Original Article

Hepatoprotective and Anti-oxidant effects of Nepeta Ispahanica Boiss extract on CCL4 induced liver Injury

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Abstract

Background: Natural products might be applicable as remedial agents with their roles in oxidative stress regulation and as natural antioxidants. In this regard, Nepeta ispahanica bioss has been utilized in traditional medicine for several functions. Despite numerous properties of the Nepeta species including their antioxidant properties, Nepeta ispahanica boiss effects against hepatic injury induced by carbon tetrachloride (CCl4) have not been studied. This study aimed to investigate the hepatoprotective effects of Nepeta ispahanica boiss on CCL4 induced acute hepatic injury in an animal model. *Materials and Methods:* The experiment used a total of 36 male Wistar rats, that were divided into six groups. Except for the intact control groups, all groups received a single intraperitoneal injection of CCl4 after pre-treatment period with distilled water, Nepeta ispahanica boiss extracts, or legalon. After 24 hours, rats were anesthetized, sacrificed and blood samples were obtained. Serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), urea and plasma levels of total protein and malondialdehyde (MDA) were evaluated. Besides, SOD and CAT enzyme activities and GSH levels were determined. Histopathological studies also were done in liver tissue samples.*Results:* After the CCl4 injection, oxidative stress-mediated necrotic acute liver injuries were observed. Also, serum ALP, AST, and ALT elevated. Hepatic lipid peroxidation and related decrease of endogenous antioxidants and antioxidative enzymes; lipid peroxidation markers, and oxidative stress markers were presented. Nevertheless, histological and biochemical markers of liver injury were reserved by the pre-treatment with Nepeta ispahanica boiss extracts.*Conclusion:* The present study confirmed that the administration of Nepeta ispahanica boiss extracts before exposure to CCl4, induced significant hepatoprotective effects. These findings verified that Nepeta ispahanica boiss has favorable properties as an antioxidant and hepatoprotective agent.

Keywords: *Nepeta ispahanica* boiss, carbon tetrachloride, hepatic injury, oxidative stress.

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Introduction

The liver performs numerous vital functions, comprising detoxification, protein synthesis, and the consumption of several nutrients [1]. Mostly, when this organ is exposed to environmental toxins, liver metabolic dysfunction may happen, that ranges from the transient increase of liver enzymes to severe hepatic fibrosis, and even liver malignant tumor [1-4]. Comparable effects are induced by Carbon tetrachloride (CCl4), an industrial solvent identified to motivate hepatic injury that is extensively utilized in experimental hepatopathy. CCl4-induced toxicity relies on exposure duration and dose. At a low dose, transient impacts happen, comprising the loss of Ca2+ sequestration, altered lipid homeostasis, and the release of numerous cytokines. Prolong exposure changes fatty acid metabolism and persuade cirrhosis, fibrosis, and malignancies [1, 5-7].

CCL4 metabolites; CCl3 and·OOCCl3 formed in liver parenchyma cells via cytochrome P450-

dependent monooxygenases which are the main effectors of hepatotoxicity [8-10].

Earlier studies indicated that some polyphenolic compounds derivative from foods or fruits showed antioxidant activities against tissue injury induced by free radicals [8, 11, 12]. Therefore, the usage of dietary polyphenols supplements is free from severe adverse effects for treatment and inhibition of hepatic injury [8, 13]. Among numerous plant families that have been recognized for their therapeutic effects, Labiatae (Mint) family (Genus Nepeta) considered quite imperative [14-16]. Some of Nepeta species are utilized as remedial plants in Iranian traditional medicine. Nepeta ispahanica Boiss. (Lamiaceae), is an endemic herbaceous plant which grows wild in Iran. As the importance of distinguishing the biological activity of essential oils and in the course of evaluating the Nepta species, the N. ispahanica essential oil was studied [16, 17]. Since Nepetaspecies have a high content of flavonoid, phenolic, terpenoid, and compounds that provide various pharmacological properties its folk utilization for therapeutic purposes justified. Numerous species are recognized for their medicinal possessions and are utilized in traditional medicine for their, antitussive, diaphoretic, antiasthmatic, antispasmodic, febrifuge, and sedative activities [18-22]. Also, Nepetaessential oils exhibit considerable antioxidant potential [21]. Previous reports have been performed to evaluate the antinociceptive effects of some species of Nepetaspecies [19, 20, 23, 24], Despite numerous properties of the Nepeta species including their antioxidant properties, based on authors knowledge anti-oxidant and hepatoprotective effects of Nepeta ispahanica have not been studied. Indeed, in this study extract from Nepeta ispahanica utilized for the first time to study the anti-oxidant and hepatoprotective effects. In this survey, we investigated the hepatoprotective properties of the hydroalchoholic extract of Nepeta ispahanica Boiss against CCl4-induced acute liver injury in male wistar rats.

Methods

Preparation of Nepeta ispahanica extract. The aerial parts of the Nepeta ispahanica were obtained from Mahan, Kerman province, Iran and were identified and authenticated by Dr.SM. Mirtadzadini, at the Department of Biology, Shahid Bahonar University of Kerman. Then the collected specimen washed, dried, powdered with a blender and soaked in methanol 80 % for 3 days with shaking and then filtered. In the following, methanol extract was evaporated in a rotary evaporator and was dried at 40 ^{o}C. The yield of extraction was 10 %. The dried extract was dissolved in water and administered to the animals.

Acute liver injury Induction. A total of 36, healthy male Wistar rat (6 weeks old upon receipt from the neurosciences research center, Kerman University of Medical Sciences, Iran) were utilized after an acclimatization period of 7 days. Four or five animals were housed in polycarbonate cage in a temperature- (24±2°C) and humidity- (30–35%) controlled room. The light-dark cycle was 12 h/12 h and animals had free access to food and water. The animals were divided into six groups (n=6) to evaluate the administration of the substance: group1 (Intact control): Distilled water (DW) orally administered for 10 days. Group 2 treated mice; distilled water (0.5 ml) for 10 days and then CCl4 administered [50% CCl4 in liquid paraffin (2.5 ml/kg BW)].

Groups 3 , 4 and 5 treated mice.: animals received different doses of the extract 50, 100, 200 individually for 10 days, then 50% CCl4 in liquid paraffin (2.5 ml/kg BW). Group 6: animals received Legalon (silymarin 70%) as a hepatoprotective standard drug (420 mg/kg BW) for 10 days then a single dose of 50% CCl4 in liquid paraffin was administered (2.5 ml/kg BW). After 24h, venous blood was collected and then the rats were anesthetized, sacrificed and all collected blood specimens were centrifuged and kept in an ultradeep freezer until analysis. Also, the separated hepatic tissues homogenized in ice-cold. Tissue homogenates were kept in a freezer under −150°C until examination.

Serum AST, ALP and ALT levels Measurement. Serum aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase enzymes (ALP), Total protein, total bilirubin, and urea were measured by spectrophotometric methods (pars azmoon, Iran).

Lipid peroxidation Measurement. The liver lipid peroxidation concentration was detected by

estimation of MDA via the thiobarbituric acid method described by Kauer et al [25] and a UV/Vis spectrophotometer to measure the absorbance of the solution at 525 nm was utilized (determined as nM of MDA/mg protein). Considering the molar absorption coefficient, the MDA value per gram of tissue was determined. The hepatic tissue protein concentration was quantified by a previously described method [26] using bovine serum albumin as an internal standard.

Measurement of hepatic antioxidant defense systems. The prepared hepatic homogenates were mixed with DW and trichloroacetic acid then centrifuged (3000 g, 15 min). The GSH content was spectrophotometrically measured through the measurement of absorbance at 412 nm by utilizing 2-nitrobenzoic acid [27].

The H2O2decomposition in the existence of CAT was analyzed at 240 nm with a spectrophotometer [28]. CAT activity was defined as the amount of enzyme required to decompose 1 nM of H2O2 per minute at 25°C and pH 7.8. The results were expressed as U/mg tissue protein.

The measurement of SOD activity was performed based on the method of Giannopolitis and Ries [29] In this method, 50 mM of potassium phosphate buffer (pH =7), 0.1 mM EDTA, 13 mM methionine, 75 mM riboflavin and 0.075 mM NBT were added to the tissue extract and spectrophotometrically measured at 560 nm.

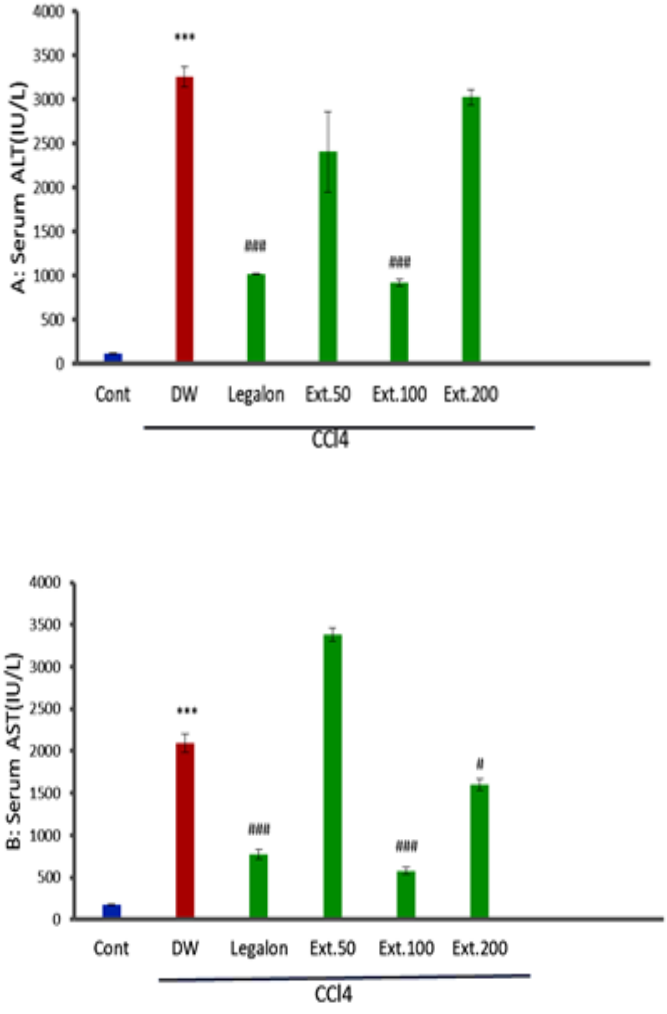
Histopathological analysis. Liver tissue samples of each group were fixed in %10 formalin, embedded in paraffin and 5 µm tissue sections were prepared then stained with hematoxylin and eosin (H&E) for histopathological examination. The histopathological slides were observed with a light microscope. Examined indexes in the slides included congestion, hydropic degradation, and Lymphocytic infiltration.

Statistical analysis. All the data were shown as mean ± standard error of the mean (SEM). Differences among means were analyzed by one-way analysis of variance and Tukey's post hoc tests using SPSS software (version 16); (for the comparison among groups). $p < 0.05$ was considered as significant level.

Results

Changes in the serum AST, ALP and ALT levels. Significant ($p < 0.01$) increase of serum ALP, AST and ALT levels were presented in the CCl4 control versus intact control. Also, significant ($p < 0.01$) decline in ALP and ALT serum levels were induced by the treatment of extracts at 100 mg/kg, and by Legalon before receiving CCl4, versus CCl4 control.

Besides, AST serum levels were decreased after administration of extracts at 100 and 200 mg/kg, and by Legalon before receiving the CCl4, in comparison to the CCl4 control (Figure 1).



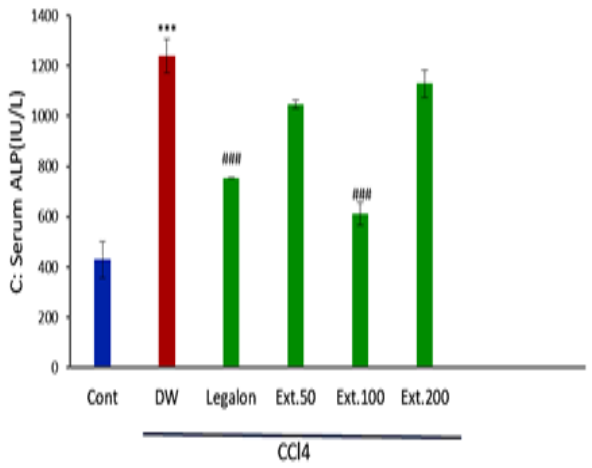


Figure 1. Effects of pretreatment with different doses (50, 100 and 200 mg) of hydroalcoholic extract of *Nepeta ispahanica* boiss on serum levels of liver enzymes; ALT (a) ،AST(b), ALP (c) in the CCl_4-induced liver injury of 6 mice in each group. Data are expressed as mean ± S.E.M. DW: deionized water; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase. ALP; Alkaline phosphatase. *: Indicates a significant difference with the control group. #: Indicates a significant difference with the DW + CCl_4 group. ***: p<0.001 #: p<0.05 ##: p<0.01 ###: p<0.001

Changes in the serum bilirubin, total protein, and urea levels. According to our results, there was a significant ($p < 0.05$) elevation in serum bilirubin and urea and a decrease in total protein level in the CCl4 control as compared with intact control. Likewise, significant ($p < 0.01$) increase in total protein and decrease in urea serum levels were induced by the treatment of extracts at 100 mg/kg, and by Legalon before receiving CCl4, versus the CCl4 control (Figure 2). Similarly, bilirubin serum levels were decreased after administration of extracts at 50, 100 and 200 mg/kg, and by Legalon before receiving CCl4, in comparison with the CCl4 control (Figure 2).

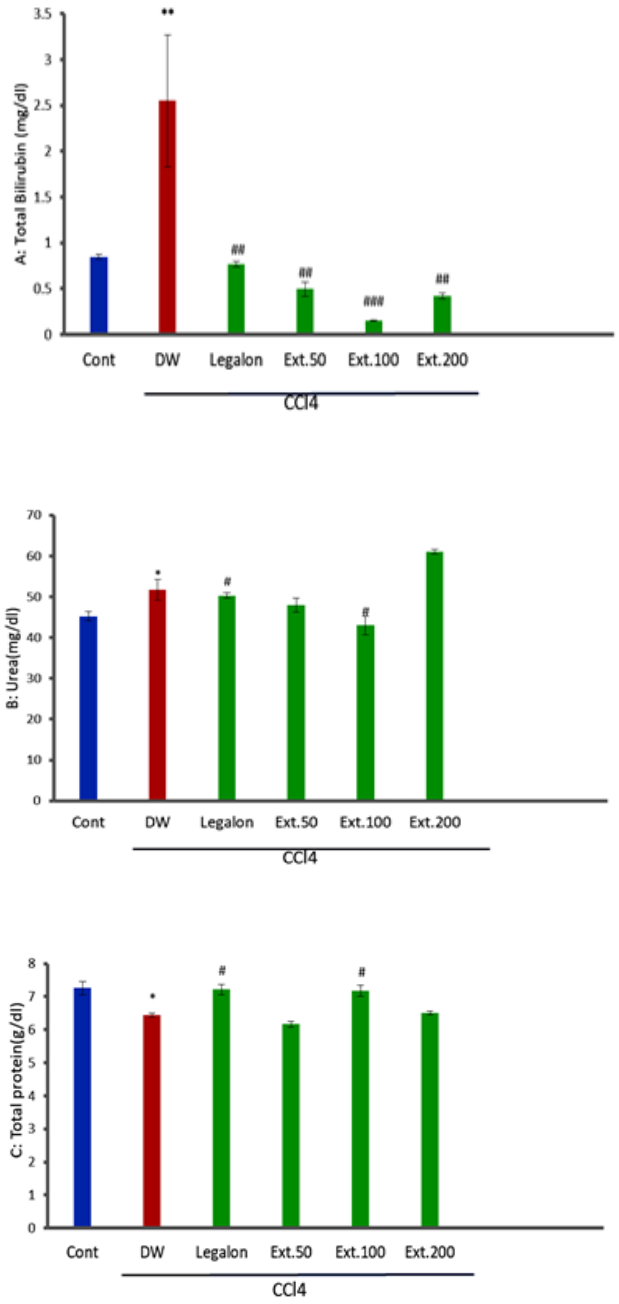


Figure 2. Effect of pretreatment with different doses (50, 100 and 200 mg) of hydroalcoholic extract of *Nepeta ispahanica* boiss on Serum bilirubin(A), urea(B) and total protein(C) in the CCl_4 induced liver injury (6 mice in each group). Data are expressed mean ± S.E.M. DW: deionized water *: Indicates a significant difference with the control group. #: Indicates a significant difference with the DW + CCl_4 group.

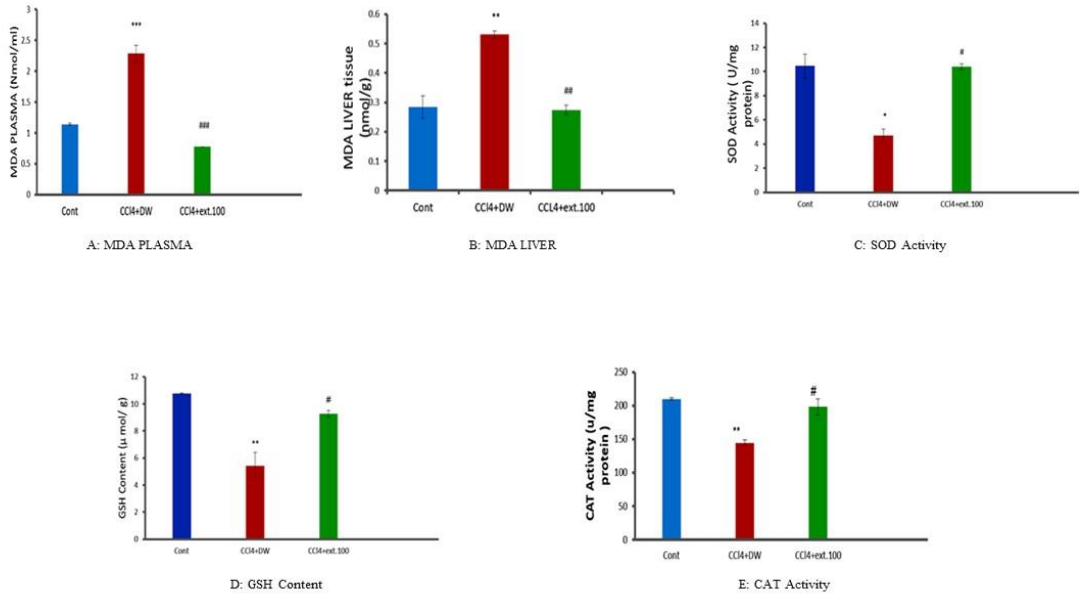


Figure 3. Effect of pretreatment (100 mg) of hydroalcoholic extract of *Nepeta ispahanica* boiss on plasma and hepatic MDA levels ، hepatic SOD ، GSH ،CAT levels in the CCl_4 induced liver injury (6 mice in each group). Data are expressed as mean ± S.E.M. DW: deionized water. MDA; Malondialdehyde. SOD; Superoxide dismutase. GSH; Glutathione. CAT; Catalase.*: Indicates a significant difference with the control group #: Indicates a significant difference with the DW + CCl_4 group

MDA: ##: p<0. 01 **: p<0. 01 ***: p<0.001 ###: p<0.001

SOD #: p<0. 05 *: p<0. 05

GSH #: p<0. 05 **: p<0.01

CAT #: p<0. 05 **: p<0.01

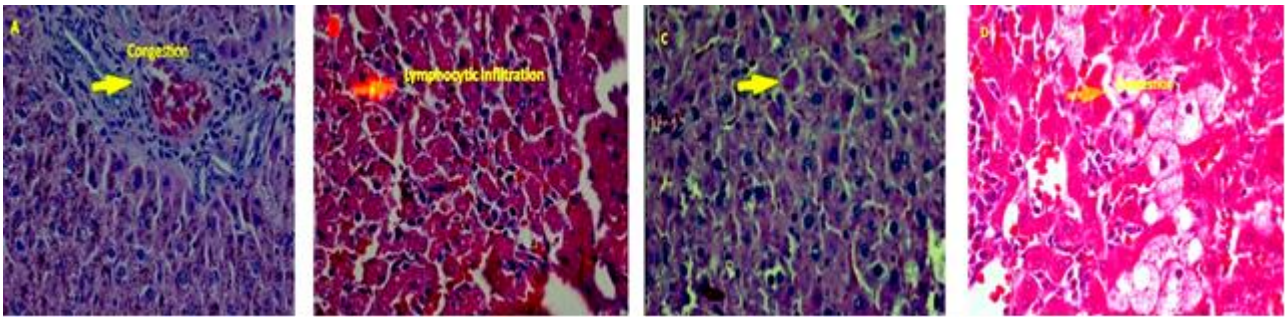


Figure 4. Histopathological changes in the rat liver sections: (A) Control group (40x), (B) CCl_4 group (40x), (C) Silymarin+ CCl_4 group (40x), (D) Extract +CCl_4 group100 mg/kg (40x).

Effects of Nepeta ispahanica boiss extract on the plasma and hepatic MDA content ،SOD and CAT Activity ،GSH Content. Based on our results, there was a significant ($p < 0.01$) increase in plasma and hepatic MDA content in the CCl4 control compared with the intact control. Nevertheless, significant ($p < 0.01$) decreases in plasma and hepatic MDA content were induced by extracts at 100 mg/kg versus CCl4 control (Figure 3A and 3B). A significant ($p < 0.05$) decline in hepatic SOD activity was presented in the CCl4 control versus the intact control. Nevertheless, significant ($p < 0.01$) increases in SOD activities detected in mice that received extract at 100 mg/kg to CCl4 control (Figure 3C). A significant ($p < 0.01$) decrease of hepatic GSH was detected in the CCl4 control versus the intact control, but these changes were significantly ($p < 0.01$) amended by pre-treatments with extracts at 100 mg/kg in compared with CCl4 control. (Figure 3D). There was a significant ($p < 0.01$) decrease in hepatic CAT activity in the CCl4 control compared with the intact control. A significant ($p < 0.05$) increase of hepatic CAT was presented in the animals that received extracts compared with the CCl4 control. This elevated CAT activity might be due to the amelioratory effects of hydroalcoholic extract of Nepeta ispahanica boiss at 100 mg/kg (Figure 3E).

Histological examination. The liver section of normal rats shows the normal hepatocytes with a prominent nucleus, cytoplasm and central vein (Figure 4 A). Liver sections of CCl4 treated rats show the histopathological changes including degeneration of hepatocytes, Congestion, hydropic degeneration and lymphocytic infiltration (Figure 4 B). Liver sections of treated rats with legalon (standard drug) and extract-treated showing central vein hepatocytes with well-preserved cytoplasm, besides tissue injuries were lower in these groups than the CCl4 group (Figure 4 C and D). Extract treatments before induction of inflammation had protective effects against the liver by decreasing the hepatocyte degeneration, and inflammation as well as fibrosis and apoptosis occurrence.

Discussion

Liver toxicity as a widespread disorder is a severe challenge to universal public health. Based on previous data, liver-protective agents are insufficient and occasionally led to serious health problems. Currently, herbal medications have gained much attention for treating liver disorders due to minimal side effects and more efficacy. In this regards natural products might be applicable in which their functions in oxidative stress have two aspects: increasing the activity of natural antioxidants like GSH and neutralizing reactive oxygen species [30].

Although abundant properties of the Nepeta species, the anti-oxidant and hepatoprotective effects of Nepeta ispahanica have not been evaluated. Thus, in this study extract from Nepeta ispahanica boiss utilized to investigate its potential hepatoprotective effects. Based on our findings the hepatoprotective effects of Nepeta ispahanica hydroalcoholic extract against CCl4-induced liver injury were validated. Indeed, after induction of CCL4- acute liver injury liver function tests, liver antioxidant enzymes levels, and histological examinations were performed to assess the antioxidant and hepatoprotective properties of this plant. In this study, legalon was utilized as a standard hepatoprotective agent. Upon legalon treatment, elevated biochemical parameters by CCl4 were reversed back to normal.

An earlier study shows that legalon displays appropriate hepatoprotective potential effects toward hepatocellular injury in experimental animal models [31]. Hydroalcoholic extract of Nepeta ispahanica has a hepatoprotective effect, which may be because of the antioxidant and anti-lipid peroxidant properties. It has flavonoids and phenols that may be responsible for its properties.

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CCl4 is metabolized by cytochrome P450 enzyme and related produces free radicals that are

highly reactive and radicals are capable of binding to proteins or lipids, thus initiating tissue lipids peroxidation, inflammation, and hepato-toxicity. Likewise, the cleavage of CCl4 leads to MDA accumulation [32, 33]. Plants Crude extracts medicinal effects including antioxidant activities has been shown in several pieces of research. In this regard, Nepeta species exhibits such properties [34-40]. indeed, phenolic compounds that exist in plants have been considered to have a high antioxidant amount and free radical scavenging capacity via the mechanism of preventing the enzymes corresponding to ROS generation and decreasing extremely oxidized ROS. Consequently, phenolic compounds may be considered as potential factors for the treatment of various oxidative stress-related disorders [41]. In a similar study, which conducted by Kraujalis et al. antioxidant activity of some Nepeta species, was investigated [39]. In this regard, Lee et al. described an association between antioxidant properties of Nepeta species and the existence of phenolic acids [42] that might be responsible for antioxidant effects that were observed in our study.

Also, a comparable study provides evidence that the essential oil of Nepeta cataria L exerts protective effects against acetaminophen-induced liver injury. They indicate that this natural resource may be considered as a hepatoprotective agent [43]. Also, in a similar study, the antioxidant activity of different extracts of N. binaludensis was evaluated. Their findings show that the extracts prepared by polar alcoholic solvents display better effects than the other extracts [44]. Indeed, antioxidative compounds such as phenols and flavonoids could induce protective impacts besides hepatocellular toxicity by decrease the lipid peroxidation and protection of glutathione depletion [30, 45, 46].

Conclusion

To the best of author knowledge, this is the first study that has both assessed the antioxidant and hepatoprotective effects of the aerial parts of Nepeta ispahanica and studied its effects on liver function tests and stress oxidative parameters. As a result of the Nepeta ispahanica use, we also demonstrated that this extract exerted anti-oxidant effects that maybe because of a synergistic action between the components present in the extract. In conclusion, our findings confirm the usage of Nepeta ispahanica boiss in oxidant conditions as a hepatoprotective agent against CCL4 hepatic injury. Further pre-clinical studies should be conducted (in vivo) to validate the Nepeta ispahanica anti-oxidant and hepatoprotective effects, as well as its safety.

Conflicts of Interest

The authors declared no conflict of interest.

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