

Effect of Pomegranate (*Punica granatum*) Seed Oil on Carbon Tetrachloride-Induced Acute and Chronic Hepatotoxicity in Rats

Duygu Yaman Gram, Ayhan Atasever, Meryem Eren¹

Departments of Pathology and ¹Biochemistry, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey

ABSTRACT

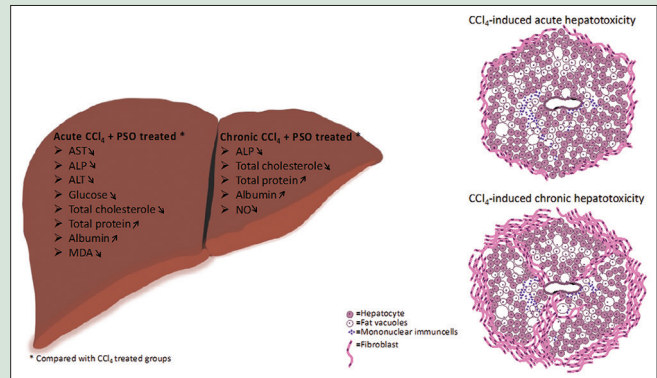
Background: Carbon tetrachloride (CCl₄) is one of the most widely used Hepatotoxin that is known to induce oxidative stress and causes hepatic damage by the formation of reactive free radicals in laboratory animals. **Objective:** This study aims to investigate the hepatoprotective role of pomegranate seed oil (PSO) on histological structure, some biochemical parameters and lipid peroxidation on CCl₄-induced acute and chronic liver injury induced rats. **Materials and Methods:** The study material comprised 80 male Wistar albino rats. They were divided into two study groups including 40 rats for acute and 40 rats for chronic hepatotoxicity induction by CCl₄. Hematoxylin and eosin staining was used to evaluate degree of steatosis, inflammation, necrosis, and fibrosis semiquantitatively. Blood serum aspartate transaminase, alanine transaminase, and alkaline phosphatase enzyme activities and glucose, triglyceride, total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, total protein, albumin and liver malondialdehyde, and nitric oxide levels were measured. **Results:** All control and only PSO given animals liver showed normal histological architecture, but in the acute CCl₄-treated animals, an intensive macro and microvesicular steatosis, mononuclear inflammatory cell infiltrations in portal area and parenchyma, and necrotic alterations; in the chronic CCl₄-treated group, additionally to acute findings mild-to-severe fibrosis with lobulation formation were observed. **Conclusion:** The results suggest that administration of PSO has partially ameliorative effects on biochemical and lipid peroxidation parameters in acute period, but it has no effect on the recovery of liver tissue damage or histopathological changes and biochemical parameters induced by CCl₄ in chronic period.

Key words: Carbon tetrachloride, hepatotoxicity, histopathology, pomegranate seed oil, rat

SUMMARY

- Antioxidant activity of Pomegranate seed oil was evaluated.
- PSO showed some antioxidative effects against CCl₄-induced oxidative stress by decreasing levels of some biochemical and lipid peroxidation parameters.

- The results of the histopathological investigation showed that prolonged usage of CCl₄ treatment have irreversible effects on hepatic architecture.



Abbreviations Used: PSO: Pomegranate seed oil; CCl₄: Carbon tetrachloride; CCl₃: Trichloromethyl; MDA: Malondialdehyde; NO: Nitric oxide; ROS: Reactive oxygen species; IM: Intramascular; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; TP: Total protein; HDL: High density lipoprotein; LDL: Low density lipoprotein.

Correspondence:

Dr. Duygu Yaman Gram,
Department of Pathology, Faculty of Veterinary
Medicine, Erciyes University, Melikgazi,
Kayseri 38039, Turkey.
E-mail: dyamangram@gmail.com
DOI: 10.4103/pr.pr_122_17

Access this article online

Website: www.phcogres.com

Quick Response Code:



INTRODUCTION

Carbon tetrachloride (CCl₄) is one of the most widely used hepatotoxic chemical agents that is known to induce oxidative stress and causes hepatic damage by the formation of reactive free radicals in laboratory animals.^[1] The well-defined model of liver necrosis and fibrosis induced by CCl₄ play a crucial role in understanding of the mechanisms of action of hepatic injury.^[2,3] CCl₄ is metabolized to trichloromethyl (CCl₃) free radical by the cytochrome P450 system and consequently, by the aid of other free radicals, lead to cellular membrane injury by covalently binding to macromolecules, which produces malondialdehyde (MDA) as a final product.^[4,5] Membrane disintegration, loss of membrane-associated enzymes and necrosis are some consequences of CCl₄-induced lipid peroxidation.^[6] Increased lipid peroxidation is believed to play a vital role of pathogenesis of many acute and chronic diseases as an underlying cause of the initiation of oxidative stress-related tissue injury and cell death.^[7] Although liver damage firstly results from the CCl₄ metabolism to CCl₃, secondary damage comprises by the inflammatory processes caused by the oxidant-induced activation of Kupffer cells^[8] and ischemic injury

lead by the formation of inflammatory prostaglandins in the circulatory system (Basu, 2003). Oxidative stress results from the overproduction and/or inadequate removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species.^[9] As ROS play a major role in the pathogenesis of both acute and chronic liver damage (Basu, 2003), changes in these enzymes are responsible for biochemical alteration and lesions of the tissues.^[10] During the inflammatory process in liver damage oxidative eruption causes excessive production of nitric oxide (NO) by hepatocytes, Kupffer cells and endothelial cells which can

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Gram DY, Atasever A, Eren M. Effect of pomegranate (*Punica granatum*) seed oil on carbon tetrachloride-induced acute and chronic hepatotoxicity in rats. Phcog Res 2018;10:124-9.

cause DNA fragmentation and lipid oxidation.^[11] Histopathologically, CCl₄ administration can result in hepatic steatosis, centrilobular necrosis, and ballooning of hepatocytes after acute exposure^[11-14] while long-term administration causes hepatitis, liver fibrosis, and cirrhosis.^[5,15] Liver fibrosis results from the excessive secretion and proliferation of extracellular matrix proteins which produced by activated hepatic stellate cells during chronic inflammation due to oxidative stress.^[16] This process is activated by several factors, including ROS, some cytokines and chemokines.^[17] Herbal drugs have gained importance, and their use is widespread because of their antioxidant properties.^[18] Many plant origin antioxidant compounds had been studied in CCl₄-induced acute^[12,14,18] and chronic^[18,19] liver injury for screening the hepatoprotective activity. *Punica granatum* is used as a medicinal plant, and it possesses an extensive therapeutic importance. Different parts of plant have been found a number of various biological effects such as antitumor,^[20] antibacterial,^[21,22] antiulcer,^[23] anti-inflammatory,^[24,25] and antioxidant^[15,26] activities. Pomegranate seed oil (PSO) contains a high concentration of conjugated fatty acids composition containing high levels of punicic acid, linoleic acid, and linolenic acid which attributes its antioxidant effects^[27,28] and its hepatoprotective effect has not yet been studied in detail. Therefore, to better understand its anti-inflammatory and antioxidant activity in the present study, we investigated effect of PSO on CCl₄-induced liver damage after acute and chronic exposure by assaying serum lipid profiles and histopathology of liver tissues in rats.

MATERIALS AND METHODS

Materials

CCl₄ was obtained from Merck (France) Ltd. (1.02222), PSO was purchased from Bukas Inc. Co., Izmir, Turkey and content of PSO is given in Table 1.

Animals

Experiments were performed using 200–250 g weighing, 80 adult male Wistar albino rats. The experiments were carried out in accordance with the Guidelines for Animal Experimentation approved by Erciyes University, Experimental Animal Ethics Committee (permit no: 11/59), and the experimental procedures were performed in Erciyes University Experimental Research and Application Center, Kayseri, Turkey. The animals were kept in a special room at a constant temperature 22°C ± 2°C and humidity (50% ± 5%) with 12-h light/dark cycles and had free access to diet and tap water.

Experimental protocol

Following an acclimatization period for 1 week, animals were divided into acute and chronic study groups, as follows;

In the acute study model;

- Group I was kept as a control group and animals received only corn oil (1 mL/kg, *n* = 10)
- Group 2, received only PSO at a dose of 0.15 mL/kg through gavage directly to the stomach for 4 weeks (*n* = 10)
- Groups 3 were injected with CCl₄ intraperitoneally (IP) at a dose of 1 mL/kg, twice in the 1st week,
- Group 4 were administered with CCl₄ at a dose of 1 mL/kg twice in the 1st week and simultaneously 0.15 mL/kg PSO through gavage directly to the stomach for 4 weeks.

In the chronic study model;

- Group 1 (control group) were administered with corn oil (0.2 mL/kg) for 12 weeks
- Group 2 were administered with 0.15 mL/kg PSO through gavage directly to the stomach for 12 weeks
- Group 3 were treated IP injection of CCl₄(0.2 mL/kg) twice a week, for 12 weeks,
- Group 4 were administered with CCl₄(0.2 mL/kg) twice a week and simultaneously 0.15 mL/kg PSO through gavage directly to the stomach for 12 weeks.

Collection and processing of samples

Rats were anesthetized with ketamine (intramuscular [IM], 50 mg/kg) and xylazine (IM., 10 mg/kg) injection and blood samples were collected by heart puncture 24 h after the last CCl₄ administration. Finally, all the animals were sacrificed by cervical dislocation and livers from all animals were removed and divided into two parts; one was placed and fixed in neutral formalin solution (10%) for the histopathological examination and the other one was homogenized after being mixed with 1:9 phosphate buffer (pH 7.2), in an ice-containing medium. The homogenates were centrifuged at +4°C, for 1 h. Obtained supernatants were transferred into Eppendorf tubes, and preserved at -80°C until analysis. Blood samples were centrifuged at 3000 rpm for 10 min and serum was taken in Eppendorf tube. All serum samples were maintained at -20°C until analysis.

Histopathological examination

Following fixation in neutral formalin solution (10%), liver tissue specimens were thoroughly rinsed overnight, under tap water. Then, all tissue samples were dehydrated in graded alcohol and cleared in xylene, and embedded in paraffin wax and sectioned (thickness, 5 µm), for histopathological evaluation. After staining with hematoxylin and eosin^[29] sections were examined with light microscope.

Liver damage scoring method

Following hematoxylin and eosin staining all sections were semiquantitatively evaluated for hepatocyte steatosis, inflammation, necrosis, and fibrosis. All liver samples were evaluated using ten different places in each section for the aforementioned parameters by two pathologists, and the mean percentile values within the group were calculated. Steatosis, inflammation, necrosis, and fibrosis were graded as 1 (mild, <33% of liver cells), 2 (moderate, 33% to 66% of liver cells), and 3 (severe, >66% of liver cells).^[30] The values obtained in each group were evaluated statistically and the statistical significance between the groups was recorded.

Biochemical analysis

All serum parameters (alanine transaminase [ALT], aspartate transaminase [AST], alkaline phosphatase [ALP], bilirubin, total protein (TP), albumin, total cholesterol, high-density

Table 1: Fatty acid composition of the pomegranate seed oil used in the trial

Fatty acid	Percentage
Myristic acid	0.09
Palmitic acid	3.29
Palmitoleic acid	0.12
Margaric acid	0.05
Stearic acid	2.13
Oleic acid	8.66
Linoleic acid	5.81
Linolenic acid	0.06
Arachidic acid	0.42
Eicosenoic acid	0.70
Punicic acid	78.67
Total	100

lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, glucose, and triglyceride) were assayed enzymatically using an autoanalyzer (Glucose Auto and Stat, GA-1122) in Gulser – Dr. Mustafa Gundogdu Central Laboratory, Erciyes University. Protein content in liver homogenates was measured by the Lowry method.^[31] MDA analyses were performed in accordance with the previously described method.^[32] NO measurements were evaluated by diazotization assay (Griess reaction).^[33]

Statistical analysis

Statistical analyses were carried out using SPSS 14.01 (License no: 9869264, SPSS Inc., Chicago, USA) for Windows software and performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. The significance of the difference between the experimental and control groups in terms of liver tissue damage score was performed with the Kruskal–Wallis test. All values were expressed as mean values \pm standard error of means.

RESULTS

Clinical findings

In the both acute and chronic administration of CCl_4 groups, clinical signs such as weakness, hunched posture, excessive salivation, ptosis, and corneal opacity were observed. No clinical signs were observed in the control and PSO groups both acute and chronic period.

Histopathologic findings

Effects of pomegranate seed oil on carbon tetrachloride-induced acute hepatotoxicity

Histopathological examination of liver tissues in the control and PSO groups showed normal hepatic lobular architecture [Figure 1a and b]. The rats treated with CCl_4 displayed spacious liver damage, characterized by diffuse macro- and microvesicular lipid vacuoles in hepatocytes, large areas of centrilobular necrosis, inflammatory cell infiltration, and loss of hepatic architecture [Figure 1c]. Necrosis, fat vacuole formation, and cell infiltration were similar in PSO-treated group [Figure 1d].

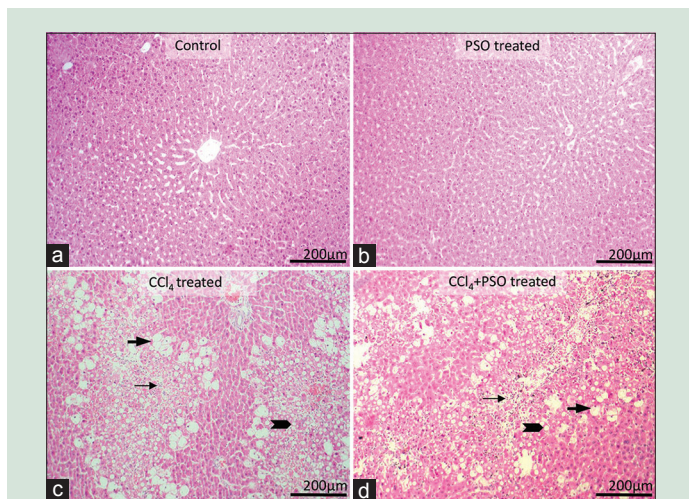


Figure 1: Histological analysis of the livers in carbon tetrachloride-induced acute hepatotoxicity; Normal appearance of the livers of the control (a) and pomegranate seed oil-treated (b) groups. The appearance of micro (arrowheads)- and macro (black thick arrows) vesicular fat vacuoles in all parenchyma and increased numbers of infiltrating mononuclear cells (black thin arrows), consisting predominantly of lymphocytes in carbon tetrachloride (c), and carbon tetrachloride + pomegranate seed oil-(d) treated groups, Liver, H and E, $\times 10$

Effects of pomegranate seed oil on carbon tetrachloride-induced chronic hepatotoxicity

Histopathological examination of liver tissues in the control and PSO groups showed normal hepatic lobular architecture [Figure 2a and b]. Appearance of lipid vacuoles in hepatocytes ranged from small discrete microvesicles to large coalescing macrovesicles in the CCl_4 -treated rats. The fibrosis throughout the lobules linked portal areas and central vein to produce pseudolobulation. Mononuclear cell infiltration, especially close to the portal area was also observed [Figure 2c]. In the PSO-treated group, histopathological findings were similar with CCl_4 administered group [Figure 2d].

The liver damage parameters were evaluated semiquantitatively in the histopathological sections of liver tissues of control and PSO groups of animals in the acute and chronic experimental groups, and the damage scores were found to be zero. Liver damage parameters were scored for

Table 2: Semiquantitative scoring system for hepatic damage in experimental groups with acute liver injury

Groups (n=10)	Histopathological findings median (25%-75%)		
	CCl_4	CCl_4 + PSO	P (Kruskal-Wallis test)
Fibrosis	0.5 (0-1)	0 (0-1)	>0.05
Steatosis	3 (3-3)	3 (2-3)	>0.05
Inflammation	3 (2-3)	2.5 (2-3)	>0.05
Necrosis	3 (2-3)	2.5 (2-3)	>0.05

PSO: Pomegranate seed oil; CCl_4 : Carbon tetrachloride

Table 3: Semiquantitative scoring system for hepatic damage in experimental groups with chronic liver injury

Groups (n=10)	Histopathological findings median (25%-75%)		
	CCl_4	CCl_4 + PSO	P (Kruskal-Wallis test)
Fibrosis	3 (2-3)	3 (1-2)	>0.05
Steatosis	3 (2-3)	3 (1-2)	>0.05
Inflammation	2.5 (1.75-3)	2 (1.75-2.25)	>0.05
Necrosis	0.5 (0-1)	1 (0-1)	>0.05

PSO: Pomegranate seed oil; CCl_4 : Carbon tetrachloride

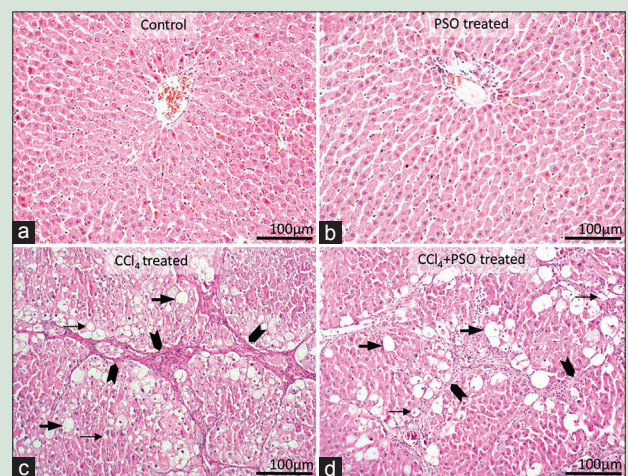


Figure 2: Histological analysis of the livers in carbon tetrachloride-induced chronic hepatotoxicity; Normal appearance of the livers of the control (a) and pomegranate seed oil-treated (b) groups. The appearance of micro (black thin arrows) and macro (black thick arrows) vesicular fat vacuoles in the parenchyma and increase in fibrous connective tissue (arrowheads) in carbon tetrachloride (c) and carbon tetrachloride + pomegranate seed oil-(d) treated groups, Liver, H and E, $\times 20$

steatosis, inflammation, necrosis, and fibrosis both acute [Table 2] and chronic [Table 3] CCl₄-treated groups, and it was showed that there was no statistically significant change between these groups.

Biochemical findings

There was a significant increase in serum glucose, LDL-cholesterol, total cholesterol levels, and ALT, AST, and ALP enzyme activities induced by CCl₄ treatment both acute and chronic trial groups [Tables 4 and 5]. The beneficial effects of treatment with PSO on the CCl₄-induced elevation of serum ALT, AST, and ALP enzyme activities, glucose, and total cholesterol levels are presented in Table 4. However, serum ALT and AST activities were not affected from PSO administration in chronic groups [Table 5]. Furthermore, PSO treatment normalized albumin and TP levels both acute and chronic groups.

The NO radicals play an import role in inducing inflammatory response.^[15] Treatment of CCl₄ caused a significant increase in NO concentration in hepatic tissue both in acute and chronic intoxication. In addition, CCl₄ treatment caused high level of oxidative damage, as evidenced by a significant elevation in hepatic MDA level [Tables 4 and 5]. Treatment with PSO caused a significant decline in MDA levels in acute CCl₄ administrated groups while it had a nonsignificant decrease in chronic groups. Treatment with PSO caused a significant decline in NO levels in chronic CCl₄-administrated groups while it had a nonsignificant decrease in acute groups.

DISCUSSION

Hepatic injury caused by CCl₄ is the most commonly used experimental models for understanding the cellular mechanism behind oxidative damage and lipid peroxidation and also the screening of hepatoprotective activity of plant extracts and drugs. Oxidative stress plays a major role in the pathogenesis of both acute and chronic liver injury caused by this well-known hepatotoxin. CCl₄ transforms into CCl₃ and CCl₃ peroxy (CCl₃O₂) free radicals, which are toxic intermediates metabolites, through the cytochrome P450 enzyme system in the nongranular endoplasmic reticulum in hepatocytes.^[3] These metabolites react with unsaturated fatty acids in the cell membrane to initiate lipid peroxidation or causes breakdown of cell membranes by binding to proteins and fats which are the causes of liver damage.^[7,34]

In the present study, for understanding the ability of PSO to protect against CCl₄ intoxication, we used an experimental model of CCl₄-induced acute and chronic hepatotoxicity models in rats. As indicated from the results, the treated rats with CCl₄ in acute injury showed centrilobular necrosis, inflammatory cell infiltration, and lipid vacuolization. These results are in agreement with Arosio (2000), who confirm that 24 h after a single IP injection of 3 mg/kg CCl₄ caused cytoplasmic vacuolization, necrosis and degenerative changes in hepatocytes, especially around vena centralis, and Grizzi (2003), who determined that a single dose of 1 mL/kg CCl₄ administration caused intense inflammatory cell

Table 4: Serum biochemical parameters and liver lipid peroxidation levels in control and experimental groups with acute liver injury

Parameters	$\bar{X} \pm S_x$				P
	Control (n=10)	PSO (n=10)	CCl ₄ (n=10)	CCl ₄ + PSO (n=10)	
AST (U/L)	166.80±17.10 ^a	165.00±5.80 ^a	320.10±15.70 ^b	209.80±29.05 ^a	<0.05
ALT (U/L)	62.00±3.72 ^a	75.40±11.90 ^{ab}	145.90±22.84 ^c	86.40±8.28 ^b	<0.01
ALP (U/L)	370.50±43.98 ^{ab}	349.80±7.86 ^b	612.30±29.80 ^c	411.50±28.11 ^a	<0.01
Glucose (mg/dL)	157.50±22.42 ^a	179.60±25.92 ^{ab}	310.80±13.48 ^c	240.60±17.52 ^b	<0.01
Triglyceride (mg/dL)	135.70±13.44	126.90±5.11	180.30±17.30	139.00±10.259	>0.05
Total cholesterol (mg/dL)	78.10±5.66 ^a	55.00±2.31 ^b	96.20±1.40 ^c	73.00±3.14 ^a	<0.05
HDL-cholesterol (mg/dL)	44.60±13.09 ^a	60.00±4.22 ^c	25.00±1.44 ^b	26.00±1.46 ^b	<0.05
LDL-cholesterol (mg/dL)	20.00±2.87 ^{ab}	16.95±0.91 ^a	30.90±1.80 ^c	26.02±1.29 ^{bc}	<0.05
Total protein (g/dL)	6.58±0.27 ^a	7.10±0.17 ^a	5.82±0.30 ^b	6.60±0.21 ^a	<0.001
Albumin (g/dL)	1.27±0.09 ^a	1.47±0.08 ^b	0.24±0.04 ^c	1.00±0.07 ^d	<0.001
MDA	1.03±0.07 ^b	0.62±0.08 ^b	1.93±0.20 ^a	1.03±0.17 ^b	<0.001
NO	35.68±4.06 ^b	34.88±3.06 ^b	53.50±3.06 ^a	38.14±4.14 ^{ab}	<0.01

^{a-d}The difference between groups in the same line with different letters is statistically significant. $\bar{X} \pm S_x$: Mean±SE. ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; MDA: Malondialdehyde; NO: Nitric oxide; PSO: Pomegranate seed oil; CCl₄: Carbon tetrachloride; SE: Standard error

Table 5: Serum biochemical parameters and liver lipid peroxidation levels in control and experimental groups with chronic liver injury

Parameters	$\bar{X} \pm S_x$				P
	Control (n=10)	PSO (n=10)	CCl ₄ (n=10)	CCl ₄ + PSO (n=10)	
AST (U/L)	174.20±16.49 ^b	171.90±6.37 ^b	1670.40±319.69 ^a	1409.40±283.14 ^a	<0.001
ALT (U/L)	80.00±7.45 ^b	62.10±3.49 ^b	1285.20±405.06 ^a	990.16±158.34 ^a	<0.01
ALP (U/L)	359.60±21.38 ^{bc}	308.30±31.43 ^c	544.90±15.01 ^a	386.80±24.78 ^b	<0.001
Glucose (mg/dL)	124.20±22.42 ^b	148.80±10.11 ^b	318.70±29.49 ^a	255.40±28.60 ^a	<0.001
Triglyceride (mg/dL)	148.00±10.60	141.50±15.91	194.50±18.10	168.20±13.48	>0.05
Total cholesterol (mg/dL)	76.30±3.65 ^b	64.80±3.28 ^c	87.70±2.15 ^a	75.10±3.98 ^b	<0.001
HDL-cholesterol (mg/dL)	31.50±1.26 ^b	44.20±4.75 ^a	23.10±1.25 ^c	28.60±2.11 ^{bc}	<0.001
LDL-cholesterol (mg/dL)	15.20±3.33 ^a	12.82±2.81 ^a	23.34±1.38 ^b	18.42±2.40 ^{a, b}	<0.05
Total protein (g/dL)	6.04±0.18 ^a	6.53±0.22 ^a	5.48±0.13 ^b	6.00±0.16 ^a	<0.01
Albumin (g/dL)	1.33±0.08 ^b	1.60±0.06 ^a	0.72±0.05 ^c	1.27±0.05 ^b	<0.001
MDA	0.98±0.12 ^{ac}	0.19±0.02 ^a	2.70±0.45 ^b	1.73±0.22 ^{bc}	<0.001
NO	49.98±4.43 ^{bc}	35.79±3.69 ^b	97.61±5.06 ^a	62.38±6.65 ^c	<0.001

^{a-c}The difference between groups in the same line with different letters is statistically significant. $\bar{X} \pm S_x$: Mean±SE. ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; MDA: Malondialdehyde; NO: Nitric oxide; PSO: Pomegranate seed oil; CCl₄: Carbon tetrachloride; SE: Standard error

infiltration mainly composed of macrophages and lymphocytes. In addition to these acute hepatotoxicity studies, there are many studies induced by CCl₄ with long-term exposure.^[5,10,15,35] In the current study, chronic CCl₄ administration caused liver damage, as demonstrated by severe necrosis, mononuclear inflammatory cells infiltration, new regenerative nodules resulted by pseudolobulation in the liver of rats. Several studies have shown that liver histology with CCl₄-treated rats in chronic intoxication was similar with our findings.^[5,10,35] Our results indicate that treatment with PSO during CCl₄ administration showed that PSO has no ameliorative effects on liver histology in acute and chronic hepatotoxicity. These findings may be related with the highly reactive molecules of CCl₄ which are leading to irreversible damage to the liver and also administration dose and duration of treatment.

Damage to hepatocytes changes serum AST and ALT transport function and membrane permeability, leading to leakage of enzymes into the circulation system from cells indicates severe damage during CCl₄ intoxication.^[36,37] Levels of serum marker enzymes of hepatic injury, ALT, AST, and ALP increased significantly in CCl₄-treated rats in both acute and chronic hepatotoxicity as an indicative of severe hepatic injury. The present study showed a decrease in serum TP and albumin levels in acute and chronic hepatotoxicity which may be due to disruption of protein synthesis by disrupting polyribosomes in the endoplasmic reticulum in the liver, as suggested by several authors.^[11,35] In the present study, the CCl₄-induced increase in serum glucose and total cholesterol levels found in acute^[4] and chronic^[35] CCl₄ groups and agrees with previous reports. It has been suggested that this increase in serum cholesterol level is thought to be due to the fatty acids^[38] and excessive circulation^[4,39] due to liver cell damage. Increase in serum glucose level probably due to the decrease in serum insulin and insulin-like growth factor-I concentrations or the decrease in glycogen synthesis in the liver due to CCl₄ intoxication.^[5] In the present study, increased serum LDL and decreased serum HDL concentration might be due to defect in their receptors as a result of liver damage in the CCl₄-treated groups which is in agreement with earlier reports.^[10,40]

Several studies have reported that liver produces large quantities of NO in CCl₄-induced hepatotoxicity in response to tissue injury and inflammation.^[5,15,41] Our findings are consistent with those studies. The increase of MDA has been considered a key feature in liver injury and reflects enhanced lipid peroxidation. We observed increased levels of MDA in the liver which are consisted with some researchers in acute^[42,43] and chronic hepatotoxicity^[5,15] treated with CCl₄.

PSO has been shown to scavenge free radicals, decrease lipid peroxidation, and inhibit lipoxygenase enzyme which is a key mediator of inflammatory process.^[44] It has been reported that punicic acid, ellagic acid, sterols, and fatty acids are the main antioxidant components in PSO.^[45] Administration of PSO led to a decline in the activities of AST, ALT, ALP, and glucose; total cholesterol; MDA; and NO levels while this treatment elevated TP and albumin levels being close to that of the control in acute hepatotoxicity. Increased levels of albumin, TP and decreased activities of serum ALP, total cholesterol, MDA, and NO levels were similar in chronic hepatotoxicity with PSO treatment. This means that constituents in PSO play an important role in scavenging the free radicals and inhibiting lipid peroxidation resulted from the CCl₄ metabolism.

The results from this study suggest that PSO has some antioxidative effects against CCl₄-induced oxidative stress by decreasing the levels of MDA and NO, which reflect the severity of liver injury in acute and chronic hepatotoxicity. However, this amelioration did not reflect on histological damage to the liver tissue of rats induced by CCl₄ treatment. It is thought to be caused by prolonged usage of CCl₄ treatment have irreversible effects on hepatic architecture. The PSO dose used in this study (0.15 mg/kg) was found to positive effects on some serum biochemical parameters and

liver MDA and NO levels. Nevertheless, dose- and duration-dependent further investigations need to be performed to understand the dose that produces the best result without any side effect.

CONCLUSION

From the present study results, it could be concluded that PSO has some antioxidative effects against CCl₄-induced oxidative stress. However, this amelioration did not reflect on histological damage to the liver tissue of rats induced by CCl₄. Further researches for the antioxidative effects of PSO and similar plant-derived antioxidative agents will provide a better understanding of the subject.

Acknowledgments

This research was summarized from a section of the PhD thesis entitled "Effects of Rosemary Extract (*Rosmarinus officinalis*) and Pomegranate (*Punica granatum*) Seed Oil on CCl₄-Induced Acute and Chronic Hepatotoxicity in Rats" and supported by the Fund of Erciyes University Scientific Research Project (Project No: TSD-38-28).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Tsukamoto H, Matsuoka M, French SW. Experimental models of hepatic fibrosis: A review. *Semin Liver Dis* 1990;10:56-65.
2. Camps J, Bargallo T, Gimenez A, Alie S, Caballeria J, Pares A, *et al.* Relationship between hepatic lipid peroxidation and fibrogenesis in carbon tetrachloride-treated rats: Effect of zinc administration. *Clin Sci (Lond)* 1992;83:695-700.
3. Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. *Crit Rev Toxicol* 2003;33:105-36.
4. Botsoglou NA, Taitzoglou IA, Botsoglou E, Lavrentiadou SN, Kokoli AN, Roubies N, *et al.* Effect of long-term dietary administration of oregano on the alleviation of carbon tetrachloride-induced oxidative stress in rats. *J Agric Food Chem* 2008;56:6287-93.
5. Gutiérrez R, Alvarado JL, Presno M, Pérez-Veyna O, Serrano CJ, Yahuaca P, *et al.* Oxidative stress modulation by *Rosmarinus officinalis* in CCl₄-induced liver cirrhosis. *Phytother Res* 2010;24:595-601.
6. Muriel P. Nitric oxide protection of rat liver from lipid peroxidation, collagen accumulation, and liver damage induced by carbon tetrachloride. *Biochem Pharmacol* 1998;56:773-9.
7. Basu S. Carbon tetrachloride-induced lipid peroxidation: Eicosanoid formation and their regulation by antioxidant nutrients. *Toxicology* 2003;189:113-27.
8. Kiso K, Ueno S, Fukuda M, Ichi I, Kobayashi K, Sakai T, *et al.* The role of Kupffer cells in carbon tetrachloride intoxication in mice. *Biol Pharm Bull* 2012;35:980-3.
9. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J, *et al.* Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007;39:44-84.
10. Khan F, Asdaq SMB, Prassana Kumar SR, Prasanna Kumar SR. Effects of few Indian medicinal herbs on carbon tetrachloride induced hepatic injury in animals. *Int J Pharm Tech Res* 2009;1:579-87.
11. Breikaa RM, Algandaby MM, El-Demerdash E, Abdel-Naim AB. Biochanin A protects against acute carbon tetrachloride-induced hepatotoxicity in rats. *Biosci Biotechnol Biochem* 2013;77:909-16.
12. Al-Jawad FH, Kadhim HM, Abbood MS, Salman NI. Protective effect of apium graveolens, cinnamomum verum in CCl₄ induced model of acute liver injury. *World J Pharm Pharm Sci* 2016;5:10-7.
13. Arosio B, Gagliano N, Fusaro LM, Parmeggiani L, Tagliabue J, Galetti P, *et al.* Aloe-emodin quinone pretreatment reduces acute liver injury induced by carbon tetrachloride. *Pharmacol Toxicol* 2000;87:229-33.
14. Atasver A, Yaman D. The effects of grape seed and colchicine on carbon tetrachloride induced hepatic damage in rats. *Exp Toxicol Pathol* 2014;66:361-5.
15. Yehia HM, Al-Olayan EM, Elkhadragey MF. Hepatoprotective role of the pomegranate (*Punica granatum*) juice on carbon tetrachloride-induced oxidative

- stress in rats. *Life Sci J* 2013;10:1534-44.
16. Gäbele E, Brenner DA, Rippe RA. Liver fibrosis: Signals leading to the amplification of the fibrogenic hepatic stellate cell. *Front Biosci* 2003;8:d69-77.
 17. Friedman SL. Hepatic fibrosis – Overview. *Toxicology* 2008;254:120-9.
 18. Abdel-Wahhab KGED, El-Shamy KA, El-Beih NAEZ, Morcy FA, Mannaa FAE. Protective effect of a natural herb (*Rosmarinus officinalis*) against hepatotoxicity in male albino rats. *Comunicata Sci* 2011;2:9-17.
 19. Chowdhury MR, Sagor MA, Tabassum N, Potal MA, Hossain H, Alam MA, *et al.* Supplementation of *Citrus maxima* peel powder prevented oxidative stress, fibrosis, and hepatic damage in carbon tetrachloride (CCl₄) treated rats. *Evid Based Complement Alternat Med* 2015;2015:598179.
 20. Kim ND, Mehta R, Yu W, Neeman I, Livney T, Amichay A, *et al.* Chemopreventive and adjuvant therapeutic potential of pomegranate (*Punica granatum*) for human breast cancer. *Breast Cancer Res Treat* 2002;71:203-17.
 21. Al-Zoreky NS. Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. *Int J Food Microbiol* 2009;134:244-8.
 22. Mahboubi A, Asgarpanah J, Sadaghiyani PN, Faizi M. Total phenolic and flavonoid content and antibacterial activity of *Punica granatum* L. var. pleniflora flowers (Golnar) against bacterial strains causing foodborne diseases. *BMC Complement Altern Med* 2015;15:366.
 23. Ghazaleh Moghaddam MS, Hassanzadeh G, Khanavi M, Hajimahmoodi M. Anti-ulcerogenic activity of the pomegranate peel (*Punica granatum*) methanol extract. *Food Nutr Sci* 2013;4:43-8.
 24. Bekir J, Mars M, Souchard JP, Bouajila J. Assessment of antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of pomegranate (*Punica granatum*) leaves. *Food Chem Toxicol* 2013;55:470-5.
 25. BenSaad LA, Kim KH, Quah CC, Kim WR, Shahimi M. Anti-inflammatory potential of ellagic acid, gallic acid and punicalagin A & B isolated from *Punica granatum*. *BMC Complement Altern Med* 2017;17:47.
 26. Kaur G, Jabbar Z, Athar M, Alam MS. *Punica granatum* (pomegranate) flower extract possesses potent antioxidant activity and abrogates fe-NTA induced hepatotoxicity in mice. *Food Chem Toxicol* 2006;44:984-93.
 27. Kaufman M, Wiesman Z. Pomegranate oil analysis with emphasis on MALDI-TOF/MS triacylglycerol fingerprinting. *J Agric Food Chem* 2007;55:10405-13.
 28. Vroegrijk IO, van Diepen JA, van den Berg S, Westbroek I, Keizer H, Gambelli L, *et al.* Pomegranate seed oil, a rich source of punicic acid, prevents diet-induced obesity and insulin resistance in mice. *Food Chem Toxicol* 2011;49:1426-30.
 29. Luna LG, editor. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. New York: Blakiston Division, McGraw-Hill; 1968. p. 258.
 30. Schwimmer JB, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, *et al.* Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* 2005;42:641-9.
 31. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265-75.
 32. Yoshioka T, Kawada K, Shimada T, Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. *Am J Obstet Gynecol* 1979;135:372-6.
 33. Tracey WR, Tse J, Carter G. Lipopolysaccharide-induced changes in plasma nitrite and nitrate concentrations in rats and mice: Pharmacological evaluation of nitric oxide synthase inhibitors. *J Pharmacol Exp Ther* 1995;272:1011-5.
 34. Manibusan MK, Odin M, Eastmond DA. Postulated carbon tetrachloride mode of action: A review. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 2007;25:185-209.
 35. Venukumar MR, Latha MS. Antioxidant activity of *Curculigo orchioides* in carbon tetrachloride-induced hepatopathy in rats. *Indian J Clin Biochem* 2002;17:80-7.
 36. Rajesh MG, Latha MS. Hepatoprotection by *Elephantopus scaber* linn. In CCl₄-induced liver injury. *Indian J Physiol Pharmacol* 2001;45:481-6.
 37. Gad SC. *Animal Models in Toxicology*. Boca Raton, FL: CRC Press; 2007. p. 1152.
 38. Santra A, Chowdhury A, Ghatak S, Biswas A, Dhali GK. Arsenic induces apoptosis in mouse liver is mitochondria dependent and is abrogated by N-acetylcysteine. *Toxicol Appl Pharmacol* 2007;220:146-55.
 39. Palaniswamy R, Raghunathan PP. Protective effect of *Bacopa monnieri* leaf extract against oxidative stress induced hepatotoxicity in rats. *Int J Pharm Pharm Sci* 2013;5:555-8.
 40. Al-Assaf AH. Preventive effect of corosolic acid on lipid profile against carbon tetrachloride induced hepatotoxic rats. *Pak J Nutr* 2013;12:748-52.
 41. Cetin E, Kanbur M, Cetin N, Eraslan G, Atasever A. Hepatoprotective effect of ghrelin on carbon tetrachloride-induced acute liver injury in rats. *Regul Pept* 2011;171:1-5.
 42. Jeon TI, Hwang SG, Park NG, Jung YR, Shin SI, Choi SD, *et al.* Antioxidative effect of chitosan on chronic carbon tetrachloride induced hepatic injury in rats. *Toxicology* 2003;187:67-73.
 43. Ashok Shenoy K, Somayaji SN, Bairy KL. Hepatoprotective effects of *Ginkgo biloba* against carbon tetrachloride induced hepatic injury in rats. *Indian J Pharmacol* 2001;33:260-6.
 44. Schubert SY, Lansky EP, Neeman I. Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. *J Ethnopharmacol* 1999;66:11-7.
 45. Jurenka JS. Therapeutic applications of pomegranate (*Punica granatum* L.): A review. *Altern Med Rev* 2008;13:128-44.