THE PROTECTIVE EFFECT OF AN AQUEOUS EXTRACT FROM *SMILAX EXCELSA* L. AGAINST CARBON TETRACHLORIDE-INDUCED LIVER INJURY IN RATS

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Abstract

**Background:** Because reactive oxygen species (ROS) contribute to the pathogenesis of various acute and chronic liver diseases, dietary antioxidants and drugs from herbal origins have been proved to be beneficial as therapeutic agents in reversing hepatotoxicity and oxidative stress. The objective of this study was to investigate the protective effect of an aqueous extract from *Smilax excelsa* L. Shoots and leaves against acute CCl₄-induced liver injury as well as the changes in antioxidative defense system in female wistar albino rats.

**Materials and Methods:** *S. excelsa* extract was administered orally in doses of 100, 200 and 400 mg/kg body weight, once daily for 9 days. Acute hepatic toxicity was induced by intraperitoneal injection of CCl₄ (1 ml/kg) on the 10th day. 24 h after CCl₄ intoxication, biochemical and histopathological analyses were undertaken on sera and liver tissues.

**Results:** CCl₄ challenge caused significant increases in the activities of liver enzymes as well as the levels of bilirubin, malondialdehyde and nitric oxide, while total serum protein levels and antioxidant defense system parameters were reduced significantly compared to the normal group. Administration of *S. excelsa* extract at a dose of 400 mg/kg resulted in a suppression of CCl₄-induced lipid peroxidation and altered oxidative stress parameters to nearly normal values in comparison to CCl₄-treated rats. Nevertheless the extract did not reduce the extent of CCl₄-induced mild liver injury, as seen by the histopathology of liver damage.

**Conclusion:** The results of this study suggest that *S. excelsa* could protect the liver tissues against CCl₄-induced oxidative stress probably by increasing antioxidative defense activities.

**Key words:** Antioxidant enzymes, carbon tetrachloride, liver injury, *Smilax excelsa*, hepatoprotective activity

Introduction

For centuries, medicinal plants have been used as remedies for human diseases because they contain phytoconstituents of therapeutic value. These phytochemicals may be useful in preventing oxidative stress-related diseases by fortifying antioxidant defense mechanisms or by acting as protective factors.

Liver diseases remain one of the serious health problems, but medical treatments for these diseases are often difficult to handle and have limited efficacy. For these reasons, developing drugs for liver diseases from plants used in traditional medicine, may lead to improved therapies (Kim et al., 2009).

Based on reports regarding the presence of polyphenols in shoots and leaves of *Smilax excelsa*, a plant species from the Black Sea region of Turkey, and the assumption that the traditional uses of *S. excelsa* might be partially attributed to its antioxidant activity (Ozsoy et al., 2008), the aim of this study was to evaluate the potential protective effect of *S. excelsa* against the oxidative stress induced by carbon tetrachloride (CCl₄) in the rat liver.

Material and Methods

**Plant Material**

*S. excelsa* L. shoots and leaves were collected in September from Istanbul in Turkey and identified by Prof. Dr. Kerim Alpinar from the Faculty of Pharmacy, Istanbul University. The leaves were separated from the other parts, washed in running tap water and dried at room temperature. Voucher specimens are deposited in the herbarium of the Faculty of Pharmacy, Istanbul University (ISTE); herbarium code number: ISTE 81928. The dried shoots and leaves were manually ground to a fine powder before extraction.

**Preparation of the Extract**

80 g of ground dried shoots and leaves were extracted with boiling water (2000 ml) for 15 min while stirring. The extracts were filtered and evaporated to dryness under reduced pressure at 40°C in a rotary evaporator (Buchi R210). The yield was 25 g. Appropriate dilutions were made before each experiment.

**Animals**

The experimental protocol described in the present study was approved by the Animal Assays Ethics Comittee of Istanbul University (HADYEK). Female wistar rats weighing 200-240 g were supplied from Istanbul University Institute of Experimental Medicine (DETAIE). Animals were acclimatized to their environment for one week prior to experimentation, housed in a room with a 12 h light/dark cycle at about 22°C and fed on standard diet with *ad libitum* access to drinking water.
Induction of Hepatic Injury

The animals were divided into 5 groups each containing 6 animals. Group 1 (Control), which served as normal control, received water and basal diet for 10 days. Group 2 (CCl₄) which served as toxin control, received water and basal diet for 10 days and was treated with CCl₄ (1 ml/kg body weight in 20 % olive oil, v/v) on the 10 th day. Three different groups of rats, groups 3, 4 and 5 were separately treated with three different doses of the leaf extract of *S. excelsa* – 100, 200 and 400 mg/kg body weight respectively, once daily for 9 days. 24 h prior to intraperitoneal administration of CCl₄ on the 10th day.

24 h after CCl₄ intoxication, the rats were anesthetized and blood samples were taken from each rat through direct intracardiac intervention and centrifuged at 3,000 x g for 10 min to separate the serum. Immediately after collecting the blood samples, the livers were excised, rinsed in ice-cold normal saline solution followed by ice-cold 0.15 M potassium phosphate buffer (pH 7.4), blotted, dried, and weighed. Part of liver tissues, 10 % w/v homogenates were prepared in ice-cold 0.15 M potassium phosphate buffer using a homogenator (Art-MICCRA D-1), and centrifuged at 13,000 rpm for 5 min at 4°C (Megafluge Hereaus1.0R). The supernatants, thus obtained were used for biochemical studies.

Biochemical Studies

Assessment of Serum Biochemical Parameters and Lipid Peroxidation

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated as prescribed by the International Federation of Clinical Chemistry (Bergmeyer, 1980; Bergmeyer et al., 1986). A unit of enzyme activity was defined as 1 μmol of NADH or NAD formed per minute under the assay conditions. Alkaline phosphatase (ALP) activity was determined in serum using p-nitrophenyl phosphate as a substrate and one unit of enzyme activity was defined as μmol p-nitrophenol formed per minute (Walter and Schütt, 1974). Serum bilirubin was estimated by the diazo method (Balistrieti and Rej, 1994). The formation of LPO products was assayed by the measurement of thiobarbituric acid reactive substances (TBARS) levels on the basis of malondialdehyde (MDA) reaction with thiobarbituric acid at 532 nm, according to Buege and Aust (1978). The values of thiobarbituric acid related substances (TBARS) were calculated using an extinction coefficient of 1.56x10² m⁻¹cm⁻¹ and expressed as nmol of MDA/g wet weight.

Serum nitric oxide (NO) was measured in terms of its products, nitrite and nitrate, by the method of Griess modified by (Fidler, 1977). Samples, after deproteinisation, were incubated with cadmium beads in order to convert nitrate to nitrite, and nitrite levels were determined spectrophotometrically at 540 nm by using a colorimetric assay based on the Griess reaction. NO products were expressed as μmoles/l serum.

Assessment of Antioxidant Status via Antioxidant Enzymes in Liver Homogenates

Superoxide dismutase (SOD) activity was assayed by the method described by Aruoma et al. (1989). Results were expressed as U/mg protein. One unit of SOD inhibits the rate of increase in absorbance at 560 nm by 50 %, under the conditions of the assay. The protein contents were determined by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard. Catalase (CAT) activity was measured by the method of Aebi (1974) and expressed as μmol H₂O₂/mg protein. Glutathione reductase (GR) activity was determined following the oxidation of NADPH at 340 nm as described by Carlberg and Mannervik (1985). The activity of glutathione peroxidase (GPx) was measured using a coupled enzyme assay system linked with GR as described by Lawrence and Burk (1976). GR and GPx activities were expressed as nmol of NADPH oxidized per min per mg protein using the molar extinction coefficient of 6.22x10⁴ m⁻¹cm⁻¹ for NADPH at 340 nm. Glutathione-S-transferase (GST) activity was assayed spectrophotometrically using 1-chloro-2,4-dinitrobenzene as substrate as described by Habig and Jacoby (1981). Specific activity was expressed as nmol conjugate formed/min/mg protein using a molar extinction coefficient of 9.6 m⁻¹cm⁻¹.

Tissue myeloperoxidase (MPO) levels were measured using a method described by Singbartl et al. (2000) with some modifications. 1 U of activity was defined as the amount of enzyme that consumes 1 μmol H₂O₂/min at 25°C. Results were expressed as units of MPO per g wet tissue.

The protein carbonyl content (PCC) was assayed by the modification of the procedure described by Reznick and Packer (1994), using dinitrophenylhydrazine (DNPH). Results were expressed as nmol of protein carbonyl per mg of protein using a molar extinction coefficient of 22,000 m⁻¹cm⁻¹ for DNPH.

The level of the reactive oxygen species (ROS) scavenger, reduced glutathione (GSH) was measured in both liver and serum by the enzymic recycling procedure using 5,5’-dithiobis-(2-nitrobenzoic acid) (DTNB) and glutathione reductase (DTNB-GSSG reductase recycling assay) as described by Anderson (1985). The oxidized glutathione (GSSG) level was measured using the same method but in presence of 2-vinylpyridine. The results were expressed as μmol of GSH or GSSG/g of wet weight.

Histopathological Assay

Dissected liver tissues were taken immediately after sacrifice and fixed in Bouin’s solution for histopathological examinations. The tissues were dehydrated and embedded in paraffin. The paraffin sections of 5 μm thickness were stained with Hematoxylin-Eosin (H&E) and Masson’s trichrome stain (Masson). All sections were examined under Olympus-CX 41 light microscope.

Statistical Analysis

Biochemical results were evaluated using an unpaired t-test and ANOVA variance analysis using the NCSS statistical computer package. The values were expressed as mean ± SD. Analysis between control and experimental groups was performed using the Mann-Whitney test. p<0.05 was considered as significant.
Results
Assessment of Serum Biochemical Parameters and Lipid Peroxidation

Results showed in Table 1, revealed that pretreatment of rats with an aqueous extract of *S. excelsa* at 100, 200 and 400 mg/kg doses, significantly attenuated the rise in serum level of AST, ALT and total bilirubin, provoked by CCl₄. Treatment with *S. excelsa* aqueous extract with three doses, prior to the CCl₄ administration decreased also the elevation of serum lipid peroxidation (as shown by the reduction of the enhanced MDA levels). Increased NO levels in the CCl₄ group were significantly reduced by *S. excelsa* at 200 and 400 mg/kg doses.

Table 1: Effect of pretreatment with *Smilax excelsa* L. leaf aqueous extract after CCl₄ intoxication, on serum biochemical parameters and lipid peroxidation in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein g/dl</th>
<th>AST U/L</th>
<th>ALT U/L</th>
<th>ALP U/L</th>
<th>Bilirubin mg/dl</th>
<th>MDA μmol/L</th>
<th>NO μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.5 ± 0.9</td>
<td>44.8 ± 16.7</td>
<td>17.5 ± 5.70</td>
<td>261.6 ± 50.0</td>
<td>0.2 ± 0.04</td>
<td>1.4 ± 0.3</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>CCl₄</td>
<td>7.2 ± 1.2</td>
<td>1616.5 ± 53.3</td>
<td>613.6 ± 105.9</td>
<td>226.7 ± 80.7</td>
<td>1.6 ± 0.3</td>
<td>3.4 ± 0.4</td>
<td>34.1 ± 14.6</td>
</tr>
<tr>
<td>S.excelsa100+CCl₄</td>
<td>7.2 ± 0.8</td>
<td>1026.5 ± 87.8</td>
<td>386.0 ± 83.7</td>
<td>234.1 ± 22.0</td>
<td>0.9 ± 0.5</td>
<td>2.5 ± 0.4</td>
<td>21.1 ± 4.7</td>
</tr>
<tr>
<td>S.excelsa200+CCl₄</td>
<td>6.9 ± 1.1</td>
<td>203.9 ± 55.4</td>
<td>64.5 ± 12.9</td>
<td>231.7 ± 23.9</td>
<td>1.1 ± 0.3</td>
<td>2.7 ± 0.6</td>
<td>4.8 ± 3.5</td>
</tr>
<tr>
<td>S.excelsa400+CCl₄</td>
<td>7.3 ± 0.6</td>
<td>203.9 ± 55.4</td>
<td>64.5 ± 12.9</td>
<td>231.7 ± 23.9</td>
<td>1.1 ± 0.3</td>
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</tr>
</tbody>
</table>

Table 2: Effect of pretreatment with *Smilax excelsa* L. leaf aqueous extract after CCl₄ intoxication, on liver tissue antioxidant enzymes in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein mg/ml</th>
<th>SOD U/mg protein</th>
<th>CAT U/mg protein</th>
<th>GR U/mg protein</th>
<th>GPx U/mg protein</th>
<th>GST U/mg protein</th>
<th>MPO U/g tissue</th>
<th>PCC nmol PC/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.0 ± 1.2</td>
<td>1.65 ± 0.2</td>
<td>85.9 ± 15.1</td>
<td>52.4 ± 9.4</td>
<td>1120.8 ± 99.8</td>
<td>785.9 ± 75.9</td>
<td>1.1 ± 0.4</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>CCl₄</td>
<td>7.6 ± 1.1</td>
<td>2.46 ± 0.7</td>
<td>60.0 ± 8.8</td>
<td>31.5 ± 4.1</td>
<td>826.8 ± 47.7</td>
<td>561.1 ± 42.7</td>
<td>30.9 ± 7.4</td>
<td>23.3 ± 3.9</td>
</tr>
<tr>
<td>S.excelsa100+CCl₄</td>
<td>6.9 ± 1.1</td>
<td>2.72 ± 0.2</td>
<td>66.1 ± 31.6</td>
<td>48.3 ± 9.3</td>
<td>1103.6 ± 88.1</td>
<td>877.4 ± 134.2</td>
<td>30.8 ± 12.9</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>S.excelsa200+CCl₄</td>
<td>6.5 ± 0.4</td>
<td>2.54 ± 0.1</td>
<td>82.3 ± 13.5</td>
<td>45.1 ± 10.9</td>
<td>1208.4 ± 100</td>
<td>545.4 ± 116.1</td>
<td>35.1 ± 9.8</td>
<td>3.6 ± 1.5</td>
</tr>
<tr>
<td>S.excelsa400+CCl₄</td>
<td>8.8 ± 1.6</td>
<td>1.75 ± 0.4</td>
<td>84.4 ± 11.7</td>
<td>62.0 ± 10.9</td>
<td>1169.0 ± 109.7</td>
<td>757.5 ± 174.5</td>
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</tbody>
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Table 2: Effect of pretreatment with *Smilax excelsa* L. leaf aqueous extract after CCl₄ intoxication, on liver tissue antioxidant enzymes in rats

P<sub>ANOVA</sub> = 0.811, 0.001, 0.001, 0.0001, 0.001, 0.0001, 0.0001, 0.0001

P<sub>ANOVA</sub> 0.001 vs Control; P<sub>ANOVA</sub> < 0.0001 vs CCl₄; P<sub>ANOVA</sub> < 0.0001 vs CCl₄; P<sub>ANOVA</sub> < 0.0001 vs CCl₄; P<sub>ANOVA</sub> < 0.0001 vs Control; P<sub>ANOVA</sub> < 0.0001 vs CCl₄; Results are given as mean ± SD.

**Key**

AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; MDA = malondialdehyde; NO = nitric oxide

Assessment of Antioxidant Status via Antioxidant Enzymes in Liver Homogenates

Pretreatment with *S. excelsa* leaf extract restored the impaired parameters of SOD, CAT, GR, GPx, GST, MPO activities as well as the levels of PCC in liver tissue (Table 2). Tissue lipid peroxidation levels measured as MDA and the levels of the glutathione system (GSH, GSSG and GSSG/GSH ratio) were also significantly reversed by pretreatment with *S. excelsa* extracts at the three doses given (Table 3).

Table 3. Effect of pretreatment with *Smilax excelsa* L. leaf aqueous extract after CCl₄ intoxication, on liver tissue antioxidant enzymes in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein mg/ml</th>
<th>SOD U/mg protein</th>
<th>CAT U/mg protein</th>
<th>GR U/mg protein</th>
<th>GPx U/mg protein</th>
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<td>66.1 ± 31.6</td>
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P<sub>ANOVA</sub> = 0.001 vs Control; P<sub>ANOVA</sub> < 0.0001 vs CCl₄; P<sub>ANOVA</sub> < 0.0001 vs CCl₄; P<sub>ANOVA</sub> < 0.0001 vs Control; P<sub>ANOVA</sub> < 0.0001 vs CCl₄; Results are given as mean ± SD.

**Key**

SOD = superoxide dismutase; CAT = catalase; GR = glutathione reductase; GPx = glutathione peroxidase; GST= glutathione-S-transferase; PCC = protein carbonyl content; MPO = myeloperoxidase
### Table 3: Effect of pretreatment with *Smilax excelsa* L. leaf aqueous extract after CCl₄ intoxication, on the formation of TBARS and glutathione system in the liver tissue in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (\text{nmol/g})</th>
<th>GSH (\text{µmol/g})</th>
<th>GSSG (\text{µmol/g})</th>
<th>GSSG/GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.7 ± 4.5</td>
<td>3.9 ± 0.3</td>
<td>0.33 ± 0.04</td>
<td>0.091 ± 0.007</td>
</tr>
<tr>
<td>CCl₄</td>
<td>60.0 ± 10.3*</td>
<td>2.2 ± 0.2*</td>
<td>0.61 ± 0.16*</td>
<td>0.156 ± 0.02*</td>
</tr>
<tr>
<td><em>S. excelsa</em>₁₀₀-CCl₄</td>
<td>40.2 ± 6.0*</td>
<td>3.5 ± 0.9*</td>
<td>0.39 ± 0.09*</td>
<td>0.091 ± 0.02*</td>
</tr>
<tr>
<td><em>S. excelsa</em>₂₀₀-CCl₄</td>
<td>41 ± 6.3*</td>
<td>2.3 ± 0.4*</td>
<td>0.27 ± 0.04*</td>
<td>0.136 ± 0.01*</td>
</tr>
<tr>
<td><em>S. excelsa</em>₄₀₀-CCl₄</td>
<td>35.1 ± 0.5*</td>
<td>3.9 ± 0.7*</td>
<td>0.25 ± 0.007*</td>
<td>0.095 ± 0.02*</td>
</tr>
</tbody>
</table>

\*\(p<0.0001\) vs control; \*\(p<0.05\) vs control; \*\(p<0.005\) vs CCl₄; \*\(p<0.01\) vs control; \*\(p<0.0001\) vs CCl₄; \*\(p<0.05\) vs CCl₄; \*\(p<0.005\) vs control; results are given as mean ± SD.

**Key**  
GSH = reduced glutathione; GSSG = oxidized glutathione; MDA = malondialdehyde

### Histopathological Assessment

The results of the light microscopic observations showed that the administration of CCl₄ produces moderate degenerative changes such as necrosis, vacuolisation and hypertrophy in hepatocytes, hyperemia, mononuclear cell infiltration, necrotic areas, increase in the dark eosinophilic cells, the rupturing in the central vein of liver tissues in the rats. The degeneration was observed around central veins in the liver tissues of the rats given CCl₄ (Fig. 1 B and C) in comparison to normal control rats (Fig. 1 A). In addition, the foamy cells around central veins were noticed in some animals of the experimental group. Similar degenerative changes were also detected in rats given *S. excelsa* extract at 100 mg/kg (Fig. 1 D), 200 mg/kg (Fig. 1 E) or 400 mg/kg (Fig. 1 F) dose together with CCl₄. The degeneration was observed to portal area from central vein in the groups given *S. excelsa* extracts. The foamy cells around central veins were also observed still in some animals of experimental groups given *S. excelsa* extracts (Fig. 1 D, E, F). Pretreatment with *Smilax excelsa* extracts in the different doses did not show a protective effect in the liver tissue of CCl₄–treated rats, morphologically.

**Figure 1:** Histological appearance of liver tissue of a control rat (A). Light micrographs of CCl₄ group (B and C), *S. excelsa* (100 mg/kg)-treated CCl₄ group (D), *S. excelsa* (200 mg/kg)-treated CCl₄ group (E), and *S. excelsa* (400 mg/kg)-treated CCl₄ group (F). Hyperemia (●) in central vein (CV), hypertrophy (▲) and vacuolisation (○) in hepatocytes, necrotic area (●), mononuclear cell infiltration (MCI), dark eosinophilic cells (●), the rupturing of the central vein (→). H&E, original magnification x200.
Discussion

Detoxification of xenobiotic and toxic substances is an important hepatic function, in the course of which large amounts of reactive oxygen intermediates are produced. The excretion of many drugs depends on the integrity of the liver and cytochrome P-450 system (Balistreri and Rej, 1994).

In this study the experimental intoxication induced by CCl₄ (Mukai et al., 2002) was used for modeling liver injury in rats. Hepatotoxicity is connected with severe impairment of cell protection mechanisms. All acute injuries and/or necrotic lesions in the liver primarily cause a marked rise in the levels of the aminotransferases, AST and ALT. Pretreatment of rats with an aqueous extract of S. excelsa attenuated the CCl₄-induced rise in serum level of transaminases and total serum bilirubin, confirming the protective effect of the extract. Hepatic damage was also supported by the elevation of lipid peroxidation (expressed as MDA) levels. Treatment with S. excelsa produced a significant attenuation in CCl₄-induced increase in lipid peroxidation.

SOD is the first antioxidant enzyme to deal with oxidative free radicals by accelerating the dismutation of superoxide to hydrogen peroxide, while CAT is a peroxisomal heme protein that catalyses the removal of hydrogen peroxide formed during the reaction catalyzed by SOD. Thus, SOD and CAT act as mutually supportive antioxidative enzymes, and provide protective defense against ROS. The increase in the activity of SOD in the liver of CCl₄-treated rats may be due to the increased accumulation of superoxide radicals, which could further stimulate lipid peroxidation. Superoxide is inactivated by SOD, the only enzyme known to use a free radical as a substrate. Any increase in SOD activity is beneficial in the event of increased free radical generation. However, a rise in SOD activity, without a concomitant rise in the activity of CAT and/or GPx might be detrimental since SOD generates hydrogen peroxide as a metabolite, which must be scavenged by CAT or GPx. Thus, a simultaneous increase in catalase and/or GPx activity, as observed in S. excelsa treated groups, is essential for an overall beneficial effect of an increase in SOD activity (Banerjee et al., 2002).

Glutathione antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. This system consisted of reduced glutathione and an array of functionally-related enzymes, one of which is glutathione reductase, responsible for the regeneration of GSH. On the other hand, GPx and GST work together with GSH in the decomposition of hydrogen peroxide or other organic hydroperoxides. If the injury is associated with a generalized increase in tissue oxidative stress, it might well be reflected in the changes in tissue glutathione antioxidant system (Mak et al., 1996). In this study, glutathione system data also suggested high cell damage induced by CCl₄ in the CCl₄-treated groups. Twenty four hours after CCl₄ administration, hepatic GSH levels decreased significantly in CCl₄ group, while GSSG levels were elevated. The decrease in the GSSG/GSH ratio, a potent indicator of tissue oxidative stress, in groups pretreated with 100, 200 and 400 mg/kg S. excelsa aqueous extract, is also in accordance with the suggestion that free radical damage is decreased in the tissues of S. excelsa treated groups.

GST is a phase II enzyme that plays a key role in cellular detoxification of xenobiotics, electrophiles and ROS through their conjugation to GSH. The decrease of hepatic GST might well be an adaptive response to the increased production of oxidized glutathione in tissues of CCl₄-treated rats (Akerboom and Sies, 1990).

Given that GPx works together with GSH in the decomposition of hydroperoxides generated from free radical-mediated reactions, the induction of GPx activity and the accompanied increase in liver glutathione level may merely be a manifest of antioxidant response to the increased oxidative stress (Mak et al., 1996). Our findings of the concomitant increase in hepatic GPx and GST activities and GSH levels in rats treated with S. excelsa aqueous extract (in the group treated with 400 mg/kg aqueous extract this increase was almost close to control group) are consistent with these observations.

Kupffer cells MPO may be an important source of oxidative damage during acute and chronic liver damage (Brown et al., 2001). The hepatic levels of MPO in the CCl₄-treated groups were higher than in the normal control group, and there was a significant difference between CCl₄-treated and control groups. Treatment with 400 mg/kg S. excelsa extract significantly reduced the levels of MPO in tissue. Thus, the results indicate that treatment with S. excelsa extract causes significant suppression of neutrophil infiltration.

Measurement of protein carbonyls has been used as a sensitive assay for oxidative damage to proteins, partly because it measures several different consequences of oxidative damage (Halliwell and Whiteman, 2004). The results showed increased protein damage by ROS in CCl₄ groups. The treatment with 400 mg/kg S. excelsa aqueous extract might have modified the oxidative status by increasing antioxidant defense.

As NO has a range of effects on a variety of biological processes, it is unclear if the rise of NO serum levels is beneficial or detrimental in the liver injury induced by hepatotoxins (Kim et al., 2009). In this study the hepatotoxin has made a significant increase in serum NO levels which has been significantly decreased at 200 and 400 mg/kg extract doses.

In view of these observations, we hypothesised that S. excelsa extract would have protective effects in an in vivo induced free radical-mediated hepatotoxicity by attenuating the CCl₄-mediated oxidative stress through increased production of free radical derivatives, as evidenced by the decreased MDA, increased activity of phase II detoxifying enzyme (GST) and antioxidative enzymes (SOD, CAT, GPx and GR), and protection against LPO and GSH depletion. Accordingly, in our other report S. excelsa shoots and leaves were proved as nephroprotective under in vivo conditions (Ozsoy et al., 2013). Our results were consistent with earlier studies which have also shown that antioxidants could comprehensively ameliorate the oxidative damage by increasing antioxidative potential (Yuan et al., 2014; Gu et al., 2014).

Despite this conclusion, the improvement in antioxidant enzymes system seem to have been incapable of neutralizing increased CCl₄ toxicity seen in the histopathology of liver cells. In the present study, the results of histological observations showed that CCl₄ leads to moderate degenerative changes mainly necrosis, hyperemia, and vacuolar degeneration in the liver of rats. Our histopathological findings were in agreement with the degenerative structural changes reported to occur in liver tissues after the application of CCl₄ (Jaramillo-Juárez et al., 2008; El Denshawy et al., 2011). In this study, pretreatment of the aqueous extracts of S. excelsa shoots and leaves did not diminished CCl₄-induced degenerative injury in liver tissue, morphologically. It was stated that sometimes there could not be strict correlation between histological findings and serum transaminase values (Kallai et al. 1964) and that entire histologic spectrum of liver disease can be seen in individuals with normal ALT values (Mofrad et al., 2003). It was also reported that ALT values do not sometimes correlate with parameters of apoptosis and oxidative stress and that the severity of the disease could be only determined by liver biopsy (Canbakan et al., 2010). Accordingly, the results of the present study showed that,

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at early stages of liver injury, biochemical findings could not always correlate with histological examination and that an antioxidant
effect could not always be along with a restored tissue structure.

In conclusion, our study demonstrated that the aqueous extract of the shoots and leaves of S. excelsa could increase
antioxidative defense system against CCl4-induced oxidative stress.

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References

Physiological Functions, 45-55, CRC Press, Florida.
5. Baltistrelli, W.F. and Rej, R. (1994). Liver function. Determination of total and conjugated bilirubin in serum (Jeddrassik and Gröf technique). In,
approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate
AOAC), 60: 594-599.
Trad. CAM, 11: 46-52.
Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology, 37: 1286-1292.
54.
Trad. CAM, 11: 85-91.