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ORIGINAL ARTICLES

Investigation of the Hepatoprotective Effects of Sesame (*Sesamum indicum* L.) in Carbon Tetrachloride-Induced Liver Toxicity

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Abstract More than 600 chemicals can cause damage in liver, one of which is carbon tetrachloride (CCl_4). Hepatoprotective agents could prevent tissue damage and reduce morbidity and mortality rates; such agents may include alternative or folkloric treatments. We investigated sesame (*Sesamum indicum* L.) for its hepatoprotective effect in CCl_4-induced experimental liver damage. To this end, 0.8 mg/kg of sesame fixed oil was provided intraperitoneally to rats whose livers were damaged by CCl_4. Tissue and blood samples were taken at the end of the experiments and evaluated histologically and biochemically. Ballooning degenerations and an increase in lipid droplets in liver parenchyma and increases in serum alanine transaminase, aspartate transaminase, and bilirubin were found in the CCl_4 group. Biochemical and histopathological findings in the sesame fixed oil treated group were not significantly different from the CCl_4 group. Sesame did not show a hepatoprotective effect in CCl_4-induced liver toxicity.

Keywords Carbon tetrachloride · Histology · Liver · Rat · Sesame · *Sesamum indicum* L. · ALT · AST · ALP

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The flowering plant *Sesamum indicum* L., known as sesame, is widely naturalized in tropical regions around the world and is cultivated for its edible seeds, which grow in pods. The sesame seed is used as a diuretic, emollient, galactogogue, and lenitive (Duke and Ayensu 1985) and as a tonic for the liver and kidneys (Bown 1995). It is taken for the treatment of premature hair loss and graying, convalescence, chronic dry constipation, dental caries, osteoporosis, Tiff joint, and dry cough. The seed contains flavonoids, cardiac glycosides, anthocyanins, saponins, and reducing sugars (Awobajo et al. 2009). The liver is the key organ of metabolism, secretion, and excretion. It is continuously and variedly exposed to xenobiotics, environmental pollutants, and chemotherapeutic agents as a result of its strategic location in the body.

Liver diseases are worldwide problems. Some compounds produce metabolites that cause liver injury in a uniform, dose-dependent fashion (Klein et al. 1989). Injury to hepatic tissue results either directly from the disruption of intracellular function or membrane integrity, or from damage affecting endothelial or bile duct cells, as seen in cholestasis or indirectly from immune-mediated membrane damage (Bharali and Dutta 2009). Factors promoting the accumulation of hepatocyte toxins include genetic

alterations in enzymes that allow the formation of the harmful metabolites of other drugs and depletion of the substrates required to detoxify the metabolite (Lee 1995). Drugs used in the treatment of liver diseases are occasionally inadequate and can have serious adverse effects. None of the drugs used in allopathic medical practice provides dependable liver protection (Bhatt and Bhatt 1996; Nagarkatti et al. 1994; Srivastava et al. 1994; Santra et al. 1998). Many medicinal plants have been tested and found to contain active substances with curative properties for a variety of diseases (Lewis 1977). Experience has demonstrated that plant drugs are relatively nontoxic, safe, and free from serious adverse effects (Momin 1987).

Antioxidant activity or inhibition of generation of free radicals is important in providing protection against hepatic damage (Bhattacharyya et al. 2003). The principal causes of carbon tetrachloride (CCl_4)-induced hepatic damage is lipid peroxidation and decreased activity of antioxidant enzymes and generation of free radicals (Nwachukwu et al. 2011). CCl_4 is a toxic agent used in experimental liver damage. It is metabolized by the mitochondrial monooxygenase system. During metabolism, an unstable trichloromethyl free radical is formed and rapidly converted to trichloromethyl peroxide (Recknagel et al. 1989; Slater 1982). These free radicals lead to the peroxidation of fatty acids found in the phospholipids making up the cell membranes. Lipid peroxide radicals, lipid hydroperoxides, and lipid breakdown products develop in this process, and each constitutes an active oxidizing agent. Consequently, cell membrane structures and intracellular organelle membrane structures are completely broken down. Structural damage spreads. Chronic administration of CCl_4 results in fibrosis and cirrhosis (Brattin et al. 1985). Lipid peroxidation is important in liver damage associated with CCl_4 (Nadkarni and D'Souza 1988; Gassó et al. 1996).

The present study was performed to assess the hepatoprotective activity of sesame seed extract against CCl_4-induced liver damage in rats. Sesame is used in folklore-based medical practices for treating liver diseases, and we wanted to validate its use.

Materials and Methods

Plant Material

Sesame (*Sesamum indicum* L.) seeds were obtained from their natural habitat in Turkey. Voucher specimens for the plant seeds have been deposited in the pharmacology laboratory of Yüzüncü Yıl University (B-01). The dried seeds were finely ground in an electric grinder and extracted by Soxhlet apparatus (Ildam, Turkey) with diethyl ether (40–50 °C) until completely exhausted. Diethyl ether was evaporated under reduced pressure by a rotary evaporator (RV 05-ST rotavapor; IKA-Werke, Staufen, Germany).

Animals

Thirty (24) male albino Sprague Dawley rats weighing 180–220 g were obtained from the Animal House of the School of Medicine, Yüzüncü Yil University. The animals were acclimatized for 2 weeks under standard conditions at a temperature 22 ± 2 °C with a 12:12 h light/dark cycle. Rats were housed in specific cages. The rats were fed with standard pellets and water ad libitum. They were kept in a controlled environment following the standard operating procedures of the Animal House with the approval of the animal ethics committee of Yüzüncü Yıl University.

Experimental Design

The CCl_4 model (Handa and Sharma 1990; Shenoy et al. 2001) was used for scheduling the dose regimen (Slater 1965; Suja et al. 2004). Intraperitoneal injection of 0.8 ml/kg CCl_4 diluted in olive oil (1:1 dilution) was used for inducing acute liver toxicity. The experimental groups were as follows (n = 6 in each group). Group 1 received isotonic saline solution (ISS) (0.5 ml/kg, i.p.). Group 2 received olive oil (0.5 mg/kg, i.p.). Group 3 received CCl_4 (0.8 mg/kg, i.p.). Group 4 received sesame fixed oil (0.8 mg/kg, i.p.) and CCl_4 (0.8 mg/kg, i.p.).

All injections were applied once a day for 7 days. CCl_4, sesame fixed oil, and olive oil were applied separately with different injectors. Body weights of the rats were measured once a day for 8 days. The percentage of daily changes in body weights was calculated. At the end of the treatment (day 8), blood samples were collected by direct cardiac puncture, and the obtained serum was used to assay aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and bilirubin. The livers of the experimental animals were extracted after killing the animals by cervical dislocation, then divided into two for biochemical and histological analyses.

The tissue levels of malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and catalase (CAT) were determined according to the methods of Wasowicz et al. (1993), Paglia and Valentine (1967), and Goth (1991), respectively.

Histological specimens were fixed in 10 % neutral-buffered formalin before routine processing in paraffin-embedded blocks. Sections (4 μm thick) were cut and stained with hematoxylin-eosin (H&E), Gomori, and Oil Red O stains.

The serum AST and ALT levels were determined with a commercial kit (Vitros DT60-II autoanalyzer; Vitros, Rochester, NY).

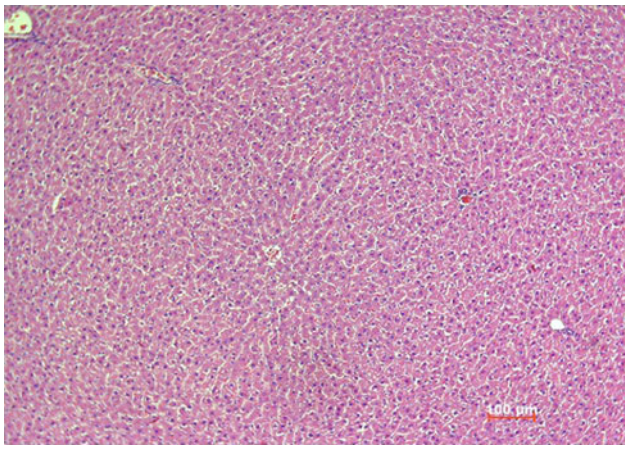

Fig. 1 H&E-stained liver tissue from the ISS group

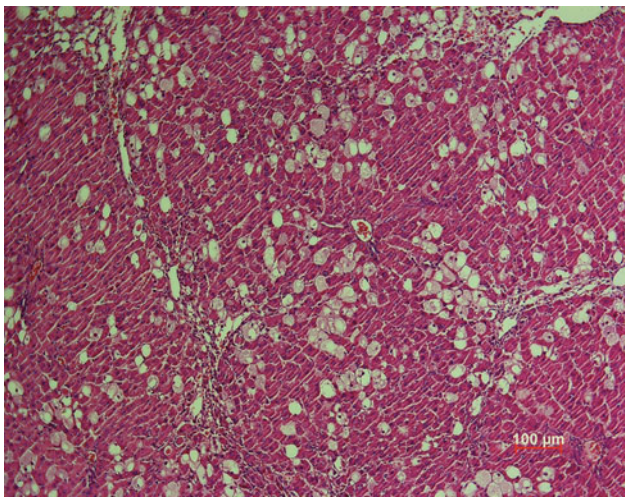

Fig. 2 H&E-stained liver tissue from the CCl_4 group

Statistical Analysis

All data were presented as mean ± standard error of the mean or as percentages. Analysis of variance was used for the statistical analysis of data. Tukey's honestly significant difference test and least significant difference test were used for determining significance. Results with $p < 0.05$ were considered as statistically significant.

Results

During the experiment, the changes in animal weight were 8.87 % in the control group, 0.69 % in the olive oil group, −7.65 % in the CCl_4 group, and −5.98 % in the CCl_4 + sesame fixed oil group.

Histological appearance of the liver stromal and parenchymal structures stained with H&E, Oil Red O, and Gomori in the ISS and olive oil groups were normal (Figs. 1, 3, 5).

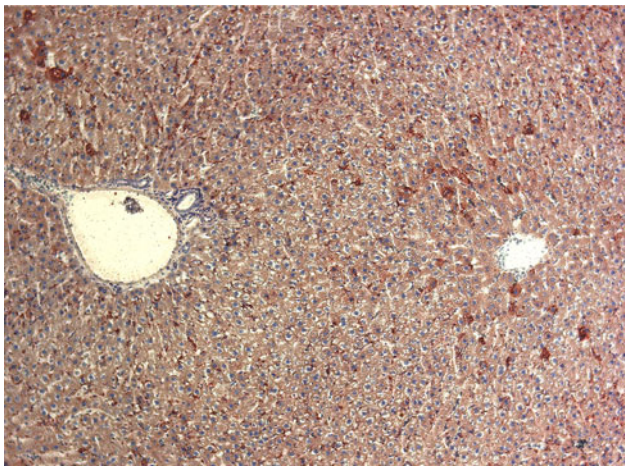

Fig. 3 Oil Red O-stained liver tissue from the ISS group

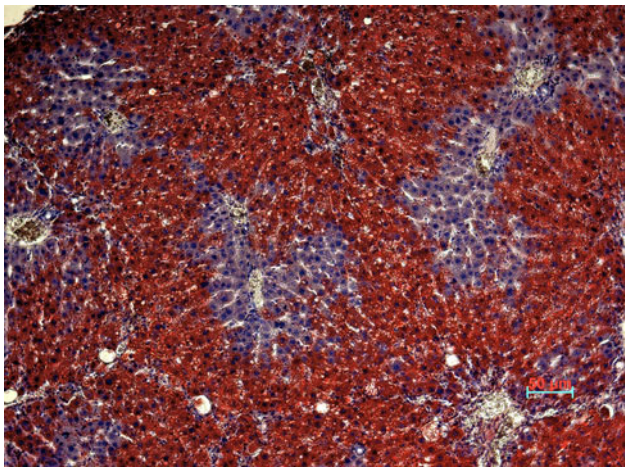

Fig. 4 Oil Red O-stained liver tissue from the CCl_4 group

In CCl_4 and CCl_4 + sesame fixed oil groups, ballooning degenerations were observed in liver parenchyma stained with H&E, especially in zone I (Fig. 2).

In Oil Red O–stained ISS and olive oil group specimens, the histological appearance of the liver stromal and parenchymal structures was normal, while increased lipid droplets were observed in the specimens of the CCl_4 and CCl_4 + sesame fixed oil groups (Figs. 3, 4).

Gomori staining of the reticular fibers in the liver stroma revealed increased collagen type III fibers in the CCl_4 and CCl_4 + sesame fixed oil groups (Figs. 5, 6).

The results of the biochemical analyses of the blood and liver tissues in all groups are presented in Tables 1 and 2. Serum ALT, AST, and bilirubin levels were significantly increased, whereas serum ALP level was decreased in the CCl_4 and CCl_4 + sesame fixed oil groups compared to the ISS and olive oil groups. Liver tissue MDA levels were significantly increased while CAT and GSH-Px levels were decreased in the CCl_4 and CCl_4 + sesame fixed oil groups compared to the ISS and olive oil groups.

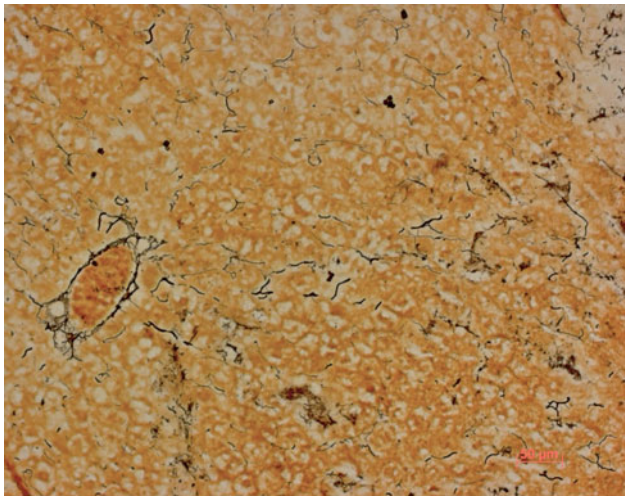

Fig. 5 Gomori-stained liver tissue from the ISS group

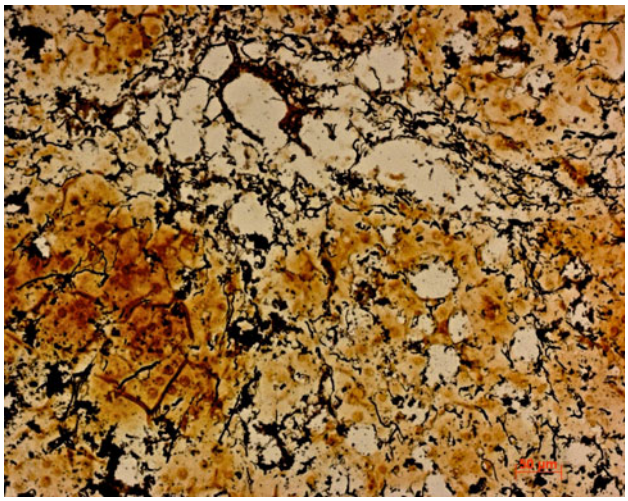

Fig. 6 Gomori-stained liver tissue from the CCl_4 group

Discussion

The liver is a vital organ present in vertebrates and some other animals. CCl_4-induced hepatic injury is commonly used as an experimental method to study the hepatoprotective effects of medicinal plant extracts and drugs (Slater 1965). The changes associated with CCl_4-induced liver damage are similar to that of acute viral hepatitis (Suja et al. 2004). The hepatotoxicity induced by CCl_4 is due to its metabolite CCl_3, a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on lipids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage (Nwachukwu et al. 2011; Bishayee et al. 1995).

Free radicals are highly reactive compounds that are created in the body during normal metabolic functions or that are introduced from the environment. Free radicals are inherently unstable because they contain "extra" energy. To reduce their energy load, free radicals react with certain chemicals in the body, and in the process, they interfere with the cells' ability to function normally. Antioxidants work in several ways: they may reduce the energy of the free radical, stop the free radical from forming in the first place, or interrupt an oxidizing chain reaction to minimize the damage caused by free radicals. To reduce their energy load, free radicals react with certain chemicals in the body, and in the process, they interfere with the cells' ability to function normally. Free radicals are believed to play a vital role in more than 60 different health conditions, including the aging process, cancer, and atherosclerosis. However, vegetables may contain other antioxidants, such as proteins, ascorbate, β-carotene, α-tocopherol, and lycopene, which could play a role in the increase of the total antioxidant activity. Especially in the case of lycopene, recent studies have shown its potential antioxidant activity because it almost completely prevented oxidative damage to DNA and liver necrosis in rats (Wei and Lee 2002; Al Hamedan and Anfenan 2002).

In the present study, the levels of these enzymes were found to increase in groups where hepatotoxicity was induced using CCl_4. Biochemical findings showed that serum ALT and AST levels were significantly lower in the ISS and olive oil groups compared to the CCl_4 and CCl_4 + sesame fixed oil groups. Increased serum ALT and AST levels suggest that sesame cannot prevent liver cell damage (Goldfrank et al. 1998).

Table 1 Serum ALT, AST, ALP, and bilirubin levels by group

Group	ALT	AST	ALP	Bilirubin
ISS	43.50 ± 2.10	177.00 ± 15.59	408.16 ± 36.86	0.05 ± 0.01
Olive oil	71.42 ± 24.76	129.42 ± 14.37	510.62 ± 39.07	0.15 ± 0.09
CCl_4	1768 ± 841.06[a,*]	1877 ± 272.40[a,**]	173.62 ± 22.76[a,**]	0.50 ± 0.18[a,*]
CCl_4 + sesame oil	1687 ± 386.27[a,*]	2246 ± 526.25[a,**]	235.50 ± 13.06[a,**]	0.57 ± 0.04[a,*]

[a] Compared to the ISS and olive oil groups

* $p < 0.05$

** $p < 0.01$

Table 2 Levels of MDA, CAT, and GSH-Px in liver tissues

Group	MDA (nmol/g protein)	CAT (kU/g protein)	GSH-Px (U/g protein)
ISS	248.54 ± 14.89	120.09 ± 6.93	0.91 ± 0.06
Olive oil	254.31 ± 13.62	115.21 ± 7.24	1.04 ± 0.07
CCl_4	363.79 ± 31.35[a,b,**]	81.81 ± 5.96[a,b,**]	0.63 ± 0.05[a,b,**]
CCl_4 + sesame oil	358.42 ± 33.09[a,b,**]	93.07 ± 4.69[a,**,b,*]	0.75 ± 0.06[b,**]

[a] Compared to the ISS group

[b] Compared to the olive oil group

* $p < 0.05$

** $p < 0.01$

Most living organisms possess enzymatic and nonenzymatic defense systems against excess production of reactive oxygen species. However, different external factors, such as CCl_4, decrease the capability of such protective systems, resulting in disturbances of the redox equilibrium that is established in healthy conditions. We found that GSH-Px enzyme activity decreased in the liver in the CCl_4 and CCl_4 sesame fixed oil groups. GSH-Px is found in the mitochondria and cytosol and reduces organic hydroperoxides and hydrogen peroxide in a reaction that involves glutathione. Compared with other herbs, because of its excellent pharmacological function, licorice has been the single most widely used compound, either by itself or in formulations, applied to treat many diseases; in particular, it is used in China to treat liver diseases. Previous studies revealed that licorice extract could significantly reduce the elevated levels of lactate dehydrogenase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and MDA and increase the reduced levels of superoxide dismutase and GSH-Px by CCl_4 in carp (Yin et al. 2011). Glutathione is not only a cofactor for glutathione S-transferase but also serves as a reductant for GSH-Px, an enzyme involved in natural protection from free radicals, in addition to CAT (Kanno et al. 2004; Linder 1984). Decreased tissue GSH-Px enzyme activity in the CCl_4 group suggests that CCl_4 caused cell membrane damage, leading to an increase in MDA level, as well as damage to cellular components such as mitochondria, which contains superoxide dismutase and GSH-Px (Fridovich 1995). Sesame fixed oil treatment in CCl_4-induced liver toxicity did not increase the levels of antioxidants in liver tissue. Sesame seeds contain flavonoids, glycosides, saponins, anthocyanins, and reducing sugars (Awobajo et al. 2009). Flavonoids have been found to possess antioxidant activity by scavenging for free radicals (Takeoka and Dao 2003). An increase in the levels of AST and ALT is an indication of the degeneration process. Comparative histopathologic study of the livers of different groups of rats supported the notion that sesame fixed oil does not have a protective potency in CCl_4-induced liver damage. Various histopathologic changes such as vacuolization and increased lipid droplets in hepatocytes seen in CCl_4-treated rats may be due to oxidative damage caused by free radical generation (Cengiz et al. 2008). The pathologic changes induced by CCl_4 were not prevented in the sesame fixed oil treated group.

In conclusion, sesame did not show hepatoprotective effect in liver toxicity induced by CCl_4. However, more studies are needed to verify and clarify the roles of the oxidative stress and antioxidant enzyme activities in the pathogenesis of CCl_4-induced toxicity.

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