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Semiprotective Effects of Hempseed Oil on Carbon Tetrachloride-induced Hepatotoxicity in Male Rats: An Ultra-short Toxicological Intervention

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KEYWORDS

Hempseed;
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ABSTRACT: This study was designed to investigate the protective activity of hempseed oil on carbon tetrachloride (CCl_4) hepatotoxicity in male rats at Islamic Azad University, Saveh Branch, Saveh, Iran in 2015. Normal control (NC) group was injected intraperitoneally (i.p.) with distilled water (0.5 ml/kg); CCl_4-intoxicated group (TCC) injected CCl_4; hempseed oil treated group (HSO) gavaged hempseed oil; TCC-HSO group was injected CCl_4 prior to intake of hempseed oil and HSO-TCC group was gavaged hempseed oil prior to being injected with CCl_4. In all treated groups, toxicity was induced by i.p. injection of CCl_4 (0.5 ml/kg) for two consecutive days and hemp seed, oil was gavaged at 8 ml/kg in respective group once daily for one week. Plasma alanine aminotransferase (ALT) and aspartate transaminase (AST) levels increased in TCC. Protection against toxicity in HSO-TCC and TCC-HSO reduced AST and ALT activities compared to TCC. Plasma alkaline phosphatase activity in TCC-HSO and HSO-TCC increased as compared with other groups. CCl_4 decreased plasma high-density lipoprotein cholesterol (HDL-C) in TCC. Hempseed oil decreased total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol, and triacylglycerols in HSO compared to NC. Hempseed oil in TCC-HSO and HSO-TCC restored TC, HDL-C, and LDL-C levels to those of NC. Atherogenic

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index was lower in HSO in comparison to TCC. Based on histopathology, hempseed oil improved CCl_4-induced-cardio- and hepatotoxicity in TCC-HSO and HSO-TCC; however, hempseed oil did no prevent CCl_4-induced nephrotoxicity. To sum up, hempseed oil has mild protective effects against CCl_4 toxicity in male rats.

INTRODUCTION

The *Cannabis sativa* L. is an annual, dioecious herb with global distribution. Nowadays, this plant is easily cultivated as a pet herb in garden or greenhouses all over the world especially for producing hashish or marihuana. It would be no exaggeration to consider this millennium-age plant as functional food factory that produces both drug-like (phytocannabinoids) and food (hempseed) components. Its licit cultivation has been prohibited in Iran due to the presence of the psychoactive drug-like phytocannabinoids and Islamic prohibition [1]. However, its fruit, hempseed, still sold in Iranian local markets as bird seed, nut, food and traditional phytomedicine.

In traditional Iranian medicine, byproducts of *Cannabis sativa* L. are used to treat inflammation, nausea, headache, stomachache, hematochezia, diarrhea, flatulence and alopecia [2]. In modern era, after great discovery of delta-9-tetrahydrocannabinol (THC) as a prototype of cannabinoids, cannabinoid receptors, and finally endocannabinoid system; the black box of *C. sativa* L. has been approximately decoded and why Iranian called it "Shahdaneh" that literally means "the shah of seeds" [2].

Hempseed contains 20%-25% protein, 20%-30% carbohydrates, 25%-35% oil, 1%-15% insoluble fiber, a rich array of minerals and perfect ratio (3:1) of two essential polyunsaturated fatty acids (PUFAs; linoleic and linolenic acids) for human nutrition [3]. Although phytic acid had, some negative effect but is a marvelous product in terms of protein, oil and antioxidant molecules [4]. In this regard, diets supplemented with 5%-10% hempseed increase total plasma PUFAs in rats [5]. The antioxidative and hepatoprotective properties of PUFAs have been reported previously [6]. According to the existing data on hemp seed oil that elucidate its nutritional value and introduce it as a functional food, its antitoxic properties need to be examined further for additional beneficial qualities and characterizations.

Carbon tetrachloride (CCl_4), an industrial solvent, is a well-established hepatotoxic widely used in scientific researches to evaluate hepatoprotective agents. However, liver is not the only target organ of CCl_4 and it causes free radical generation in an array of tissues [7, 8]. The CCl4 can influence homeostasis of hepatocellular calcium through the induction of hypomethylated ribosomal RNA and cause in the inhibition of protein synthesis [9]. This classic organotoxic is activated by cytochrome (CYP) 2E1 to form the trichloromethyl radical, CCl_3* which binds to cellular molecules (nucleic acid, protein, lipid) impairing crucial cellular processes such as lipid metabolism, with the potential outcome of steatosis [10, 11]. "Adduct formation between CCl_3* and DNA is thought to function as initiator of hepatic cancer "[11]. This radical can also react with oxygen to form the trichloromethyl peroxy radical CCl_3OO* as a highly reactive species that initiates the chain reaction of lipid peroxidation, which attacks and destroys PUFAs, in particular, those associated with phospholipids [11, 12]. "This radical affects the permeability's of mitochondrial, endoplasmic reticulum, and plasma membranes, resulting in the loss of cellular calcium sequestration and homeostasis that can contribute heavily to subsequent cell damage" [12].

Recently, besides physicians, patient's interest increased to use phytomedicine and herbal remedies in liver complications [13]. In orthodox medi-

cine, several nutritional, pharmacological and toxicological properties of hempseed have been evidently investigated [1, 2, 14-16].

In continuum of therapeutic and antitoxic effects of hempseed, the main purpose of this study was to clarify effect of hempseed oil on CCl_4-induced organotoxicity in male adult rats.

MATERIALS AND METHODS

Plant material

Whole hempseed, the fruit of *C. sativa* L., was purchased from the herbal market and washed three times with clean water and once in ethanol to remove any extraneous contamination. To extract oil, hempseed nuts were first shelled and then boiled in water to come apart. Table salt was often added to hasten this process. As the nuts broke, the oil floated to the surface of the water. Once the watercooled, the hemp oil was skimmed from the surface of mixture.

Animal subjects

Male Sprague-Dawley rats (180-200 gr) were purchased from Razi Vaccine and Serum Research Institute (Karaj, Iran) and housed individually in stainless cages and resided under standard laboratory conditions (22±1 °C, 12 h lighting period) and had free access to chow pellets and fresh water.

All animal procedures were performed in accordance with Ethics Committee of our institute that follow recommendations for the proper care and use of laboratory animals [17] at Islamic Azad University, Saveh Branch in 2015.

Experimental design

Carbon tetrachloride (BDH Chemicals, England), was purchased from Razipakhsh, Iran. All solutions were prepared in pyrogen-free 0.9% NaCl solution that served as the vehicle before administration. Dosages of drugs were adopted from previous studies with minor modifications [2, 15, 16 and 18].

Thirty male rats were divided into 5 groups (six rats for each). Normal control (NC) group was kept in standard condition. (TCC) group was i.p. injected with 0.5 ml/kg CCl_4 in two last days. Hempseed oil treated (HSO) group received orally hempseed oil (8 ml/kg) for one week; TCC-HSO group was i.p. injected with CCl_4 (0.5 ml/kg) two days prior to orally receiving hempseed oil (8 ml/kg) for one week, and HSO-TCC group orally received hempseed oil (8 ml/kg) for one week, then i.p. was injected with CCl_4 (0.5 ml/kg) for two consecutive days. After one week of CCl_4 or hempseed oil administration, blood samples were collected via cardiac puncture and rats were killed under deep anesthesia with i.p. ketamine (80 mg/kg)/xylazine (40 mg/kg) cocktail injection.

Clinical chemistry

All plasma samples were separated by centrifugation at 1400 × *g* at 4°C for 15 min, aliquoted and stored at –20 °C until chemical analysis. The plasma levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triacylglycerides (TGs) were enzymatically determined with a commercial diagnostic kit (ELI TECH Diagnostic, French). Plasma high-density lipoprotein cholesterol (HDL-C) was determined by immunoinhibition method (ELI TECH Diagnostic, French). Very low-density lipoprotein-cholesterol (VLDL-C) was calculated by formula: VLDL-C = TGs/5 [19]. Atherogenic index (AI) was calculated according to the following equation: AI = (TC-HDL-C)/HDL-C [20]. Coronary heart disease index (CHD) was calculated according to the following equation: HDL-C/TC [21].

Plasma AST, ALT and ALP activities and the levels of albumin, direct bilirubin and total bilirubin were determined by biochemical kits (Pars Azmon Co., Tehran, Iran) using an autoanalyzer (Heitachi 896, Germany). The level of indirect bilirubin was calculated by subtracting direct bilirubin level from total bilirubin level.

Histopathological analyses

The selected tissues (heart, kidney and liver) were fixed in 10% neutral buffered formalin (pH 7.4), dehydrated and embedded in paraffin wax to obtain 5 µm thick hematoxylin and eosin stained sections. For histopathological examination, the major hepatic lesions like fatty degeneration, presence of large and small vacuoles in hepatocytes, hemorrhage and hyperemia, necrosis of hepatocytes, expansion of sinusoidal space were considered. Kidneys were also evaluated for the presence of cortical or medullary degeneration, necrosis or degeneration of epithelium, and glomerular atrophy or hyperemia while hyperemia and myocardial necrosis were two major lesions considered in heart. All sections were investigated under a light microscope. Based on the aforementioned lesions, tissue injuries were scored on a semi-quantitive scale of 1-3 grades. 1: lesser than 25% of studied section has been pathologically injured; 2: between 25 to 50% of studied section has been pathologically injured and 3: greater than 25% of studied section has been pathologically injured.

STATISTICAL ANALYSIS

All data were analyzed using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA), and presented as mean ± standard errors of the mean (SEM). The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. $P<0.05$ was considered as significant level.

RESULTS

Biochemical results

The effects of hempseed oil on canonical hepatic biomarkers in CCl_4-intoxicated rats are summarized in Table 1. The ALT and AST activities were significantly increased after the injection of CCl_4 in TCC group as compared with NC. Protection against hepatotoxicity in HSO-TCC group and treatment of hepatotoxicity in TCC-HSO group received hempseed oil occurred throughout reduced AST and ALT activities as compared to TCC group. The sole administration of hempseed oil in HSO group significantly conserved normal AST and ALT activities when compared to TCC group. Treatment of hepatotoxicity with hempseed oil in TCC-HSO group restored AST and ALT activities to normal levels that observed in NC group as compared to HSO-TCC group ($P>0.05$). AST/ALT ratio significantly declined in TCC and HSO-TCC groups compared to NC group. The intake of hempseed oil in HSO group and treatment of CCl_4-induced hepatotoxicity in TCC-HSO significantly increased AST/ALT ratio in comparison to TCC group ($P<0.05$). The ALP enzyme activity in NC, HSO, and TCC groups were the same ($P>0.05$), however, its activity in TCC-HSO and HSO-TCC was significantly increased compared with three other groups. Moreover, no alterations have occurred on the levels of direct bilirubin, indirect bilirubin and total bilirubin among studied groups ($P>0.05$).

Table 1. The effect of oral hempseed oil intake on plasma clinical chemistry in carbon tetrachloride-induced hepatotoxicity in male rats.

Group	ALP (U/L)	ALT (U/L)	AST (U/L)	AST/ALT ratio	Direct bilirubin (mg/dL)	Indirect bilirubin (mg/dL)	Total bilirubin (mg/dL)
NC	9.0±4.00 [a]	55.5±10.50 [a]	89.0±8.00 [b]	1.63±0.165 [b]	0.09±0.000	0.05±0.045	0.14±0.045
TCC	11.4±0.60 [a]	182.0±11.30 [b]	189.6±1.72 [a]	0.11±0.003 [a]	0.25±0.104	0.07±0.017	0.32±0.103
HSO	10.6±1.25 [a]	74.8±12.14 [a]	103.3±6.29 [b]	1.40±0.120 [bc]	0.07±0.028	0.17±0.062	0.24±0.076
TCC-HSO	588.5±166.35 [b]	86.1±11.10 [a]	80.1±13.95 [b]	1.03±0.256 [bc]	0.05±0.016	0.20±0.102	0.25±0.113
HSO-TCC	410.0±220.0 [b]	133.0±10.00 [a]	165.0±6.50 [b]	0.49±0.053 [ac]	0.02±0.005	0.07±0.010	0.09±0.005

Data are presented as the mean ± SEM. There are significant differences between groups with different superscript letters in a column at $P<0.05$.

Table 2 shows that CCl_4 significantly decreased plasma HDL-C level in TCC group compared to the NC group while TC and LDL-C levels tended to be decreased in TCC group compared to NC group ($P>0.05$). Furthermore, short-term intake of hempseed oil in HSO group positively modified lipid profile of rats by decreasing TC, LDL-C, VLDL-C, TG, CHD, and AI as compared with lipid profiles of NC group ($P<0.05$) (Table 2). Intake of hempseed oil in both TCC-HSO and HSO-TCC groups could restore TC, HDL-C, LDL-C, CHD, and AI to corresponding levels in NC group ($P<0.05$). In addition, the TG and VLDL-C were no statistically significant difference among groups ($P>0.05$). Atherogenic Index was significantly lower in HSO group in comparison to TCC ($P<0.05$) but no significant changes was observed among the other groups ($P>0.05$).

Table 2. The effect of oral hempseed oil intake on plasma lipid and lipoprotein profiles in carbon. tetrachloride-induced hepatotoxicity in male rats.

Group	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	TG (mg/dL)	CHD (mg/dL)	AI
Control	89.0±1.00 [a]	45.0±3.00 [a]	34.6±2.60 [b]	9.4± 0.60	47.0±3.00	1.9±0.11[ab]	0.9±0.11 [ab]
TCC	69.0± 8.53 [a]	27.6±1.50 [b]	31.4± 7.73 [b]	9.9± 0.24	49.6±6.20	2.5±0.34 [a]	1.5±0.34 [a]
HSO	66.0±5.41 [a]	42.0±2.32 [a]	15.4±2.43 [a]	8.6±1.06	43.0±5.32	1.5±0.04 [b]	0.5±0.04 [b]
TCC-HSO	80.8± 3.83 [a]	42.0±1.36 [a]	30.4± 2.7 [b]	8.3±0.59	41.8±2.97	1.9± 0.06 [ab]	0.9±0.07 [ab]
HSO-TCC	92.0±15.00 [a]	43.0± 5.00 [a]	37.5±15.7 [b]	11.5± 4.30	57.5± 21.50	2.2±0.60 [ab]	1.2±0.60 [ab]

Data are presented as the mean ± SEM. There were significant differences between groups with different superscript letters in a column at $P<0.05$.

Histopathological results

Histopathological results (Figure 1) showed changes occurred in kidney, liver and heart tissues of all studied groups. In this line, heart tissues showed normal architecture in NC group and overt necrosis (grade 3) in TCC group. Surprisingly, degeneration and mild myocardial necrosis (grade 2) have been detected in HSO group. Treatment with hempseed oil in TCC-HSO group improved mildly CCl_4-induced cardiotoxicity and degeneration and myocardial necrosis (grade 2) were major lesions in this group. Pretreatment with hempseed oil in HSO-TCC prevented from severity of CCl_4-induced cardiotoxicity and moderate degeneration and myocardial necrosis (grade 2) occurred in this group. Under the photomicroscope, normal cellular architecture with distinct hepatic cells, sinusoi-

dal spaces, and a central vein was seen in NC group. However, overt CCl_4-inuced hepatoxicity (grade 3) has been observed in TCC group that exhibited severe histopathological changes such as fatty change, hyperemia, hemorrhagia, and necrosis of hepatocytes (Figure 1). Mild fatty liver, necrosis, and hyperemia in hepatocytes with severity grade 1 have occurred in HSO group while intake of hempseed oil after CCl_4-induced hepatotoxicity in TCC-HSO group led to decrease of lesions to grade two level and necrosis and degeneration of hepatocytes have been detected in this group. Sever hyperemia; fatty degeneration and mild centrilobular necrosis have been determined in HSO-TCC group. Intake of hempseed in this group decreased CCl_4-induced hepatotoxicity to the level of grade 2. Kidneys of NC group were normal while hyperemia, cell swelling, epithelial degeneration, glomerular atrophy and pyknotic cells (grade 3) were seen in the kidneys of TCC group. Hyperemia, inflammation of tubular epithelium, tubular obstruction and pyknotic cells (grade 1) have been found in HSO group. Degeneration and necrosis of tubular epithelium, hyperemia, and necrosis at grade 2 level were seen in TCC-HSO group. Preventive effects of hempseed oil on CCl_4-induced nephrotoxicity have not been found in HSO-TCC group and lesions like severe and overt necrosis of tubular epithelium in grade 3 have been seen in this group (Figure 1) (Table 3).

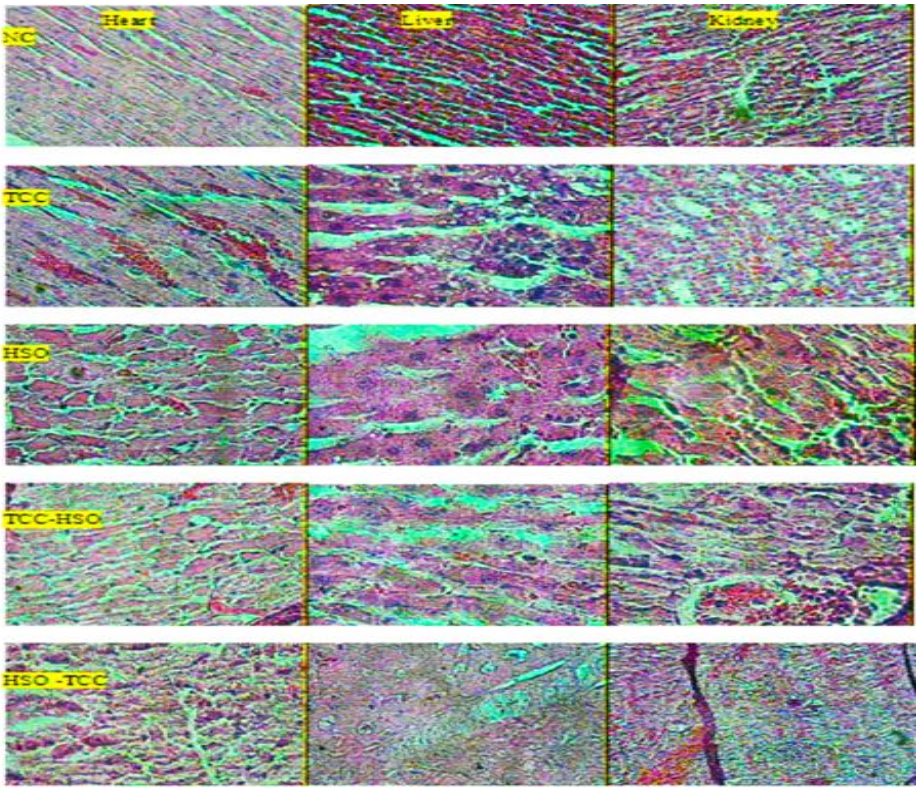

Figure 1. The effect of oral hempseed oil intake on histopathology of carbon tetrachloride-induced hepatotoxicity in male rats. Tissue sections were stained with haematoxylin and eosin (×100 or ×200).

Table 3. histopathological analysis.

Groups	Heart	Kidney	Liver
Control	Normal	Normal	Normal
TCC	Necrosis (grade 3)	Atrophy and pyknotic cells (grade 3)	Hepatoxicity (grade 3)
HSO	Degeneration and mild necrosis (grade 2)	Pyknotic cells (grade 1)	Necrosis (grade 1)
TCC-HSO	Degeneration and necrosis (grade 2)	Necrosis (grade 2)	Lesions (grade 2)
HSO-TCC	Moderate degeneration and necrosis (grade 2)	Necrosis (grade 3)	Necrosis (grade 2)

DISCUSSION

Carbon tetrachloride as an applicable candidate for other halogenated hydrocarbons has been one of the most broadly studied hepatotoxicants to date [22]. The CCl_4 affected secretion of VLDL and HDL [23]. Acute poisoning with CCl_4 manifested as a multiorgan disorder [24]. Consequences of high doses of CCl_4 in nonspecific toxicity consist central nervous system depression and respiratory failure resulting in death [25]. Liver is very vulnerable to this messy hepatotoxin and a single CCl_4 exposure can quickly lead to severe centrizonal necrosis and steatosis. Organ function tests are useful to evaluate amount or severity of CCl_4 toxicity. In this line, assaying activities of several enzymes like AST, ALT and ALP has been frequently used to determine severity CCl_4 toxicity [9, 26 and 27]. The current study is one of the first studies that consider lipid and lipoprotein profiles as endpoints that reflect an integrated function of several organs in the presence of CCl_4 toxicity.

We motivated to screen putative protective impact of hempseed oil against CCl_4-induced organ toxicity in a rat model. An array of derangements including increased ALT and AST activities, mild elevation of bilirubin and TGs levels and considerable decline of HDL-C level confirmed that CCl_4 (0.5 ml/kg) injections for two consecutive days have been reliable and efficient regimen to be employed for ultra-short challenge of toxicity. However, the lake of significant change of ALP, TC, and LDL-C levels in this toxicity model compared with control animal declined its validity *per se*.

In contrast to the alteration of biochemical parameters in our model of toxicity, an acceptable level of cardio-, nephro-, and hepatotoxicity were that occurred following injection of CCl_4 makes this model obsolete faithful and trusted at least at histological level. The sole intake of hempseed in rats did not adversely change enzymatic parameters and bilirubin content. Surprisingly, force-feeding of hempseed oil (8 ml/kg) for one week improved lipid profile of rats. In this sense, the levels of TC, HDL-C, LDL-C, VLDL-C and TGs decreased in hempseed oil treated group in comparison to control group. Free access to normal chow diet and whole hempseed could significantly decrease LDL-C and increase HDL-C in rats during 20 d [2]. Levels of TC and TG levels no significantly decreased in rats during 20 d [2]. Marijuana seed a variety of hempseed that contaminated to high levels of phytocannabinoids significantly increased TC and LDL-C levels in guinea pigs fed marijuana seed for two months [14]. Whole hempseed in the levels of 1%, 2% and 10% of chow diet for 3 wk have been improved lipid profile in ovariectomized rats [16]. Hempseed may prevent unwanted dyslipidemia that usually occurred post ovariectomy due to the presence of phytoestrogens like β-sitosterol in hempseed [16]. In addition, intake of hempseed oil decreased the

levels of AI and CHD as compared with control animals in the present study. Hempseed has the potential beneficially to influence heart disease [28]. Whole hempseed has 30.0% oil composed of saturated fatty acids (13.0%), unsaturated fatty acids (87.0%), PUFAs (64.2%), and 20.9% mono-unsaturated fatty acids [1].

Moreover, hempseed oil has perfect n3/n6 ratio as essential PUFAs (linoleic and linolenic acids). These PUFAs improve lipid profile and decreases the risk of atherosclerosis or coronary heart diseases in human and animal nutrition [1, 28]. Whole hempseed and hempseed oil positively modify lipid profile and atherosclerotic indices (AI and CHD). Hempseed oil restored TC, HDL-C, LDL-C, CHD, and AI levels in CCl_4-intoxicated rats to reference interval levels in our normal control animals and previous studies [1, 2 and 16].

One of the most striking features of this study is its message about the histopathology of selected organs. In this regards, degeneration and mild myocardial necrosis (grade 2), mild fatty liver and necrosis and hyperemia in hepatocytes with severity (grade 1), and hyperemia, inflammation of tubular epithelium, tubular obstruction and pyknotic cells of kidneys (grade 1) have been found in HSO group that received hempseed oil. Our prepared hempseed oil was olive-colored may be due to the presence of chlorophyll and other components; however, we did not bleach it to conserve its antioxidants and other useful components. We prepared fresh hemp seed oil and conserved it in cold smoky containers to prevent rancidity because high level of PUFAs makes it highly nutritious but chemically unstable [29]. We did not perform phytochemical analysis of prepared hempseed; however, we did not find any reports regarding the presence of antinutrients in hempseed that lead to the lesions that we have been detected in heart, liver, and kidney [30, 31].

Hempseed oil may contain traces of phytocannabinoids and these substances can lead some kinds of hepatic complications like liver steatosis throughout activation of receptor of cannabinoid CB1 [32, 33]. The phytocannabinoid content of Iranian varieties of hempseed is not reported yet. Apparently, hempseed oil intake played preventive and/or therapeutic roles against CCl_4-induced toxicity and improved liver function throughout decreasing ALT and AST activities. However, ALP activity as a hepatobiliary biomarker increased in CCl_4-intoxicated groups received hempseed oil in comparison to NC and TCC groups may be due to biliary obstruction that usually occurred in animals that fed high-fat diet like hempseed oil [34]. Three plasma hepatic marker enzymes (ALT, AST, and ALP) were routinely evaluated for hepatotoxicity. The oil of *Cannabis sativa* (0.5-1 ml) was demonstrated caused remarkable decreased on AST, ALT, ALP as well as billirubin concentrations in the serum [35]. ALT and AST are aminotransferase enzymes that transfer amino group into alpha-ketoacid. Most ALT functionality is in hepatocytes and less in other cells. ALT is a cytoplasmic enzyme however, AST is a mitochondrial one that has isoenzyme in other cells beside hepatocytes such as myocardiocytes, myocytes, kidney, etc. [18]. The ALP is a hydrolase enzyme responsible for dephosphorylation of molecules including nucleotides, proteins, and alkaloids, its activity reflects health of biliary system, and high ALP levels can show that the bile ducts are obstructed. Intake of hempseed in CCl_4-intoxicated animals mildly improved toxicities of heart, liver, and kidneys; however, pretreatment with hempseed oil only prevented from toxicities evaluated with histopathological findings in heart and liver tissues (*vide supra*). The concurrent decrement of HDL-C and LDL-C (P>0.05), VLDL-C (P>0.05) levels in the presence of CCl_4 toxicity emphasize that this toxicant may inhibit cholesterol biosynthesis.

CONCLUSIONS

Anti-hyperlipidemic action of hempseed participates in the protective or therapeutic effects against CCl_4 toxicity. In addition, hemp seed in-

take exerted semiprotective effects on CCl_4-induced hepatotoxicity in an ultra-short intervention and long-term preventive or therapeutic effects of hempseed highly recommended and requested to elucidate its organoprotective effects against various toxicities.

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REFERENCES

1. Yousofi M., Saberivand A., Becker L.A., Karimi I., 2011. The effects of *Cannabis sativa* L. seed (hemp seed) on reproductive and neurobehavioral end points in rats. Dev Psychobiol. 53, 402-412.

2. Karimi I., Hayatghaibi H., 2006. Effect of Cannabis *Sativa* L. seed (hempseed) on serum lipid and protein profiles of rat. Pak J Nutr. 5, 585-588.

3. Deferne J.L., Pate D.W., 1996. Hemp seed oil: A source of valuable essential fatty acids. J Int Hemp Assoc. 3, 4-7.

4. Galasso1 I., Russo1 R., Mapelli S., Ponzoni E., Brambilla I. M., Battelli G., Reggiani R. 2016. Variability in seed traitsina collection of *Cannabis sativa* L. Genotypes. Front Plant Sci.7, 688.

5. Richard M.N., Ganguly R., Steigerwald S.N., Al-Khalifa A., Pierce G.N. 2007. Dietary hempseed reduces platelet aggregation. J Thromb Haemost. 5, 424-5.

6. Chavan T., Khadke S., Harke S., Ghadge A., Karandikar M., Pandit V., Ranjekar P., Kulkarni O., Kuvalekar A. 2013. Hepatoprotective effect of polyunsaturated fatty acids against repeated subacute acetaminophen dosing in rats. Int J Pharm Bio Sci. 4(2), 286 – 295.

7. Ganie1 S.A., Haq E., Hamid A., Qurishi Y., Mahmood Z., Zargar B.A., Masood A., Zargar M.A., 2011. Carbon tetrachloride induced kidney and lung tissue damages and antioxidant activities of the aqueous rhizome extract of *Podophyllum hexandrum*. BMC Complement Altern Med. 11, 17.

8. Ojo1 O.A., Ojo A.B., Ajiboye1 B., Fadaka1 A., Imiere1 O.D., Adeyonu1 O., Olayide1 I. 2016. Protective influence of *Ficus asperifolia* Miq leaf extract on carbon tetrachloride (CCl_4)-induced testicular toxicity in rat's testes. J App Pharm Sci. 6(06), 037-041.

9. Hesami Z., Jamshidzadeh A., Ayatollahi M., Gramizadeh B., Vahdati A. 2016. The comparative effects of human mesenchymal stem cell and platelet extract on CCl_4-Induced liver toxicity in rats. Jundishapur J Nat Pharm Prod. Inpress (Inpress), e36818.

10. Yoshioka H., Onosaka S., 2016. Zinc sulfate pretreatment prevents carbon tetrachloride-induced lethal toxicity through metallothionein-mediated suppression of lipid peroxidation in mice. Fundam Toxicol Sci. 3(4), 151-156.

11. Boll M., Lutz1W. D., Becker1 E., Stampfl A., 2001. Mechanism of carbon tetrachloride-induced hepatotoxicity. Hepatocellular damage by reactive carbon tetrachloride metabolites. Z Naturforsch. C Bio Sci. 56(7-8), 649–659.

12. Weber L.W., Boll M., Stampfl A., 2003. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol. 33(2), 105-136.

13. Fogden E., Neuberger J., 2003. Alternative medicines and the liver. Liver Int. 23, 213-220.

14. Hayatghaybi H., Karimi I., 2007. Hypercholesterolemic effect of drug-type Cannabis *sativa L.*

seed (marijuana seed) in guinea pig. Pak J Nut. 6, 56-59.

15. Karimi I., Hayatghaibi H., Yousofi J., Saberivand A., Zavareh S., 2007. Effects of nondrug type *Cannabis sativa* L. (hempseed) on hematological parameters in guinea pig. J Cardiova Hemato Dis - Drug Targets. 7, 288-290.

16. Saberivand A., Karimi I., Becker L. A., Moghaddam A., Azizi-Mahmoodjigh S., Yousefi M., Zavareh S., 2010. The effects of *Cannabis sativa* L. seed (hempseed) in the ovariectomized rat model of menopause. Methods find Exp Clin Pharmacol. 32, 467-473.

17. NIH publication no. 85-23. Institutional administrator's manual for laboratory animal care and use. Revised 1985.

18. Ujah O.F., Ujah I. R., Johnson J.T., Ekam V.S., Udenze E.C.C., 2013. Hepatoprotective property of ethanolic leaf extract of *Moringa oleifera* on carbon tetrachloride (CCl_4) induced hepatotoxicity. J Nat Prod Plant Resour. 3(2), 15-22.

19. Friedewald W.T., Ley R. I., Fradrickson D.S., 1972. Estimation of low density lipoprotein cholesterol in plasma without the use of preparative ultracentrifuge. Clin Chem. 18, 499-502.

20. Lee R., Niemann D., Nutritional Assessment, 2th ed., Mosby Missou: USA. 1996.

21. Kinosian B., Glick H., Garland G., 1994. Cholesterol and coronary heart disease: predicting risks by levels and ratios. Ann Intern Med. 121, 641-664.

22. Weber L.W.D., Boll M., Stampfl A., 2003. Hepatotoxicity and mechanism of action of halaoalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicolol. 33, 105-136.

23. Musa E.M., EL Badwi S.M., Jah Elnabi M.A., Osman E.A., Dahab M.M., 2012. Hepatoprotective and toxicity assessment of *Cannabis sativa* seed oil in Albino rat. IJCBS. 1, 69-76.

24. Gitlin N., Clinical aspects of liver disease caused by industrial and environmental toxins. In: Hepatology. A Textbook of Liver Disease. Zakim D., Boyer T. D., Eds., Philadelphia, USA, WB Saunders. 1996. Pp. 1018-1050.

25. Boll M., Weber L.W., Beker E., Stampfl A., 2001. Hepatocyte damage induced by carbon tetracholoride: inhibited lipoprotein secretion and changed composition. Z Naturoforsch C. 56(3-4), 283-90.

26. Peng W.H., Tien Y.C., Huang C.Y., Huang T.H., Liao J.C., Kuo C.L., Lin Y.C., 2010. *Fraxinus rhynchophylla* ethanol extract attenuates carbon tetrachloride-induced liver fibrosis in rats via down-regulating the expressions of uPA, MMP-2, MMP-9 and TIMP-1. J Ethnopharmacol. 127, 606-613.

27. Abdel Salam O.M.E., Sleem A.A., Omara E., 2013. Micronised purified *flavonoid* fraction alleviates the carbon tetrachloride-induced hepatic injury. Comp Clin Pathol. 22, 1145-1154.

28. Rodriguez-Leyva D., Pierce G.N., 2010. The cardiac and haemostatic effects of dietary hempseed. Nutr Metabol. 7, 32.

29. Deman J.M., Chemical and physical properties of fatty acids, in fatty acids in Foods and their Health Implications, 2nd ed., Marcel Dekker, Inc. Basel: Ching Kuang Chow. 2000. Pp. 17-49.

30. Leizer C.C., Ribnicky D., Poulev A., Dushenkov S., Raskin I., 2000. The composition of *hemp seed* oil and its potential as an important source of nutrition. J Nutraceutical Func Med Foods. 2, 35-53.

31. Callaway J.C., Pate D.W. Hempseed oil. In: Chapter 5: Gourmet and Health-Promoting Specialty Oils. Robert A., Kamal-Eldin M., Kamel-Eldin A., Eds., Urbana, USA, American Oil Chemists Society Press. 2009. pp. 185-213.

32. Lehmann T., Sager F., Brenneisen R., 1997. Excretion of cannabinoids in urine after ingestion of cannabis seed oil. J Anal Toxicol. 21, 373-375.

33. Parfieniuk A., Flisiak R., 2008. Role of cannabinoids in chronic liver diseases. World J Gastroenterol. 14, 6109-6114.

34. Muriel P., 1995. High fat diet and liver damage induced by biliary obstruction in the rat. J Appl Toxicol. 15, 125-8.

35. Ritesh K.R., Suganya A., Dileepkumar H.V., Rajashekar Y., Shivanandappa T., 2015. A single acute hepatotoxic dose of CCl_4 causes oxidative stress in the rat brain. Toxicol Reports. 2, 891-895.