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Research Article

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Protective Effects of *Cuscuta* sp. against Cardiorenal Injury in Bile Duct-Ligated Rats

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Abstract

Objective: Bile duct ligation (BDL) obstructs bile flow, resulting in bile and toxic substances buildup that causes liver damage. This study investigated the protective effects of *Cuscuta* sp. methanol extract (CUS) against cardiorenal injury in bile duct-ligated rats.

Materials and Methods: Rats were categorised into four groups: Control (C), CUS, BDL, and BDL+CUS. The C and BDL groups received saline, whereas the other groups received oral 250 mg/kg CUS. After 28 days, blood, kidney, and heart tissue samples were collected for biochemical and histological analyses. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), direct bilirubin (DB), and total bilirubin (TB) levels were analysed to determine liver function, while Transforming growth factor-beta (TGF-β) and hydroxyproline (HYP) levels were evaluated for fibrosis, and 8-hydroxy-2-deoxyguanosine (8-OHdG) levels alongside Na^+/K^+-ATPase activity were analysed to assess oxidative stress and membrane injury in the heart and renal tissues.

Results: AST, ALT, DB, and TB levels were significantly elevated in the BDL group compared with the C group; however, the levels were distinctly lower in the BDL+CUS group than in the BDL group. Additionally, in both tissues, TGF-β, HYP, and 8-OHdG levels were higher in the BDL group than in the C group, but decreased in the BDL+CUS group, with Na^+/K^+-ATPase activity being lower in BDL group compared with the C group and significantly increased in BDL+CUS group.

Conclusion: CUS has protective effects against oxidative damage and offers antioxidant and anti-inflammatory benefits against cholestasis-induced tissue injury.

Keywords Bile duct ligation · Fibrosis · Cardiorenal injury · *Cuscuta* sp.

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INTRODUCTION

The liver is a crucial organ for the regulation of metabolic balance. Obstruction of the bile ducts blocks the flow of bile. This causes bile and/or toxic substances to accumulate in the liver.[1] The accumulation of hydrophobic bile acids causes oxidative damage to the liver by producing oxygen free radicals.[2,3] Damage to parenchymal cells in an early phase of cholestasis may be responsible for inflammatory and fibrinogenic effects on non-parenchymal cells.[4] After liver injury, activation of hepatic stellate cells (HSCs) and unbalanced collagen synthesis are major factors involved in the pathogenesis of fibrosis.[5] Fibrosis is an important pathological process in liver cirrhosis.[6]

The serious complications of cirrhosis worsen patient prognosis. One of these complications is portal hypertension. The main factor contributing to the increase in portal pressure is the increased resistance of blood to the flow out of the portal system. The liver is bypassed because of portosystemic shunts due to portal hypertension, and vasodilators like nitric oxide, carbon monoxide, and endogenous cannabinoids remain to circulate throughout the body. The damage to hepatocytes causes an increased release of these substances into the splanchnic circulation, leading to arterial vasodilation.[7] Hyperdynamic circulation syndrome, defined by depleted, low blood pressure in arteries, systemic vascular resistance, increased cardiac output, and high heart rate is seen in the advanced stages of portal hypertension, and this is accepted as a pre-stage for multi-organ failure.[8,9] While the arterial blood pressure decreases with the increase in the production or activity of vasodilator factors, vasoconstrictor mechanisms (Renin-angiotensin system, arginine vasopressin, etc.) are activated to compensate for the arterial pressure; however, as a result, renal perfusion decreases, and ultimately kidney damage occurs as hepatorenal syndrome occurs.[10,11] In addition to changes in splanchnic and systemic circulation, inflammation plays a significant role in the progression of hepatorenal syndrome.[12]

As cirrhosis advances, systemic vascular resistance diminishes to such an extent that an increase in cardiac output is no longer sufficient to ensure proper organ perfusion. This condition is referred to as cirrhotic cardiomyopathy and is closely associated with persistent portal hypertension.[10] Cardiac dysfunction due to cirrhosis may occur in patients without known heart disease. Pathogenic mechanisms include vasodilation, decreased myocardial beta-adrenergic function, and impaired sodium and calcium transport.[13,14] In this syndrome, in addition to hyperdynamic circulation, there are impaired contractile responsiveness to stress and/or abnormal diastolic relaxation, and electrophysiological dysfunctional.[14]

The bile duct ligation (BDL) method shows morphological changes similar to human biliary cirrhosis, and it has been widely used as an experimental model of hepatic cholestasis and fibrosis in rats.[15,16] The role of oxygen radicals in the initiation and persistence of tissue damage in obstructive jaundice has been demonstrated through various experimental and clinical investigations.[17-20] Therefore, eliminating free radicals as a strategy for treating cholestatic liver injury could help reduce oxidative damage and fibrosis after biliary obstruction.[21]

Cuscuta species are parasitic plants that are usually yellow, orange, and red. These species carry alkaloids, carotenoids, fatty acids, flavonoids, steroids/sterols, triterpenes, and other compounds as secondary metabolites. Some studies have shown that different species of this genus have antiproliferative, antioxidant, anti-inflammatory, hepatoprotective, antimicrobial, and anxiolytic activities, and anticancer and antiviral effects in ovarian and breast cancer.[22]

Accordingly, this study assessed the potential protective or deterrent effects of *Cuscuta* sp. on cardiorenal injury due to bile duct ligation-induced cholestasis in rats.

MATERIALS AND METHODS

Preparation of Plant Extracts

The "IKŞUT" or "Küsküt" plant, which grows in various regions of Turkey and has become a part of the culture of Mardin and its region, has been identified with the region and its culture, especially in the treatment of liver disorders and physiological jaundice in newborn babies and mothers. For this reason, the plant was obtained from an herbalist in the Mardin region.[23] In May 2018, aerial parts of *Cuscuta* sp. were acquired from a Mardin herbalist. Dr. Ahmet Doğan identified the plant, and once it was turned into a herbarium sample, it was registered by giving it a herbarium number in the Faculty of Pharmacy of Marmara University (MARE No: 22668). The dehydrated aboveground parts of the plant were ground into a powder, weighed to a precise quantity (757.06 g), and macerated in 1600 ml of methanol. The following filtration, the resulting methanol extract's solvent was dried out in a rotary evaporator. The obtained methanol extract (CUS) (10.83%) was stored at +4°C until analysis. The decision to use the methanol extract in this study was based on the findings of Koca-Caliskan et al.[24], who demonstrated that the methanol extract of similar *Cuscuta* sp. exhibited stronger hepatoprotective effects than the water extract. Since the methanol extract used in the studies by Koca-Caliskan et al. working with similar

Cuscuta species was active at these doses, we decided that the methanol extract dose should be 250 mg/kg in this study.[24]

Animals

In this study, 200-300 g male Sprague-Dawley rats (n=28) were used. Marmara University (M.U) Research Centre for Experimental Animals supplied the animals. M.U. The Animal Experiments Ethics Committee approved all experimental protocols (25.11.2020, Decision No:60.2020.mar). The animals were housed in a place with a steady temperature of 22±2 °C, 50%±5 humidity with a 12 h light/dark cycle and were fed standard pellet feed and tap water *ad libitum*.

Experimental Design

The rats were divided into 4 groups. Bile duct ligation (BDL) and BDL+CUS groups underwent the BDL procedure, whereas the C and CUS groups underwent a sham operation. The BDL and BDL+CUS groups contains 8 rats, whereas the C and CUS groups' 6 rats. Animals in the C and BDL groups received 1 mL/kg of saline orally, whereas the CUS and BDL+CUS groups received 250 mg/kg of CUS extract dissolved in saline orally every day for 28 days.

On the 28th day of the study, rats were decapitated, and blood, heart, and kidney tissue samples were obtained. Blood samples were examined for the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and direct and total bilirubin (DB and TB). In heart and kidney tissues, transforming growth factor-β (TGF-β), 8-hydroxyguanosine (8-OHdG), sodium-potassium ATPase (Na^+/K^+-ATPase), and hydroxyproline (HYP) levels were assessed. Additionally, using a light microscope, heart and kidney tissue sections were histologically analysed.

Induction of Bile Duct Ligation

Rats were anaesthetised using ketamine (100 mg/kg) and chlorpromazine (0.7 mg/kg). The bile duct ligation procedure was performed accordingly Criado et al.'s procedure.[25] The common bile duct located between the liver lobes and the duodenum was exposed and cleared off surrounding tissues, and then ligated with double silk sutures. The initial ligature was placed below where the hepatic ducts converge, while the subsequent one was placed above the opening of the pancreatic ducts. Ultimately, the duct was resected between the two ligatures. In the sham group, the bile duct was similarly exposed, but no ligation was performed.

Biochemical Analysis

Evaluation of Serum Biochemical Parameters

To evaluate BDL-induced liver injury, serum AST, ALT, DB, and TB levels were quantified using commercial kits (BT Laboratory, Shanghai).

Determination of Tissue Biochemical Parameters

The tissue samples were homogenised in PBS (pH 7.4) and centrifuged at 1000xg for 15 min at +4°C to obtain the upper phase. All parameters to be examined in tissues were measured using commercial kits. The tissue TGF-β levels were evaluated using a kit (AFG company, EK720060), following the instructions provided by the manufacturer. After homogenisation of the tissues, protein determination was performed with BCA in the upper phase. Na^+/K^+-ATPase levels in tissue were evaluated using a kit (AFG company, EK720668) according to the manufacturer's instructions. After homogenisation of the tissues, protein determination was performed with BCA in the upper phase. 8-OHdG levels in tissue were evaluated using a kit (AFG company, EK720424) according to the manufacturer's instructions. After homogenisation of the tissues, protein was determined using BCA in the upper phase. Hydroxyproline levels in tissue were determined using a commercial kit (AFG company, EK720734) following the instructions provided by the manufacturer. After homogenisation of the tissues, protein was determined using BCA in the upper phase.

Light Microscopic Preparation

For light microscopic analysis, samples from the heart and kidney were preserved in 10% formaldehyde, subjected to a graded alcohol dehydration process, cleared with toluene, and embedded in paraffin. 5 µm thick paraffin sections were stained with haematoxylin and eosin for histopathological analysis. The sections were then inspected and captured using a digital camera (Olympus DP72, Tokyo, Japan) of a photomicroscope (Olympus BX51, Tokyo, Japan). For histopathological examination, one section from each animal in the experimental group was obtained, and at least five similar areas in each section were examined. Stained heart and kidney samples were scored semiquantitatively using a scale ranging from 0 to 3 (0: none; 1: mild; 2: moderate; 3: severe) for each criterion summarised in Table 1.[26,27] The maximum scores were 6 for the heart and 9 for the kidneys.

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). All data are presented as the mean ± standard error of the mean. One-way

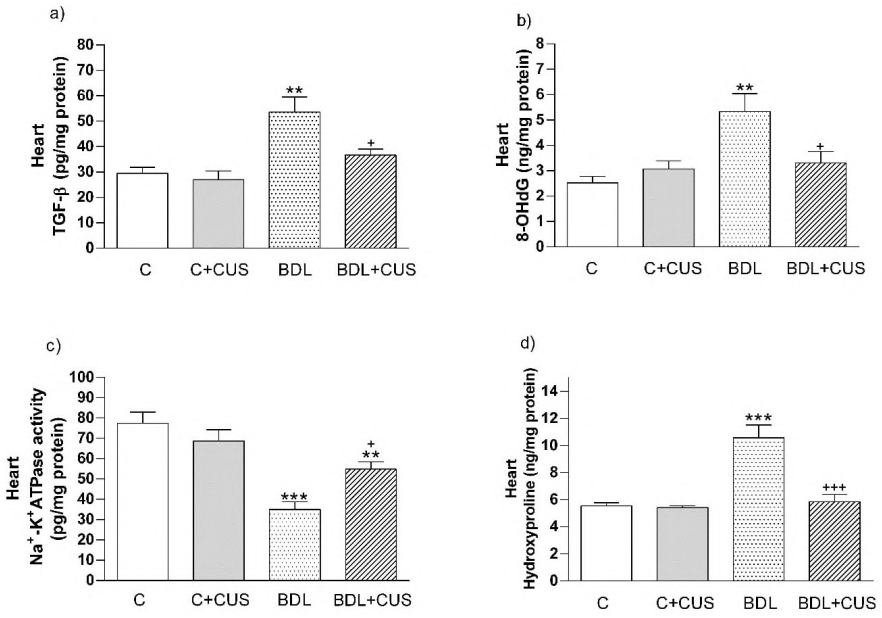

Figure 1. TGF-β (a), 8-OHdG (b), Na^+/K^+-ATPase (c), and HYP (d) levels of heart tissue. TGF-β: Transforming growth factor, 8-OHdG: 8-hydroxy-2'-deoxyguanosine, Na^+/K^+-ATPase: Sodium-potassium adenosine triphosphatase, HYP: Hydroxyproline, C: Control group, BDL: Bile duct ligation. CUS: *Cuscuta* sp. extract. One-way analysis of variance ANOVA was used for statistical analysis. **p<0.01 and ***p<0.001; Compared to the control group, +p<0.05 and +++p<0.001; Compared to BDL group. Values are presented as mean and standard error.

Table 1. The microscopic scoring criteria for heart and kidney damage.

Tissue	Appearance
Heart	Disorganisation of cardiomyocytes
	Vascular congestion and haemorrhage
Kidney	Degeneration of Bowman's space and glomeruli
	Degeneration of the proximal and distal tubules
	Vascular congestion and interstitial oedema

Scores for each criterion are given as 0, none; 1, mild; 2, moderate; 3, severe. At least five microscopic areas were examined to score each specimen.

Table 2. Serum AST, ALT, DB, TB levels.

Serum	C	C+CUS	BDL	BDL+CUS
AST (U/L)	134.3 ± 4.5	132.4 ± 6.0	427.0 ± 24.8***	298.3 ± 5.2***,+++
ALT (U/L)	53.5 ± 7.3	56.8 ± 6.1	152.0 ± 5.0***	86.3 ± 7.9 *,+++
Direct bilirubin (mg/dL)	0.4 ± 0.2	0.4 ± 0.2	5.0 ± 0.3***	4.0 ± 0.3***
Total bilirubin (mg/dL)	0.4 ± 0.1	0.5 ± 0.1	5.4 ± 0.4***	3.9 ± 0.4***,+

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, DB: Direct bilirubin, TB: Total bilirubin, C: Control group, BDL: Bile Duct Ligation, CUS: *Cuscuta* sp. extract. One-way analysis of variance ANOVA for statistical analysis. *p<0.05, ***p<0.001 compared to Control group, +p<0.05, +++p<0.001 compared to BDL group. Values are presented as mean and standard error.

analysis of variance (ANOVA) was used to create data groups, and p<0.05 values were considered significant.

RESULTS

Serum Biomarkers of Liver Function

Table 2 shows that there was a significantly rise (p<0.001) in AST, ALT, DB, and TB levels in the BDL group compared with the C group. In contrast, the BDL+ CUS group showed a marked reduction in these levels compared with the BDL group (p<0.05 for TB, p<0.001 for AST and ALT).

Biochemical Parameters of Heart Tissue

Heart tissue TGF-β, 8-OHdG, and hydroxyproline levels were significantly elevated in the BDL group compared with the C group (p<0.01 for TGF-β and 8-OHdG, p<0.001 for HYP). In addition, these levels were significantly reduced in the BDL+ CUS group compared with the BDL group (p<0.05 for TGF-β and 8-OHdG, p<0.001 for HYP) (Figure 1). Compared with the C group, the Na^+/K^+-ATPase activity in the heart tissues of the BDL group was significantly reduced (p<0.001), but it was significantly increased (p<0.05) in the CUS-treated BDL group (Figure 1).

Biochemical Parameters of Renal Tissue

While there were significantly higher TGF-β, 8-OHdG, and hydroxyproline levels in the renal tissues of the BDL group compared with the C group (p<0.05 for 8-OHdG, p<0.01 for TGF-β and HYP), in the BDL+CUS group, these values were lower than those in the BDL group. (p<0.05 for 8-OHdG and HYP , p<0.01 for TGF-β) (Figure 2).Na^+/K^+-ATPase activity in the

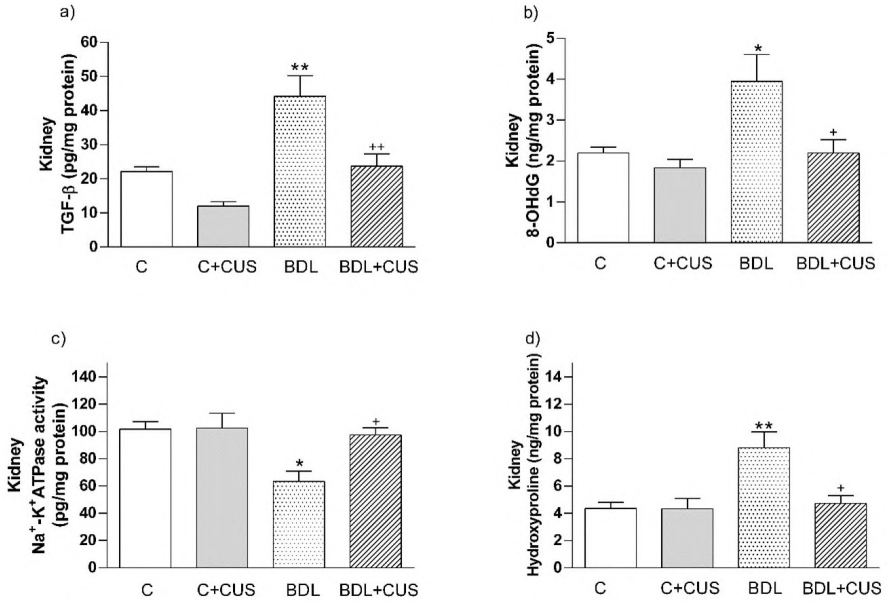

Figure 2. TGF-β (a), 8-OHdG (b), Na^+/K^+-ATPase (c), and HYP (d) levels in kidney tissue. TGF-β: Transforming growth factor, 8-OHdG: 8-hydroxy-2'-deoxyguanosine, Na^+/K^+-ATPase: Sodium-potassium adenosine triphosphatase, HYP: Hydroxyproline, C: Control group, BDL: Bile duct ligation. CUS: *Cuscuta* sp. extract. One-way analysis of variance ANOVA was used for statistical analysis. *p<0.05, **p<0.01; Compared to the control group, +p<0.05, ++p<0.01; Compared to BDL group. Values are presented as mean and standard error.

BDL group's renal tissue was significantly reduced (p<0.05) in comparison with the C group, but it was significantly increased in the BDL+CUS group (p<0.05) (Figure 2).

Histopathological Evaluation on Heart Tissue Samples

Regular cardiomyoctes and capillaries were observed in groups C (Figure 3A) and C+CUS (Figure 3B). In BDL group, severe vascular congestion and degenerated cardiomyocytes were noted (Figure 3C). Quite regular cardiomyoctes and mild vascular congestion were noted in the BDL+CUS group (Figure 3D).

In heart tissue, none of the criteria for microscopic scoring were observed in the C and C+CUS groups. BDL group showed significantly higher scores than the C group (p<0.001), indicating more severe cardiac damage. In BDL+CUS group, a significant reduction in severity was noted compared with the BDL group (p<0.01) (Figure 4A).

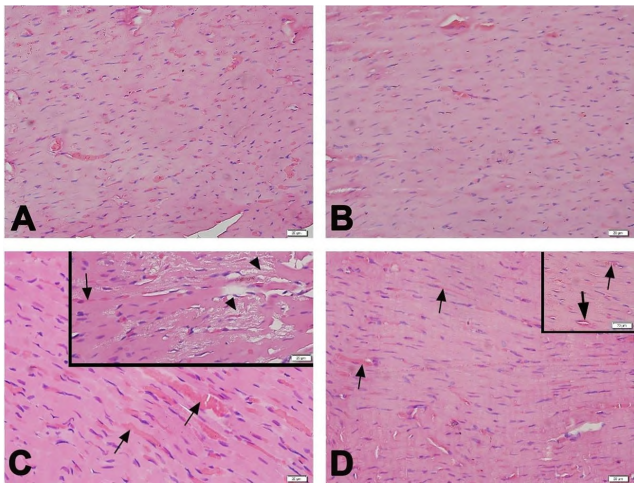

Figure 3. Representative photomicrographs of the heart in the experimental groups. Regular cardiac muscle with cardiomyocytes and capillaries observed in the Control (A) and C+CUS (B) groups. Strong vascular congestion (arrow) and degenerated cardiomyocytes (arrowhead) are observed in the BDL group (C). In the BDL+CUS group, regular cardiomyoctes and moderate vascular congestion (arrow) are noticed (D). Haematoxylin and eosin staining. Scale bars: 20 µm.

Histopathological Evaluation on Kidney Tissue Samples

Regular kidney structure in Bowman's spaces, glomeruli, and tubular epithelium (Figure 5A) was observed in the C group. The C+CUS group exhibited relatively normal kidney morphology, including Bowman's space and tubular epithelium, with moderate glomerular and interstitial congestion (Figure 5B). In BDL group, moderate interstitial and glomerular vascular congestion and slight tubular epithelium degeneration were noted (Figure 5C). BDL+CUS group showed mild tubular epithelial degradation and mild glomerular and interstitial vascular congestion (Figure 5D). In kidney tissue, none of the criteria for microscopic scoring were observed in the C group. The BDL group showed significantly higher scores than the C group (p<0.001), indicating more severe renal damage. In BDL+CUS group, a significant reduction in

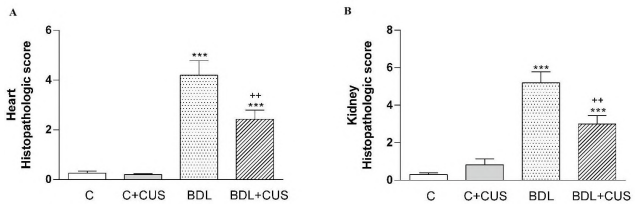

Figure 4. Total histopathological scores of the heart (A) and kidney (B) tissues. C: Control group; BDL: Bile duct ligation. CUS: *Cuscuta* sp. extract. One-way analysis of variance ANOVA was used for statistical analysis. ***p<0.001; Compared to the control group, ++p<0.01; Compared to BDL group. Values are presented as mean and standard error.

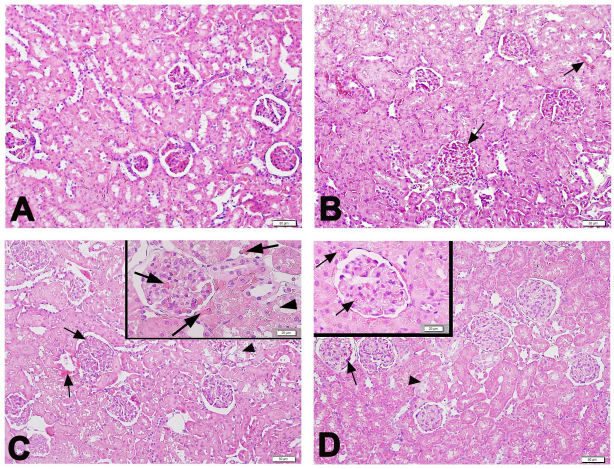

Figure 5. Representative photomicrographs of the kidneys in the experimental groups. Regular structure of kidney is noticed in the C group (A). Mild glomerular and interstitial congestion (arrow) and regular tubules are noticed in the C+CUS group (B). Moderate interstitial and glomerular vascular congestion (arrow) and mild tubular epithelium degeneration (arrowhead) are noticed in the BDL group (C). In the BDL+CUS group, moderate interstitial and glomerular vascular congestion (arrow) and tubular epithelium degeneration (arrowhead) are noticed (D). Haematoxylin and eosin staining; scale bars: 50 µm and insets: 20 µm.

the severity was noted compared with the BDL group (p<0.01) (Figure 4B).

DISCUSSION

One of the most dangerous consequences of cirrhosis is hepatorenal syndrome- acute kidney injury, which affects up to half of hospitalised patients and has a high mortality rate. Recent developments have supported the important role of systemic inflammation and circulatory abnormalities in the kidney, in addition to systemic and splanchnic circulatory changes, in the pathogenesis of hepatorenal syndrome.[28] In patients with cirrhosis, abnormalities in heart tissue structure and function have been found, with disturbances of systolic and diastolic contraction, electrophysiological repolarization changes with a long QT interval, and enlargement or hypertrophy of the heart chambers.[29-31] In some studies, it has been said that histological anomalies such oedema of cardiomyocytes, mild diffuse fibrosis, atypical pigmentation, ventricular dilatation, and hypertrophy have been linked to cirrhosis.[32] In the present study, we found that BDL caused both cardiac and renal tissue damage, as evidenced by biochemical and histopathological results. TGF-β, 8-OHdG, and hydroxyproline levels in these tissues were increased, whereas Na^+/K^+-ATPase activity was decreased. In addition, histopathological evidence in the kidney tissues BDL group revealed moderate interstitial and glomerular vascular occlusion and mild tubular epithelial degeneration, whereas severe vascular congestion and degenerated cardiomyocytes were observed in the heart tissues. Bile duct ligation induces a significant pathological effect on kidney and heart morphology. On the other hand, treatment with *Cuscuta* sp. alleviated histological changes in both heart and kidney tissues, reducing cardiomyocyte degeneration and vascular congestion in the heart and diminishing interstitial and glomerular vascular congestion in the kidney, resulting in less severe pathological changes compared with the BDL group. Our results show that BDL induces significant cardiac and renal damage, as reflected by higher scores in the BDL group compared with the C group. *Cuscuta* sp. treatment significantly reduced the severity of cardiac and renal injury. Biochemical and histological results indicate a protective effect of *Cuscuta* sp. against BDL-induced cardiorenal damage, possibly due to its antioxidant and anti-inflammatory properties.

The two most widely used experimental models for liver fibrosis are carbon tetrachloride administration and bile duct ligation. The advantage of BDL model is that it causes changes in kidney function in a short time.[8] Matyas et al., it was shown that BDL is an appropriate experimental model to examine the pathophysiology of cirrhotic cardiomyopathy at a preclinical level.[33] Accordingly, we used this method to investigate the effects of the methanolic extract of *Cuscuta* sp. on kidney and heart function due to cholestasis. The lack of specific treatment for hepatic cardiomyopathy highlights the increasing need for drug therapies to address the cardiac and vascular complications associated with liver failure.[33] Many studies have shown that *Cuscuta* species have a variety of biological activities, including antioxidant, antimicrobial, immunostimulatory, antibacterial, anti-inflammatory, hepatoprotective, antiproliferative, and antiulcer activities.[22,34-36] In our study, histopathological findings showed severe vascular congestion and degeneration in cardiomyocytes with BDL, which were ameliorated with CUS treatment.

It is well known that ALT, AST, and TB levels, which indicate liver damage, increase in cholestasis.[24] In the present study,

ALT, AST, DB, and TB levels were measured to assess liver function and the presence of cholestasis. Koca-Çalışkan et al. examined the effects of *Cuscuta arvensis* methanolic extract on acetaminophen-induced liver toxicity and showed that the extract protects against tissue damage with its antioxidant effects and reduces serum ALT, AST, and TB levels, thus regulates liver functions.[24] Similarly, in the present study, ALT, AST, DB, and TB levels, which increased with BDL, decreased with CUS treatment.

TGF-β has been synthesised by all cell types in the kidney and has been suggested to be an important profibrotic mediator.[37] TGF-β was measured in both heart and kidney tissues as a marker to observe the development of fibrosis, given its role as a key profibrotic mediator, and hydroxyproline levels, which indicate collagen accumulation, were also measured for the same purpose. In a previous study, BDL-induced renal dysfunction in mice was associated with increased hydroxyproline levels in kidney tissue were observed.[38] As a result, increased TGF-β and hydroxyproline levels in renal and heart tissue due to BDL led to the observation of fibrosis. In our study, the decrease in the BDL-induced increase in TGF-β and hydroxyproline levels in heart tissue following *Cuscuta* sp. treatment suggests that the methanolic CUS extract used in our study reduces tissue damage through its antioxidant and anti-inflammatory effects.

Membrane-bound Na^+/K^+-ATPase is critical for cellular transport but is very vulnerable to free radical reactions and lipid peroxidation. As a result, a decrease in Na^+/K^+-ATPase activity indicates oxidative membrane damage.[39] In another study to investigate the effects of *Cuscuta chinensis* in an experimental acute renal failure model, it was observed that ischaemia/reperfusion-induced acute renal failure decreased Na^+/K^+-ATPase expression in the renal medulla. However, treatment with *Cuscuta chinensis* significantly increased Na^+/K^+-ATPase expression levels.[40] In the present study, we found that BDL reduced the Na^+/K^+-ATPase activity in kidney and heart tissues, which was measured as an indicator of oxidative stress and membrane damage. However, in both tissues, we observed a significant increase in these levels following CUS administration.

It has been acknowledged that the tissue 8-OHdG level is a helpful marker for evaluating DNA damage caused by oxidative stress. In a study by Huang et al., liver injury induced by the BDL method, it was shown that 8-OHdG levels increased.[41] Matono et al. also agreed with these results and emphasised that 8-OHdG is an oxidative marker in rats with BDL.[42] Serum 8-OHdG levels are increased in various cardiovascular insults, such as interstitial cardiac fibrosis, myocardial infarction, and left ventricular remodelling following cardiac hypertrophy.[43] In our study, BDL induced increased renal and cardiac 8-OHdG levels, which were measured as an indicator of oxidative DNA damage, and these levels were reduced by CUS treatment. The reduced 8-OHdG levels demonstrated that *Cuscuta* sp. can prevent the oxidative damage of BDL-induced renal and cardiac problems.

In a study conducted to investigate the pathogenesis of cholemic nephropathy due to cirrhosis, interstitial inflammation, significant necrosis, and tubular dilatation were observed in the histopathological examination of kidney tissue in animals with bile duct ligation.[44] Sheibani et al. examined cirrhosis-induced changes histologically and observed histopathological changes such as intracellular plug formation in the kidney tissue of BDL and myocardial fibrosis and myocardial hypertrophy in the heart tissue.[45] Consistent with these results, histopathological examination showed the presence of moderate interstitial and glomerular vascular occlusion and mild tubular epithelial degeneration in kidney tissue and severe vascular occlusion and degenerated cardiomyocytes in heart tissue in the BDL group. We observed that the treatment group exhibited improved findings.

A previous study reported that *Cuscuta* species contain many phenolic compounds, especially flavonoids.[22] Similarly, our analysis of the *Cuscuta* extracts showed that it has high phenolic content and antioxidant and anti-inflammatory activities.[46] Phenolic compounds and/or medicinal plants rich in phenolic compounds have protective effects against hepatorenal and cardiac injuries.[47,48] Antioxidant and anti-inflammatory compounds found in plants have also been reported to be effective against hepatorenal[49,50] and cardiac damage.[51-53] Therefore, phenolic compounds together with other compounds found in the plant may be responsible for these effects of CUS, which was previously reported to have antioxidant and anti-inflammatory activities.

Our histological findings support the biochemical findings and indicate that there was significant damage to the renal and heart tissues of the BDL group, whereas this damage was reduced in CUS-treated BDL rats. Accordingly, this improvement in tissues is due to the antioxidant and antifibrotic effects of *Cuscuta* species and that the plant should be examined in further studies.

CONCLUSION

In this study, we demonstrated the biochemical and histological effects of bile duct ligation-induced cholestasis in the heart and kidneys. Our findings led us to believe that *Cuscuta* sp. can alleviate these damages. However, more detailed studies are needed. Therefore, based on the

subsequent investigations, we concluded that it may play a part for treating cardiorenal injury caused by cholestasis.

Ethics Committee Approval	The Animal Experiments Ethics Committee approved all experimental protocols (25.11.2020, Decision No:60.2020.mar).
Peer Review	Externally peer-reviewed.
Author Contributions	Conception/Design of Study- G.S., B.N.H., D.O.; Data Acquisition- G.S., B.N.H., D.O., A.S., O.A., S..EP.; Data Analysis/Interpretation- G.S., O.C., F.E., F.K.M.; Drafting Manuscript- G.S., B.N.H., A.S., O.A., S.E.P.; Critical Revision of Manuscript- G.S., D.O., A.S., O.C., F.E., F..KM.; Final Approval and Accountability- G.S., B.N.H., D.O., A.S., O.C., F.E., OA, SEP, F.K.M.
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