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

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ABSTRACT: This study was designed to investigate the protective activity of hempseed oil on carbon tetrachloride (CCL₄) 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); CCL₄-intoxicated group (TCC) injected CCL₄; hempseed oil treated group (HSO) gavaged hempseed oil; TCC-HSO group was injected CCL₄ prior to intake of hempseed oil and HSO-TCC group was gavaged hempseed oil prior to being injected with CCL₄. In all treated groups, toxicity was induced by i.p. injection of CCL₄ (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. CCL₄ 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
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 (CCl₄), 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 CCl₄ 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, CCl₄⁺ 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 CCl₄⁺ 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 CCl₃OO⁺ 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 medic-
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 CCl₄-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 Razipakhs, 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 CCl₄ 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 CCl₄ (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 CCl₄ (0.5 ml/kg) for two consecutive days. After one week of CCl₄ 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-quantitative 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 CCl₄-intoxicated rats are summarized in Table 1. The ALT and AST activities were significantly increased after the injection of CCl₄ 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 CCl₄-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.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALP (U/L)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>AST/ALT ratio</th>
<th>Direct bilirubin (mg/dL)</th>
<th>Indirect bilirubin (mg/dL)</th>
<th>Total bilirubin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>9.0±4.00</td>
<td>a</td>
<td>55.5±10.50</td>
<td>a</td>
<td>89.0±8.00</td>
<td>1.6±0.165</td>
<td>0.0±0.000</td>
</tr>
<tr>
<td>TCC</td>
<td>11.4±0.60</td>
<td>a</td>
<td>182.0±11.30</td>
<td>a</td>
<td>189.6±1.72</td>
<td>0.11±0.003</td>
<td>0.25±0.104</td>
</tr>
<tr>
<td>HSO</td>
<td>10.6±1.25</td>
<td>a</td>
<td>74.8±12.14</td>
<td>a</td>
<td>103.3±6.29</td>
<td>1.40±0.120</td>
<td>0.07±0.028</td>
</tr>
<tr>
<td>TCC-HSO</td>
<td>588.5±166.35</td>
<td>b</td>
<td>86.1±11.10</td>
<td>b</td>
<td>80.1±13.95</td>
<td>1.03±0.256</td>
<td>0.08±0.016</td>
</tr>
<tr>
<td>HSO-TCC</td>
<td>410.0±220.0</td>
<td>b</td>
<td>133.0±10.0</td>
<td>b</td>
<td>165.0±6.50</td>
<td>0.49±0.053</td>
<td>0.02±0.005</td>
</tr>
</tbody>
</table>

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 CCl₄ 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.

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

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 CCl₄-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 CCl₄-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 CCl₄-induced hepatotoxicity (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 CCl₄-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. Severe hyperemia; fatty degeneration and mild centrilobular necrosis have been determined in HSO-TCC group. Intake of hempseed in this group decreased CCl₄-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 CCl₄-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.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Heart</th>
<th>Kidney</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>TCC</td>
<td>Necrosis (grade 3)</td>
<td>Atrophy and pyknotic cells (grade 3)</td>
<td>Hepatotoxicity (grade 3)</td>
</tr>
<tr>
<td>HSO</td>
<td>Degeneration and mild necrosis (grade 2)</td>
<td>Pyknotic cells (grade 1)</td>
<td>Necrosis (grade 1)</td>
</tr>
<tr>
<td>TCC-HSO</td>
<td>Degeneration and necrosis (grade 2)</td>
<td>Necrosis (grade 2)</td>
<td>Lesions (grade 2)</td>
</tr>
<tr>
<td>HSO-TCC</td>
<td>Moderate degeneration and necrosis (grade 2)</td>
<td>Necrosis (grade 3)</td>
<td>Necrosis (grade 2)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Carbon tetrachloride as an applicable candidate for other halogenated hydrocarbons has been one of the most broadly studied hepatotoxicants to date [22]. The CCl₄ affected secretion of VLDL and HDL [23]. Acute poisoning with CCl₄ manifested as a multorgan disorder [24]. Consequences of high doses of CCl₄ 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 CCl₄ exposure can quickly lead to severe centrizonal necrosis and steatosis. Organ function tests are useful to evaluate amount or severity of CCl₄ toxicity. In this line, assaying activities of several enzymes like AST, ALT and ALP has been frequently used to determine severity CCl₄ 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 CCl₄ toxicity. We motivated to screen putative protective impact of hempseed oil against CCl₄-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 CCl₄ (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 CCl₄ 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. Hemptseed has the potential beneficially to influence heart disease [28]. Whole hemptseed 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, hemptseed 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 hemptseed and hemptseed oil positively modify lipid profile and atherosclerotic indices (AI and CHD). Hemptseed oil restored TC, HDL-C, LDL-C, CHD, and AI levels in CCl4-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 hemptseed oil. Our prepared hemptseed 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 hemptseed; however, we did not find any reports regarding the presence of antinutrients in hemptseed that lead to the lesions that we have been detected in heart, liver, and kidney [30, 31].

Hemptseed 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 hemptseed is not reported yet. Apparently, hemptseed oil intake played preventive and/or therapeutic roles against CCl4-induced toxicity and improved liver function throughout decreasing ALT and AST activities. However, ALP activity as a hepatobiliary biomarker increased in CCl4-intoxicated groups received hemptseed 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 hemptseed 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 hemptseed in CCl4-intoxicated animals mildly improved toxicities of heart, liver, and kidneys; however, pretreatment with hemptseed 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 CCl4 toxicity emphasize that this toxicant may inhibit cholesterol biosynthesis.

CONCLUSIONS

Anti-hyperlipidemic action of hemptseed participates in the protective or therapeutic effects against CCl4 toxicity. In addition, hemp seed in-
take exerted semiprotective effects on CCl₄-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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