sepsis-induced organ damage.

Keywords

Daucus carota; Sepsis; Hepatoprotective; Black carrot; Nephroprotective

1. Introduction

In 2016, the guide published by the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) defined the concept of sepsis as the dysregulated response of the host organism to the infection caused by apathogenic agent that results in organ dysfunction of organs such as the kidney or liver [1, 2]. The kidneys are among the earliest organs to sustain damage during sepsis, and acute kidney injury (AKI) occurs in nearly two-thirds of patients with septic shock, with half of these cases AKI developing prior to hospital admission. Consequently, AKI can be considered an early indicator of sepsis. Sepsis-associated acute kidney injury (S-AKI) is a common, life-threatening complication in hospitalised and critically ill patients. Current reports show that the incidence of S-AKI in septic patients ranges from 10% to 67%, with mortality rates ranging from 11% to 77% [3, 4].

Sepsis-associated liver injury (SALI) is another major organ injury with no exact pathophysiology, although it may involve inflammatory cellular responses, endotoxin-induced damage, hepatic microcirculatory dysfunction and disruptions in bilirubin-bile acid metabolism. SALI typically presents as either hypoxic hepatitis (HH) or cholestasis. HH is frequently associated with circulatory and respiratory failure, predisposed primarily by acute heart failure and septic shock. Even though the definitive incidence of sepsis-associated liver injury is unknown, a recent study reported detection of liver injury in 29.8% of sepsis patients and an in-hospital mortality rate of

43.5% [5–8].

The high morbidity and mortality of sepsis in all existing hospital units, and especially in intensive care units and emergency services, makes it an extremely important condition. Therefore, its treatment should be as urgent and aggressive as the seriousness of the case. In Turkey, as in the rest of the world, the incidence of sepsis has nearly doubled in the last decade, causing both the loss of workforce, financial losses, and losses as mortality and morbidity [9]. This situation points to the urgent need for new sepsis treatments, including those derived from traditional medicinal plants.

One potential candidate is *Daucus carota* L. ssp. commonly known as wild carrot, which belongs to the Apiaceae (Umbelliferae) family. It is a hardy, spiny-fruited plant that grows in arid regions of Europe. Various studies have reported that it possesses antibacterial, antifungal, anti-inflammatory, antisteroidogenic, antiseptic, expectorant, diuretic, vasodilator and spasmolytic actions, as well as hepatoprotective properties [10, 11]. Black carrot (*D. carota* L. ssp. *sativus* var. *atrorubens* Alef.) is a root vegetable cultivated extensively worldwide. This vegetable is esteemed for its substantial nutritional profile, notably high in anthocyanins and phenolic acids, which confer significant health benefits and nutraceutical properties. In recent years, black carrots have emerged as a subject of growing interest within the scientific community in several countries, including Germany, Turkey and Australia.

Known for their rich anthocyanin content, black carrots have attracted scientific interest due to their phenolic compounds, which contribute significantly to their antioxidant properties. Anthocyanins, which impart the intense purple colour to black carrots and are predominantly found in their outer parts, represent the largest group of water-soluble pigments in the plant kingdom. However, beyond their pigmentation properties, anthocyanins offer several health benefits, including reduced risks of coronary heart disease and stroke, antitumor properties, anti-inflammatory effects and improved cognitive behaviour [12]. Previous research on the antioxidant and anti-inflammatory properties of black carrots confirmed the anti-inflammatory effects of black carrot and its by-products. Once absorbed during digestion, black carrot significantly reduced the secretion of specific pro-inflammatory markers, such as Interleukin 8 (IL-8), Monocyte Chemoattractant protein 1 (MCP-1), Vascular endothelial growth factor (VEGF) and Intercellular Adhesion Molecule 1 (ICAM-1), particularly in endothelial cells treated with Tumor necrosis factor alpha (TNF-α) [13].

Similarly, the antioxidant properties of black carrot have been demonstrated in numerous previous *in vivo* studies [14, 15]. For example, black carrot oil extract reversed the reduction in the activity of antioxidant liver enzymes, specifically superoxide dismutase and catalase, induced by Carbon tetrachloride (CCl_4) treatment. Overall, the antioxidants present in black carrot appear to function as primary antioxidants or free radical scavengers, thereby preserving the activity of these protective enzymes. However, the available literature does not contain studies on the efficacy of black carrot as a treatment in bactericidal or infection cases. The aim of the present study was to evaluate its potential as a therapeutic agent in an experimental sepsis model established in rats following intraperitoneal injection of a black carrot extract.

2. Materials and methods

2.1 Experimental model

This study included 32 sexually mature Wistar Albino-type rats of both sexes, weighing 240–280 grams. All animals were housed at 22 °C room temperature, 45–75% humidity, under 12 h circadian rhythms of daylight and dark and received water and feed ad libitum. The study duration was 7 days, and the animals were weighed daily.

2.2 Preparation of the black carrot extract

Fresh fruits (300 g) were ground with a blender and extracted in 3 L of methanol by maceration at room temperature, followed by filtration. The solvent was removed using a rotary evaporator, yielding 1.704 g of dried mass. It was prepared for the next stages of the study. The black carrot solution for intraperitoneal administration was prepared by dissolving the extracted material to provide a dose of 100 mg/kg in 3 mL of sodium chloride (NaCl).

2.3 The cecal ligation and perforation procedure:

In a sterile environment, a 3 cm midline laparotomy was carried out to expose the cecum and the adjacent intestine. The cecum was securely tied at its base below the ileocecal valve using a 3.0 silk suture, and a single puncture was made with a 22-gauge needle. To confirm the perforation, the cecum was gently pressed to release a small amount of faecal matter. The cecum was then repositioned into the peritoneal cavity, and the abdominal incision was closed using a 4.0 polyglactin 910 suture. After surgery, the animals were given time to recover before being returned to their cages. In the sham-operated group, only the laparotomy was performed under aseptic conditions, without ligating or puncturing the cecum. In this model, rats were classified as septic 5 h after the CLP procedure. All treatments were administered within the first hour following surgery [16].

2.4 Experimental groups

The experimental groups were as follows: Group I (the Control group, n = 8) did not receive any treatment. Group II (CLP group, n = 8) underwent only the cecal ligation and perforation (CLP) procedure and was followed up until day 7. Group III (0.9% NaCl group, n = 8) underwent the CLP procedure and was treated by intraperitoneal injection of 3 mL isotonic NaCl, once every 24 h for 7 days after the CLP procedure. Group IV (Black carrot group. N = 8) underwent the CLP procedure and was treated by intraperitoneal injection of 3 mL black carrot solution in isotonic NaCl once every 24 h for 7 days after CLP. At the end of day 7, all animals were sacrificed for blood samples and kidney and liver biopsies.

2.5 Biochemical methods

Serum was obtained by centrifuging the collected blood samples in plain biochemical tubes at 3000 rpm for 10 min. The obtained serum samples were transferred to Eppendorf tubes and stored at −80 °C until analysis. Biochemical tests

included analysis for C-reactive protein (CRP), procalcitonin (PCT), lactic acid, urea, direct bilirubin, glucose, alanine transaminase (ALT) and aspartate transaminase (AST) using the following kits, according to the manufacturers' instructions: CRP (SunRed Biotechnology Company, Shanghai, China, Rat C-Reactive Protein ELISA Kit, Lot: 202103), PCT (SunRed Biotechnology Company, Shanghai, China, Rat PCT ELISA Kit, Lot: 202103), lactic acid (Elabscience, Houston, TX, USA, Lactic Acid Colorimetric Assay Kit, lot: XER7DSUZVA), urea (Elabscience, Houston, TX, USA, Urea Colorimetric Assay Kit, Urease method, Lot: 1DM623TVH1), direct bilirubin (Elabscience, Houston, TX, USA, Direct Bilirubin Colorimetric Assay Kit (Chemical Oxidation Method), Lot: LKGTB2WGS7), glucose (Elabscience, Houston, Texas, United States, Glucose Colorimetric Assay Kit, glucose oxidase, peroxidase method, 9J2AXD26RC), ALT (Elabscience, Houston, TX, USA, alanine aminotransferase/glutamate-pyruvate transaminase (ALT/GPT) Activity Assay Kit, Lot: P74NUM73CY), and AST (Elabscience, Houston, TX, USA, Rat AST (Aspartate Aminotransferase) enzyme-linked immunosorbent assay (ELISA Kit, Lot: H2PIYCYR3M).

2.6 Histopathological methods

Histological staining included hematoxylin eosin (H&E staining), anti-endothelial nitric oxide synthases (Anti-eNOS), anti-isoform nitric oxide synthases (Anti-iNOS), and Anti-desmin. The stained tissues were examined by light microscopy at 4×, 20× and 40× magnifications.

2.6.1 Histological tissue processing method and histological analyses

Liver and kidney tissues collected from sacrificed animals underwent standard histological processing and were embedded in paraffin. For sectioning, 5 μm thick slices were prepared using a Leica RM2145 microtome (RWD Life Science Co., Ltd., San Diego, CA, USA). The sections were subsequently stained with both photochromic and immunohistochemical dyes and analysed using an Olympus BX-51 light microscope (Leica Microsystems Inc., Deerfield, MA, USA).

2.6.2 Sectioning for light microscopy

Following the embedding process, tissue blocks were allowed to equilibrate at room temperature for one day. They were then stored at +4 °C for 1 h prior to sectioning. Sections of 5 μm thickness were cut with a Leica RM 2145 microtome (Leica Microsystems Inc., Deerfield, MA, USA) and subsequently mounted on polylysine-coated slides. To ensure proper spreading, sections were preheated in a 37 °C water bath.

2.7 Histochemical methods

2.7.1 Deparaffinization process

To prepare tissues for light microscopy, paraffin was removed by heating the slides at 57 °C for 1 h. After heating, the slides were allowed to cool and were then immersed in xylene overnight to complete the deparaffinisation process.

2.7.2 Hematoxylin-eosin staining method

Deparaffinized tissue sections were stained with hematoxylin-eosin stain. Following a 30 min immersion in xylene, the slides were mounted with Entellan and examined. The staining protocol yielded blue nuclei and pink cytoplasm.

2.7.3 Immunohistochemical staining methods

For immunohistochemical analysis, sections were placed on polylysine-coated slides and allowed to dry and adhere. After incubation at 60 C for 1 h, the slides were cooled and underwent deparaffinisation in xylene overnight. Immunohistochemical staining was carried out using primary antibodies against iNOS (Bioss, bs-2072R), VEGF (Bioss, 2079R) and desmin (Bioss, 1026R).

The samples taken from the rat kidneys and livers were examined for organ-specific pathologies, including cast formation, erythrocyte extravasation, brush border loss, dilatation and congestion in hepatic sinusoids, inflammation and dilatation in the portal area, tubular dilatation, and congestion in the central vein, assessed using a visual and pathological scoring system ranging from 0 to 5. A score of 0 was assigned to tissue most resembling normal, while a score of 5 indicated the tissue most affected by inflammation.

2.8 Statistical analyses

All statistical analyses were performed using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA) with Tukey's multiple comparison test. An alpha value of 0.05 was accepted. Statistical significance was considered for $p < 0.05$, $p < 0.005$, $p < 0.001$. All data were presented as mean $\pm$ the standard error of the mean (SEM). One-way analysis of variance (ANOVA) and Tukey's multiple comparison tests were used to calculate variances among the four groups. A p value < 0.05 was considered statistically significant. For graphical representation of the data, column graphs were chosen as the most appropriate for inter-group comparisons. The obtained graphs and p-values were interpreted separately for each parameter.

3. Results

3.1 Biochemical results

Samples taken for biochemical analyses were analysed for biochemical c-reactive protein (CRP), procalcitonin (PRC), lactic acid (LA), urea and direct bilirubin (DBIL), glucose, alanine transaminase (ALT) and aspartate transaminase (AST).

The multi-group analysis for CRP revealed statistically significant differences between the control group and the CLP and CLP + 0.9% NaCl groups ($p < 0.05$ and $p < 0.001$, respectively), whereas the CRP levels in the black carrot group were similar to the control group. The PRC values showed statistically significant differences between the control group and both the CLP and black carrot groups ($p < 0.05$ for both) (Fig. 1).

Comparison of the LA values between the groups revealed significant differences between the CLP group and the CLP + 0.9% NaCl and black carrot groups ($p < 0.005$ and $p < 0.05$, respectively) and between the control group and the CLP

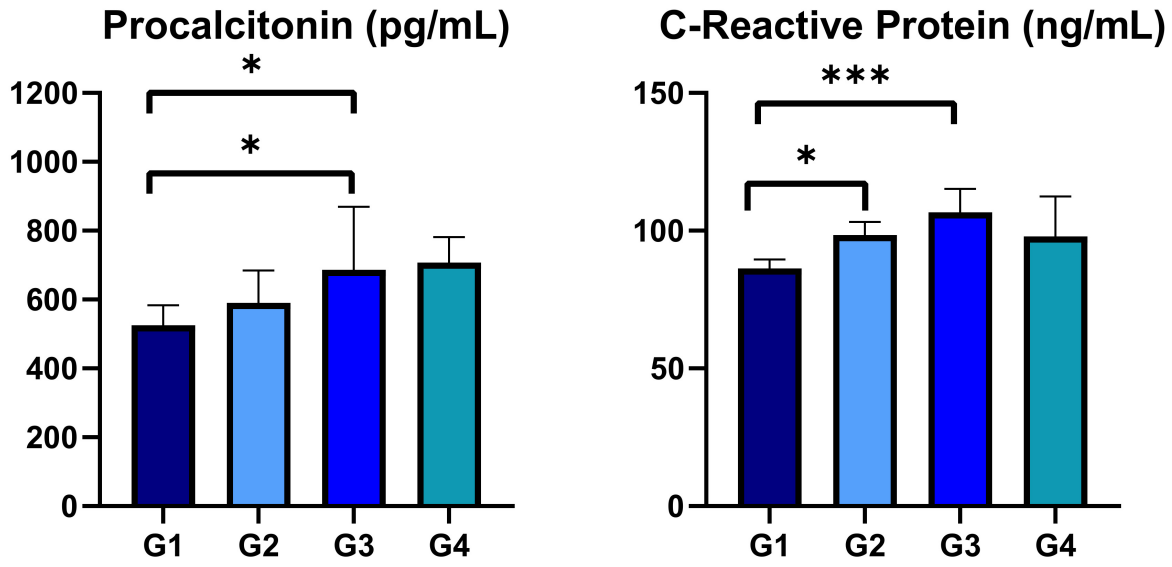

FIGURE 1. The biochemical results of C-Reactive Protein (CRP) and Procalcitonin (PRC) levels relationship between the groups in the comparison of blood samples (*$p < 0.05$, ***$p < 0.001$, values that do not exert a statistical significance are not shown in the figure).

group ($p < 0.05$). No statistical differences were found for glucose and urea values among the groups. No significant differences were noted in the direct bilirubin tests among the groups (Fig. 2).

Significant differences were also found between the control group and the CLP and CLP + 0.9% NaCl groups for AST values ($p < 0.05$ for both). Comparisons of the CLP and CLP + 0.9% NaCl groups with the black carrot group revealed significant differences ($p < 0.05$ for both). In terms of ALT values, statistical differences were determined between the control group with the CLP and CLP + 0.9% NaCl groups ($p < 0.005$ and $p < 0.05$, respectively). There was also a statistically significant difference when comparing the CLP and CLP + 0.9% NaCl groups to the black carrot group for ALT values ($p < 0.001$ and $p < 0.005$, respectively). Also significant differences noted in lactate dehydrogenase (LDH) values between the control group and the CLP group ($p < 0.05$) (Fig. 3).

3.2 Histopathological results

The liver tissues of the control group had a normal histological structure. The vena centralis located in the centre of the classical liver lobule in the organ surrounded by Glisson's capsule, a tight connective tissue structure, was observed to be in a normal histological structure. Remark cords were detected extending radially from the vena centralis. Sinusoids were in normal structure in the laterals of the hepatocytes. In the portal hepatic triangular structures, normal morphologies were observed in the arteria hepaticus interlobularis, vena interlobularis and ductus biliferi (Fig. 4).

The liver tissues of the CLP and the CLP + 0.9% NaCl groups, when compared to the control group, showed a dilatation in the vena centralis located in the centre of the classical liver lobule ($p < 0.005$). Similarly, sinusoids accompanying the hepatocytes were larger than those observed in the control group (**$p < 0.005$, ***$p < 0.001$). Clumps were observed due to inflammation in the liver parenchymatous structure. Cell clusters related to the polymorphonuclear leukocyte (PMNL) infiltration were observed in these clumps. In the portal hepatic triangular structure, dilated morphologies were observed in the arteria hepaticus interlobularis, vena interlobularis and ductus biliferi (Fig. 4).

Only minimal dilatation was observed in the vena centralis located in the centre of the classical liver lobule in the CLP + black carrot group compared to the control group ($p < 0.05$). The sinusoidal structures accompanying the Remark cords were smaller in the CLP + black carrot group than in the CLP and CLP + 0.9% NaCl groups ($p < 0.001$), but were larger than in the control group ($p < 0.05$). No clumps due to inflammation in the liver parenchymatous structure were detected in the CLP + black carrot group. In the portal hepatic triangle, the structures of the ateria hepaticus interlobularis, vena interlobularis and ductus biliferi were normal; however, they were minimally positive for PMNL infiltration (Fig. 4).

The kidney sections of the control group showed a separation of the cortex and medulla. The fibrous capsule located in the upper outer part of the cortex was intact. Cortical nephrons were located close to the capsule surrounding the kidney in the cortex, whereas juxtaglomerular nephrons were observed in the medullary fields. The renal corpuscles and the parietal and visceral leaves of the Bowman's capsule showed normal histological morphology. The proximal tubule, distal tubule, the loop of Henle, and collecting tubules also showed normal histological structure, and the characteristic structure and features of epithelial cells were preserved (Fig. 5).

The kidney sections belonging to the CLP and CLP + 0.9% NaCl groups showed separation of the cortex and medulla, and the fibrous capsule structure surrounding the cortex was intact. The Bowman's capsule in the glomerular structures was

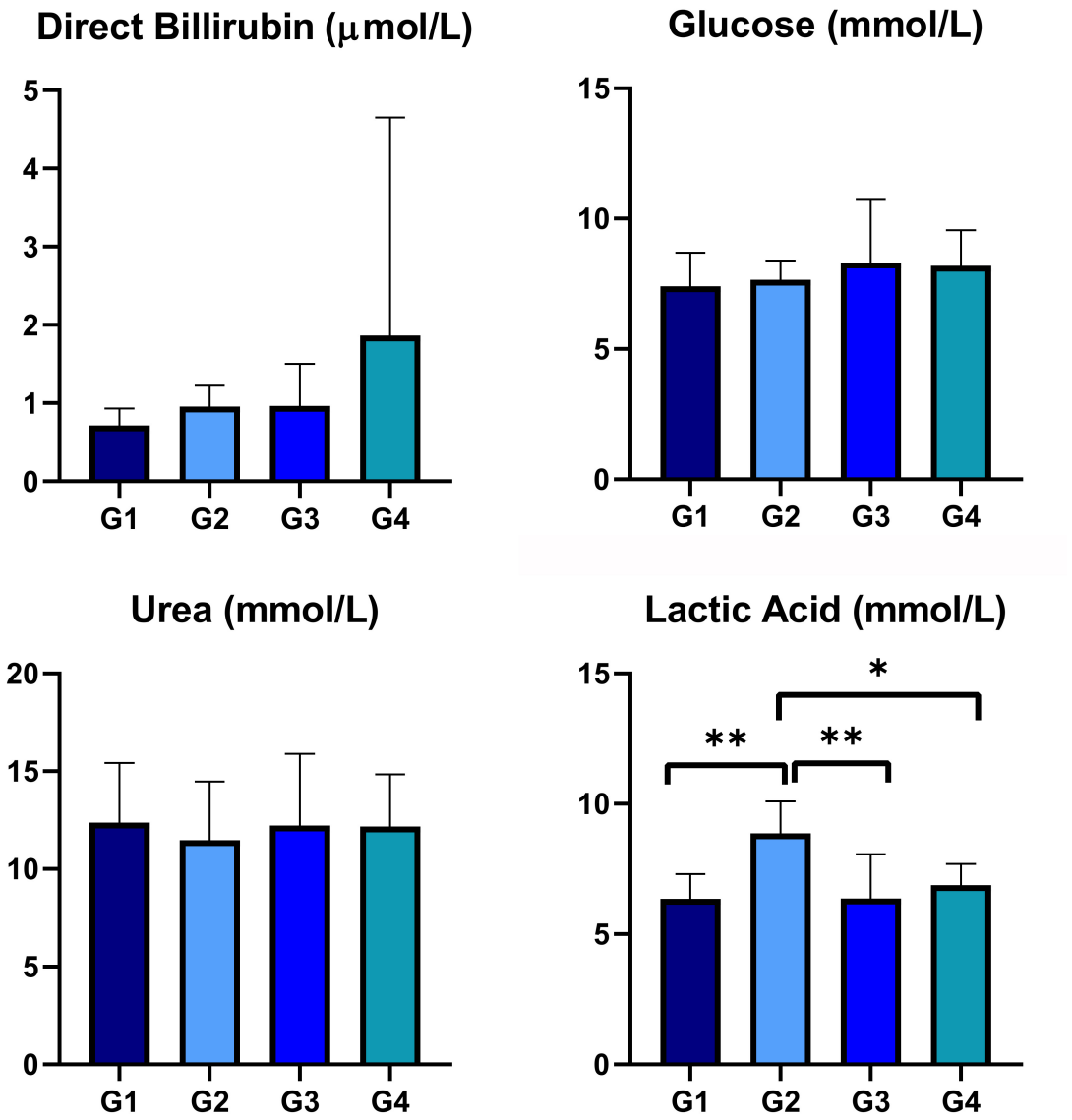

FIGURE 2. The biochemical results of direct bilirubin, lactic acid, glucose and urea levels relationship between the groups in the comparison of blood samples (*$p < 0.05$, **$p < 0.005$, values that do not exert a statistical significance are not shown in the figure).

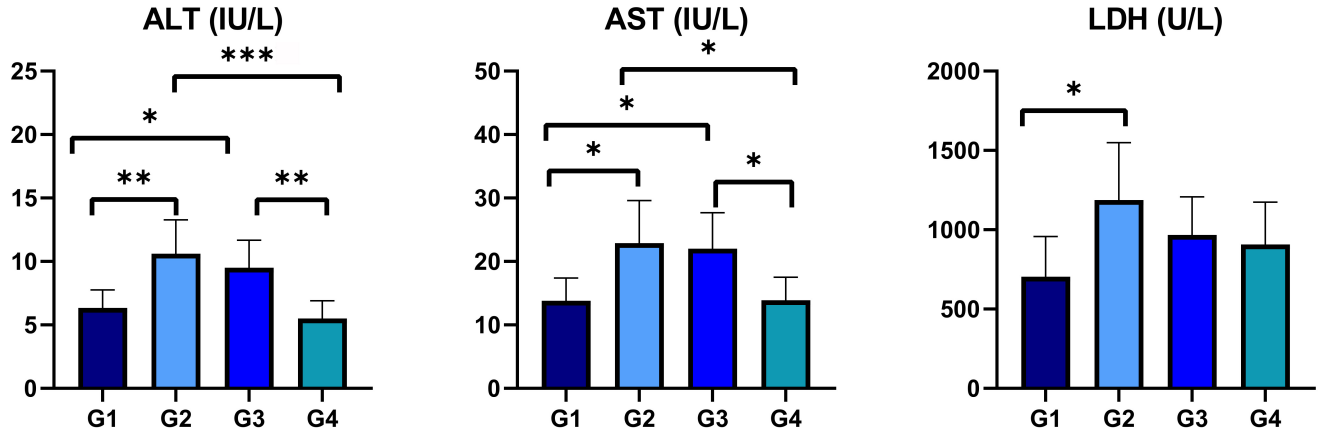

FIGURE 3. The biochemical results of lactate dehydrogenase (LDH), alanine transaminase (ALT) and aspartate transaminase (AST) levels relationship between the groups in the comparison of blood samples (*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, values that do not exert a statistical significance are not shown in the figure).

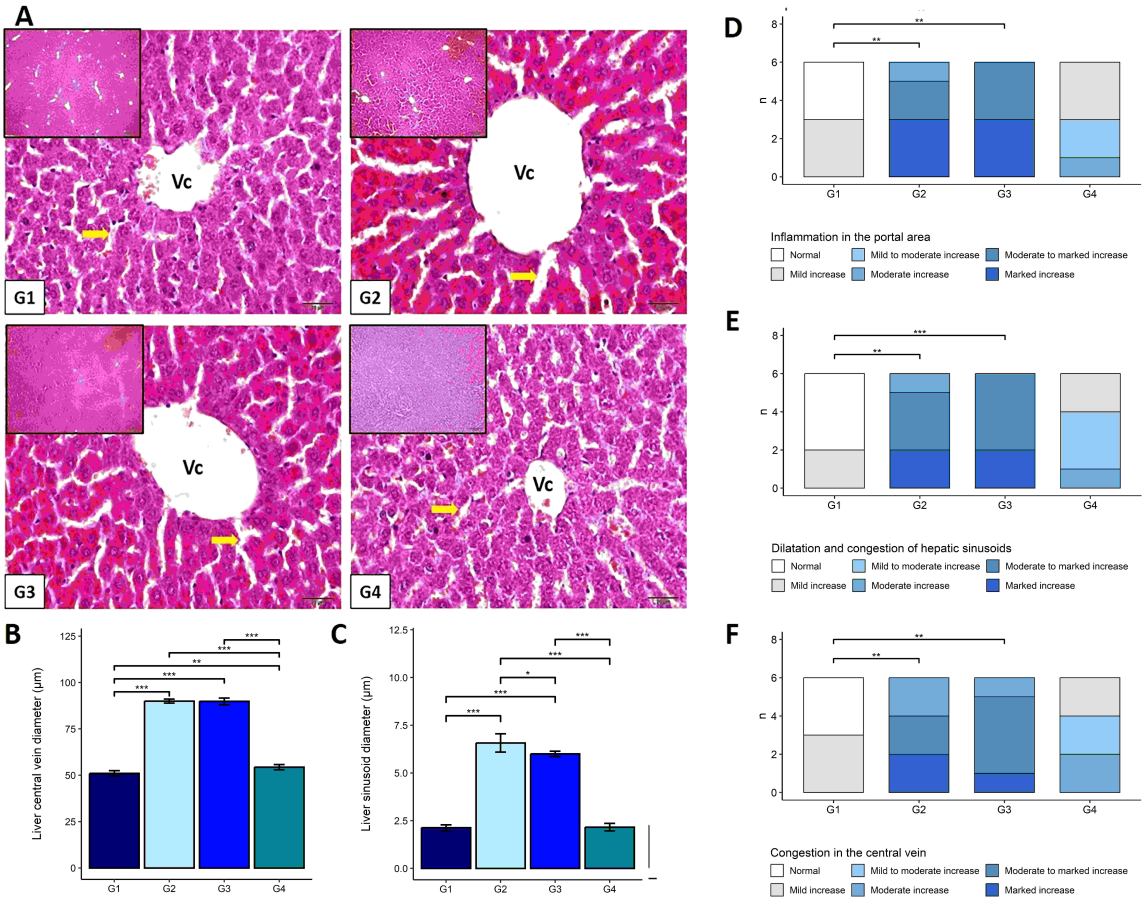

FIGURE 4. Histopathological appearance of the liver tissues of the control and experimental sepsis model groups. H&E staining, 40×-magnification (Small photos 4×-magnification). Vc, Vena centralis; yellow arrow, liver sinusoid. (A) Liver histopathological evaluation findings. (B) Statistical evaluation of liver central vein diameter measurements. (C) Statistical evaluation of liver sinusoid diameter measurements. (D) Statistical evaluation of histopathological scoring for inflammation in the portal area. (E) Statistical evaluation of histopathological scoring for hepatic sinusoid dilatation and hemorrhage. (F) Statistical evaluation of histopathological scoring for central vein hemorrhage (*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, values that do not exert a statistical significance are not shown in the figure).

enlarged. PMNL infiltration and congestion were observed in the cortical and medullary regions, especially in the peri-glomerular and peritubular areas. Dilatation was evident in the proximal and distal tubules around the glomerular structures (**$p < 0.005$, ***$p < 0.001$), as well as a loss of brush border in proximal tubular cells, and vacuolization in places ($p < 0.05$). A rare accumulation of proteinaceous matter was observed in some tubular cells, as well as cell debris in the tubular lumen (Fig. 5).

The kidney sections of the CLP + black carrot group showed separation of the cortex and medulla. Compared to the sepsis group, the CLP + black carrot group showed significantly less loss of the brush border in the proximal tubule structures, significantly decreased dilatation of the proximal and the distal tubule, and significantly fewer infiltrated PMNL cells. It was determined that the accumulation of proteinaceous material in the tubules, glomerular changes, and erythrocyte extravasation significantly decreased. Less dilatation, edema, and signs of PMNL infiltration were evident in the afferent and efferent vessels forming the glomerular clump. There is a minimal deletion in the proximal and distal tubular and minimally-dilated epithelial cells seen around the glomerular structures (Fig. 5).

3.3 Immunohistochemical results

The expression of the iNOS enzyme is increased in almost all liver cells in response to various stimuli. However, iNOS is most intensely expressed in Kupffer cells compared to other liver cells. A higher immunopositivity was detected in the CLP and CLP + 0.9% NaCl groups compared to the control group, whereas a decrease was observed in this immunoreactivity by the black carrot application. In the livers of the control group, anti-VEGF yielded an immunopositive reaction in the hepatocytes, endothelium of hepatic arteries, terminal hepatic venules, sinusoid walls and bile duct epithelial cells. This immunopositivity was substantially higher in the CLP and CLP + 0.9% NaCl groups and was decreased by the black carrot application (Fig. 6).

In the anti-VEGF staining, an immunoreaction could not be detected in the glomeruli and tubular structures in the control group, where as an intense expression pattern was observed

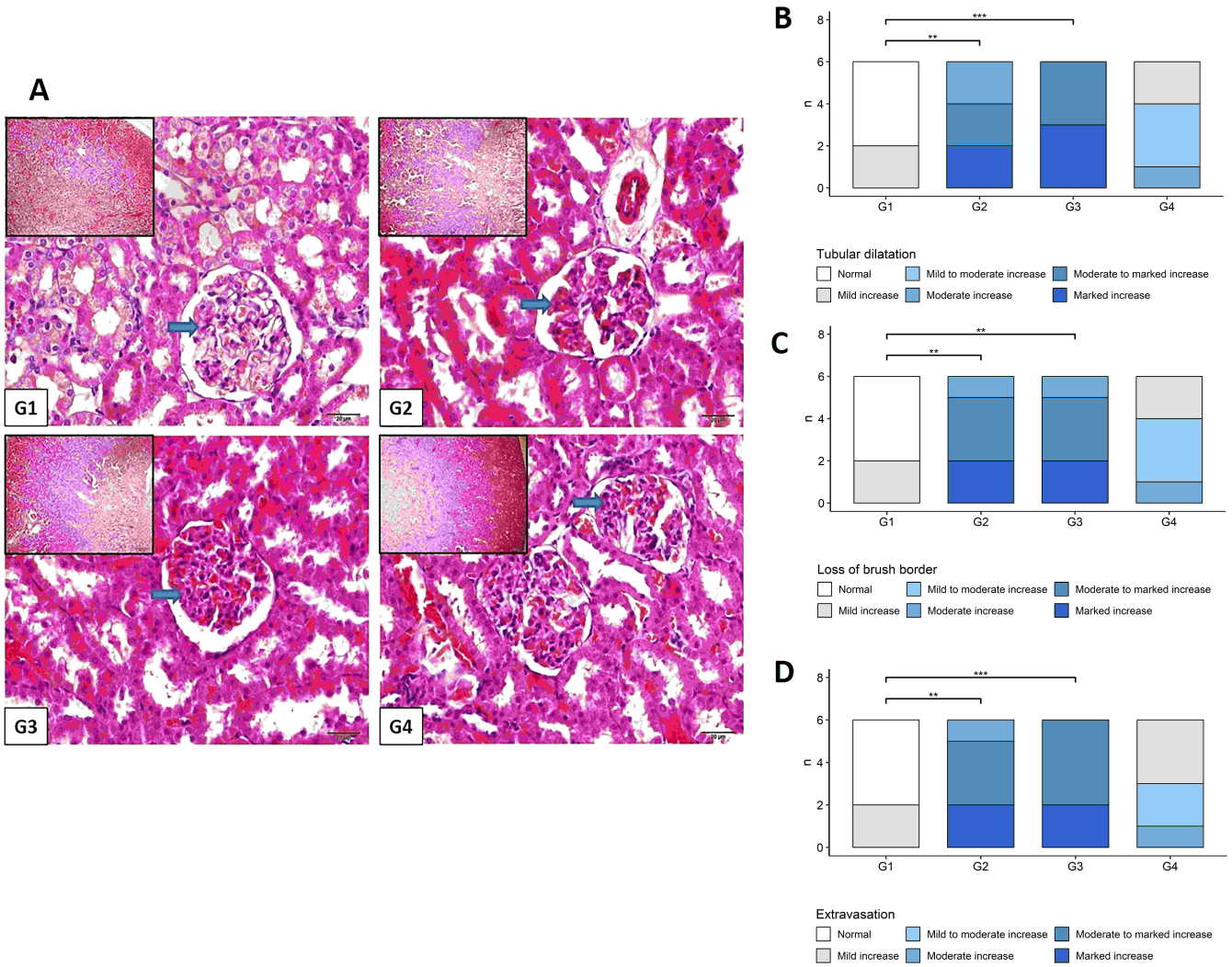

FIGURE 5. Histopathological appearance of the kidney tissues of the control and experimental sepsis model groups. H&E staining, 40×-magnification (Small photos 4×-magnification). Blue arrow, glomerulus. (A) Kidney histopathological evaluation findings. (B) Statistical evaluation of histopathological scoring for tubular dilatation. (C) Statistical evaluation of histopathological scoring for loss of brush border. (D) Statistical evaluation of histopathological scoring for extravasation (**$p < 0.005$, ***$p < 0.001$, values that do not exert a statistical significance are not shown in the figure).

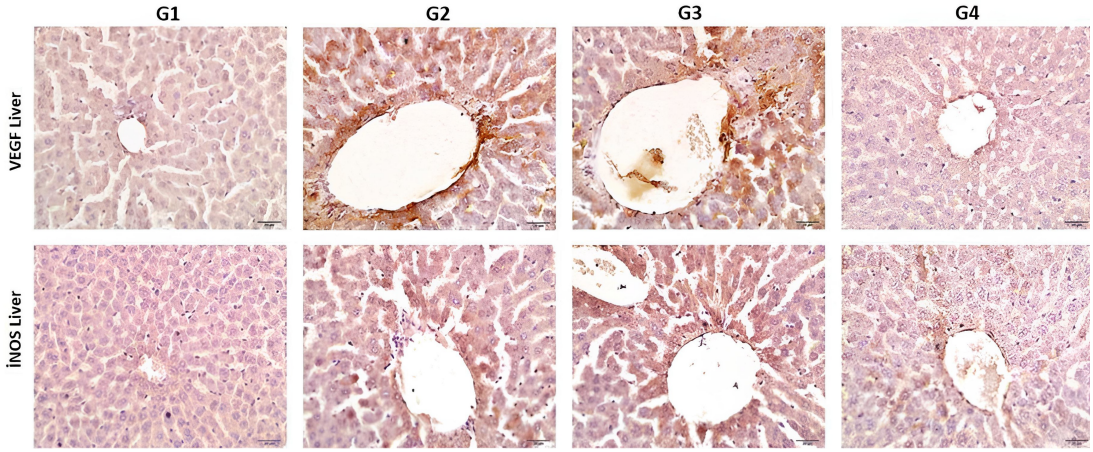

FIGURE 6. Liver histopathological appearance of the control and experimental sepsis model groups. Anti-Isoform nitric oxide synthases (Anti-iNOS, Vascular Endothelial Growth Factor (Anti-VEGF) staining for liver tissues. 40× magnification.

in the glomerular mesangial tissue and the proximal and distal tubules in the CLP and CLP + 0.9% NaCl groups. By contrast, the black carrot group showed a higher immunoreaction than in the control group but a lower immunoreaction than in the CLP and the CLP + 0.9% NaCl groups. The anti-iNOS staining showed no glomerular iNOS expression in the control group, although a few positive cells were found for iNOS in the interstitium and in a few tubular cells. In the CLP and CLP + 0.9% NaCl groups, an intense expression pattern was observed in the glomerular mesangial tissue and proximal and distal tubules. The black carrot group showed a similar staining pattern to that of the control group. In the control group, anti-desmin staining showed a minimal staining pattern in the epithelial cells of the Bowman parietal and visceral leaf and the distal and proximal tubular cells. In the CLP and CLP + 0.9% NaCl groups, the staining pattern increased due to the possible thickening of the basal laminae. Desmin expression was lower in the black carrot group and showed a similar staining pattern to that of the control group (Fig. 7).

4. Discussion

Sepsis is the whole systemic response of metabolism to an external factor or toxins secreted by pathogens. Sepsis can occur due to an infectious cause, or for autoimmunological or other reasons following a cytokine storm. Various experimental sepsis methods and models have been created for studies on sepsis [17, 18].

In the present study, CLP was found suitable as an experimental sepsis model. The main reasons for choosing the experimental sepsis model with the CLP method include its simple procedure, being a preferred procedure as reported in the literature, low costs, and is the closest method to sepsis suitable for the human body. The present study aimed to see the effects of black carrot extract on organ damage, treatment, and mortality in sepsis. This effect was investigated histologically with the biochemical values made with blood samples and also with sections taken from liver and kidney tissues [19–21].

In a study by Banks *et al.* [22], post-mortem liver biopsy was performed on 22 of 57 patients admitted to the service or intensive care unit with the diagnosis or pre-diagnosis of septicemia. In 16 of these patients, hyperplasia of Kupfer cells, inflammation in the liver portal area, congestion in the vena centralis, and focal liver cell necrosis were detected. Inflammation in the portal area of the liver was expressed as the main damage to the liver in patients who died as a result of sepsis. In the present study, a significant similarity in inflammation in the portal area was observed in the CLP and CLP + 0.9% NaCl groups. Greater dilatation of the vena centralis, located in the centre of the classical liver lobule, was detected in these groups compared to the CLP + black carrot and control groups. Although minimal PMNL infiltration was observed in the CLP + black carrot group, the structures of the arteria hepaticus interlobularis, vena interlobularis, and ductus biliferi in the portal hepatic triangle appeared normal, similar to those in the control group.

In an experimental sepsis model conducted by Fatemi *et al.* [23], the subjects received gamma-irradiated chervil oil and its hepatoprotective effects were investigated in rats. Glutathione, AST and ALT values in rats receiving this treatment were reported to have improved well-being based on biochemistry. In the present study, subjects which received the black carrot extract showed statistically significant differences in the AST and ALT values between the CLP and the CLP + 0.9% NaCl groups. The histological findings examined in our study demonstrated considerable differences in liver function and cellular damage markers between the CLP + black carrot group and the CLP or CLP + 0.9% NaCl groups. Furthermore,

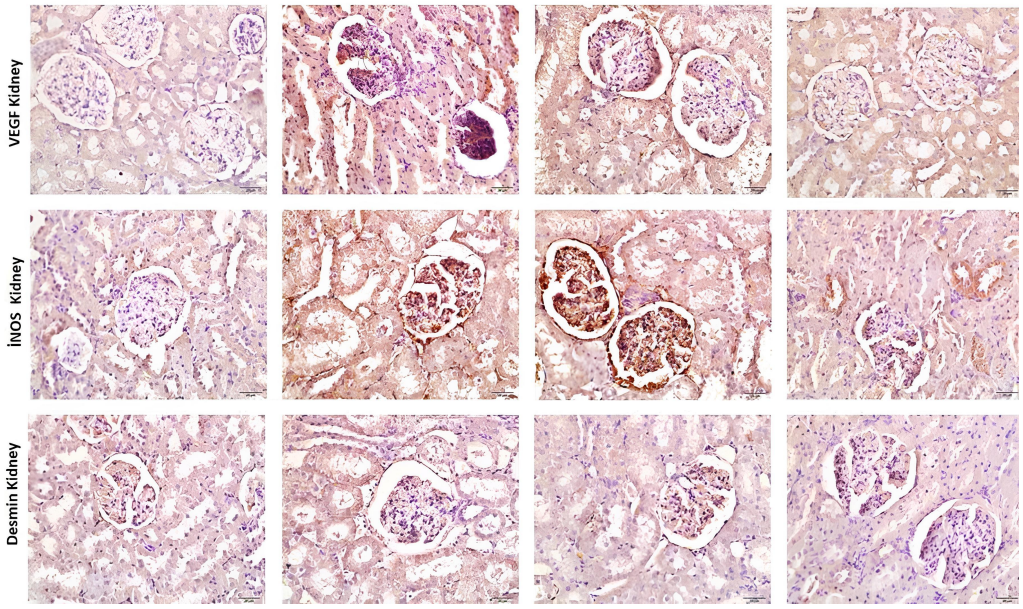

FIGURE 7. Kidney histopathological appearance of the control and experimental sepsis model groups. Anti-isoform nitric oxide synthases (Anti-iNOS), Vascular endothelial growth factor (Anti-VEGF) and Anti-Desmin staining for kidney tissues. 40× magnification.

both the biochemical analysis of AST and ALT, as well as the histological data, indicated that black carrot may have hepatoprotective effects.

In an experimental study by Kamlesh and colleagues evaluating the antioxidant and hepatoprotective effects of methanolic extracts of *Daucus carota* seeds, oxidative stress was induced in rat groups by administering thioacetamide at a dose of 100 mg/kg s.c., a known potent hepatotoxin and carcinogen that causes significant liver damage. Two test groups were given *D. carota* seed extract (DCSE) at doses of 200 mg/kg and 400 mg/kg, and liver function was evaluated on the 8th day by measuring AST, ALT and ALP levels. The study found that treatment with DCSE significantly reversed the thioacetamide-induced increases in biochemical parameters such as serum glutamik piruvik transaminaz (SGPT), serum glutamik-oksaloasetik transaminaz (SGOT), and alkaline phosphatase (ALP) levels, demonstrating the membrane-stabilizing activity and hepatoprotective effect of the extract [24]. In our study, evaluation of the biochemical samples for AST and ALT values revealed a similarity between the control group and the black carrot group. However, significant differences were found when comparing the black carrot group with the CLP and CLP + 0.9% NaCl groups, supporting the hepatoprotective effect of black carrot. Similarly, in a study by Tafail *et al.* [25], which examined the protective effects of *Daucus carota* root extract on carbon tetrachloride-induced hepatotoxicity in Balb C mice, reported a significant reduction in AST and ALT levels in the group treated with black carrot, paralleling the findings of our study.

Garofolo *et al.* [26] reviewed kidney and liver damage following sepsis, detailing histological changes observed in sepsis cases. These researchers specifically highlighted acute tubular necrosis in the kidney, resulting from a hypotension-induced decrease in secondary renal perfusion, which leads to tubular dilatation and brush border loss. In the present study, a significant difference was identified in the CLP + black carrot group compared to the CLP and CLP + 0.9% NaCl groups, particularly in terms of tubular dilatation and brush border loss. This finding aligns with those reported in the literature.

Al Toufaily *et al.* [27] conducted a study to investigate the nephroprotective effects of *Daucus carota* and *Cannabis sativa* in animal models, specifically addressing cisplatin-induced nephrotoxicity, given the widespread use of cisplatin in the treatment of various tumors. The findings of the study reveal that *Daucus carota* administration resulted in a notable decrease in urea levels, whereas the reduction in serum creatinine levels was comparatively less significant. These results suggest that both *Daucus carota* and *Cannabis sativa* may provide a protective effect against cisplatin-induced nephrotoxicity, potentially by mitigating the inflammatory response. In our study, although histopathological findings supported the nephroprotective effect, no significant reduction in serum urea levels was detected. This discrepancy with the literature is thought to be attributable to differences in treatment protocols. In the research conducted by Al Toufaily *et al.* [27], treatments commenced 5 days prior to cisplatin injection and continued daily for 5 days post-injection, resulting in a significant decrease in serum urea levels. Conversely, our study did not involve any treatment prior to CLP administration; instead, a black carrot solution was administered every 24 h for 7 days following CLP.

5. Limitations

This study has some limitations. The small sample size may limit the generalizability of the findings, and future research with larger cohorts is needed. While biochemical and histological analyses were conducted, molecular mechanisms underlying black carrot's effects were not explored. Finally, the study focused on short-term outcomes (7 days), and long-term effects remain unknown.

6. Conclusions

In our study, serum CRP and PRC values, along with biochemical markers indicating organ function and histological examinations, demonstrated that CLP is an effective experimental sepsis model. The biochemical data revealed that AST and ALT levels, which indicate liver function, were notably lower in groups treated with black carrot compared to those that did not receive the treatment. This suggests that black carrot may have hepatoprotective, anti-inflammatory, and antioxidant effects. These findings were further supported by histological data.

According to the biochemical data obtained from the tests, no significant differences were found in kidney function tests. However, histological data showed a marked reduction in inflammation at the cellular level. We believe these findings are valuable and will contribute to future studies employing different treatment methodologies.

In conclusion, the present study indicates that black carrot possesses anti-inflammatory and antioxidant properties at cellular and biochemical levels, with a particularly significant hepatoprotective effect. When combined with findings from the literature, it is evident that black carrot extracts reduce tissue inflammation. However, this reduction is insufficient for complete recovery. Future research employing various treatment methods and dosages of black carrot is anticipated to be necessary to further explore these effects.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

AUTHOR CONTRIBUTIONS

MSK, KG, EG, ME—Project Design, Interpreting data and Writing the manuscript. CT; CŞT, MAŞ—Follow-up, therapy and treatment of rats. YU—Preparation of samples for histological examination and evaluation. EÖÇ, FK—Obtaining and implying the extract. EC, EDS—Evaluation and examination of biochemical results. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of Ege University with the number 2018-064 on 19 September 2018.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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