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ORIGINAL ARTICLE

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

Duygu Yaman Gram, Ayhan Atasever, Meryem Eren[1]

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

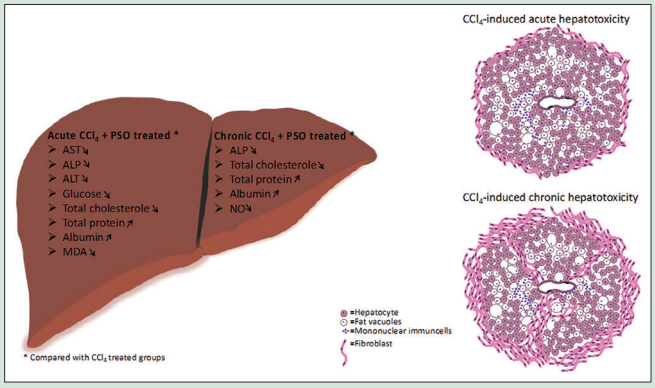
ABSTRACT

Background: Carbon tetrachloride (CCl_4) 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 CCl_4-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 CCl_4. 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 CCl_4-treated animals, an intensive macro and microvesicular steatosis, mononuclear inflammatory cell infiltrations in portal area and parenchyma, and necrotic alterations; in the chronic CCl_4-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 CCl_4 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 CCl_4-induced oxidative stress by decreasing levels of some biochemical and lipid peroxidation parameters.
- The results of the histopathological investigation showed that prolonged usage of CCl_4 treatment have irreversible effects on hepatic architecture.



Abbreviations Used: PSO: Pomegranate seed oil; CCl_4: Carbon tetrachloride; CCl_3: Trichloromethyl; MDA: Malondialdehyde; NO: Nitric oxide; ROS: Reactive oxygen species; IM: Intramuscular; 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

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INTRODUCTION

Carbon tetrachloride (CCl_4) 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 CCl_4 play a crucial role in understanding of the mechanisms of action of hepatic injury.[2,3] CCl_4 is metabolized to trichloromethyl (CCl_3) 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 CCl_4-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 CCl_4 metabolism to CCl_3, 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

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cause DNA fragmentation and lipid oxidation.[11] Histopathologically, CCl_4 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 CCl_4-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 CCl_4-induced liver damage after acute and chronic exposure by assaying serum lipid profiles and histopathology of liver tissues in rats.

MATERIALS AND METHODS

Materials

CCl_4 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 CCl_4 intraperitoneally (IP) at a dose of 1 mL/kg, twice in the 1st week,
- Group 4 were administered with CCl_4 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 CCl_4(0.2 mL/kg) twice a week, for 12 weeks,
- Group 4 were administered with CCl_4(0.2 mL/kg) twice a week and simultaneously 0.15 mL/kg PSO through gavage directly to the stomach for 12 weeks.

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

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 CCl_4 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

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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 ± 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].

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; CCI_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; CCI_4: Carbon tetrachloride

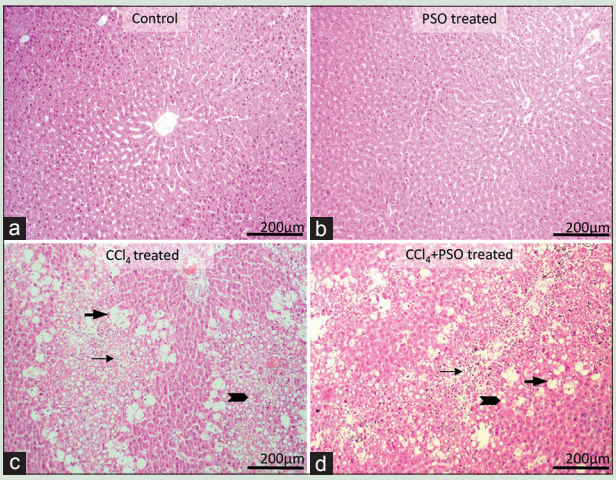

Figure 1: Histological analysis of the livers in carbon tetrachloride-induced acute hepatotoxicity; Normal appearance of the livers of the control (a) and pomegranate seed oil-treated (b) groups. The appearance of micro (arrowheads)- and macro (black thick arrows) vesicular fat vacuoles in all parenchyma 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, ×10

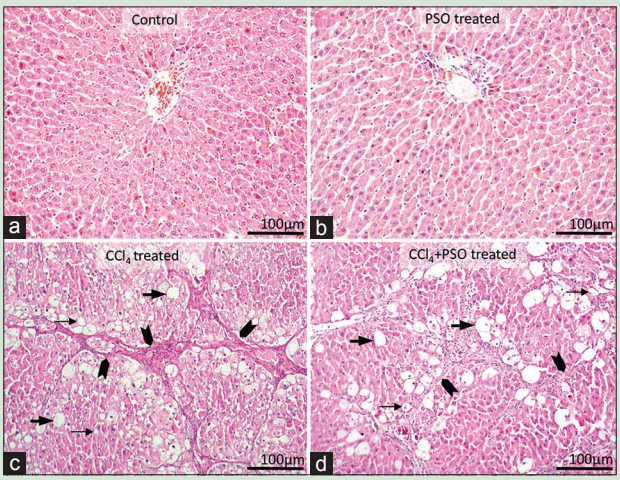

Figure 2: Histological analysis of the livers in carbon tetrachloride-induced chronic hepatotoxicity; Normal appearance of the livers of the control (a) and pomegranate seed oil-treated (b) groups. The appearance of micro (black thin arrows) and macro (black thick arrows) vesicular fat vacuoles in the 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, ×20

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steatosis, inflammation, necrosis, and fibrosis both acute [Table 2] and chronic [Table 3] CCl_4-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 CCl_4 treatment both acute and chronic trial groups [Tables 4 and 5]. The beneficial effects of treatment with PSO on the CCl_4-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 CCl_4 caused a significant increase in NO concentration in hepatic tissue both in acute and chronic intoxication. In addition, CCl_4 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 CCl_4 administrated groups while it had a nonsignificant decrease in chronic groups. Treatment with PSO caused a significant decline in NO levels in chronic CCl_4-administrated groups while it had a nonsignificant decrease in acute groups.

DISCUSSION

Hepatic injury caused by CCl_4 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. CCl_4 transforms into CCl_3 and CCl_3 peroxyl (CCl_3O_2) 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 CCl_4 intoxication, we used an experimental model of CCl_4-induced acute and chronic hepatotoxicity models in rats. As indicated from the results, the treated rats with CCl_4 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 CCl_4 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 CCl_4 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	$\bar{X}\pm S_{\bar{x}}$				*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[a,b]	145.90±22.84[c]	86.40±8.28[b]	<0.01
ALP (U/L)	370.50±43.98[a,b]	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[a,b]	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[a,b]	16.95±0.91[a]	30.90±1.80[c]	26.02±1.29[b,c]	<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[a,b]	<0.01

[a-d]The difference between groups in the same line with different letters is statistically significant. $\bar{X}\pm S_{\bar{x}}$: 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; CCI_4: 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	$\bar{X}\pm S_{\bar{x}}$				*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[b,c]	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[a,c]	0.19±0.02[a]	2.70±0.45[b]	1.73±0.22[b,c]	<0.001
NO	49.98±4.43[b,c]	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. $\bar{X}\pm S_{\bar{x}}$: 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; CCI_4: Carbon tetrachloride; SE: Standard error

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infiltration mainly composed of macrophages and lymphocytes. In addition to these acute hepatotoxicity studies, there are many studies induced by CCl_4 with long-term exposure.[5,10,15,35] In the current study, chronic CCl_4 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 CCl_4-treated rats in chronic intoxication was similar with our findings.[5,10,35] Our results indicate that treatment with PSO during CCl_4 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 CCl_4 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 CCl_4 intoxication.[36,37] Levels of serum marker enzymes of hepatic injury, ALT, AST, and ALP increased significantly in CCl_4-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 CCl_4-induced increase in serum glucose and total cholesterol levels found in acute[4] and chronic[35] CCl_4 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 CCl_4 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 CCl_4-treated groups which is in agreement with earlier reports.[10,40]

Several studies have reported that liver produces large quantities of NO in CCl_4-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 CCl_4.

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 CCl_4 metabolism.

The results from this study suggest that PSO has some antioxidative effects against CCl_4-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 CCl_4 treatment. It is thought to be caused by prolonged usage of CCl_4 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 CCl_4-induced oxidative stress. However, this amelioration did not reflect on histological damage to the liver tissue of rats induced by CCl_4. Further researches for the antioxidative effects of PSO and similar plant-derived antioxidative agents will provide a better understanding of the subject.

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Conflicts of interest

There are no conflicts of interest.

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