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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 antulcerigenic. 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 (CCl₄) toxicity.

Materials and methods: The experiment included control, CCl₄, SO, and CCl₄ + SO treatment groups. Control and SO group rats were fed a diet without CCl₄. CCl₄ and CCl₄ + SO treatment groups received 0.5 mL/kg CCl₄ diluted in olive oil (1:1 dilution) intraperitoneally injection twice per week. The CCl₄ + 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 CCl₄ group were significantly (*p* < 0.05) increased compared with the control, whereas the HDSB levels of the CCl₄ + SO group resulted in marked decreases (*p* < 0.05) compared with the CCl₄ group. In addition, the results showed that SO-supplemented diet restored the CCl₄-induced MDA and ADS towards to control. Hepatoprotection of SO is further substantiated by the almost normal histologic findings in the CCl₄ + SO group against degenerative changes in the CCl₄ group.

Discussion and conclusion: It was concluded that SO has a hepatoprotective effect and antioxidant capacity against CCl₄ 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 antulcerigenic (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.
(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 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 (O2•−) 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 supplementation used in this study. The objective of this study was to determine healthful potentials of SO against CCl4-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 protein (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), metaphosphoric 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 (KH2PO4), carbon tetrachloride (tetra-chloromethane, CCl4), 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 ± 2°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 Ethnic 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 Kütcegiz, 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 (CCl4):** The rats received 0.5 mL/kg CCl4 intraperitoneally and fed with standard pellet diet ad libitum. The dose of 0.5-mL/kg CCl4 diluted in olive oil (1:1 dilution)
intraperitonally injection which caused oxidative stress and hepatotoxicity on administration (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 CCl4 + 1000 mg/kg SO): The rats received 0.5 mL/kg CCl4 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 50d 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 intraperitonally injection. The blood samples were obtained from a 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).

**Histopathological examination**

Tissue samples from organs were collected in 10% neutralized formaldehyde after sacrifice. After fixation, samples were placed in 70% ethyl alcohol and stained with hematoxylin and eosin (Thermo Shandon, Cheshire, UK).

**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 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 H2O2 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.

**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 ≤ 0.05.

**Results**

Following the exposure of experimental groups, the effects of CCl4 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 CCl4 and SO-supplemented 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 CCl4-treated group were significantly increased compared with the control group, whereas these enzyme levels of SO supplemented + CCl4 (CCl4 + 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 CCl4-induced imbalance between MDA and fluctuated ADSCs towards normal particularly in erythrocytes, liver, brain, kidney, and spleen. In addition, the increased MDA content due to oxidative stress induced by CCl4 in the all tissues was found to be decreasing in the tissues of the SO-treated groups. On the contrary, CCl4 caused fluctuations in the antioxidant defence system which determines the level of oxidative stress condition in the rats. The healing effects of
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 noticeable histopathological changes (Figure 1). Evident histopathological changes were observed consistently in livers of all rats of the CCl4-treated group. The predominant lesions were focal disseminated hydropic degeneration and coagulation necrosis in hepatocytes and

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Parameters</th>
<th>Control mean ± SD</th>
<th>CCl4 mean ± SD</th>
<th>SO mean ± SD</th>
<th>SO+CCl4 mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte</td>
<td>GSH mg/mL</td>
<td>75.21 ± 0.13</td>
<td>72.88 ± 0.42a</td>
<td>75.34 ± 0.41</td>
<td>75.73 ± 0.84d</td>
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<tr>
<td></td>
<td>MDA nmol/mL</td>
<td>1.41 ± 0.20</td>
<td>2.92 ± 0.52a</td>
<td>1.75 ± 0.40</td>
<td>1.89 ± 0.34cd</td>
</tr>
<tr>
<td></td>
<td>GST U/mL</td>
<td>14.92 ± 2.64</td>
<td>24.58 ± 4.67a</td>
<td>14.72 ± 2.58</td>
<td>15.03 ± 1.08d</td>
</tr>
<tr>
<td></td>
<td>GPx U/mL</td>
<td>176.25 ± 30.29</td>
<td>160.88 ± 17.05</td>
<td>167.05 ± 19.27</td>
<td>163.05 ± 21.30</td>
</tr>
<tr>
<td></td>
<td>GR U/mL</td>
<td>2.41 ± 0.56</td>
<td>2.13 ± 0.48</td>
<td>2.47 ± 0.52</td>
<td>2.39 ± 0.67</td>
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<tr>
<td></td>
<td>SOD U/mL</td>
<td>2105.46 ± 90.08</td>
<td>2079.21 ± 82.97</td>
<td>2064.77 ± 35.29</td>
<td>2068.26 ± 24.31</td>
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<tr>
<td></td>
<td>CAT U/mL</td>
<td>232.91 ± 32.91</td>
<td>244.98 ± 45.54</td>
<td>275.76 ± 50.42</td>
<td>246.2 ± 33.08</td>
</tr>
<tr>
<td>Liver</td>
<td>GSH mg/g</td>
<td>71.2 ± 2.93</td>
<td>67.63 ± 2.08a</td>
<td>67.01 ± 4.43</td>
<td>65.29 ± 1.42cd</td>
</tr>
<tr>
<td></td>
<td>MDA nmol/g</td>
<td>41.01 ± 5.78</td>
<td>59.36 ± 5.56a</td>
<td>43.71 ± 8.32</td>
<td>57.85 ± 5.78</td>
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<tr>
<td></td>
<td>GST U/g</td>
<td>84.47 ± 8.37</td>
<td>108.14 ± 8.45a</td>
<td>83.24 ± 11.68</td>
<td>82.99 ± 12.73d</td>
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<tr>
<td></td>
<td>GPx U/g</td>
<td>141.55 ± 15.80</td>
<td>114.45 ± 5.43a</td>
<td>135.20 ± 15.30</td>
<td>132.95 ± 7.68d</td>
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<tr>
<td></td>
<td>GR U/g</td>
<td>35.42 ± 2.02</td>
<td>31.75 ± 2.25a</td>
<td>33.30 ± 3.03</td>
<td>33.06 ± 1.28</td>
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<tr>
<td></td>
<td>SOD U/g</td>
<td>2019.87 ± 73.67</td>
<td>1983.59 ± 76.16</td>
<td>1924.50 ± 35.83</td>
<td>1961.30 ± 45.18</td>
</tr>
<tr>
<td></td>
<td>CAT U/g</td>
<td>336.51 ± 34.70</td>
<td>397.56 ± 57.60a</td>
<td>326.96 ± 65.25</td>
<td>335.39 ± 52.33</td>
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<tr>
<td>Brain</td>
<td>GSH mg/g</td>
<td>18.28 ± 1.63</td>
<td>19.66 ± 3.67</td>
<td>19.77 ± 3.88</td>
<td>19.98 ± 2.29</td>
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<tr>
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<td>MDA nmol/g</td>
<td>41.29 ± 4.17</td>
<td>70.91 ± 8.51a</td>
<td>43.71 ± 8.32</td>
<td>57.85 ± 5.78</td>
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<td>61.40 ± 11.35a</td>
<td>52.66 ± 11.68</td>
<td>51.55 ± 6.97</td>
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<td>GPx U/g</td>
<td>163.57 ± 30.46</td>
<td>154.79 ± 21.86</td>
<td>162.45 ± 21.83</td>
<td>165.14 ± 23.23</td>
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<td>GR U/g</td>
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<td>52.03 ± 2.34</td>
<td>51.04 ± 2.69</td>
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<tr>
<td></td>
<td>SOD U/g</td>
<td>2147.03 ± 44.03</td>
<td>2161.02 ± 45.02</td>
<td>2140.58 ± 77.85b</td>
<td>2138.52 ± 93.50c</td>
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<tr>
<td></td>
<td>CAT U/g</td>
<td>268.69 ± 48.16</td>
<td>308.78 ± 50.29</td>
<td>276.39 ± 41.93</td>
<td>279.18 ± 44.22</td>
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<tr>
<td>Kidney</td>
<td>GSH mg/g</td>
<td>70.52 ± 1.77</td>
<td>59.10 ± 2.89a</td>
<td>59.7 ± 4.43</td>
<td>59.7 ± 2.89</td>
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<tr>
<td></td>
<td>MDA nmol/g</td>
<td>115.06 ± 10.86</td>
<td>151.76 ± 12.32a</td>
<td>128.33 ± 19.18</td>
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<td>61.40 ± 7.17a</td>
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<tr>
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<td>GPx U/g</td>
<td>157.70 ± 12.05</td>
<td>145.07 ± 18.72</td>
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<td>149.33 ± 24.45</td>
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<td>GR U/g</td>
<td>32.83 ± 0.33</td>
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<td>32.00 ± 0.23</td>
<td>31.51 ± 1.64</td>
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<td>279.18 ± 44.22</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD. Significantly different from control rats at p < 0.05 (one way ANOVA).

aCCl4 significantly different from control.
bSO significantly different from control.
cSO+CCl4 significantly different from control.
dSO+CCl4 significantly different from CCl4.

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 noticeable histopathological changes (Figure 1). Evident histopathological changes were observed consistently in livers of all rats of the CCl4-treated group. The predominant lesions were focal disseminated hydropic degeneration and coagulation necrosis in hepatocytes and
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 pyknotic 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, 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 CCl₄. The lesions in the liver of rats that received CCl₄ + SO were conspicuously less than those in the rats that received CCl₄ 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 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 CCl₄-induced oxidative stress and hepatotoxicity biomarkers in the rat model.

As shown in Table 1, CCl₄ 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 CCl₄-treated rats. The reasons for such effect of CCl₄ 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 CCl₄ group rats may be due to the hepatocellular necrosis leading to leakage of the enzyme to the blood stream (Wang & Zhai, 1988). Thus, when CCl₄ may lead to the release of these enzymes into plasma because of autolytic breakdown or cellular necrosis, the SO supplement imparts protection against CCl₄-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

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

**Figure 2.** CCl₄-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.

**Figure 3.** SW oil + CCl₄-treated group: Note that there is no hydropic degeneration and coagulation necrosis in the hepatocytes and fibrosis. Haematoxylin–eosin, bar = 100 μm.
lipid peroxidation. Further, it is known that have a high potential to initiate free radicals chain reactions of were fluctuated at significant levels in CCl4-treated rats, SOD, GR, GPX, GST, and CAT activities and GSH levels 1996). Increased MDA content is an important indicator of product of peroxidized polyunsaturated fatty acids (Halliwell, lipid peroxidation in tissues and MDA is a major oxidating/Cl4 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 polysaturated 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 CCl4-treated rats, generally, an increase whereas the administration of SO supplementation restored the CCl4-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 CCl4. The fluctuated ADS activities may reflect an adaptive change against CCl4-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 has been reported that essential oils of SO were obtained by supercritical carbon dioxide (SC-CO2) extraction and steam distillation. The antioxidant activities of SC-CO2 extraction and steam distillation extracts were tested by means of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Essential oils extracted by SC-CO2 and steam distillation showed different compositions in different species. SO essential oils obtained by SC-CO2 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). CCl4-induced rats showed severe and common degenerative and necrotic changes. The pathological findings, observed in the case of CCl4 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 CCl4-induced hepatotoxicity and the potent effect of CCl4 on the excessive generation of free radicals in CCl4-induced liver damage which will provoke a massive increase of lipid peroxidation in liver. The histo-pathological changes of our findings, observed in the case of CCl4, 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 CCl4 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 CCl4 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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