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Original Article

Capparis spinosa L. Reduces Cisplatin-induced Acute Liver Injury in Male Rats: Pretreatment and Single Dose Therapy

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ABSTRACT

Cisplatin as a chemotherapeutic agent causes liver injury by increasing inflammatory production. *Capparis spinosa* L. as a source of natural antioxidants can clear this production. The present study was designed to assess the effects of pretreatment as well as treatment with a single dose of hydroalcoholic extract of Capparis seed on cisplatin-induced liver damage in rats. Forty-eight male Sprague-Dawley rats were divided into six groups (control group, Cis (cisplatin) group, 200 C/S (*C. spinosa*), Cis + 50 C/S 1-day, Cis + 100 C/S 1-day, and 100 C/S + Cis groups). Biochemical and histopathological assessment were done. Statistical analyses were performed with Graph Pad Prism Statistics software 9.1.2. The level of significance was set at $p< 0.05$. Liver function tests, antioxidant and inflammatory parameters and quantitative parameters of histopathological changes were measured. Significant changes in the pathology results were obvious. The diameter of central vein, portal vein, and bile duct, the thickness of the hepatic artery wall, and hepatic sinusoids were significantly increased in the Cis and 200 C/S-fed groups, compared to the controls, and also changes in favor of improvement were evident in the treatment groups compared to the Cis and 200 C/S groups. By increasing the time interval between cisplatin injection and testing, as well as using the western blotting method to measure the level of antioxidant and inflammatory markers, we may have significant biochemical and antioxidant results. Based on pathology results, single-dose treatment with *C. spinosa* seed extract may be beneficial in the cisplatin-induced liver damage.

INTRODUCTION

Liver diseases have been growing in the world in recent decades, exchanging to one of the most usual reasons of malady and fatality [1], and chronic liver diseases (CLDs) signify a main communal healthiness problematic in the world [2]. Presently, the main reason of acute liver diseases (ALDs) is viral hepatitis, whereas alcohol and viral hepatitis are the major reasons of CLDs. These processes vary and in the futurity drug-induced liver injury (DILI) will be progressively known as a reason for acute hepatitis [3]. More than thousand medications are thought to inure liver harm, only 353 medications have persuasively been related to liver injury [3].

Cisplatin, one of the most effective chemotherapeutic medications, is prescribed to treat a wide range of tumors. Contrary to its useful antineoplastic results, cisplatin has several unpleasant side effects on various tissues, such as nephrotoxicity, hepatotoxicity, cardiotoxicity, and ototoxicity. These side effects restrict its usage in clinical oncology as a potent chemotherapeutic medication [4,5]. Hepatotoxicity and nephrotoxicity are the dose-restriction complications in chemotherapy with cisplatin. The amount of cisplatin accumulation in the liver is significant, although this is less than in the kidney. However, nephrotoxicity has been studied very well, hepatotoxicity has yet to be examined as well [5].

Numerous studies have clarified that the production of reactive oxygen species (ROS), including superoxide anion and hydroxyl radical play role in the mechanism of cisplatin toxicity, which results in an increment in lipid peroxidation (LPO), decrease in the amount of protein bound sulfhydryl groups and glutathione [4,5]. Thus, it is very important to find a way to hamper the dose-restriction complications of cisplatin at its tumoricidal doses for harmless clinical usage [4,5].

Persian Medicine (PM), as an alternative medicine, that has been used amongst Iranian people since the golden years, proposes numerous remedial strategies to cure liver diseases, ranging from lifestyle amendment to herbal treatment [6]. *C. spinosa L.*, which is named "Kabbar", "Shapleh", "Lagay", and etc., is a member of the Capparidaceae family. *C. spinosa* is a perennial spiny bush, usually known as caper. Caper genus includes more than 250 species, which are widespread in different regions such as the Mediterranean, Western and Central Asia areas, and different regions of Iran [7-9].

Different portions of *C. spinosa* have been handled as a traditional herbal therapy that has useful effects on human healthiness. Phytochemical studies have revealed numerous bioactive combinations such as spermidine, rutin, quercetin, kaempferol, stigmasterol, tocopherols, and carotenoids [7,10]. The essential pharmacological actions of caper are hepatoprotective, antidiabetic, antihypertensive, hypolipidemic, antioxidant, antimutagenic, anti-allergic, antibacterial, antiviral, antifungal, immunomodulatory, anti-apoptotic, and anti-inflammatory activities [7,9,10]. The various sections of the plant contain a high diversity of active secondary metabolites with numerous known biological functions [7], for example, caper berry (seed) is a good source of antioxidant combinations such as free phenolics, flavonoids, and carotenoids [11,12]. But according to our literature review, there is no study to assess the hepatoprotective effects of hydroalcoholic seed extract of *C. spinosa* on drug induced liver injury. Herein, we induced liver damage in rats via cisplatin injection and assessed the effect of hydroalcoholic seed extract of *C. spinosa* in different treatment methods and doses on liver function tests, inflammatory, antioxidant, and particularly quantitative changes of pathology markers.

MATERIALS AND METHODS

Preparation of C. spinosa Hydroalcoholic Seed Extract

Fruits of caper were collected from the Moghan-Pars Abad of Ardabil, Iran. The herbarium code 3969 has obtained from traditional medicine and material medical research center, Shahid Beheshti University of Medical Sciences, Tehran. Iran [13]. We separated seeds from washed fruits and dried in the shade at a temperature of 25 to 30 °C. In a study which conducted for the standardization of Capparis seeds, cold press oil and hydroalcoholic extracts of Capparis seeds were prepared in three concentrations of 50, 100, and 200 and the power of inhibiting free radicals of these three compounds was checked. The results obtained from this study showed that the hydroalcoholic extract of Capparis seeds had the greatest ability to inhibit free radicals [14], and according to these results, we used the hydroalcoholic extract of Capparis seeds in our plan. So, five hundred g of the plant seeds were powdered and inserted in the percolator and was treated with ethanol (70%) in a tightly closed container for three days. Then, the mixture was filtered and the solvent was removed under the laboratory hood. This process was repeated three times, and finally the last step, instead of alcohol, distilled water was added to the percolator containing the seed powder. Finally, the last task in each step was to collect the dried extract.

Animal Study

Male Sprague-Dawley rats (250±20) gr were obtained from Razi Vaccine and Serum Research Institute. Two days before the study, the animals were housed in a pathogen-free, environment standard humidity (40-70%) with a 12-hour light-dark timer, and thermal conditions (22±2 °C) with ad libitum access to chow diet and water. The ethic committee at the Zanjan University of Medical Sciences approved the use of animals and experimental procedures (IR.ZUMS.REC.1399.352). All procedures were applied according to the ARRIVE guidelines. The animals were distributed into six groups, each containing eight rats. The control group (n = 8) had ad libitum access to diet and water without cisplatin and *C. spinosa* seed extract intake. One group (n = 8) received 200 mg/kg of the *C. spinosa* seed extract (C/S) dissolved in distilled water through a

nasogastric tube two times per day for two weeks for toxicity assessment (200 C/S). The other group, as a pretreatment group, received 100 mg/kg of the *C. spinose* seed extract dissolved in distilled water two times per day for two weeks; finally, five days before the last day of the study, after receiving the last dose of the extract, single-dose cisplatin with a dose of 7.5 mg/kg was injected intraperitoneally to induce liver injury (100 C/S + Cis) [4,5]. On the same day, three other group received cisplatin in the same manner, of which two groups received 50 mg/kg and 100 mg/kg of the *C. spinose* seed extract dissolved in distilled water only twice daily on the same day (Cis + 50 C/S 1-day and Cis + 100 C/S 1-day) and the Cis group that did not received any treatment. Before experiment, the rats were anesthetized by intraperitoneal injection of a ketamine-xylazine cocktail (xylazine 10 mg/kg and ketamine 100 mg/kg), then blood samples were collected from retro orbital sinus into heparinized microtubes, and allowed to stand for 30 minutes at 37° C. Blood samples were centrifuged at 1500 g for 20 minutes at 4° C in a SiGmA 3-16K centrifuge, and the isolated serum was stored in a freezer at -70° C for final analysis. The liver tissues were quickly harvested and one part of the liver tissue was instantly fixed in 10% phosphate buffered formaldehyde for histological and immunohistochemical studies. Also, another part of the liver was homogenized in lysis buffer containing protease and phosphatase inhibitor cocktails for the biochemical determinations. At the end of the study, all animals were euthanized with CO2 gradient.

Biochemical Assessment

Blood levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (Alp) were measured by spectrophotometry method (Pars Azmon Co. Kit, Tehran, Iran) and an autoanalyzer device model (MINDRAY BS-200 analyzer (MINDRAY, Shenzhen, China)). Serum Gamma–Glutamyl Transferase (GGT) level was measured according to the method of Szasz. Albumin was measured by BROMOCRESOL GREEN method, Bilirubin-Total by Diazotized Sulfanilic acid method, and lactate dehydrogenase by Lactate Dehydrogenase Activity Assay Kit. Pt and Ptt test were measured by Pars Azmoon kits (Tehran, Iran).

Antioxidant markers including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and malondialdehyde (MDA) activity were measured using a calorimetrically enzymatic assay kit (ZellBio GmbH, Germany) in each gram of liver tissue. In addition, inflammatory markers such as interleukin-6 (IL-6), IL-1β, and TNF-α were measured in each gram of liver tissue using DuoSet ELISA (R&D Systems, Inc.) kits.

Liver Histopathology

The hematoxylin and eosin (H&E) stain method was used for histological analysis. Parts of the hepatic lobes were removed from each animal, and they were fixed in a 10% formaldehyde buffer as mentioned before. The samples were then dehydrated in increasing grades of alcohol before being embedded in paraffin. Sections at 5 Mm thickness were taken, stained with hematoxylin and eosin. Images were captured at ×400 magnification, and were analyzed by the image-portlab software.

Statistical Analysis

Statistical analyses were performed with Graph Pad Prism Statistics software 9.1.2. All data were expressed as means ± SD. The level of significance was set at $p < 0.05$. Normal distribution of the variables was checked by the Kolmogorov Smirnov Test. One-way analysis of variance followed by Bonferroni post-hoc test was used due to the normal distribution for comparison among the various groups.

RESULTS

Biochemical Markers

Results of serum AST, ALT, Alp, Alb, T. Bil, GGT, LDH, and result of Pt, Ptt are shown in Figure 1. Serum AST ($p = 0.005$), Alb ($p = 0.005$), GGT ($p = 0.003$), and LDH ($p = 0.009$) were significantly different among the groups. Although many of the changes are not statistically significant, but decreased Alb levels and increased Pt, Alt, and LDH levels are evident in the Cis group compared to the control group. However, AST, Alp and GGT serum level was increased in the control group compared with the Cis one. Also, we found a reduced of Alb level and an increase in Pt, Ptt, Alt, Ast, GGT, T. Bil, and LDH levels in the 200 C/S group compared with control group. Changes in favor of recovery were somewhat evident in the Cis + 50 C/S 1-day group and Cis + 100 C/S 1-day group.

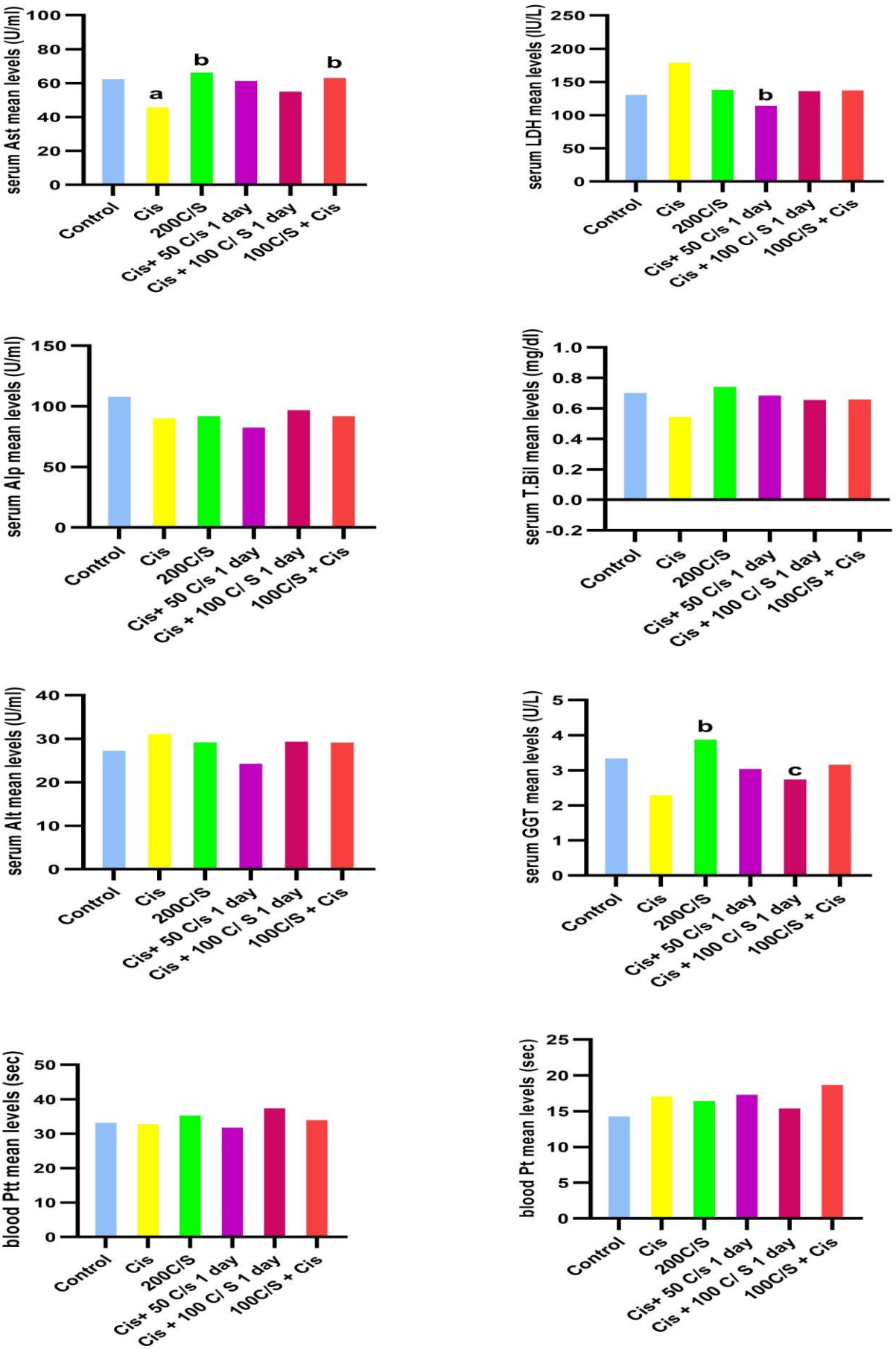

Fig. 1 Biochemical parameters in the studied groups

For example, serum LDH levels were increased in rats that received Cis and significantly treated with 50 mg/kg of *C. spinosa* seed extract for one day compared with the Cis group (p = 0.004), but there were no noticeable changes in the 100 C/S + Cis group. Alb: albumin; T. Bil: total bilirubin; GGT: gamma glutamyl transferase; Alt: alanine aminotransferase; AST: aspartate aminotransferase; Alp: alkaline phosphatase; LDH: lactate dehydrogenase; Pt: prothrombin time; Ptt: partial thromboplastin time; Cis: rats received 7.5 mg/kg of cisplatin to induce cirrhosis; 200C/S: rats received 200 mg/kg of *C. spinosa* seed extract two times per day for two weeks; Cis + 50C/S 1-day: cirrhotic rats received 50 mg/kg of *C. spinosa* seed extract two times for a day; Cis + 100C/S 1-day: cirrhotic rats received 100 mg/kg of *C. spinosa* seed extract two times for a day; 100C/S + Cis: rats received 100 mg/kg of *C. spinosa* seed extract two times per day for two weeks then 7.5 mg/kg Cisplatin single dose. Analysis was performed by ANOVA test followed by Bonferroni post-hoc analysis for between group comparisons.
a: Significantly different from the control group; b: Significantly different from the cis group; c: Significantly different from the 200C/S

Reduction of Alb levels and elevation of Pt, Alt, and LDH levels in the Cis group compared to the control group. Increment in Pt, Ptt, Alt, Ast, GGT, T. Bil, and LDH levels in the 200 C/S group compared with controls. Changes in favor of improvement in the Cis + 50 C/S 1-day group and Cis + 100 C/S 1-day group. For example, significant reduction of serum LDH levels in the Cis + 50 C/S 1-day group compared with the Cis group (p = 0.004).

Liver Inflammatory and Antioxidant Markers

As shown in Figure 2 and Figure 3, tissue inflammatory and antioxidant levels were not statistically significant among the groups except for SOD and IL-1β (p = 0.02 and $p < 0.001$ respectively). Increased SOD and GPX levels are evident in the 200 C/S and Cis + 100 C/S 1-day groups, as well as there is an increase in CAT level only in the Cis + 100 C/S 1-day group. But tissue IL-1β was significantly decreased in the Cis + 50 C/S 1-day group compared with the Cis group (p =0.006). In

addition, our findings indicated the elevation of MDA levels in the Cis and 200 C/S groups and its reduction in the treatment groups. Also, among the inflammatory markers, we have an increase in the levels of IL-6, IL-1β, and TNF-α in the Cis and 200 C/S groups and their decrease in the treatment groups.

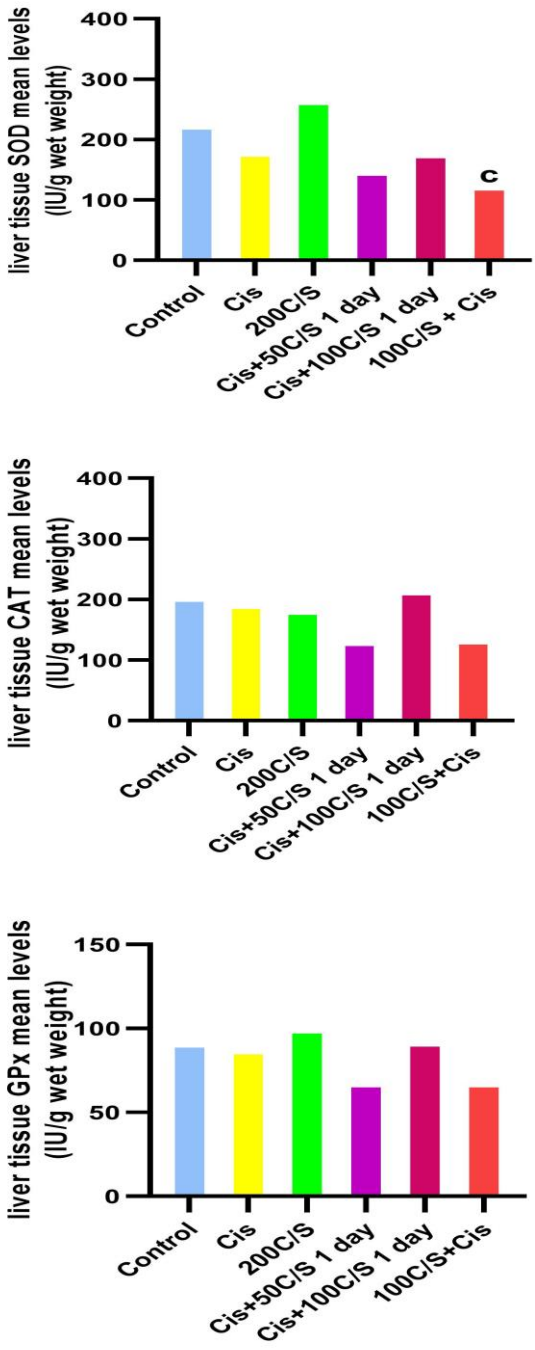

Fig. 2 Tissue antioxidant parameters in the studied groups

SOD: superoxide dismutase; GPx: glutathione peroxidase; CAT: catalase; Cis: rats received 7.5 mg/kg of cisplatin to induce cirrhosis; 200C/S: rats received 200 mg/kg of *C. spinosa* seed extract two times per day for two weeks; Cis+50C/S 1-day: cirrhotic rats received 50 mg/kg of *C. spinosa* seed extract two times for a day; Cis + 100C/S 1-day: cirrhotic rats received 100 mg/kg of *C. spinosa* seed extract two times for a day; 100C/S + Cis: rats

received 100 mg/kg of *C. spinosa* seed extract two times per day for two weeks then 7.5mg/kg Cisplatin single dose. Analysis was performed by ANOVA test followed by Bonferroni post-hoc analysis for between group comparisons

c: Significantly different from the 200C/S

Elevation of SOD and GPX levels in the 200 C/S and Cis + 100 C/S 1-day groups, and increment of CAT levels only in the Cis + 100 C/S 1-day group

MDA: malonedialdehyde; TNF-α: tumor necrosis factor alpha; IL-6: interleukin-6; IL-1β: interleukin-1 beta; Cis: rats received 7.5 mg/kg of cisplatin to induce cirrhosis; 200C/S: rats received 200 mg/kg of C. spinosa seed extract two times per day for two weeks; Cis+50C/S 1-day: cirrhotic rats received 50 mg/kg of C. spinosa seed extract two times for a day; Cis+100C/S 1-day: cirrhotic rats received 100 mg/kg of C. spinosa seed extract two times for a day; 100C/S + Cis: rats received 100 mg/kg of C. spinosa seed extract two times per day for two weeks then 7.5mg/kg Cisplatin single dose. Analysis was performed by ANOVA test followed by Bonferroni post-hoc analysis for between group comparisons

b: Significantly different from the cis group; d: Significantly different from the Cis+50C/S 1-day

Significant decrement of IL-1β level in the Cis + 50 C/S 1-day group compared with the Cis group, increment of MDA and tissue inflammatory markers in the Cis and 200 C/S groups and their decrement in the treatment groups

Histopathological Assessment

Figure 4 and Figure 5 show the histopathological graph of the liver in the studied groups. Results indicated a significant difference in central vein diameter (CV), portal vein diameter (PV), hepatic artery diameter (HA), thickness of the hepatic artery wall, bile duct diameter (BD), and dilated hepatic sinusoids among the groups. Central vein's diameter was significantly increased in the Cis and 200 C/S-fed groups, compared to the controls ($p = 0.001$ and $p = 0.008$, respectively). However, daily intake of C. spinosa seed extract decreased this diameter in the Cis + 50 C/S 1-day groups compared with the Cis and 200 C/S groups ($p = 0.003$ and $p = 0.023$, respectively).

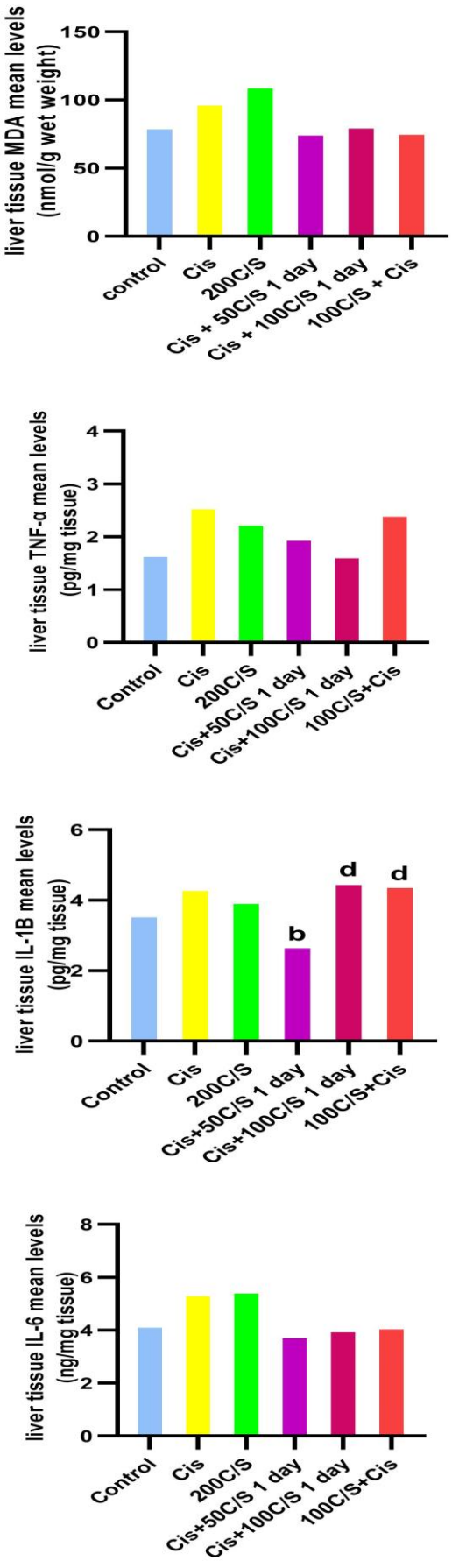

Fig. 3 Tissue inflammatory parameters in the studied groups

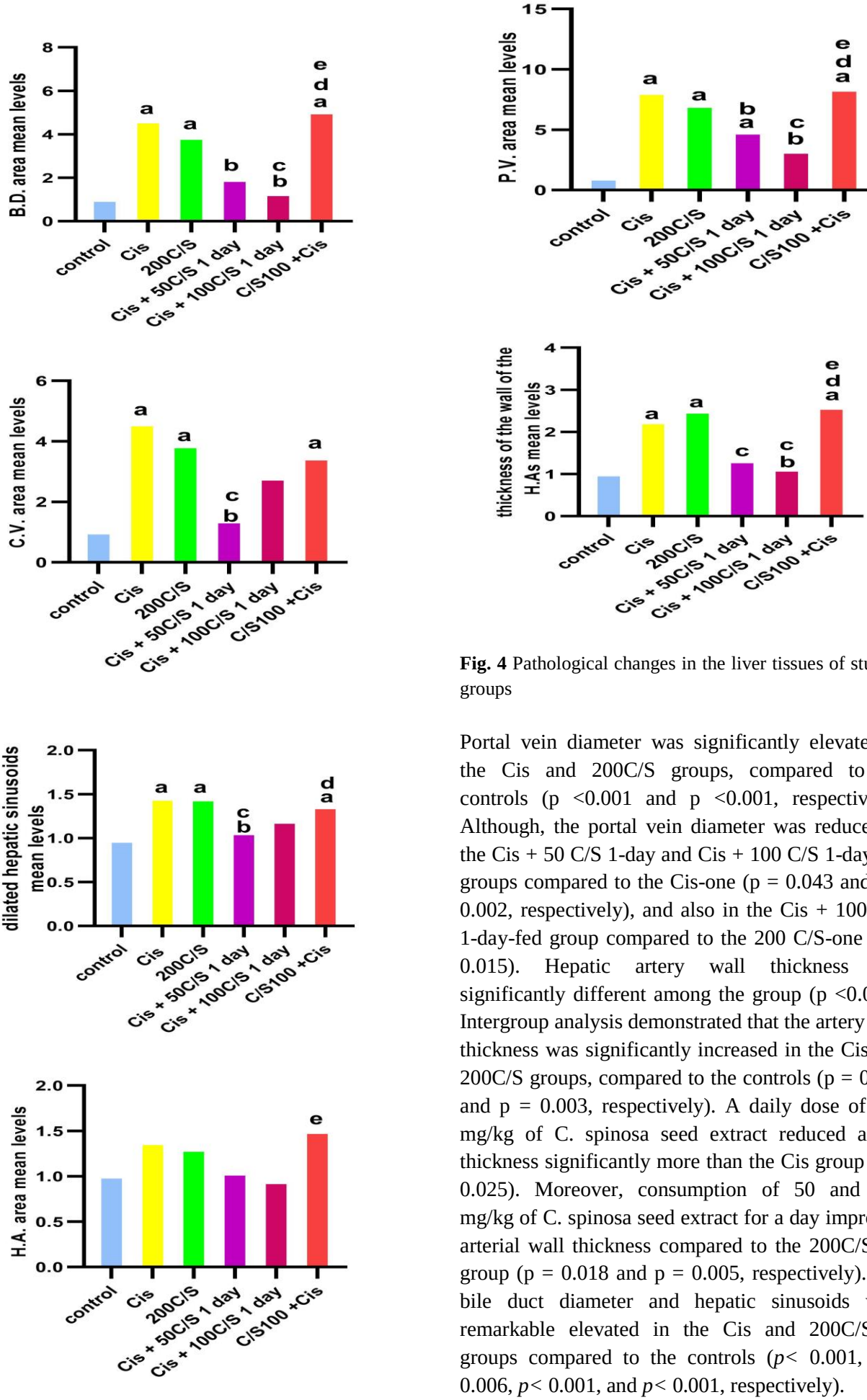

Fig. 4 Pathological changes in the liver tissues of studied groups

Portal vein diameter was significantly elevated in the Cis and 200C/S groups, compared to the controls ($p < 0.001$ and $p < 0.001$, respectively). Although, the portal vein diameter was reduced in the Cis + 50 C/S 1-day and Cis + 100 C/S 1-day-fed groups compared to the Cis-one ($p = 0.043$ and $p = 0.002$, respectively), and also in the Cis + 100 C/S 1-day-fed group compared to the 200 C/S-one ($p = 0.015$). Hepatic artery wall thickness was significantly different among the group ($p < 0.001$). Intergroup analysis demonstrated that the artery wall thickness was significantly increased in the Cis and 200C/S groups, compared to the controls ($p = 0.013$ and $p = 0.003$, respectively). A daily dose of 100 mg/kg of C. spinosa seed extract reduced artery thickness significantly more than the Cis group ($p = 0.025$). Moreover, consumption of 50 and 100 mg/kg of C. spinosa seed extract for a day improved arterial wall thickness compared to the 200C/S-fed group ($p = 0.018$ and $p = 0.005$, respectively). The bile duct diameter and hepatic sinusoids were remarkable elevated in the Cis and 200C/S-fed groups compared to the controls ($p < 0.001$, $p = 0.006$, $p < 0.001$, and $p < 0.001$, respectively).

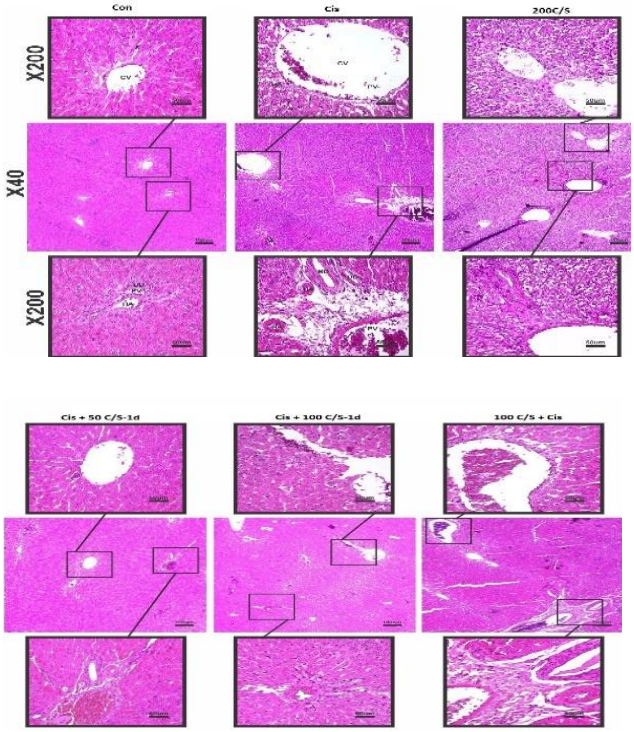

Fig. 5 Photo micrograph of rat liver tissues H&E staining Dilatation and congestion of the central vein and variable degenerative changes in the hepatocytes, dilatation and congestion of the portal vein, thickening of the wall of the hepatic artery, and dilated hepatic sinusoid in the Cis and 200C/S group, and evident improvement of the liver structure and normalization of the hepatocytes, sinusoids and portal area in the treatment group.

However, intake of 50 and 100 mg/kg of C. spinosa seed extract per day decreased bile duct diameter compared to the Cis group ($p = 0.011$ and $p = 0.002$), and intake of 100 mg/kg of C. spinosa seed extract per day decreased this diameter compared to the 200 C/S-fed group ($p = 0.014$), and intake of 50 mg/kg of C. spinosa seed extract per day improved dilated hepatic sinusoid compared to the Cis and 200 C/S group ($p = 0.003$ and $p = 0.004$).

CV: central vein; PV: portal vein; HA: hepatic artery; BD: bile duct; Cis: rats received 7.5 mg/kg of cisplatin to induce cirrhosis; 200C/S: rats received 200 mg/kg of C. spinose seed extract two times per day for two weeks; Cis + 50C/S 1-day: cirrhotic rats received 50 mg/kg of C. spinose seed extract two times for a day; Cis + 100C/S 1-day: cirrhotic rats received 100 mg/kg of C. spinose seed extract two times for a day; 100C/S + Cis: rats received 100 mg/kg of C. spinose seed extract two times per day for two weeks then 7.5mg/kg Cisplatin single dose. Analysis was performed by ANOVA test followed by Bonferroni post-hoc analysis for between group comparisons.

a: Significantly different from the control group; b: Significantly different from the cis group; c: Significantly different from the 200C/S; d: Significantly different from the Cis + 50C/S 1-day; e: Significantly different from the Cis + 100C/S 1-day Significant elevation in diameter of BD, CV, PV, and thickness of the wall of HAs, and dilation of hepatic sinusoids and noticeable increment of HA diameter in the Cis and 200C/S group compared with control group, and significant therapeutic responses in almost all of them

DISCUSSION

Liver injury is common during chemotherapy and to treat this complication, several treatments as pretreatment, single dose, and treatment after chemotherapy are recommended. In the present study, we investigated the effect of hydroalcoholic extracts of *C. spinosa* seeds as a single dose and pretreatment against Cisplatin-Induced liver damage. As mentioned before, in our study, in the serum results, we had a series of undesirable and desirable significant changes and a series of good non-significant changes. For example, decreased Alb level and increased Pt, Alt, and LDH levels were evident in the Cis group compared to the control group. These findings were consistent with other similar studies [4,5,15,16], which implied that during cisplatin administration to rats, such changes may be occur. On the other hand, serum AST, Alp and GGT level were not increased in the Cis group. According to previous reports, we know that ALT is more sentient test in acute liver damage, whereas AST is more sentient in chronic damage [15]. On the other hand, Omar *et al*. revealed that the evident changes in the enzymes act triggered by cisplatin are owing to their leak from hepatocytes, which could be a secondary incident to the liver injury caused by cisplatin [5]. Perhaps an increase in these serum markers would have been evident if the time interval between Cisplatin administration and testing had been extended. Also, we had a decrease in Alb level and an increase in Pt, Ptt, Alt, Ast, Alp, GGT, T. Bil, and LDH levels in the 200 C/S group, which may indicate the hepatotoxic properties of C. spinosa in high doses, and these findings were in agreement with Fanoudi *et al* [17]. Similar to other studies, we also had significant changes in serum

markers in the treatment groups [16], serum LDH levels were significantly increased in rats received Cis and treated with 50 mg/kg of C. spinosa seed extract for one day compared with the Cis group (p =0.004). Among tissue inflammatory and antioxidant markers, tissue IL-1 β was significantly decreased in the Cis + 50 C/S 1-day group compared with the Cis group (p = 0.006), but we also had acceptable non-significant results that were somewhat consistent with the result of other similar studies [16, 18]. But most of all, the drastic and significant changes in the pathology results were obvious. Of all the items measured as pathology results, central vein diameter, portal vein diameter, thickness of the hepatic artery wall, bile duct diameter, and hepatic sinusoids were significantly increased in the Cis and 200 C/S-fed groups, compared to the controls, which we had similar results in limited studies [4,5,15]. In all the mentioned items, changes in favor of improvement were evident in the treatment groups compared to the Cis and 200 C/S groups. Therefore, according to the present study and similar studies, the cisplatin group indicated noticeable influence on the liver structures and functions. Cisplatin remarkably develop hepatotoxicity in rats, certified by substantial rises in serum ALT and AST acts, and this rising is associated with necrosis and lipid agglomeration of hepatocyte. Cisplatin toxicity is credited to augmented free radical generation and reduced anti-oxidant protection mechanisms. The other feasible comment about toxic effects due to cisplatin usage is the induction of a cascade of inflammatory reactions, these inflammatory reactions may be linked to the oxidative stress that creates a significant pathogenic role in tissue harm resulted from cisplatin. In the cisplatin group, important histological changes containing of congestion, dilatation, epithelial vacuolization, and infiltration of mononuclear cells in the liver tissues happened. There was congestion and dilatation in the central vein and the hepatic sinusoids with disjunction of the endothelial coating of the central vein. Also, dilated congested portal vein with epithelial hyperplasia of bile duct and thickening of the hepatic artery wall and acidophilic exudate is obvious [4, 5, 15]. So several reported dose restricting side effects of cisplatin prevent the increment of cisplatin dose in several instances for better efficiency. Numerous studies have revealed that the usage of various anti-oxidants and anti-inflammatory agents is efficient versus harmful effects of cisplatin on liver and kidney [4,5,19]. Medicinal plants have got significance in healthcare systems all over the world for their confirmed and efficient remedial traits [20]. Truly, liver damages has become one of the most solemn health difficulties, and the accessible synthetic medications (interferon and corticosteroids) are costly and may lead to further injury [21]. Herbal medicine may help as a possible remedy for the prevalent liver problems due to their safety, facile accessibility, affordable, and friendship with the environment [20]. Many researchists have described how friendly natural biomolecules, including phenolic compounds, carotenoids, and polysaccharides, were effective in hindering organ pathologies due to reactive oxygen species. In addition, there is a rising precedency for natural antioxidant rather than synthetic molecules due to the safety of the natural sources [21]. Capparis species are seeded for their pharmaceutical traits and as well as food consumption. *Capparis sp.* is a member of the Capparidaceae family. Caper is bred plentifully in automotive dry areas of Asia, Africa, Saudi Arabia, and Europe, particularly in the Mediterranean domain [22,23]. Previous informations has recommended that numerous portions of the caper (leaves, flower buds, fruits, seeds, roots, and bark) can be applied to cure several ailments, including liver disorder, diabetes, hypertension, rheumatism, headache, and kidney disease. All these therapeutical specifications are owing to several useful combinations, including minerals, carotenoids, tocopherols, phenolic compounds, glucosinolates, phytosterols, and unsaturated fatty acids. From a nutritional perspective, C. spinosa berries, which contain the seeds, have protein (2%), lipids (1%), carbohydrates (5%), dietary fibers (3%), and a proportionate amount of vitamin C. Phenolic combinations, as the most potent bio antioxidants in plants, can also be detected in great amounts in the eatable portions of Capparis such as flower buds, fruits, leaves, and roots [16,24]. Natural antioxidants in C. spinosa can clear injurious free radicals from our body [24]. Perhaps increasing the body's natural antioxidant guarding or taking dietary antioxidant supplements can decrease the venture of chronic diseases or avoid diseases progress [24]. Based on the studies conducted in 2015 and 2018, it was

found that the alcoholic extract of C. spinosa has numerous biologically active chemical groups such as alkaloids, glycosides, carbohydrates, tannins, phenolics, flavonoids, triterpenoids, volatile oil, and fatty acids, whiles the aqueous extract contains steroids, glycosides, carbohydrates, flavonoids, and saponins [7, 10]. And on the other hand, in a new study in 2022, the hydroalcoholic extract of Capparis seed had a higher antioxidant level than cold press oil and n-hexane oil. According to these results, we chose the hydroalcoholic extract of Capparis seed to investigate its effect on liver damage caused by cisplatin. One of the most significant overall traits of Caper in Persian Medicine is its Anti poison (as named Teriaq) trait, which it may be similar to antioxidant effects [8]. On the other hand, Caper processed with vinegar can be beneficial in relieving liver obstruction, especially if consumed with a little olive oil just before mealtimes [8, 13]. In a new study similar to the present study, good nephroprotective effects were seen in treatment by *C. spinosa* seed extracts (CSEE) [19]. And also in another similar study, Tir *et al*. revealed the hepatoprotective and nephroprotective effects of CSSE, and their primary phytochemical screening indicated that CSSE comprised a great amount of phenolic combinations with high hepatoprotective and nephroprotective acts [16]. Also, in another study with the purpose of appraising the anti-inflammatory and hepatoprotective effects of methanolic extracts from fruits and leaves of *C. spinosa*, Aichour *et al*. determined that C. spinosa leaf extract (CSLE) and fruit extract (CSFE) have an anti-inflammatory and hepatoprotective effects, but a little better for the leaf extract [25]. On the other hand, another study found that hydroalcoholic extract of *C. spinosa* root bark presented hepatoprotective effects in mice with liver injury due to CCL4 [26]. Kalantari *et al*. determined that C. spinosa and quercetin are efficient for the avoidance of liver injury by t-BHP induction in mice [18]. In addition, previously, the beneficial effects of daily C. spinosa fruit pickle consumption on liver enzyme tests were reported in a small randomized clinical trial [27]. Also, in a number of studies, the effects of C. spinosa on other diseases have been proven. For example, C. spinosa down regulated inflammatory genes in patients with Alzheimer's disease [28]. Or, Eddouks *et al*. revealed that the antihyperglycemic effect of C. spinosa may be related to the prevention of basal endogenous glucose generation and the amelioration of insulin sensitivity in multi low dose streptozotocin-induced (MLDS) diabetic mice [29]. In a similar study, Rahmani revealed that intake of Caper fruit extract propel to a substantial reduction in blood sugar and also a significant decline in blood triglycerides in diabetic rats [30]. In another animal study, capers extract by enhancement the thickness of the epithelium papillae, decreasing the thickness of the mucus lining, increment the number of blood vessels, and mast cells, and diminished expression of nitric oxide synthases (INOS) can lead to mending mouth ulcers in rats [31]. In our knowledge, this is the first study on the effect of C. spinosa seed extract on liver enzymes, inflammatory and antioxidant markers with a histopathological sight in cirrhosis to determine the best effective dosage. The present study is an animal model that is not generalizable to the humans. Herein, we assessed the liver tissue inflammatory and antioxidant markers by the ELISA method. However, the best method for measuring tissue protein level is western blotting that is suggested in future. Also, in subsequent studies, changes in favor of cirrhosis in serum markers are better seen by increasing the time interval between cisplatin injection and testing.

CONCLUSION

According to the result of this study, cisplatin-induced liver damage is mainly evident with pathological changes, and single-dose treatment with C. spinosa seed extract in two different doses may be has a good effect on these cisplatin-induced changes, Therefore, C. spinosa seed extract may be of help to prevent liver toxicity revealed by cisplatin chemotherapy. In the future, a study with increasing the interval between cisplatin administration and final experiments, as well as measuring the level of tissue proteins by western blotting, is needed to obtain accurate results to generalize these results to the human population.

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Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Ethics Approval

The ethic committee at the Zanjan University of Medical Sciences approved the use of animals and experimental procedures (IR.ZUMS.REC.1399.352). All procedures were applied according to the ARRIVE guidelines. This article does not contain any studies with human participants performed by any of the authors.

Consent for publication

Not applicable

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