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ORIGINAL ARTICLE

Protective effect and antioxidant role of sweetgum (*Liquidambar orientalis*) oil against carbon tetrachloride-induced hepatotoxicity and oxidative stress in rats

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Abstract

Context: Sweetgum oil (SO) obtained from the *Liquidambar orientalis* Mill (Hamamelidaceae) tree has been used in Turkish folk medicine for centuries as an antiulcerigenic. Some studies have reported the antibacterial and antioxidant activities of SO; however, its effect on hepatic and oxidative stress complications is still unexplored.

Objective: This study investigates the hepatoprotective effect and the antioxidant role of SO against carbon tetrachloride (CCl_4) toxicity.

Materials and methods: The experiment included control, CCl_4, SO, and CCl_4 + SO treatment groups. Control and SO group rats were fed a diet without CCl_4. CCl_4 and CCl_4 + SO treatment groups received 0.5 mL/kg CCl_4 diluted in olive oil (1:1 dilution) intraperitonally injection twice per week. The CCl_4 + SO group also received 1000 mg/kg SO-supplemented feed for 50 d. Blood and tissue samples were used for the determination of hepatic damage serum biomarkers (HDSBs) levels, antioxidant defense system constituents (ADSCs), and malondialdehyde (MDA) contents. In addition, the liver was evaluated for histopathological changes.

Results: According to the results, the HDSBs levels of the CCl_4 group were significantly ($p < 0.05$) increased compared with the control, whereas the HDSB levels of the CCl_4 + SO group resulted in marked decreases ($p < 0.05$) compared with the CCl_4 group. In addition, the results showed that SO-supplemented diet restored the CCl_4-induced MDA and ADS towards to control. Hepatoprotection of SO is further substantiated by the almost normal histologic findings in the CCl_4 + SO group against degenerative changes in the CCl_4 group.

Discussion and conclusion: It was concluded that SO has a hepatoprotective effect and antioxidant capacity against CCl_4 toxicity.

Keywords

Antioxidant defense system, histopathology, malondialdehyde, serum enzymes

History

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Introduction

Epidemiological studies consistently show that increased consumption of plant-based antioxidant-rich foods, i.e., fruits, vegetables, whole grains, and nuts, is associated with the reduced risk of several chronic diseases. Clinical testings have demonstrated that a nut-containing diet, low in saturated fat and cholesterol, while high in poly and monounsaturated fatty acids, has a beneficial effect on plasma lipids and lipoproteins when compared with either a low fat or average American diet (Griel & Kris-Etherton, 2006).

Liquidambar orientalis Mill (Hamamelidaceae) is known for its balsamic exudations (Davis, 1982). *Liquidambar orientalis*, a medical plant known as ''Sığla Ağacı'' in Turkey, is wide spread in the southwestern coastal district of Turkey especially in Köyceğiz, Fethiye, Marmaris, and Ula. SO is a resinous exudate, locally named as ''Sığla Yağı'', obtained from the wounded trunk of *Liquidambar orientalis* (Davis, 1982; Hill, 1952). The plant material has been used in Turkish folk medicine for centuries for its antiulcerigenic (Gurbuz et al., 2013). Antibacterial activity of SO was determined previously by using *in vitro* techniques (Sagdic et al., 2005). In addition, the antioxidant activity of SO was determined by DPPH assay *in vitro* techniques (Topal et al., 2008). SO has *in vitro* antioxidant such as LDL protection effect (Andrikopoulos et al., 2003). SO has protective activity on many bacteria species, phytopathogenic fungi, and nematode (Kim et al., 2008). Further, SO decreased Alzheimer's disease generated in mice (Jeon et al., 2011) and human lymphocyte cell proliferation *in vitro* (Karadeniz et al., 2013). Previous researchers have found styrene and cinnamyl alcohol as the major components of storax (Fernandez, 2005; Kim et al., 2008). Fernandez (2005) determined that Asian storax has 70.4% styrene as a major component whereas Lee et al.

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(2009) determined that the major component of storax is 45.07% *trans*-cinnamyl alcohol. Such compounds can activate the phase II detoxification enzymes, which can remove toxic elements from our system. Therefore, consuming such phytochemicals is beneficial to human health. In addition, many natural compounds are now known to have a modulator role on physiological functions and biotransformation reactions involved in the detoxification process, thereby affording protection from cytotoxic, genotoxic, and metabolic actions of environmental toxicants (Saha & Das, 2003). Numerous studies demonstrate that a great number of medicinal and aromatic herbs, as well as fruits and leaves of some berry plants, biosynthesize phytochemicals possessing antioxidant activity and may be used as a natural source of free radical scavenging compounds (Sacchetti et al., 2005; Yu et al., 2005). In addition, a great number of spices and aromatic herbs contain chemical compounds exhibiting antioxidant properties. These properties are attributed to a variety of active phytochemicals including vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignans, simple phenols, and phenolic acids (Liu & Ng, 2000).

The reactive oxygen species (ROS) are known to play a major role in either the initiation or the progression of carcinogenesis by inducing oxidative stress (Gulcin, 2006). Peroxides and superoxide anion (O_2^-) produce cytotoxicity/genotoxicity in the cellular system (Gulcin, 2010; Gulcin et al., 2008). ROS and nitrogen species are formed in the human body and endogenous antioxidant defenses are not always sufficient to counteract them completely. A large number of studies support the hypothesis that oxidative damage to DNA, lipids, and proteins may contribute to the development of cardiovascular disease, cancer, and neurodegenerative diseases (Halliwell, 1996). Therefore, diet-derived antioxidants may be particularly important in protecting against chronic diseases (Halliwell, 1996; Vendemiale et al., 1999).

There is a growing interest of natural products in human diet, both due to the possible negative effects of synthetic food additives on human health and due to the increased consumer perception of this problem in recent years. As far as our literature survey could ascertain, no studies have so far been reported on hepatoprotective role and antioxidant capacity of the SO suplementation used in this study. The objective of this study was to determine healthful potentials of SO against CCl_4-induced oxidative stress by evaluating their *in vivo* hepatoprotective role and antioxidant role. Thus, in the present study, we have extensively studied the antioxidant activity of SO using *in vivo* models. For this aim, the treatments of SO were done orally in food-containing SO (1000 mg/kg). As the effect of the plants is well characterized in the nutrition, it is widely consumed by human in Turkish folk medicine. The preventive potential and antioxidant capacity of the SO was evaluated by liver histopathological changes, measuring HDSBs such as aspartate aminotransferase (AST), alanin aminotransferase (ALT), γ-glutamyltranspeptidase (GGT), lactate dehydrogenase (LDH), total proteine (TP), total cholesterol (TC), ADSCs such as reduced glutathione (GSH), glutathione reductase (GR), superoxide dismutase (SOD), glutathione *S*-transferase (GST), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) contents in various tissues of rats. The serum biomarkers were chosen due to their importance as an index of hepatotoxicity and kidney defunction. The antioxidant activity of SO on some phase II detoxification ADSCs, such as GSH, GR, SOD, GST, CAT, and GPx, and MDA contents in the various tissues was evaluated in the following experiment.

Materials and methods

Chemicals

Thiobarbituric acid (TBA), butylatedhydroxytoluene (BHT), trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), reduced glutathione (GSH), metphosphoric acid, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), trihydroxymethylaminomethane (Tris), 1-chloro-2,4-dinitrobenzene (CDNB), oxidized glutathione (GSSG), β-nicotinamide adenine dinucleotide phosphate reduced (NADPH), potassium dihydrogen phosphate (KH_2PO_4), carbon tetrachloride (tetrachloromethane, CCl_4), and sodium chloride (NaCl) of technical grade used in this study were supplied by Sigma Chemical Co. (St. Louis, MO). Kits for antioxidant enzymes analysis were supplied by Randox Laboratories Ltd (Crumlin, UK).

Animals

Wistar albino male rats 4–6 months of age with an average weight of 200–300 g were provided by the Experimental Animal Research Center, Yüzüncü Yıl University, and were housed in four groups, each group containing six rats. The animals were housed at $20 \pm 2^{\circ}C$ in a daily light/dark (~16/8 h) cycle. All animals were fed a group wheat–soybean-based diet and water *ad libitum* in stainless cages, and received human care according to the criteria outlined in the ''Guide for the Care and Use of Laboratory Animals'' prepared by the National Academy of Science and published by the National Institute of Health. The ethic regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments. This study was approved by The Ethic Committee of the Yüzüncü Yıl University with B.08.6.YÖK.2.YY.0.05.0.06/300-42 protocol number.

Preparation of foods

Briefly, SO was provided from a local producer in Köycegiz, a major SO-producing province of Muğla-Turkey. A voucher specimen is kept at the Department of Biology, Faculty of Sciences, Yuzuncu Yil University, Van, Turkey, for the future reference. SO was adjusted at the rate of 1000 mg/kg of rat food.

Experimental design

The rats were randomly divided into four groups, each containing six rats.

Group I (*control*): The rats received tap water and fed with standard pellet diet as *ad libitum*.

Group II (CCl_4): The rats received 0.5 mL/kg CCl_4 intraperitonally and fed with standard pellet diet *ad libitum*. The dose of 0.5-mL/kg CCl_4 diluted in olive oil (1:1 dilution)

intraperitonally injection which caused oxidative stress and hepatotoxicity on administeration (Kim et al., 2009).
Group III (1000 *mg/kg SO*): The rats received tap water and fed with 1000 mg/kg containing diet supplementation.
*Group IV (*0.5 *mL/kg BW* CCl_4 + 1000 *mg/kg SO*): The rats received 0.5 mL/kg CCl_4 diluted in olive oil (1:1 dilution) intraperitonally injection and fed with 1000 mg/kg SO-containing diet supplementation.

Preparation of tissues supernatant and erythrocyte pellets

At the end of the 50 d experiment, the rats were anesthetized by injecting ketamine (5 mg/100 g body weight) intraperitoneally.The blood samples were obtained from a cardiac puncture, using a syringe for the determination of serum marker enzyme levels and biochemical analysis. The serum samples were obtained by centrifuging blood samples at 4000 *g* for 15 min at 4°C, and enzyme levels were measured in these serum samples. For biochemical analysis, blood samples were put immediately into silicon disposable glass tubes with EDTA as an anticoagulant and were centrifuged at 4000 *g* for 15 min at 4°C and erythrocyte pellets were obtained. Then, the pellets were washed three times with physiological saline (0.9% NaCl).

The tissues such as brain, kidney, spleen, and liver were disected and put in petri dishes. After washing the tissues with physiological saline (0.9% NaCl), samples were taken and kept at −78°C during the analysis. The tissues were homogenized for 5 min in 50 mM ice-cold KH_2PO_4 solution (1:5 w/v) using stainless steel probe homogenizer (20 kHz frequency ultrasonic, Jencons Scientific Co., Bedfordshire, UK) and centrifuged at 7000 *g* for 15 min. All processes were carried out at 4°C. Supernatants and erythrocyte pellets were used to determine ADS constituents and MDA contents (Celik et al., 2009; Dogan & Celik, 2012; Yurt & Celik, 2011).

Biochemical analysis

The erythrocyte and tissue MDA concentrations were determined using the method described by Jain et al. (1989) based on the TBA reactivity. The erythrocyte and tissues GSH concentrations were measured using the method described by Beutler et al. (1963). GST was assayed by following the conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm as described by Mannervik and Guthenberg (1981). GR activity was assayed according to Carlberg and Mannervik (1975) as the absorbance of NADPH decreases at 340 nm. GPx activity was measured using a method described by Paglia and Valentine (1967). GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is converted into a reduced form with a concomitant oxidation of NADPH. The resulting decrease in absorbance at 340 nm can be measured using a spectrophotometer. SOD activity was measured at 505 nm by calculating inhibition percentage of formazan dye formation (McCord & Fridovich, 1969). CAT activity was determined using the method described by Aebi (1974) based on the rate of H_2O_2 consumption and the decrease in absorbance at 240 nm.

Measurement of enzyme levels

Serum marker enzyme activities such as AST, ALT, GGT, LDH enzyme levels, TC, and TP levels were measured by an auto-analyzer (BM/HITACHI-911, Hitachi Inc., Tokyo, Japan), using the kits.

Histopathological examination

Tissue samples from organs were collected in 10% neutralized formaldehyde after sacrification. After fixation, samples were dehydrated in alcohol, cleared in xylene, and embedded in paraffin wax. Sections were cut at 5 μm and stained with hematoxylin and eosin (Thermo Shandon, Cheshire, UK). Microscopically, degenerations in livers and kidneys were graded. Hepatocellular degeneration in livers is as follows: slight (degree 1): mild hepatocellular swelling due to hydropic degeneration and fatty changes only in centrilobular areas. Moderate (degree 2): clear hepatocellular swelling in both centrilobular and midzonal areas. Severe (degree 3): diffuse and severe hepatocellular swelling, cytoplasmic paleness, and rupture (Ortatatli et al., 2005).

Analysis of data

All data were expressed as mean ± standard deviation (SD). The statistical analyses were done using the Minitab 13 program for MS Windows (Minitab, State College, PA). Means and SDs were calculated according to the standard methods for all parameters. One-way analysis of variance (ANOVA) statistical test was used to determine the differences between means of the experimental groups accepting the significance level at $p \leq 0.05$.

Results

Following the exposure of experimental groups, the effects of CCl_4 and the SO-supplemented diet on liver damages index and antioxidative role were evaluated such as liver histopathological changes, HDSBs, ADSCs, and MDA contents of blood and various tissue samples from control and treated rats. The results of experiment showed that the treatment of rats with CCl_4 and SO-containing diet supplementation caused liver histopathological changes, changes in the level of serum biomarkers, MDA content, and ADSCs in comparison with control rats. According to the results, serum liver damage enzymes such as AST, ALT, GGT, and LDH levels of the CCl_4-treated group were significantly increased compared with the control group, whereas these enzyme levels of SO supplemented + CCl_4 (CCl_4 + SO-treated) resulted in marked decrease. There was no significant difference in the TP and TC levels between control and the other groups (Table 1). Also, administration of SO supplementation restored the CCl_4-induced imbalance between MDA and fluctuated ADSCs towards near normal particularly in erythrocytes, liver, brain, kidney, and spleen. In addition, the increased MDA content due to oxidative stress induced by CCl_4 in the all tissues was found to be decreasing in the tissues of the SO-treated groups. On the contrary, CCl_4 caused fluctuations in the antioxidant defence system which determines the level of oxidative stress condition in the rats. The healing effects of

Table 1. Effect of CCl_4 and SO oil on serum liver damage biomarker and body weight of rats (mean ± SD).

	Control mean ± SD		CCl_4 mean ± SD		SO mean ± SD		SO+CCl_4 mean ± SD	
Parameters	Beginning	Final	Beginning	Final	Beginning	Final	Beginning	Final
Body weight (g)	280.8 ± 21.5	344.8 ± 19.2*	277.0 ± 13.5	313.5 ± 17.4*	265.0 ± 38.9	350.3 ± 47.5*	240.0 ± 29.7	275.0 ± 21.9*
Food intake* (g/Week)	424.5 ± 50.2	459.0 ± 58.0	401.5 ± 26.2	439.0 ± 86.3	458.0 ± 59.4	463.0 ± 83.4	351.5 ± 77.1	444.5 ± 53.0
AST U/L	86.0 ± 11.7		347.3 ± 22.5[a]		91.3 ± 11.6		187.7 ± 17.5[cd]	
ALT U/L	33.5 ± 1.9		398.5 ± 22.3[a]		45.5 ± 6.5[b]		116.7 ± 7.6[cd]	
GGT U/L	0.9 ± 0.1		2.5 ± 0.6[a]		1.8 ± 0.3[d]		1.9 ± 0.4	
LDH U/L	1092.5 ± 77.6		1851.5 ± 165.5[a]		1389.3 ± 116.6[b]		1680.0 ± 88.9[cd]	
TC mg/dl	48.5 ± 6.6		37.5 ± 6.5[a]		59.7 ± 7.8[b]		48.0 ± 8.2[d]	
TP mg/dl	5.9 ± 0.4		5.5 ± 0.5		6.3 ± 0.3		5.8 ± 0.8	

Each value represents the mean ± SD. Significantly different from control rats at $p < 0.05$ (one way ANOVA).
*Significantly different from the beginning.
[a]CCl_4 significantly different from control.
[b]SO significantly different from control.
[c]SO + CCl_4 significantly different from control.
[d]SO + CCl_4 significantly different from CCl_4.

Table 2. Determination of antioxidant capacity of SO oil against CCl_4 exposed in rats.

Tissue	Parameters	Control X ± SD	CCl_4 X ± SD	SO X ± SD	SO+CCl_4 X ± SD
Erythrocyte	GSH mg/mL	75.21 ± 0.13	72.88 ± 0.42[a]	75.34 ± 0.41	75.73 ± 0.84[d]
	MDA nmol/mL	1.41 ± 0.20	2.92 ± 0.52[a]	1.75 ± 0.40	1.89 ± 0.34[cd]
	GST U/mL	14.92 ± 2.64	24.58 ± 4.67[a]	14.72 ± 2.58	15.03 ± 1.08[d]
	GPx U/mL	176.25 ± 30.29	160.88 ± 17.05	167.05 ± 19.27	163.05 ± 21.30
	GR U/mL	2.41 ± 0.56	2.13 ± 0.48	2.47 ± 0.52	2.39 ± 0.67
	SOD U/mL	2105.46 ± 90.08	2079.21 ± 82.97	2064.77 ± 35.29	2068.26 ± 24.31
	CAT U/mL	232.91 ± 32.91	244.98 ± 45.54	275.76 ± 50.42	246.2 ± ±33.08
Liver	GSH mg/g	71.2 ± ±2.93	67.63 ± 2.08[a]	67.01 ± 4.43	65.29 ± 1.42[cd]
	MDA nmol/g	41,01 ± 5.78	59.36 ± 5.56[a]	43.71 ± 8.32	57.85 ± 5.78[c]
	GST U/g	84.47 ± 8.37	108.14 ± 8.45[a]	83.24 ± 11.68	82.99 ± 12.73[d]
	GPx U/g	141.55 ± 15.80	114.45 ± 5.43[a]	135.20 ± 15.30	132.95 ± 7.68[d]
	GR U/g	35.42 ± 2.02	31.75 ± 2.25[a]	33.30 ± 3.03	33.06 ± 1.28[c]
	SOD U/g	2019.87 ± 73.67	1983.59 ± 761.36	1924.50 ± 35.83[b]	1961.30 ± 45.18
	CAT U/g	336.51 ± 34.70	397.56 ± 57.60[a]	312.69 ± 65.25	335.39 ± 32.53[d]
Brain	GSH mg/g	18.28 ± 1.63	19.66 ± 3.67	19.77 ± 3.88	19.98 ± 2.29
	MDA nmol/g	39.28 ± 4.17	70.91 ± 8.51[a]	38.10 ± 4.95	39.92 ± 2.61[d]
	GST U/g	57.23 ± 10.24	72.95 ± 11.35[a]	62.49 ± 8.34	65.05 ± 6.97
	GPx U/g	163.57 ± 30.46	154.79 ± 21.86	162.45 ± 21.83	165.14 ± 23.23
	GR U/g	32.83 ± 0.33	29.09 ± 3.17[a]	32.00 ± 2.83	31.51 ± 1.64
	SOD U/g	2165.24 ± 47.09	2161.02 ± 32.72	2083.89 ± 42.00[b]	2096.52 ± 11.70[cd]
	CAT U/g	2.16 ± 0.61	6.21 ± 1.36[a]	2.16 ± 017	2.37 ± 0.50
Kidney	GSH mg/g	70.52 ± 1.77	59.10 ± 2.89[a]	65.37 ± 4.91[b]	66.14 ± 3.49[cd]
	MDA nmol/g	115.06 ± 10.86	151.76 ± 12.32[a]	128.33 ± 19.18	140.32 ± 14.97[c]
	GST U/g	38.53 ± 4.64	61.40 ± 7.17[a]	40.51 ± 6.28	51.55 ± 5.70[cd]
	GPx U/g	157.70 ± 12.05	145.07 ± 18.72	158.82 ± 24.65	149.33 ± 24.45
	GR U/g	53.21 ± 4.33	46.38 ± 3.30[a]	52.03 ± 2.34	51.04 ± 2.69[d]
	SOD U/g	2147.03 ± 44.03	2100.49 ± 40.82	2147.10 ± 42.98	2113.18 ± 11.22
	CAT U/g	269.69 ± 48.16	308.78 ± 50.29	276.39 ± 41.93	279.18 ± 44.22
Spleen	GSH mg/g	72.75 ± 2.54	73.98 ± 1.55	74.18 ± 1.58	72.22 ± 2.05
	MDA nmol/g	158.67 ± 25.60	199.90 ± 14.15[a]	162.01 ± 21.81	161.15 ± 20.23[d]
	GST U/g	34.68 ± 3.99	47.20 ± 6.38[a]	35.57 ± 4.87	34.89 ± 3.64[d]
	GPx U/g	151.20 ± 16.11	143.42 ± 11.04	157.26 ± 27.11	155.50 ± 33.95
	GR U/g	12.10 ± 0.60	12.94 ± 2.37	11.74 ± 1.57	12.53 ± 1.27
	SOD U/g	2258.75 ± 63.55	2179.14 ± 56.44[a]	2140.58 ± 77.85[b]	2138.52 ± 93.50[c]
	CAT U/g	20.28 ± 1.39	34.43 ± 8.95[a]	21.12 ± 1.47	20.65 ± 3.03[d]

Each value represents the mean ± SD. Significantly different from control rats at $p<0.05$ (one-way ANOVA).
[a]CCl_4 significantly different from control.
[b]SO significantly different from control.
[c]SO+CCl_4 significantly different from control.
[d]SO+CCl_4 significantly different from CCl_4.

the SO could have been determined by these fluctuations (Table 2).

With regard to liver histopathological changes, the livers of rats from the control group had no noticable histopathological changes (Figure 1). Evident histopathological changes were observed consistently in livers of all rats of the CCl_4-treated group. The predominant lesions were focal disseminehydropic degeneration and coagulation necrosis in hepatocytes and

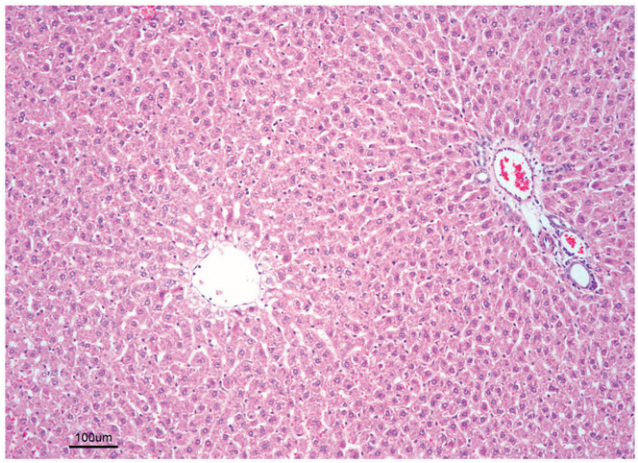


Figure 1. Control group: The liver of control group rats shows normal architecture of lobules. Hematoxylin–eosin, bar = 100 μm.

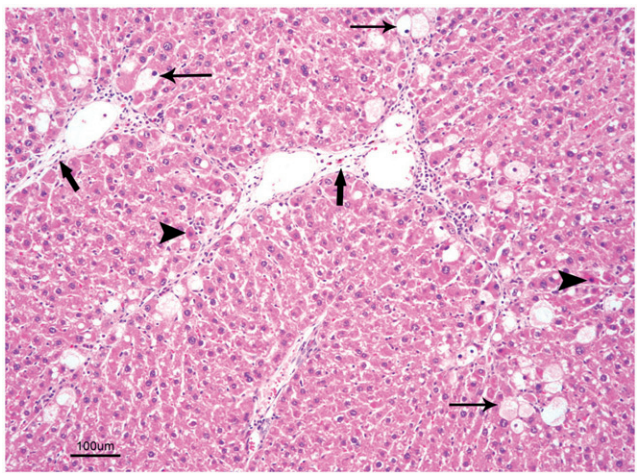


Figure 2. CCL_4-treated group: Hydropic degeneration (fine arrows) and coagulation necrosis (arrow heads) in the hepatocytes, and fibrous bands extending from periacinar regions to parenchyma. Hematoxylin–eosin, bar = 100 μm.

fibrosis (Figure 2). These changes were seen in almost in all lobules and were more pronounced in periacinar and periportal areas. Most hepatocytes in the degenerative and necrotic regions had pycnotic nuclei, and were so swollen that several cells had ruptured. The sinusoids were shrunken or completely plugged due to swollen hepatocytes. Fibrosis was also observed in periacinar and portal regions associated with lymphoid cell infiltrations. In addition, fine fibrous bands extending from periacinar regions to parenchyma were observed. In addition, some hepatocytes had moderate to severe cytoplasmic vacuolation, indicating fatty change. Because of all these alterations, the normal architecture of the hepatic parenchyma was distorted (Figure 2). SO supplementation ameliorated or reversed substantially the changes induced by CCl_4. The lesions in the liver of rats that received CCl_4 + SO were conspicuously less than those in the rats that received CCl_4 only. The livers from the rats in this group showed an occasional apoptotic and lightly degenerative hepatocytes, no extensive hydropic degeneration and necrotic changes, and fibrosis seen in any of the livers. Moreover, the

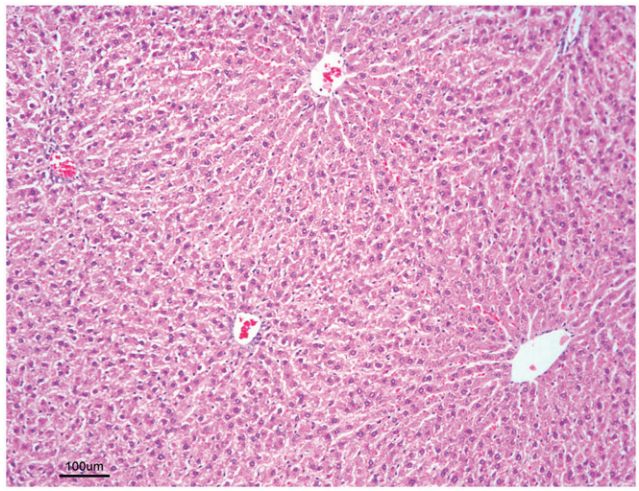


Figure 3. SW oil + CCL_4-treated group: Note that there is no hydropic degeneration and coagulation necrosis in the hepatocytes and fibrosis. Haematoxylin–eosin, bar = 100 μm.

architecture of lobules was not disrupted and the hepatocytes mostly had normal appearance (Figure 3).

Discussion

Today, natural products rather than synthetic drugs are used as treatment. Replacing the synthetic food additives with natural antioxidants is also increasing their efforts to change. Functional foods through their specific components or prophylactic or therapeutic effect have mainly antioxidant, anticancer, and antitumor substances. The present study was designed to testify the potential of hepatoprotective and antioxidant activities of SO oil by using CCl_4-induced oxidative stress and hepatotoxicity biomarkers in the rat model.

As shown in Table 1, CCl_4 caused a significant elevation in the levels of AST, ALT, GGT, and LDH in comparison with those of control rats whereas SO supplementation caused a significant decrease in the serum marker enzymes in comparison with those of CCl_4-treated rats. The reasons for such effect of CCl_4 and the SO supplementation were not certainly understood at present. However, it is known that several soluble enzymes in blood serum have been considered as indicators of the hepatic dysfunction and damage. Further, ALT and AST levels are also of value indicating the existence of liver diseases, as this enzyme is present in large quantities in the liver. ALT increases in serum when cellular degeneration or destruction occurs in this organ (Hassoun & Stohs, 1995). The increase in plasma LDH activity in CCl_4 group rats may be due to the hepatocellular necrosis leading to leakage of the enzyme to the blood stream (Wang & Zhai, 1988). Thus, when CCl_4 may lead to the release of these enzymes into plasma because of autolytic breakdown or cellular necrosis, the SO supplement imparts protection against CCl_4-induced oxidative injury that may result in the development of liver damage. In addition, LDH increase in SO group rats may be due to the hepatocellular permeability leading to leakage of the enzyme to the serum (Karadeniz et al., 2013). Although the treatment, materials of studies, and

the setting of studies are different, this result is in accordance with our result.

The present study demonstrated that the SO could have antioxidative role in rats. This was obvious from our observation that by the consequence of additional SO treatment *in vivo*, the concentration of MDA in the tissues differed from that of CCl_4-exposed group (Table 2). According to the obtained results, while the concentrations of MDA increased in the erythrocytes, liver, brain, kidney, and spleen of rats treated with CCl_4, the MDA contents of the tissue significantly decreased in the SO-supplemented group compared with the CCl_4 group. The reasons for such effect of CCl_4 and the SO additions are not understood until now. Nevertheless, the increased MDA content might have resulted from an increase of ROS as a result of stress condition in the rats with CCl_4 intoxication. Studies have shown that CCl_4 is potent liver oxidant and causes severe damage in vital organs like liver (Recknagel, 1983; Turkdogan et al., 2003). On one hand, the excessive generation of free radicals in CCl_4-induced liver damage will provoke a massive increase of lipid peroxidation in liver (Chidambara Murthy et al., 2005). On the other hand, it is known that the elevation of lipid peroxidation after consumption of some xenobiotics and following superoxide overproduction that produces dismutation singlet oxygen and H_2O_2 can be easily converted later into the reactive ·OH. Both single oxygen and OH radicals have a high potential to initiate free radicals chain reactions of lipid peroxidation. Further, it is known that ·OH can initiate lipid peroxidation in tissues and MDA is a major oxidating product of peroxidized polyunsaturated fatty acids (Halliwell, 1996). Increased MDA content is an important indicator of lipid peroxidation (Freeman & Crapo, 1981). Meanwhile, SOD, GR, GPX, GST, and CAT activities and GSH levels were fluctuated at significant levels in CCl_4-treated rats, generally, an increase whereas the administration of SO supplementation restored the CCl_4-induced imbalance between the fluctuated antioxidant systems to near normal levels, particularly in erythrocytes, liver, brain, kidney, and spleen tissues. Until now, the reasons for such effect of functions of plant supplementation are not understood. However, oxidative stress can affect the activities of protective enzymatic antioxidants in organisms exposed to CCl_4. The fluctuated ADS activities may reflect an adaptive change against CCl_4-induced ROS toxicity (Chidambara Murthy et al., 2005). However, the increased activities of ADS are known to serve as protective responses to eliminate xenobiotics (Smith & Litwack, 1980). Thus, the existence of an inducible antioxidant system may reflect an adaptation of organisms. Also, the reasons for such effect of addition of SO may be due to antioxidant activity of SO (Andrikopoulos et al., 2003; Topal et al., 2008). It have been reported that essential oils of SO were obtained by supercritical carbon dioxide (SC-CO_2) extraction and steam distillation. The antioxidant activities of SC-CO_2 extraction and steam distillation extracts were tested by means of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Essential oils extracted by SC-CO_2 and steam distillation showed different compositions in different species. SO essential oils obtained by SC-CO_2 extraction showed higher antioxidant activity than steam distillation extracts (Topal et al., 2008). Further, it has been reported that SO has *in vitro* LDL protection and antioxidant effect (Andrikopoulos et al., 2003).

CCl_4-induced rats showed severe and common degenerative and necrotic changes. The pathological findings, observed in the case of CCl_4 toxicity, are similar to previous reports (Chidambara Murthy et al., 2005; Recknagel, 1983; Turkdogan et al., 2003). These results of our investigation are in accordance with those of Turkdogan et al. (2003), Recknagel (1983), and Chidambara Murthy et al. (2005) who have reported that the CCl_4-induced hepatotoxicity and the potent effect of CCl_4 on the excessive generation of free radicals in CCl_4-induced liver damage which will provoke a massive increase of lipid peroxidation in liver. The histopathological changes of our findings, observed in the case of CCl_4, are similar to the previous reports. SO-treated rats showed significantly less histological abnormalities including hydropic degeneration, bile-duct proliferation, and periportal fibrosis when compared with SO-treated rats. Thereby, SO-protective role in countering the hepatotoxicity induced by CCl_4 arises. So far, no study examining the preventive role of SO-supplemented food *in vivo* has been performed on rats for histological abnormalities, serum biomarkers, antioxidant defense systems, and MDA content as a containing diet supplementation. Therefore, we had no chance to compare our results with the previous ones.

Conclusion

This study demonstrated that CCl_4 exposure gave rise to lipid peroxidation via inducing the ROS and had different effects on various tissues of rats on their antioxidant defense enzyme systems. This can result from adaptation of different qualities of cell physiology for different tissues. According to the data obtained by the survey of SO tissues of the liver, it can be concluded that there is a protective feature and it has antioxidant activity. SO has protective effects on liver, but to better understand the molecular mechanism and nature of this process and to reach a conclusion, more studies are needed. The protective and antioxidant effects of SO on liver need to be studied more on the rats *in vivo*.

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Declaration of interest

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References

Aebi H. (1974). Catalase. In: Bergemeyer HU, ed. *Methods of Enzymatic Analysis*. New York, London: Academic Press, 673–84.

Andrikopoulos NK, Kaliora AC, Assimopoulou AN, Papapeorgiou VP. (2003). Biological activity of some naturally occurring resins, gums and pigments against *in vitro* LDL oxidation. *Phytother Res* 17:501–7.

Beutler E, Dubon OB, Kelly M. (1963). Improved method for the determination of blood glutathione. *J Lab Clin Med* 61:882–8.

Carlberg I, Mannervik B. (1975). Purification and characterization of the flavoenzyme glutathione reductase from rat live. *J Biol Chem* 250: 5475–80.

Celik I, Temur A, Isık I. (2009). Hepatoprotective role and antioxidant capacity of pomegranate (*Punicagranatum* L.) flowers infusion against trichloroacetic acid-exposed in rats. *Food Chem Toxicol* 47: 145–9.

Chidambara Murthy KN, Rajesha J, Vanitha A, et al. (2005). Protective effect of *Dunaliella salina*, a marine micro alga, against carbon tetrachloride-induced hepatotoxicity in rats. *Hepatol Res* 33:313–19.

Davis PH. (1982). *Flora of Turkey and East Eagean Island*. Vol. 4. Edinburgh: Edinburgh University Press, 264.

Dogan A, Celik I. (2012). Hepatoprotective and antioxidant activities of grape seeds against ethanol-induced oxidative stress in rats. *Br J Nutr* 107:45–51.

Fernandez X. (2005). Chemical composition of the essential oils from Turkish and Honduras *Styrax*. *Flavour Frag J* 20:70–3.

Freeman BA, Crapo JD. (1981). Hyperoxia increases oxygen radical production in rat lung and lung mitochondria. *J Biol Chem* 256: 10986–92.

Griel AE, Kris-Etherton PM. (2006). Tree nuts and the lipid profile: A review of clinical studies. *Brit J Nutr* 96:S68–78.

Gulcin I. (2006). Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). *Toxicology* 217:213–20.

Gulcin I, Tel AZ, Kireci E. (2008). Antioxidant, antimicrobial, antifungal, and antiradical activities of *Cyclotrichium niveum* (Boiss.) Manden and Scheng. *Int J Food Propert* 11:450–71.

Gulcin I. (2010). Antioxidant properties of resveratrol: A structure–activity insight. *Innov Food Sci Emerg* 11:210–18.

Gurbuz I, Yesilada E, Demirci B, et al. (2013). Characterization of volatiles and anti-ulcerogenic effect of Turkish sweetgum balsam (*Styrax liquidus*). *J Ethnopharmacol* 148:332–6.

Halliwell B. (1996). Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free Radical Res* 25:57–74.

Hassoun EA, Stohs SJ. (1995). Comparative studies on oxidative stress as a mechanism for the fetotoxic of TCDD, endrin and lindane in C57BL/6J and DBA/2J mice. *Teratology* 51:186–92.

Hill AF. (1952). *Economic Botany: A Textbook of Useful Plants and Products*. 2nd ed. New York: McGraw Hill, 172p.

Jain SK, McVie R, Duett J, et al. (1989). Erythrocyte membrane lipid peroxidation and glycolylated hemoglobin in diabetes. *Diabetes* 38: 1539–43.

Jeon S, Bose S, Hur J, et al. (2011). A modified formulation of Chinese traditional medicine improves memory impairment and reduces Aβ level in the Tg-APPswe/PS1dE9 mouse model of Alzheimer's disease. *J Ethnopharmacol* 137:783–9.

Karadeniz B, Ulker Z, Alpsoy L. (2013). Genotoxic and cytotoxic effects of storax *in vitro*. *Toxicol Ind Health* 29:181–6.

Kim J, Seo SM, Lee SG, et al. (2008). Nematicidal activity of plant essential oils and components from coriander (*Coriandrum sativum*), Oriental sweetgum (*Liquidambar orientalis*), and valerian (*Valeriana wallichii)* essential oils against pine wood nematode (*Bursaphelenchus xylophilus*). *J Agric Food Chem* 56:7316–20.

Kim SH, Cheon HJ, Yun N, et al. (2009). Protective effect of a mixture of *Aloe vera* and *Silybum marianum* against carbon tetrachloride-induced acute hepatotoxicity and liver fibrosis. *J Pharmacol Sci* 109: 119–27.

Lee YS, Kim J, Lee SG, et al. (2009). Effects of plant essential oils and components from Oriental sweetgum (*Liquidambar orientalis*) on growth and morphogenesis of three phytopathogenic fungi. *Pestic Biochem Phys* 93:138–43.

Liu F, Ng TB. (2000). Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sci* 66:725–35.

Mannervik B, Guthenberg C. (1981). Glutathione *S*-transferase (Human Plasenta). *Method Enzymol* 77:231–5.

McCord JM, Fridovich I. (1969). Superoxide dismutase, an enzymatic function for erythrocuprein (hemocuprein). *J Biol Chem* 244:6049–53.

Ortatatli M, Oguz H, Hatipoglu F, Karaman M. (2005). Evaluation of pathological changes in broilers during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure. *Res Vet Sci* 78:61–8.

Paglia DE, Valentine WN. (1967). Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 70:158–69.

Recknagel RO. (1983). A new direction in the study of carbon tetrachloride hepatotoxicity. *Life Sci* 33:401–8.

Sacchetti G, Maietti S, Muzzoli M, et al. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chem* 9: 621–32.

Sagdic O, Ozkan G, Ozcan M, Ozcelik SA. (2005). Study on inhibitory effects of Siğla tree (*Liquidambar orientalis* Mill. var. *orientalis*) storax against several bacteria. *Phytother Res* 19:549–51.

Saha P, Das S. (2003). Regulation of hazardous exposure by protective exposure modulation of phase II detoxification and lipid peroxidation by *Camellia sinensis* and *Swertia chirata*. *Teratogen Carcin Mut* 1: 313–22.

Smith GJ, Litwack G. (1980). Roles of ligandin and the glutathione *S*-transferases in binding steroid metabolites, carcinogens and other compounds. *Rev Biochem Toxicol* 2:1–47.

Topal U, Sasaki M, Goto M, Otles S. (2008). Chemical compositions and antioxidant properties of essential oils from nine species of Turkish plants obtained by supercritical carbon dioxide extraction and steam distillation. *Int J Food Sci Nutr* 59:619–34.

Turkdogan MK, Ozbek H, Yener Z, et al. (2003). The role of *Urtica dioica* and *Nigella sativa* in the prevention of carbon tetrachloride induced hepatotoxicity in rats. *Phytother Res* 17:942–6.

Vendemiale G, Grattagliano I, Altomare E. (1999). An update on the role of free radicals and antioxidant defense in human disease. *Int J Clin Lab Res* 29:49–55.

Wang X, Zhai W. (1988). Cellular and biochemical factors in bronchoalveolar lavage fluids of rats exposed to fenvalerate. *Zhongguo Yaolixue Yu Dulixue Zoghi* 2:271–6.

Yu LL, Zhou KK, Parry J. (2005). Antioxidant properties of cold pressed black caraway, carrot, cranberry, and hemp seed oils. *Food Chem* 91: 723–9.

Yurt B, Celik I. (2011). Hepatoprotective effect and antioxidant role of sun, sulphited-dried apricot (*Prunus armeniaca* L.) and its kernel against ethanol-induced oxidative stress in rats. *Food Chem Toxicol* 49:508–13.