

Hepatoprotective Effect of *Zizyphus vulgaris* on Carbon Tetrachloride (CCl₄) Induced Liver Damage in Rats as Animal Model

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Abstract

Background: Finding protective agents with fewer adverse effects against toxin-induced liver injuries, as a key detoxifier and excretor organ, have always been a concern for researchers. Carbon tetra-chloride (CCl₄)-induced liver damage has been introduced as an experimental model of liver damage. This study aimed to investigate the protective effect of ethanolic extract of *Zizyphus vulgaris* (ZV) against hepatic injury induced by CCl₄ in laboratory rats. **Materials and Methods:** Fifty healthy male Wistar rats (200±20 g) were randomly divided into 5 groups (n=10) as following for a 45days study: Base, which received 1 cc/Kg olive oil intraperitoneally (IP) twice a week and 0.5cc distilled water orally; Control, which received 0.5 cc/kg olive oil+0.5 cc/Kg CCl₄ IP+0.5cc distilled water orally; experimental groups, which received 0.5 cc/kg olive oil+0.5 cc/Kg CCl₄ IP+0.5cc distilled water plus ZV extract in dosages of 200 mg/Kg (group E200), 400 mg/Kg (group E400) and 600 mg/Kg (group E600) PO. Levels of aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), alkaline-phosphatase (Alk-P), Albumin, total-protein, and bilirubin were measured as well as pathological assessment of the liver samples for scoring of portal inflammation and hepatocellular necrosis. **Results:** Results of this study revealed that although there was a significant decrease in liver enzymes of the ZV treated groups (P<0.05) there were insignificant differences in protein and albumin concentrations between the 5 experimental groups. In addition, ZV treatment reduced hepatic necrosis and portal inflammation compared with the control group. **Conclusion:** ZV showed hepatoprotective impact against CCl₄-induced liver injury according to both serological and pathological investigations. [GMJ. 2013;2(3):88-94]

Keywords: *Zizyphus vulgaris*; Carbon tetra-chloride; hepatoprotective effect; rat

Introduction

Liver plays the role of detoxifier and excretor of destructive agents against body intoxication. The toxins are then converted into the intermediate reactive radicals and ex-

hibit their hepatotoxic impacts. However, liver injury occurs followed by histopathological changes including degeneration, necrosis and atrophy of liver parenchymal cells with interstitial connective tissue [1].

Experimental liver damage induced by carbon

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tetrachloride (CCl₄) has been widely investigated and the profile of damage even after a single administration has been well established [2]. Cell damage caused by free radicals has been reported as the predominant mechanism of liver injury by CCl₄ [3]. In literature, It has been demonstrated that lipid peroxidation which is also induced by CCl₄ can be inhibited by natural antioxidants [2,4]. Thus, identification of oxidation inhibitors, especially among natural products, in order to prevent cell damage could lead to important new methods for prevention and even treatment of liver damage.

Zizyphus vulgaris(ZV), from the *Zizyphus* genus, is one of the well known medicinal herbs which has been commonly consumed as a food and a medicine in some of Iranian tribes as well as traditional Chinese medicine [5]. For instance, different components of this plant such as fruit, leaf and seeds were used as immunity stimulants, anti- tumor activators, and as a treatment for insomnia and anxiety[6, 7]. Previous researches revealed that ZV possesses palliative, hypnotic, hypotensive, anti-hyperlipidemic, anti-hypoxic, anti-hypothermic and anti-oxidative influences as well as some effects on central nervous system[5, 8,9] (e.g. Hippocampal formation, inhibition of glucose mediated excitatory pathway and coordination of movements) [9]. Hepatoprotective effects of some other members of this genus have also been reported [10-12].

Searching for hepatoprotective agents with fewer side effects, lower prices, and more availability, have always been a concern for researchers. The present study was conducted to determine the hepatoprotective effect of ZV, a widely accessible herbal medicine, against CCl₄- induced liver damage in laboratory rats.

Materials and Methods

Preparation of Plant Extract

Zizyphus vulgaris (ZV) plant was collected from the rural areas of Yasuj, Kohgiluyeh & Boyer-Ahmad province of Iran on June 2010. It was authenticated by the research center of agriculture and natural sources of Yasuj University of medical sciences, Yasuj, Iran (Herb-

al No.151). A mixture of all plant organs were dried under room temperature ($25 \pm 2^\circ\text{C}$) and made into powder by using mortar and piston. The extract was obtained from the powder (500 g) through soxhlet extraction with 80% ethanol. The extract was concentrated into a semi-solid material by using rotary evaporator at $< 50^\circ\text{C}$ temperature. The extract was dissolved in distilled water to 200, 400 and 600 mg/ml and administered to rats orally at doses of 200,400 and 600 mg/kg body weight.

Animals, Carbon tetrachloride-induced hepatotoxicity and Hepatoprotective activity Fifty healthy male Wistar rats (200 ± 20 g) were obtained from animal house of Shiraz university of medical sciences, Iran, and randomly divided into 5 groups (n=10) as following: Base group that received olive oil, 1 cc/Kg intraperitoneally (IP), twice a week, and 0.5 cc distilled water orally, simultaneously. The control group which was given a mixed solution of 0.5 cc/kg olive oil and 0.5 cc/ Kg CCl₄ (Sigma-Aldrich™, Milano, Italy) by IP injection and 0.5 cc distilled water orally, simultaneously, in the same day as the prior group. Three experimental groups were given the olive oil-CCl₄ solution as described for the control group plus ZV extract which was given to the first, second and the third experimental groups, in dosages of 200 mg/Kg (group E200),400 mg/Kg (group E400) and 600 mg/Kg (group E600) PO, respectively. The rats were weighted weekly and according to the new weights, the extract and CCl₄ dosage were altered. The animals were housed in standard cages and kept in well ventilated location under 12 h light/dark cycles, with food and water ad-libitum. This study was performed based on the guidelines for the use and care for laboratory animals. After 45 days, the rats were anesthetized by ether. Blood samples were taken by cardiac puncture and livers were excised and fixed in buffered formaldehyde (pH=7.2) for further evaluations.

Assessment of liver functions

Blood samples were separated into serum for the analysis of biochemical parameters of liver. Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (Alk-P), Albumin, total

protein, and bilirubin were assayed by using clinical test kits (Randox, Randox Laboratories Ltd., UK).

Pathological Assessment of liver damage

Followed by weighting, specimens were embedded in paraffin, sliced into 15µm sections, and stained with hematoxylin-eosin (H&E) for blinded histological assessment. The degree of portal inflammation and hepatocellular necrosis were assessed semi-quantitatively according to the reported method by Frei et al (1984) and changes were graded as follows: 0 (absent); I (minimal); II (mild); III (severe) [13]. The histological evaluation performed in nonconsecutive, randomly chose histological fields (×200).

Statistical analysis

All data are expressed as mean ± standard deviation (SD). Results were statistically analyzed by Mann-Whitney U test for significant difference between group means (P value < 0.05 considered as statistically significant).

Results

Results of this study revealed that there were no significant differences in protein and albumin concentrations between the 5 experimental groups (table-1). Nonetheless, the highest decrease of total protein and albumin among stated groups were achieved by

group E600 and group E200 (P<0.05). There were significant differences in total and direct bilirubin levels of the control group versus group E400 and group E600. The level of this marker in group E400 and E600 were insignificantly lower than the base group as well. In Table-2, the effect of ZV on liver enzymes is shown. Alk-P, ALT and AST levels in group E200, E400, and E600 illustrated a significant decrease in contrast with Control group (P<0.05). Histopathological findings (Table-3, Figure-1) analyzed by Mann-Whitney U test demonstrated that in groups E200, E400 and E600, portal inflammation were decreased significantly in comparison to the control group (P< 0.001, P= 0.01 and P= 0.004, respectively); however, the differences among ZV treated groups were not noticeable. Necrosis scores were also lower than Controls by groups E200, E400 and E600, and the differences were significant (P<0.01 vs. control). There were no considerable differences among the three ZV received groups for necrosis and portal inflammation. Histopathological analyses described normal cells with intact cytoplasm and nuclei and prominent central vessel in base group.

Discussion

Liver is a vital organ which has a key role in metabolism and detoxification[14]. So many endogenous and exogenous agents get ex

Table 1. Effect of Zizyphus vulgaris on plasma concentrations of proteins, albumin, total and direct bilirubin in CCl4-induced liver injury; mean ± standard deviation of total protein, albumin and bilirubin in the Base group that received the vehicles (olive oil and distilled water), the control group that received CCl4 other than vehicles, groups E200, E400 and E600 that received 200 mg/Kg, 400mg/Kg and 600mg/Kg of ZV extract, respectively, plus vehicles and CCl4. N=10.

Groups	Proteins (mg %)	Albumin (mg %)	Total bilirubin (µmol/L)	Direct bilirubin (µmol/L)
Base	7.11±0.37	3.82±13	0.28±0.07	0.16±0.13
Control	7.76±49	3.76±0.021	0.48±0.21 a	0.25±0.17 a
Group E200	7.06±0.09	3.66±0.09	0.42±0.05	0.22±0.09
Group E400	6.90±0.25	3.75±0.91	0.30±0.08 b	0.10±0.11 b
Group E600	6.65±0.91	3.70±0.28	0.27±0.17 b	0.07±0.12 c

a P< 0.05 vs. Base group

b P< 0.05 vs. control group

c P< 0.01 vs. control group

posed to it and may destroy its structure and cause problems. On the other hand, many chemical compounds were investigated to find hepatoprotective agents against destructive damages to the liver, but still researches keen to find more efficient medicines with less adverse effects. Nowadays, herbal medicine has received more attention due to their fewer side effects and abundance. The current study was conducted to determine the hepatoprotective effect of ZV against CCl4-

induced liver damage. CCl4 catabolised radicals induce lipid peroxidation, damage the liver cells' membranes and organelles, lead to swelling and necrosis of hepatocytes, and result in the release of cytosolic enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), into the circulating blood [5]. Many chemical compounds, such as fatty acids, teri terpenoid acids, flavonoid ,saponin and cyclopeptide alkaloids were isolated from

Table 2. Effect of Zizyphus vulgaris on liver enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (Alk-P) in CCl4-induced liver injury; mean± standard deviation of AST, ALT, Alk-P, in the Base group that received the vehicles (olive oil and distilled water), the control group that received CCl4 other than vehicles, groups E200, E400 and E600 that received 200 mg/Kg, 400mg/Kg and 600mg/Kg of ZV extract, respectively, plus vehicles and CCl4. n=10.

Groups	Alk-P (IU)	AST (IU)	ALT (IU)
Base	177±15.9	139±16.2	80±12
Control	355±14.5a	413±12.9a	287±10.3a
Group E200	315±13.7b	357±5.1 b	231±15.3 b
Group E400	301±10.3 b	338±13.1 b	222±15.7 b
Group E600	296±15.6 b	332±16.3 b	214±17.6 b

a P< 0.05 vs. Base group

b P< 0.05 vs. control group

Table 3. Effect of Zizyphus vulgaris on histopathological findings of livers in CCl4-induced liver injury; Quantitative summary of the histopathological observations of the liver samples of the Base group that received the vehicles (olive oil and distilled water), the control group that received CCl4 other than vehicles, groups E200, E400 and E600 that received 200 mg/Kg, 400mg/Kg and 600mg/Kg of ZV extract, respectively, plus vehicles and CCl4. n=10. The liver samples were fixed in 10% neutral-buffered formalin prior to paraffin-embedding, and stained with H&E. The histopathological changes were graded according to the following criteria: 0, absent; I, minimal; II, mild; III, severe.

Histopathologic grading	Base	Control*	Group E200	Group E400	Group E600
<i>Portal Inflammation</i>					
Grade 0	10	0	0	0	0
Grade I	0	0	3	2	3
Grade II	0	1	5	5	4
Grade III	0	7	2	3	3
<i>Necrosis</i>					
Grade 0	8	0	0	0	0
Grade I	2	0	4	4	6
Grade II	0	2	5	4	3
Grade III	0	6	1	2	1

* Two animals were died during the study and their livers were not analyzed.

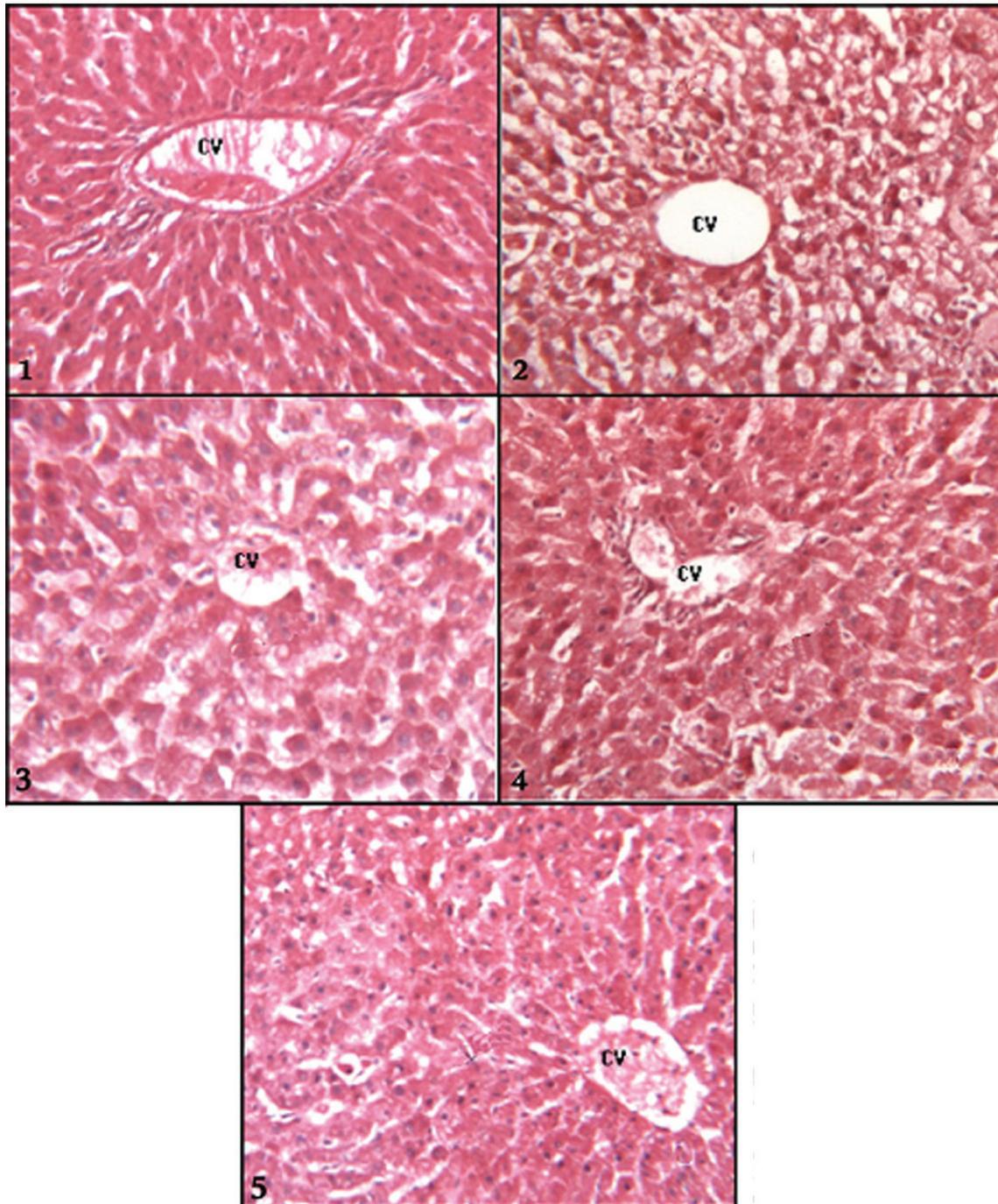


Figure 1. The microscopic images of liver sections from rats treated with Carbon tetrachloride (CCl₄), the post-doses of ZV at 200 (group E200), 400 (group E400), and 600 (group E600) mg/kg, the group which received no other treatment than CCl₄, and the base group that received the vehicles (olive oil and distilled water) . Livers were examined for portal inflammation and necrosis. (1) Liver section of Base group which shows normal structures; (2) liver section of the control group; (3) liver section of the experimental group E200; (4) from livers of group E400; (5) liver section of the group E600.

this plant, of which some of the therapeutic effects of ZV were attributed to [15,16]. For example, Sheng Guo et al (2009) suggested that tri terpenoid is responsible for many biological effects such as cytotoxic, anti-microbial, anti-complementary, anti-inflammatory, anti-HIV, cyclooxygenase 2 inhibitory and inhibitory effect on human melanoma[6]. Moreover, Zhao et al (2006) declared the sedative effect, by decreasing monoaminergic system activity, and hypnotic influence for saponin and fatty oil[8]. According to the outcome of the present study, serological assessments have shown that the levels of hepatic enzymes in control group were enhanced due to CCl₄ administration in comparison with the base group while ALT, AST and Alk-P in the groups E200, E400, and E600 decreased significantly with a dose- dependent trend; although not significantly, group E600 with higher ZV extract administration exhibited lower liver enzyme levels. Results showed an increase in rates of total and direct bilirubin in control group that received CCl₄ in comparison with base group which could mostly be due to hepatocyte destruction and absence of protective agents while ZV receiving groups had lower bilirubin concentration suggestive of noticeable hepatoprotective activity of this medicinal herb; besides, though reduction of total and direct Bilirubin were observed in all the three ZV treated groups, the decrease were significant in groups E400 and E600 which could again implicate the dose-dependent hepatoprotective effect of the agent . Histopathological assessment revealed that ZV diminished portal inflammation and necrosis of liver considerably. There are many published studies on hepatoprotective effects of other members of the *Zizyphus* family (e.g. *Zizyphus spina-christi*, *Zizyphus jujuba*) in which antioxidative and anti-inflammatory activities

were introduced as the dominant mechanism of hepatoprotection [10,12,17]. The antioxidative and anti-inflammatory mechanisms are assumed to be the mechanisms which are involved in the hepatoprotective effect of ZV; however, based on a Google-scholar literature review, studies on ZV protective impact on drug-induced liver damage were still lacking. As a limitation of this study, considering the mechanism of hepatotoxicity of CCl₄, assessment of markers of oxidative stress such as the level of Glutathione peroxidase and Superoxide dismutase could be performed which were not done in this study.

Briefly, results of this experimental study claims that ZV extract possessed protective effects against CCl₄ induced liver damage and perhaps other types of hepatic injury. It is also assumed that this protective effect can be found in other members of the *Zizyphus* family. However, more investigations are still required to evaluate efficacy of ZV on liver damage with different etiologies, and to determine any possible adverse effects of this herbal medicine.

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Conflicts of Interest

None

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