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# Dodder (*Cuscuta* sp.) Extract Ameliorates Liver Fibrosis in Bile Duct-Ligated Rats

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## ABSTRACT

**Objective:** The aim is to examine the possible protective effect of *Cuscuta* sp. extract against liver damage induced by biliary obstruction in rats.

**Materials and Methods:** To induce biliary obstruction, the bile duct ligation (BDL) method was used. Sprague Dawley rats were allocated to 4 groups: Control (C), *Cuscuta* (CUS), bile duct ligation (BDL), and bile duct ligation + *Cuscuta* (BDL+CUS). Control and BDL rats were given physiological saline (SF), while CUS and BDL+CUS groups were administered 250 mg/kg of *Cuscuta* extract by oral gavage. At the end of 28$^{th}$ day, the rats were decapitated, serum and tissue samples were collected, and aspartate aminotransferase (AST), alanine transaminase (ALT), and direct and total bilirubin (DB and TB) levels were determined in blood samples. In liver tissues, transforming growth factor-β (TGF-β), 8-hydroxyguanosine (8-OHdG), hydroxyproline, and sodium–potassium ATPase ($Na^+/K^+$-ATPase) levels were determined.

**Results:** Serum samples of rats with cholestasis had high ALT, AST, DB, and TB levels, while TGF-β, 8-OHdG, and hydroxyproline concentrations were found to be significantly high in tissues. Hepatic $Na^+/K^+$-ATPase levels were decreased through biliary obstruction. Biochemical parameters were drastically reversed by *Cuscuta* care; also, this was supported histologically.

**Conclusion:** Results showed that *Cuscuta* extract, through its antioxidant and anti-inflammatory properties, provided protection against oxidative injury by biliary obstruction. Also, these results confirm the traditional use of *Cuscuta* sp. as hepatoprotective.

**Keywords:** Liver damage, Dodder, Inflammation, Oxidative injury

## INTRODUCTION

Chronic liver disease is the most important disease globally (1). Fibrosis is a wound healing state that develop as a result of a response to liver injury due to different etiological causes, and are also a dynamic process involving unbalanced synthesis and destruction of extracellular matrix (2-5). Chronic liver disease is caused by liver fibrosis, hepatocyte damage, aggregation of platelet and inflammatory cells, stimulation of Kupffer cells, and finally discharge of cytokines and growth factors, respectively (6,7).

Obstruction in the bile duct preventing the flow of bile acids causes the accumulation of toxic bile components, which leads to the formation of reactive oxygen/nitro-

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gen derivatives in the liver (8). *In vitro* studies have shown that these harmful derivatives are formed when hepatocytes are treated with bile acids (9). Mitochondria is the main organelle from which reactive products are produced. Free radicals originating from hepatic mitochondria cause damage to liver cells and constitute the underlying causes of fibrosis (10). Considering that these factors have a momentous impact on pathogenesis, suppression of radical formation can prevent inflammation and fibrosis. Although various models have been used to develop liver fibrosis, bile duct ligation has the most clinical relevance.

The adverse effects of drugs lead researchers to look for new drugs. Recently, drugs obtained from natural sources have been studied and new alternatives with anti-fibrotic properties continue to be sought. It has been reported that some herbs are used in traditional medicine for liver-biliary disorders. Along these lines, one of these herbs is the *Cuscuta* species, used by the public in the treatment of jaundice in Turkey (11). The species of *Cuscuta*, known by the name Bostanbozan, are perennial, chlorophyll-free, and parasitic plants (12,13). *Cuscuta* species are used in the treatment of jaundice in Denizli, Diyarbakır, and Manisa, as well as in the province of Mardin. It has also been reported that these species have traditional uses as diuretics, carminative, laxatives, and cholagogues (14-16). They contain several secondary metabolites, such as flavonoids (quercetin, hiperoside etc.), alkaloids (cuscutamine, lupanine etc.), steroids and sterols (campesterol, sesamin etc.), triterpenes (Lupeol, ursolic acid etc.), carotenoids (Lutein, lycopene), fatty acids (oleic acid, linolenic acid etc.), and other compounds (cuscutalin, amarvelin etc.) (17).

In this experiment, the aim is to examine the possible therapeutic effect of *Cuscuta* sp. extract against liver damage induced by bile duct ligation (BDL).

## MATERIALS AND METHODS

### Animals and Ethics

In this experiment, all procedures related to experimental animals were performed according to NIH guidelines (NIH Publications No. 8023, revised 1978). Male Sprague Dawley rats (weighing 200-300 g) were kept at a constant temperature of 22±2°C, 50%±5 humidity, and a light/dark cycle (12/12h). Animals were allowed ad libitum feeding. This experiment was guided with the approval of Animal Experiments Ethics Committee (03/03/2021, Decision No: 40.2021mar).

### Groups and Experimental Protocol

Rats were arbitrarily separated into 4 groups. The Control (C) and *Cuscuta* (CUS) groups were sham operated; Bile duct ligation (BDL) and BDL+CUS groups were subjected to the BDL procedure. C, CUS, and BDL+CUS groups consisted of 12 animals, while the BDL group had 14 animals. Animals in the C and BDL groups were given 1 ml of saline orally, while methanol extract dissolved in distilled saline was given by oral gavage at a dose of 250 mg/kg for 28 days in the CUS and BDL+CUS groups. The *Cuscuta* dose was based on previous studies (15). Animals were weighed twice, at the beginning and end of the study (before decapitation). On the 28$^{th}$ day, the rats were decapitated, and blood and liver tissue samples were collected. Aspartate aminotransferase (AST), alanine transaminase (ALT), and direct and total bilirubin (DB and TB) levels were analyzed in blood samples, while transforming growth factor-β (TGF-β), 8-hydroxyguanosine (8-OHdG), and hydroxyproline and sodium-potassium ATPase ($Na^+/K^+$-ATPase) were determined in liver tissues. In addition, histological examinations of liver tissue sections were performed under a light microscope.

### Bile Duct Ligation for Induction of Cholestasis

Under anesthesia (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine; i.p.), midline laparotomy was performed. By exposing the common bile duct, a double ligation was applied. One of the ligatures was underneath the hepatic duct junctions and the other one was over the access to the pancreatic ducts. Bile ducts were cut between ligations (18).

### Formulation of Plant Extract

The aerial parts of *Cuscuta* sp. were purchased from an herbalist in the Midyat district of Mardin in May 2018. The plant was identified by Asst. Prof. Dr. Ahmet Doğan; a sample of the plant was made into a suitable herbarium sample and recorded by assigning it an herbarium number in the Herbarium of the Faculty of Pharmacy of Marmara University (MARE No: 22668).

After the dried aerial parts of the plant were dusted, approximately 757.06 g was weighed and macerated with 1600 mL of methanol for 10 days. The solvent of methanol extract obtained after filtration was vaporized using a rotary evaporator (Rotavapor® R-300). The methanol extract obtained with a yield of 10.83% was kept at +4°C until analysis.

### Biochemical Analysis

### Determination of the DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity of Plant Extract

The antioxidant level of *Cuscuta* sp. extract was designated in compliance with the method of Zou et al (19). The percent free radical scavenging of the extract was computed according to the formula below.

DPPH radical scavenging percent (%): (A control - A sample / A control) x 100

The Control contained $DPPH^{\cdot}$ solution and dimethyl sulfoxide (DMSO). The sample contained $DPPH^{\cdot}$ solution and sample solutions. The inhibition concentrations ($IC_{50}$) of extract that scavenge 50% of DPPH radical were calculated by plotting an inhibition graph based on concentrations of extract using the GraphPad Prism 6 program. Ascorbic acid was used as a reference standard. Measurements were repeated three times.

### ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) Radical Cation Scavenging Activity Determination of Plant Extract

To produce $ABTS^{\cdot+}$ used in the determination of total antioxidant capacity of the extract, 7 mM ABTS was mixed with 2.45 mM $K_2S_2O_8$ and left in the dark for 16 hours at room tempera-

ture (23°C) to complete reaction. The $ABTS^{\cdot+}$ solution was diluted with an ethanol (96%) solvent of analytical purity to give an optical density value of 0.700±0.050 at 734 nm. From the stock solution prepared in DMSO at a concentration of 5 mg/mL from extract, solutions at different concentrations were prepared by making 1:8 dilutions. 10 µl of each prepared solution was moved to the assay plates. 190 µl $ABTS^{\cdot+}$ solution was added. Immediately, the solution was placed at room temperature and kept there for thirty minutes; optical density was measured at 734 nm. Trolox was used as a standard and outcomes were expressed as $IC_{50}$ value. The test was repeated three times (19).

**Anti-inflammatory Activity of Plant Extract**

For the detection of anti-inflammatory activity, Phosrithong et al.'s method was used with revisions (20, 21). 20 µL of ethanol, 20 µL of BBS (Borate buffered saline), and 25 µL of type 5 soy lipoxygenase solution (20.000 U/mL) were added to 10 µL of extract/standard at different concentrations. After the mixture was incubated for five minutes at 25°C, 100 µL of 0.6 mM linoleic acid solution was added, the mixture was thoroughly mixed, and the absorbance change at 234 nm was noted for six minutes. Indomethacin was used as a reference standard. Percent (%) inhibition was computed with the formula below.

% Inhibition: [(A control – A sample) / A control] × 100

A dose-response curve was charted to conclude $IC_{50}$ values. $IC_{50}$ is outlined as enough concentration to obtain 50% of the maximum anti-inflammatory activity. All analyses were executed in triplicate.

**Determination of Total Phenol Compound of Plant Extract**

The total phenol content (TFB) of the extract was determined using the Folin-Ciocalteau solution, according to Gao's method (21.22). Solutions of various concentrations were prepared by dilutions made from stock solution prepared in DMSO at a concentration of 5 mg/mL from extract. 10 µL of these solutions were taken into microplates, and then 20 µL of Folin-Ciocalteau solution, 200 µL of ultrapure water, and 100 µL of 15% $Na_2SO_4$ were added, respectively. Solutions were read against a blank formed by replacing only extract with the same amount of DMSO, with the other components remaining constant at 765 nm optical density. For standard curve plot, optical density corresponding to each concentration was measured using gallic acid (500-0.977 µg/mL). The amount of TFB of extract was calculated from this graph and scores were determined as mg GAE (Gallic Acid Equivalent)/g plant extract. The measurements of extract were repeated three times and the measurements of standard curve were repeated five times.

**Examination of the Serum Samples**

Liver enzymes (ALT and AST), TB, and DB were surveyed to assess BDL-induced liver injury using commercial kits (BT Laboratory, Shanghai) (23).

**Examinations of Tissue Samples**

$Na^+/K^+$-ATPase activity in tissue were determined using a commercial kit (AFG, EK720668) in accordance with the manufacturer's procedure. After tissue samples were homogenized in a phosphate-buffered saline (PBS) with pH 7.4, they were centrifugated at 3000 rpm at +4°C and used aiming at upper phase. The measuring range of the kit is 50-1000 pg/mL, and the sensitivity is 20 pg/mL. Standard solution in the range of 900 pg/mL-75 pg/mL was set by diluting stock standard in the kit (1350 pg/mL).

TGF-β levels in the tissue were determined using a commercial kit (AFG, EK720060) in accordance with the manufacturer's procedure. After tissue samples were homogenized in PBS (pH 7.4), they were centrifugated at 3000 rpm at +4°C and used aiming at upper phase. The measuring range of the kit is 10 ng/mL-200 ng/mL, and the sensitivity is 3 ng/mL. The standard solution in the range of 150 ng/mL-12.5 ng/mL was set by diluting stock standard (225 ng/mL) in the kit.

Hydroxyproline levels in the tissue were determined in accordance with manufacturer's procedure using a commercial kit (AFG, EK720734). After tissue samples were homogenized in PBS (pH 7.4), they were centrifugated at 3000 rpm at +4°C and used aiming at upper phase. The measuring range of the kit is 1 ug/mL-20 ug/mL and the sensitivity is 0.5 ug/mL. Standard solution in the range of 15 ug/mL-1.25 ug/mL was set by diluting the stock standard (22.5 ug/mL) in the kit.

8-OHdG concentration in the tissue was determined using a commercial kit (AFG, EK720424) in accordance with the manufacturer's procedure. After the tissue samples were homogenized in PBS (pH 7.4), they were centrifugated at 3000 rpm at +4°C and used aiming at upper phase. The measuring range of the kit is 0.625 ng/mL-20 ng/mL, and the sensitivity is 0.078 ng/mL. The standard solution in the range of 15 ng/mL-1.25 ng/mL was set by diluting stock standard (22.5 ng/mL) in the kit.

**Light Microscopic Preparation**

For light microscopic examinations, samples from the liver were fixed with 10% formaldehyde, diluted alcohol was then cleared using toluene, and they were embedded in paraffin and cut in 5 µm in thickness. Liver tissue slices were stained with hematoxylin for histopathological evaluation, then inspected and shot with the digital camera (Olympus, Tokyo, Japan) of a photomicroscope (Olympus BX51, Tokyo, Japan).

**Statistical Analysis**

GraphPad Prism 5.0 (GraphPad Software, San Diego; CA; USA) was utilized to perform statistical evaluations. All numeric records were expressed as means±SEM. ANOVA was used to compare groups of data followed by Tukey's multiple comparison tests. Values of $p<0.05$ were considered significant.

## RESULTS

**Anti-Inflammatory, Antioxidant Activities and Total Compound Contents of Dodder (*Cuscuta sp.*) Methanol Extract**

Low $IC_{50}$ value obtained in the measurements (the concentration that removes 50% of the radical or halts the enzyme's activity by 50%) implies high activity. When Table 1 is examined,

CUS extract showed remarkable antioxidant activity against the DPPH radical, with an $IC_{50}$ value of 125.5 µg/ml. The obtained extract also exhibited significant antioxidant activity against the ABTS radical, with an $IC_{50}$ value of 138.9 µg/ml. It was observed to have noteworthy anti-inflammatory activity against the 5-LOX (5-Lipoxygenase) enzyme, with an $IC_{50}$ value of 103.5 µg/ml. The total amount of phenolic compounds of dodder plant extract was found to be 38.58 mg/g, equivalent to gallic acid in g extract (Table 1).

**Cirrhosis Caused by Liver Damage and Related Urine Color Changes**

As shown in Figure 1, changes occurred due to cirrhosis developing as a result of liver damage.

**Evaluation of Body Weight**

When body weights were compared at the beginning (t1) and the end of study (t2), there were significant differences between the groups' own t1 and t2 values (***p<0.001), and it was seen that there was weight gain in all groups. However, there was no statistically significant difference between both t1 and t2 of groups (Table 2). It is expected that there will be weight gain due to acid that develops in the body, so we measured the weights to observe this, but there was an increase in other groups because there was normal physiological weight gain in the process.

**Findings of Serum Samples**

The serum AST, ALT, DB, and TB levels of the BDL group were found to be significantly greater (p<0.001) compared to the C

**Table 1.** Antioxidant/anti-inflammatory activity and total phenolic compound content of methanol extract-obtained *Cuscuta* sp.

| Experiments | *Cuscuta* sp. methanol extract | Ascorbic Acid | Trolox | Indomethacin |
|---|---|---|---|---|
| **DPPH radical scavenging activity ($IC_{50}$ µg/mL)** | 125.5±0.14[b] | 17.6 ±0.37[a] | | |
| **ABTS radical scavenging activity ($IC_{50}$ µg/mL)** | 138.9±0.28[b] | | 13.00 ±0.21[a] | |
| **Anti-lipoxygenase activity ($IC_{50}$ µg/mL)** | 103.5±4.72[b] | | | 22.39±0.26[a] |
| **Total phenolic compound content (mg GAE/g extract)*** | 38.58±0.39[b] | | | |

* Total phenolic content was expressed as gallic acid equivalent (GAE).
Each value in the table is given as mean±SD (n=3). Different letter superscripts in the same line indicate a statistically significant difference (p<0.05).

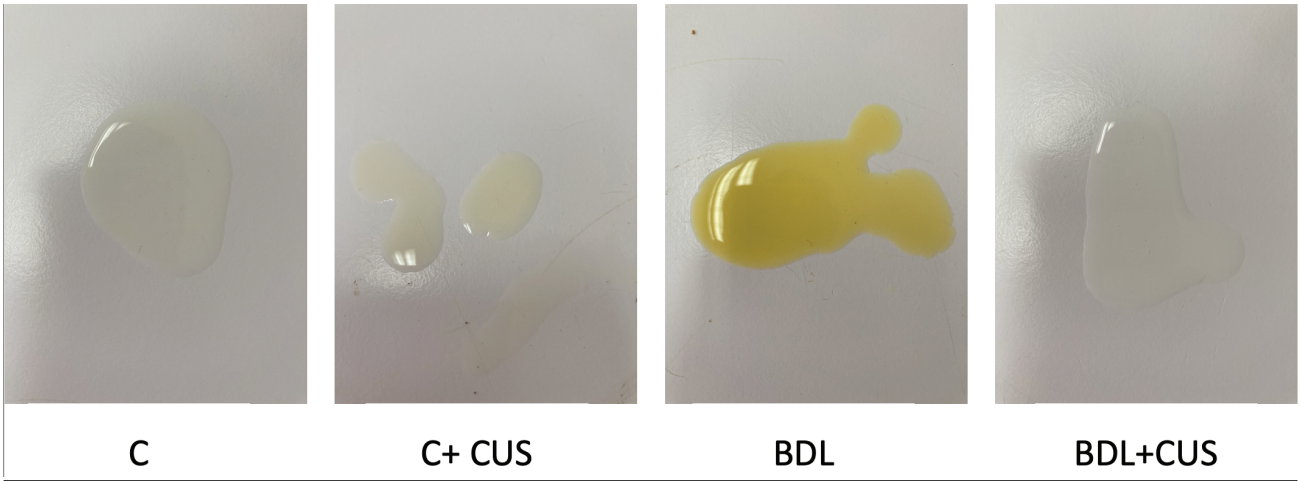


Figure 1. The urine colors of treated groups at the end of the experiment. C: Control, BDL: Bile duct ligation, CUS: *Cuscuta* sp.

**Table 2.** The body weights (g) of groups measured at the beginning of experiment (t1) and at the end of experiment (t2).

| Body Weights | C | CUS | BDL | BDL+CUS |
|---|---|---|---|---|
| t1 | 224±7.1 | 220±4.6 | 235±4.7 | 239±5.1 |
| t2 | 279±6.3 *** | 266±10.1*** | 280±10.5*** | 285±5.8*** |

C: Control, BDL: Bile duct ligation, CUS: *Cuscuta* sp. ***p<0.001: comparisons between t1 and t2

**Table 3.** Serum AST, ALT, DB, TB values.

| Serum | C | CUS | BDL | BDL+CUS |
|---|---|---|---|---|
| AST (U/L) | 137.8±5.5 | 133.0±7.6 | 435.5±28.8$^{***}$ | 304.3±4.318$^{***,+++}$ |
| ALT (U/L) | 55.3±4.8 | 52.3±4.4 | 142.0±5.6$^{***}$ | 91.3±5.8$^{***,+++}$ |
| DB (mg/dl) | 0.4±0.2 | 0.4±0.2 | 4.8±0.3$^{***}$ | 3.5±0.3$^{***,++}$ |
| TB (mg/dl) | 0.4±0.04 | 0.5±0.04 | 5.5±0.4$^{***}$ | 4.2±0.32$^{***,++}$ |

Serum AST (Aspartate Aminotranferase), ALT (Alenine transaminase), DB (Direct Bilirubin), TB (Total Bilirubin) values. *p<0.05, **p<0.01, ***p<0.001 compared to control group, +p<0.05, ++p<0.01, +++p<0.001 compared to BDL group.

group, while the AST, ALT, DB, and TB levels of the treated group were significantly lower than the BDL group (p<0.01-0.001). However, the serum AST, ALT, DB, and TB levels of CUS-treated BDL group were found to be significantly (p<0.001) greater than those of the C group (Table 3).

**Biochemical Findings of Liver**

TGF-β, 8-OHdG, and hydroxyproline levels were significantly greater (p<0.05, p<0.01) in the liver tissues of the BDL (saline-treated) group compared to the C group, while these levels were significantly lower in the CUS-treated BDLgroup compared to the BDL group (p<0.05-0.01) (Figures 2a, 2b, 2d).

$Na^+/K^+$-ATPase activity were found to be significantly lower (p<0.05) in the liver tissues of the BDL group compared to the C group. On the other hand, $Na^+/K^+$-ATPase activity tended to increase, but this was not significant (Figure 2c).

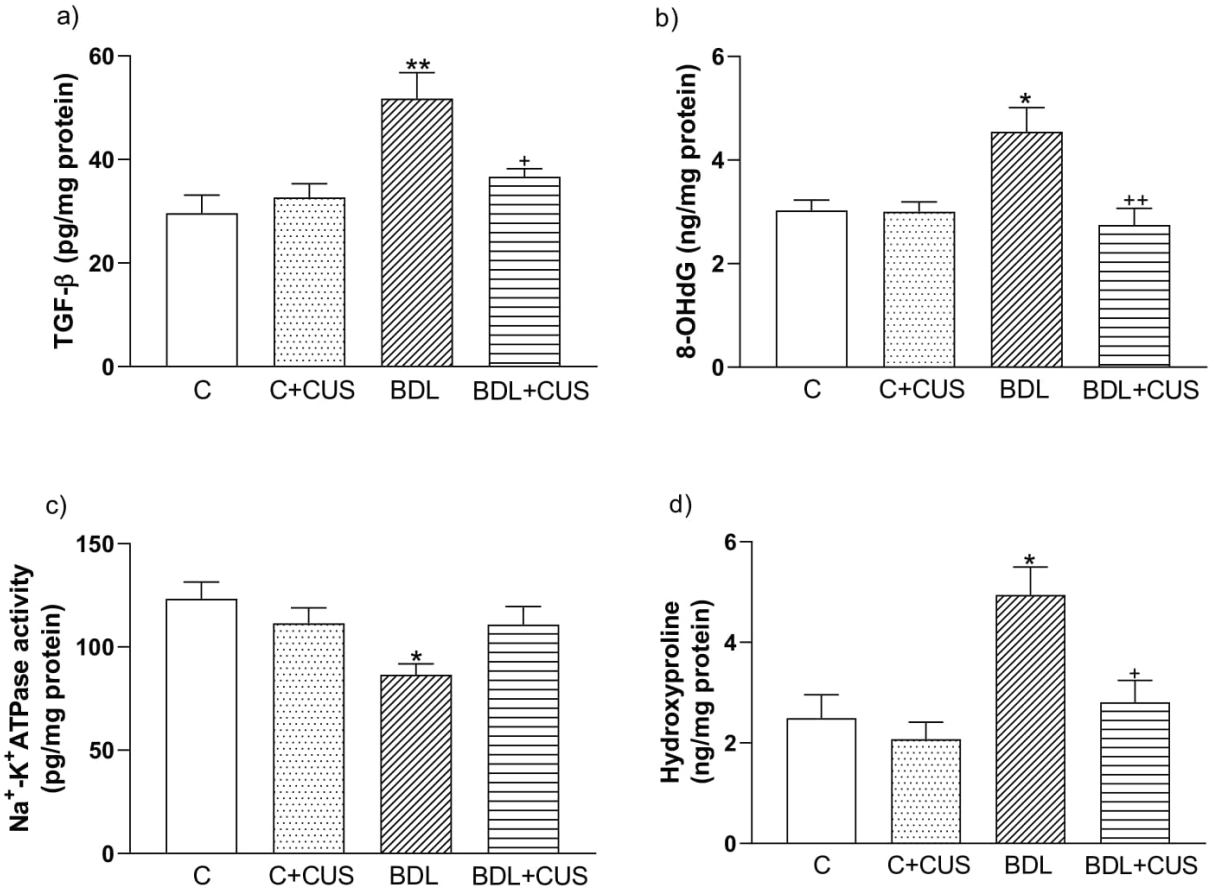


Figure 2. TGF-β, 8-OHdG, $Na^+/K^+$-ATPase, and Hydroxyproline results of liver tissue. TGF-β: Transforming growth factor, 8-OHdG: 8-hydroxy-2'-deoxyguanosine, $Na^+/K^+$-ATPase: Sodium-Potassium adenosine triphosphatase, C: Control group, BDL: Bile duct ligation, CUS: *Cuscuta* sp. ANOVA for statistical analysis. *p<0.05, **p<0.01; Compared to control group, +p<0.05, ++p<0.01; Compared to BDL group.

**Histological Evaluations**

The light microscopic assessment of the control group (Figure 3a) showed a steady form of liver parenchyma through intact hepatic cells, sinusoids, and the portal tract. A steady form of liver parenchyma through intact hepatic cells and portal tract, mild sinusoidal obstruction was detected in the C+CUS group (Figure 3b). Disorganized hepatic cords, severe increase of degenerated hepatocytes, bile duct proliferation, and inflammatory cell infiltration were seen in the saline-treated BDL group (Figure. 3c). Moderate decrease in the count of deteriorated hepatocytes, disorder of hepatic cords, drop in bile duct proliferation (ductular reaction), and inflammatory cell infiltration were observed in the BDL+CUS group (Figure. 3d).

**DISCUSSION**

The liver is a vital organ that plays an important role in the configuration and secretion of bile, as well as the excretion of toxic substances through bile ducts (24). Liver fibrosis is a reversible wound-healing response characterized by extracellular matrix (ECM) deposition and develops after liver injury (25). Delay in treatment can lead to life-threatening hyperammonemia, hepatic encephalopathy, and cirrhosis with high morbidity and mortality rates (26-28).

Different experimental studies have been conducted to slow or reverse the development of liver fibrosis; however, no effective treatment has been found for clinical use (29). There are many studies using anti-fibrogenic agents. However, in traditional

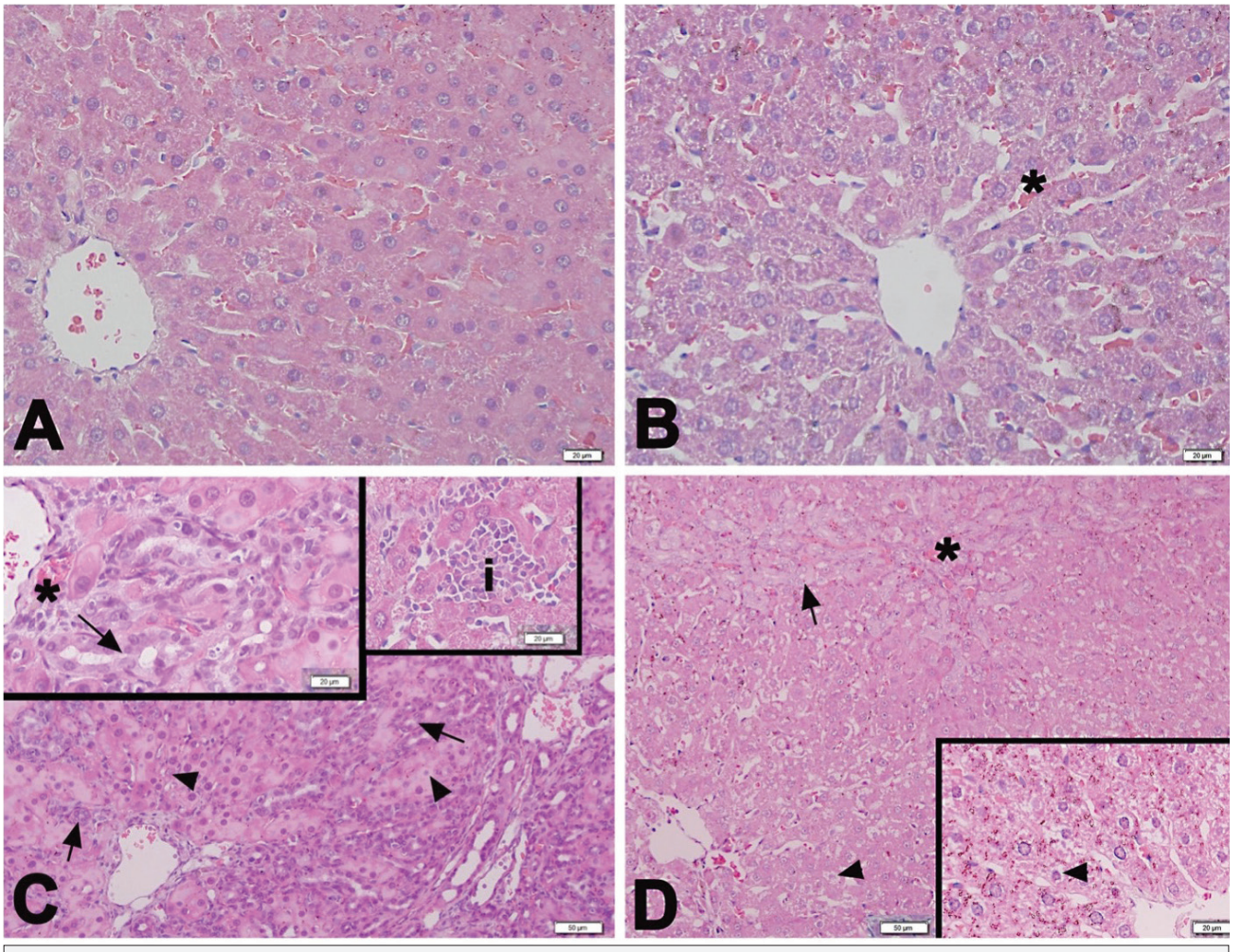


Figure 3. The representative light micrographs of liver samples in experimental groups. Normal liver parenchyma with hepatocytes, sinusoids, and portal tracts are seen in the C group (A). Normal liver parenchyma with hepatocytes, portal tracts, and mild sinusoidal congestion (*) are seen in the C+ CUS group (B). Disorganization of hepatic cords, sinusoidal congestion (*), increased number of degenerated hepatocytes (arrowhead), bile duct proliferation within portal tract and within acini (arrow), and inflammatory cell infiltration (i) in parenchyma (C) are seen in the BDL group. Mild sinusoidal congestion (*), disorganization of hepatic cords, decrease in degenerated hepatocytes (arrow), and decreased bile duct proliferation (arrow) are seen in the BDL+CUS group (D). Hematoxylin and eosin staining, Scale bars: A, B and insets in C and D: 20 µm, C and D: 50 µm. BDL: Bile duct ligation, CUS: *Cuscuta* sp., C: Control

medicine, natural products that are less toxic and cause side effects are also used instead of chemical anti-fibrotic drugs. These natural products are still in the experimental stage and are counted as therapeutic interventions (30).

Today, medicinal plants and products are being researched in the treatment of various diseases (31). In a detailed literature review, different types of *Cuscuta* (dodder) were found to have various biological effects, such as antitumor, antimicrobial, hepatoprotective, anticonvulsant, immunostimulant, antioxidant, α-glucosidase inhibitor, anti-inflammatory, diuretic, analgesic, antipyretic, neuroprotective, antiulcer, antispasmodic, antihypertensive, cardiotonic, and muscle relaxant (12,13,32). Similarly, in Turkey, it has been reported that some plants in traditional medicine are used in liver-biliary disorders. These species are used by the public in the treatment of jaundice (12,32). One of these species is the *Cuscuta* species (i-iv). Accordingly, in this study, liver damage was developed by creating cholestasis with BDL, and the treatment effect was investigated with an extract prepared from a traditional plant, the *Cuscuta* species.

Blood ALT and AST concentrations are well known as sensitive indicators of potential tissue damage, mainly liver toxicity (33). ALT and AST are known as aminotransferases involved in gluconeogenesis (34,35). AST is found in both cytosol and mitochondria of hepatocytes, while ALT is found only in cytosol (36-39). These enzymes leak into blood as a result of damage to hepatocytes (40). Metabolic factors such as drug use, toxins, viruses, ischemia, and autoimmune liver damage may affect liver parenchyma and increase AST and ALT values (23). Bilirubin, which is an important indicator of liver disease due to cholestasis, is the final breakdown product of hemoglobin and is directly or indirectly present in serum (41). According to scientific studies, direct bilirubin concentration increases due to BDL and in addition to the ALT, AST and bilirubin concentration in the BDL group were found to be remarkably greater than in the control group (42-44). In this study, the ALT, AST, and bilirubin concentration in the BDL group were found out to be greater than in the control group. According to another study on the curative effects of dodder plant extract on hepatotoxicity in rats, it was detected that ALT, AST, and bilirubin concentration were lower in groups treated with dodder plant extract compared to the model group (32). In agreement with these previous studies, in our study, the ALT, AST, and bilirubin levels of the BDL group were greater than those of the control group. Furthermore, in the BDL+CUS group, treatment with dodder plant extract, although not as high as control levels, significantly decreased these levels.

Sodium/potassium adenosine triphosphatase enzyme takes part in the structure and physiology of liver cells by providing sodium and potassium balance in all cell membranes (45). According to studies, it has been observed that BDL reduces the levels of $Na^+/K^+$-ATPase in liver tissue (46,47). In our study, the $Na^+/K^+$-ATPase activity of liver tissues of the BDL group were found to be lower than those of the control group. Shin et al demonstrated that decrease in renal $Na^+/K^+$-ATPase enzyme synthesis in a rat model of ischemia/reperfusion, while it was reversed by using dodder plant extract (48). In our study, the obtained hepatic $Na^+/K^+$-ATPase activities consistent with the literature were found to be significantly lower in cholestatic rats compared to the control group, while $Na^+/K^+$-ATPase activities were observed to be increased in the group treated with dodder plant extract.

Although free radicals are formed continuously in organisms during metabolic activities, they are scavenged by the endogenous antioxidant system. However, they have very harmful effects when they occur at a level that exceeds antioxidant system capacity. They are known to damage macromolecules, especially lipids, proteins, and nucleic acids, and are responsible for the pathogenesis of many diseases (49). The most widely used oxidative DNA damage indicator is 8-OHdG (50,51). Studies in cholestatic rats, and increased levels of 8-OHdG in liver tissue demonstrate oxidative damage in tissue due to BDL (52-54). In parallel with this information, in our study, the 8-OHdG levels of the liver tissues of the BDL group were found to be greater than those of the control group. According to a study, Ganoderma lucidum, an antioxidant and medicinal plant, ameliorates high 8-OHdG enzyme levels in liver tissues due to BDL (55). Likewise, in our study, a significant increase was seen in the concentration of 8-OHdG in the BDL group, and a significant reduction was observed in the level of the relevant damage marker in the rats treated with an antioxidant-effective dodder plant extract.

TGF-β is a regulator that has an essential role in the physiological and pathological changes of the liver, as well as in liver damage, inflammation, and fibrosis (56). Various studies have reported that TGF-β concentration is greater in the BDL group than in the control group and contributes to development of fibrosis as a proinflammatory cytokine (57-59). In parallel with this information, TGF-β concentration of liver tissues in the BDL group was found to be greater than in the control group in our study. According to a study examining its possible therapeutic effects on liver damage, the application of dodder plant extract was reported to be a hepatoprotective agent and alleviated an increase in TGF-β levels (21). In our study, it was found that TGF-β level was significantly greater in the BDL group compared to the control group. In addition, a diminution in TGF-β concentration was seen in the group treated with dodder plant extract.

Active hepatic stellate cells transforming into myofibroblasts increase hydroxyproline levels, which then cause collagen protein production and fibrosis (60,61). Consequently, hydroxyproline is used as a marker to assess the level of liver damage. According to studies on liver fibrosis in the BDL rat model, the hydroxyproline level of the BDL group was found to be significantly greater than in the control group (62-64). Similarly, in our study, the hydroxyproline concentration of liver tissues of the rats with BDL was found to be significantly greater, while a significant drop was observed in the hydroxyproline concentration of CUS treatment. It is thought that, as shown in our *in vitro* analyses, the anti-inflammatory properties of the CUS extract contribute to this effect, and thus it protects the tissue by

suppressing both TGF-β and collagen formation. Our histological findings also supported biochemical results, as significant damage was seen in the liver tissue of the BDL group and this damage was healed in liver tissue of the BDL group treated with dodder plant extract (32).

*In vitro* studies in the current study also supported *in vivo* studies. It has been found that the *Cuscuta* species have good antioxidant and anti-inflammatory activity as well as total phenolic content. A review study also confirmed that the plant has a DPPH radical scavenging effect and a good total phenol content (13). It has been reported that *Cuscuta* species are rich in phenolic compounds, especially flavonoids (17). Phenolic compounds have a hepatoprotective effect (65). Therefore, this group of compounds may be responsible for the hepatoprotective effect of the *Cuscuta* sp.

As a result, through the findings of the study, it has been seen that dodder plant extract has a possible protective effect against liver fibrosis caused by the BDL technique. This protective effect of dodder plant extract can be explained by its antioxidant, anti-inflammatory, and hepatoprotective effects. For this reason, it is thought that dodder plant extract may have a promising role in the treatment of liver fibrosis, with the support of clinical studies in future.

## CONCLUSION

Our biochemical and histological results, obtained with the investigated parameters, suggest that *Cuscuta* sp. methanol extract may be beneficial for liver diseases and needs more detailed studies in the future. Accordingly, we concluded that it may have a role in the treatment of liver fibrosis if supported by future clinical studies. Also, these results confirm the traditional use of *Cuscuta* sp. in the treatment of liver disease.

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## REFERENCES

1. Lai M, Afdhal NH. Liver Fibrosis Determination. Gastroenterol Clin North Am 2019; 48(2): 281-9.
2. Han JM, Kim HG, Choi MK, Lee JS, Park HJ, Wang JH, et al. Aqueous extract of *Artemisia iwayomogi* Kitamura attenuates cholestatic liver fibrosis in a rat model of bile duct ligation. Food Chem Toxicol 2012; 50(10): 3505-13.
3. Noll C, Raaf L, Planque C, Benard L, Secardin L, Petit E, et al. Protection and reversal of hepatic fibrosis by red wine polyphenols in hyperhomocysteinemic mice. J Nutr Biochem 2011; 22(9): 856-64.
4. Tsukada S, Parsons CJ, Rippe RA. Mechanisms of liver fibrosis. Clin Chim Acta 2006; 364(1-2): 33-60.
5. Xu R, Zhang Z, Wang FS. Liver fibrosis: Mechanisms of immune-mediated liver injury. Cell Mol Immunol 2012; 9(4): 296-301.
6. Dulundu E, Ozel Y, Topaloglu U, Toklu H, Ercan F, Gedik N, et al. Grape seed extract reduces oxidative stress and fibrosis in experimental biliary obstruction. J Gastroenterol Hepatol 2007; 22(6): 885-92.
7. Işeri SÖ, Şener G, Saglam B, Ercan F, Gedik N, Yeğen BÇ. Ghrelin alleviates biliary obstruction-induced chronic hepatic injury in rats. Regul Pept 2008; 146(1-3): 73-9.
8. Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, et al. The role of oxidative stress and antioxidants in liver diseases. Int J Mol Sci 2015; 16(11): 26087-124.
9. Sokol RJ, Devereaux M, Khandwala R, O'Brien K. Evidence for involvement of oxygen free radicals in bile acid toxicity to isolated rat hepatocytes. Hepatology 1993; 17(5): 869-81.
10. Videla LA, Rodrigo R, Orellana M, Fernandez V, Tapia G, Quiñones L, et al. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. Clin Sci 2004; 106(3): 261-8.
11. Koca-Caliskan U, Yilmaz I, Taslidere A, Yalcin FN, Aka C, Sekeroglu N. *Cuscuta* arvensis Beyr "dodder": *In Vivo* Hepatoprotective Effects Against Acetaminophen-Induced Hepatotoxicity in Rats. J Med Food 2018; 21(6): 625-31.
12. Erecevit Sönmez P, Kirbağ S, İnci Ş. Antifungal and antibacterial effect of dodder (*Cuscuta campestris*) used for hepatitis treatment of mothers and newborn infants in Province Mardin in Turkey. Yuz Yil Univ J Agric Sci 2019; 29(4): 722-30.
13. Noureen S, Noreen S, Ghumman SA, Batool F, Bukhari SNA. The genus *Cuscuta* (Convolvolaceae): An updated review on indigenous uses, phytochemistry, and pharmacology. Iran J Basic Med Sci 2019; 22(11): 1225-52.
14. Şekeroğlu N, Koca U, Meraler SA. Geleneksel Bir Halk İlacı:Iksut. YYÜ TAR BİL DERG 2012; 22(1): 56-61.
15. Bulut G, Biçer M, Tuzlaci E. The folk medicinal plants of Yüksekova (Hakkari-Turkey). J Pharm Istanbul Univ 2016; 46(2): 115-24.
16. Sari AO, Üniversitesi E, Fakültesi F, Bölümü B, Anabilim B. Ege ve Güney Marmara Bölgelerinde Halk İlacı Olarak Kullanılan Bitkiler. Anadolu 2010; 20(2): 1-21.
17. Ahmad A, Tandon S, Xuan TD, Nooreen Z. A Review on Phytoconstituents and Biological activities of *Cuscuta* species. Biomed Pharmacother 2017; 92: 772-95.
18. Criado M, Flores O, Ortíz MC, Hidalgo F, Rodríguez-López AM, Eleno N, et al. Elevated glomerular and blood mononuclear lymphocyte nitric oxide production in rats with chronic bile duct ligation: Role of inducible nitric oxide synthase activation. Hepatology 1997; 26(2): 268-76.
19. Zou Y, Chang SKC, Gu Y, Qian SY. Antioxidant activity and phenolic compositions of lentil (*Lens culinaris* var. *morton*) extract and its fractions. J Agric Food Chem 2011; 59(6): 2268-76.
20. Phosrithong N, Nuchtavorn N. Antioxidant and anti-inflammatory activites of *Clerodendrum* leaf extracts collected in Thailand. Eur J Integr Med 2016; 8(3): 281-5.
21. Şen A, Yıldırım A, Bitis L, Doğan A. Antioxidant and anti-inflammatory activity of capitula, leaf and stem extracts of *Tanacetum cilicicum* (Boiss.) Grierson. Int J Second Metab 2019; 6(2): 211-22.
22. Gao X, Ohlander M, Jeppsson N, Björk L, Trajkovski V. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. J Agric Food Chem 2000; 48(5): 1485-90.

23. Limdi JK, Hyde GM. Evaluation of abnormal liver function tests. Postgrad Med J 2003; 79: 307-12.
24. Strazzabosco M. Transport systems in cholangiocytes: Their role in bile formation and cholestasis. Yale J Biol Med 1997; 70(4): 427-34.
25. Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. Annu Rev Pathol Mech Dis 2011; 6: 425-56.
26. Goldbecker A, Buchert R, Berding G, Bokemeyer M, Lichtinghagen R, Wilke F, et al. Blood-brain barrier permeability for ammonia in patients with different grades of liver fibrosis is not different from healthy controls. J Cereb Blood Flow Metab 2010; 30(7): 1384-93.
27. Khan A, Ayub M, Khan WM. Hyperammonemia Is Associated with Increasing Severity of Both Liver Cirrhosis and Hepatic Encephalopathy. Int J Hepatol 2016; 2016.
28. Zhangdi HJ, Su SB, Wang F, Liang ZY, Yan YD, Qin SY, et al. Crosstalk network among multiple inflammatory mediators in liver fibrosis. World J Gastroenterol 2019; 25(33): 4835-49.
29. Tahan G, Akin H, Aydogan F, Ramadan SS, Yapicier O, Tarcin O, et al. Melatonin ameliorates liver fibrosis induced by bile-duct ligation in rats. Can J Surg 2010; 53(5): 313-8.
30. Gedik N, Kabasakal L, Şehirli Ö, Ercan F, Sirvanci S, Keyer-Uysal M, et al. Long-term administration of aqueous garlic extract (AGE) alleviates liver fibrosis and oxidative damage induced by biliary obstruction in rats. Life Sci 2005; 76(22): 2593-606.
31. Singh U, Singh S, Kochhar A. Therapeutic potential of antidiabetic nutraceuticals. Phytopharmacology 2012; 2(1): 144-69.
32. Koca-Caliskan U, Yilmaz I, Taslidere A, Yalcin FN, Aka C, Sekeroglu N. *Cuscuta arvensis* Beyr "dodder": *In Vivo* Hepatoprotective Effects Against Acetaminophen-Induced Hepatotoxicity in Rats. J Med Food 2018; 21(6): 625-31.
33. Ramaiah SK. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food Chem Toxicol 2007; 45(9): 1551-7.
34. Kobayashi A, Yokoyama H, Kataoka J, Ishida T, Kuno H, Sugai S, et al. Effects of spaced feeding on gene expression of hepatic transaminase and gluconeogenic enzymes in rats. J Toxicol Sci 2011; 36(3): 325-37.
35. Qian K, Zhong S, Xie K, Yu D, Yang R, Gong D-W. Hepatic ALT isoenzymes are elevated in gluconeogenic conditions including diabetes and suppressed by insulin at the protein level Kun. Diabetes Metab Res Rev 2015; 31(6): 562-71.
36. Horio Y, Tanaka T, Taketoshi M, Uno T, Wada H. Rat cytosolic aspartate aminotransferase: Regulation of its mRNA and contribution to gluconeogenesis. J Biochem 1988; 103(5): 805-8.
37. Jadaho SB, Yang RZ, Lin Q, Hu H, Anania FA, Shuldiner AR, et al. Murine Alanine Aminotransferase: cDNA Cloning, Functional Expression, and Differential Gene Regulation in Mouse Fatty Liver. Hepatology 2004; 39(5): 1297-302.
38. Tomkiewicz C, Muzeau F, Edgar AD, Barouki R, Aggerbeck M. Opposite regulation of the rat and human cytosolic aspartate aminotransferase genes by fibrates. Biochem Pharmacol 2004; 67(2): 213-25.
39. Yang RZ, Park S, Reagan WJ, Goldstein R, Zhong S, Lawton M, et al. Alanine aminotransferase isoenzymes: Molecular cloning and quantitative analysis of tissue expression in rats and serum elevation in liver toxicity. Hepatology 2009; 49(2): 598-607.
40. Raval N, Kalyane D, Maheshwari R, Tekade RK. Surface Modifications of Biomaterials and Their Implication on Biocompatibility. Biomaterials and Bionanotechnology. Elsevier Inc 2019; 639-674.
41. Jo J, Yun JE, Lee H, Kimm H, Jee SH. Total, direct, and indirect serum bilirubin concentrations and metabolic syndrome among the Korean population. Endocrine 2011; 39(2): 182-9.
42. Lim JH, Kim TW, Song IB, Park SJ, Kim MS, Cho ES, et al. Protective effect of the roots extract of *Platycodon grandiflorum* on bile duct ligation-induced hepatic fibrosis in rats. Hum Exp Toxicol 2013; 32(11): 1197-205.
43. Şener G, Kabasakal L, Şehirli Ö, Ercan F, Gedik N. 2-Mercaptoethane sulfonate (MESNA) protects against biliary obstruction-induced oxidative damage in rats. Hepatol Res 2006; 35(2): 140-6.
44. Yada K, Ishibashi H, Mori H, Morine Y, Zhu C, Feng R, et al. The Kampo medicine "daikenchuto (TU-100)" prevents bacterial translocation and hepatic fibrosis in a rat model of biliary atresia. Surg 2016; 159(6): 1600-11.
45. Atalay S, Soylu B, Aykaç A, Oğünç AV, Çetinel Ş, Ozkan N, et al. Protective effects of St. John's wort in the hepatic ischemia/ reperfusion injury in rats. Turkish J Surg 2018; 34(3): 198-204.
46. Muriel P. Interferon-α preserves erythrocyte and hepatocyte atpase activities from liver damage induced by prolonged bile duct ligation in the rat. J Appl Toxicol 1995; 15(6): 449-53.
47. Muriel P, Suarez OR, Gonzalez P, Zuñiga L. Protective effect of S-adenosyl-l-methionine on liver damage induced by biliary obstruction in rats: a histological, ultrastructural and biochemical approach. J Hepatol 1994; 21(1): 95-102.
48. Shin S, Lee YJ, Kim EJ, Lee AS, Kang DG, Lee HS. Effect of *Cuscuta chinensis* on renal function in ischemia/reperfusion- induced acute renal failure rats. Am J Chin Med 2011; 39(5): 889-902.
49. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nat Rev Drug Discov 2021; 20(9): 689-709.
50. Atmaca E, Aksoy A. Oksidatif DNA Hasarı ve Kromatografik Yöntemlerle Tespit Edilmesi. YYU Veteriner Fakultesi Dergisi 2009; 20(2): 79-83.
51. Li P, Ramm GA, Macdonald GA. Value of the 8-oxodG/dG ratio in chronic liver inflammation of patients with hepatocellular carcinoma. Redox Biol 2016; 8: 259-70.
52. Huang YT, Hsu YC, Chen CJ, Liu CT, Wei YH. Oxidative-stress-related changes in the livers of bile-duct-ligated rats. J Biomed Sci 2003; 10(2): 170-8.
53. Maeda K, Koda M, Matono T, Sugihara T, Yamamoto S, Ueki M, et al. Preventive effects of ME3738 on hepatic fibrosis induced by bile duct ligation in rats. Hepatol Res 2008; 38(7): 727-35.
54. Matono T, Koda M, Tokunaga S, Sugihara T, Ueki M, Murawaki Y. The effects of the selective mineralocorticoid receptor antagonist eplerenone on hepatic fibrosis induced by bile duct ligation in rat. Int J Mol Med 2010; 25: 875-82.
55. Aydin S, Aytac E, Uzun H, Altug T, Mansur B, Saygili S, et al. Effects of *Ganoderma lucidum* on obstructive jaundice-induced oxidative stress. Asian J Surg 2010; 33(4): 173-80.
56. Fabregat I, Moreno-Càceres J, Sánchez A, Dooley S, Dewidar B, Giannelli G, et al. TGF-β signalling and liver disease. FEBS J 2016; 283: 2219-32.
57. Asakawa T, Yagi M, Tanaka Y, Asagiri K, Kobayashi H, Egami H, et al. The herbal medicine Inchinko-to reduces hepatic fibrosis in cholestatic rats. Pediatr Surg Int 2012; 28(4): 379-84.
58. Du JX, Sun MY, Du GL, Li FH, Liu C, Mu YP, et al. Ingredients of Huangqi decoction slow biliary fibrosis progression by inhibiting the activation of the transforming growth factor-beta signaling pathway. BMC Complement Altern Med 2012; 12: 1-11.
59. Sharawy MH, Abdel-Rahman N, Megahed N, El-Awady MS. Paclitaxel alleviates liver fibrosis induced by bile duct ligation in rats: Role of TGF-β1, IL-10 and c-Myc. Life Sci 2018; 211: 245-51.
60. Mu M, Zuo S, Wu RM, Deng KS, Lu S, Zhu JJ, et al. Ferulic acid attenuates liver fibrosis and hepatic stellate cell activation via inhibition of TGF-β/Smad signaling pathway. Drug Des Devel Ther 2018; 12: 4107-15.

61. Wang R, Song F, Li S, Wu B, Gu Y, Yuan Y. Salvianolic acid A attenuates CCl4-induced liver fibrosis by regulating the PI3K/AKT/mTOR, Bcl-2/Bax and caspase-3/cleaved caspase-3 signaling pathways. Drug Des Devel Ther 2019; 13: 1889-900.
62. Lee TY, Chang HH, Chen JH, Hsueh ML, Kuo JJ. Herb medicine Yin-Chen-Hao-Tang ameliorates hepatic fibrosis in bile duct ligation rats. J Ethnopharmacol 2007; 109(2): 318-24.
63. Nan J-X, Park E-J, Kim Y-C, Ko G, Sohn DH. *Scutellaria baicalensis* inhibits liver fibrosis induced by bile duct ligation or carbon tetrachloride in rats. J Pharm Pharmacol 2010; 54(4): 555-63.
64. Tian X, Zhao C, Guo J, Xie S, Yin F, Huo X, et al. Carvedilol Attenuates the Progression of Hepatic Fibrosis Induced by Bile Duct Ligation. Biomed Res Int 2017; 2017.
65. Yadav RK, Nandy BC, Maity S, Sarkar S, Saha S. Phytochemistry, pharmacology, toxicology, and clinical trial of Ficus racemosa. Pharmacogn Rev 2015; 9(17): 73-80.