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Original Research

Hepatoprotective properties for *Salvia cryptantha* extract on carbon tetrachloride-induced liver injury

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Abstract: The present study was designed to determine the possible hepatoprotective effects of *Salvia cryptantha* (black weed) plant extract against carbon tetrachloride (CCl4)-induced hepatic injury in rats. Animals were grouped as follows: control group (Group I), CCl4 group (Group II), olive oil group (Group III), CCl4 + *S. cryphantha* 200 mg/kg group (Group IV), and CCl4 + *S. cryptantha* 400mg/kg group (Group V). Rats were injected intraperitoneally with CCl4 diluted in olive oil (50% v/v) at a dose of 1ml/kg body weight. Bax and Caspase3 were determined by immunohistochemical staining, while apoptotic index was evaluated using TUNEL assay. Total mRNA was isolated from liver tissues, and the levels of BCL2, Caspase3, SOD, CAT, and glutathione peroxidase (GPx) were determined by using PCR, while MDA level were determined using a colorimetric assay. The antioxidant and anti-apoptotic gene transcripts were decreased in all of the control and treatment groups, while Caspase3 levels were not statistically different. The *S. cryptantha* plant extract treatment was also found to improve SOD, GPx, and catalase levels, while reducing the serum levels of MDA. The extract of *S. cryptantha* supplementation had a protective effect against CCl4-induced liver damage. *S. cryptantha* extract as a supplement may be useful as a hepato-protective agent to combat the toxic effects caused by CCl4 and other chemicals.

Key words: Carbon tetrachloride; TUNEL; Caspase3; BCL2; Liver injury; *Salvia cryptantha.*

Introduction

Many important steps in modern medicine have been taken for the treatment of many diseases. These treatments strategies are supported by alternative medical approaches. Interestingly, novel therapeutic approaches using compounds derived from plants for the treatment of cancer, neurological diseases, liver diseases, and many other diseases are currently underway. It is known that liver is the main organ for whole body detoxification (1) and liver diseases have high mortality rates worldwide. In the literature, there are many studies concerning liver disease such as alcoholism (2), nonalcoholic fatty liver disease (3), chronic liver disease (4), polycystic liver disease (5), cancer, and HIV. The increase in the number of studies on these diseases is based on its widespread occurrence in the society. Despite the frequent appearance of these symptoms, high mortality and morbidity, medical treatment is still limited. In addition to the lack of treatment options, synthetic drugs used for the treatment of liver diseases have been reported to show significant side effects in patients (2).

The need for the development of novel therapeutic approaches and medicines with minimal side effects are a priority in the treatment of liver diseases. Acute and chronic injuries to the liver are usually caused by ROS (reactive oxygen species) (6). Over-production of ROS damage primary metabolites such as carbohydrates, proteins, lipids, and nucleic acids. In parallel, enzymes such as SOD, catalase, glutathione peroxidase, as well as non-enzymatic phenolic and terpenic antioxidant substances, have a protective effect on the primary metabolites in cells through the reduction of free radical damage. Although many antioxidant substances are synthetically available, they have been shown to have serious side effects. Therefore, identification and characterization of novel hepato-protective medicinal plants with high antioxidant capacity is of great interest.

Apoptosis and necrosis may occur in cells due to injuries caused by xenobiotic substances and drugs (7). In the apoptosis mechanism, the apoptotic Bax protein is often synthesized more than BcL-2, and is localized around the mitochondria. The cytochrome C is released into the cytosol and activates the caspase pathway. This process continues until the activation of Caspase3 (8). Interestingly, plants showing liver protection activity have been shown to reduce apoptotic damage in the liver (9).

It is known that many medical plants, especially those with antioxidant properties, have large bioactive capacities (10-12). Like lipid peroxides, plants with antioxidant activity can detoxify molecules that cause significant damage to the liver tissue and reduce the levels of antioxidant enzymes. This, in turn, protects the body against damage caused by ROS. In addition, there are several reports in the literature that demonstrate the role of phytochemicals and their antioxidant/hepato-protective effects (13,14).

One of the most important families of medicinal plants that contain phytochemicalsis Lamiaceae, which include phenolics and terpenes. Plants belonging to this family have been used in the treatment of many diseases among the people for centuries. The genus *Salvia*, which has ~700 species and is represented by 88 species and 45 endemics in Turkish flora, is one of the most widespread members of the Lamiaceae family (15). Hepato-protective studies using species belonging to the Lamiaceae family are available but limited (11,12) *S. cryptantha* is an aromatic herb belonging to Lamiaceae. The wild growing species is endemic to the rocky lands and chalky hills of Turkey, and is widely distributed from 700 to 2500 m (16). Antioxidant and phytochemical studies with this species have shown that these plants have strong antioxidant activities (17, 18).

Interestingly, there are no reports available that have investigated the hepato-protective nature of *S. cryptantha* and its effects on antioxidant levels and apoptotic/anti-apoptotic genes. Therefore, we aimed to investigate the hepato-protective effects of the *S. cryptantha* extract against CCl4-induced liver damage using histopathology and molecular-based methods.

Materials and Methods

Sources of plants

For the collection of *S. cryptantha,* the "*Flora of Turkey and the East Aegean Island*" was used (19). *S. cryptantha* was collected at the flowering stage from Adana (Akçatekir)-Turkey (May 26, 2013). The identification of *S. cryptantha* was performed by Dr. Mustafa Pehlivan and numbered individually (Voucher no: MPH2013-5).

Preparation of plant extracts

The aerial parts *S. cryptantha* were air dried in a dark room before the extraction procedures were initiated. Air-dried *S. cryptantha* samples were pulverized using a mortar and pestle. For the extraction, 40 grams of each plant powder was used and the standard Soxhlet extraction method was followed. For preparation of extraction, the plant material placed in the Soxhlet apparatus was treated with Ethanol for 6 hours at boiling temperature. After extraction, organic solvents were eliminated by using a rotary evaporator (ISOLAB, Germany) at 50 °C, and the remaining solid material was preserved at + 4 ˚C until use (20).

Animals and study groups

This study was performed after approval by the local ethical committee (issue number). A total of 30 Sprague-Dawley male rats of 265-280 grams 10 weeks of age were used in the present study. Animals in all groups were weighed before starting the experiment and weekly weights were noted. Rats were injected intraperitoneally with CCl4 diluted in olive oil (50% v/v) at a dose of 1ml/kg bodyweight. Animals were divided randomly into five groups (each group bearing six rats) as follows: control group (Group I), Group II (CCl4), olive oil group (Group III); CCl4 + *S. cryptantha* 200mg/kg group (Group IV) and CCl4 + *S. cryptantha* 400mg/kg group (Group V). Group I was not subjected to any application; Group II was administered 5 mL/kg olive oil orally every day during the 7 days; Group III was administered 1 mL/kg CCl4 intraperitoneally (no treatment); Group IV was given 200mg/kg *S. cryptantha* plant extract orally every day for 7 days; Group V was given 400mg /kg *S. cryptantha* plant extract orally every day for 7 days. Generally, in literature, researcher who tested protective activity of Salvia species determined the doses in ranges of 50-1000 mg/kg. Doses used in the study were calculated according to Yue et al. (21).

Animals were kept at standard environmental conditions (23 ± 2 °C temperature, humidity 55 ± 10%, and 12 hours light / dark), and fed by standard methods of water and food. At the end of the study, rats in all groups were anesthetized with xylazine (10 mg / kg) and ketamine (75 mg / kg) and decapitated.

Immunohistochemical evaluations

Tissue sections (5–6 μm) were cut from paraffin blocks and transferred to microscope slides. The tissues were deparaffinized and subjected to antigen retrieval using citrate buffer, pH 6 and a microwave. After retrieval, the tissues were cooled for 20 minutes at room temperature and washed with PBS (Phosphate Buffered Saline), P4417, Sigma-Aldrich, USA) for three times for 5 minutes and then incubated with hydrogen peroxide block solution for 5 minutes to prevent endogenous peroxidase activity (Hydrogen Peroxide Block, TA-125-HP, Lab Vision Corporation, USA). After washing with PBS for 3 x 5 minutes, the tissue was incubated for 5 minutes with Ultra V Block (TA-125-UB, Lab Vision Corporation, USA) solution to prevent background staining and then incubated with 1/200 diluted primary antibodies (Bax mouse monoclonal IgG, Santa Cruz Biotechnology, sc–7480, California, USA; and caspase 3 Rabbit polyclonal IgG, Abcam**,** ab2302, London, UK) at room temperature for 60 minutes. The tissues were washed three times with PBS for 5 minutes followed by secondary antibody incubation (biotinylated Goat Anti-Poliyvalent (anti-mouse / rabbit IgG) TP–125-BN, Lab Vision Corporation, USA) at room temperature for 30 minutes. The tissues were then washed three times with PBS for 5 minutes and incubated with Streptavidin Peroxidase (TS-125-HR, Lab Vision Corporation, USA) at room temperature for 30 minutes and allowed

Table 1. Primers pairs used in the amplification of selected genes.

GAPDH	Sense	TCCCTCAAGATTGTCAGCAA
	Antisense	AGATCCACAACGGATACATT
SOD1	Sense	GCAGAAGGCAAGCGGTGAAC
	Antisense	TAGCAGGACAGCAGATGAGT
CAT	Sense	GCGAATGGAGAGGCAGTGTAC
	Antisense	GAGTGAGTTGTCTTCATTAGCACTG
GPX1	Sense	GCTCACCCGCTCTTTACCTT
	Antisense	GATGTCGATGGTGCGAAAGC
CASP3	Sense	GAGCTTGGAACGCGAAGAAA
	Antisense	TAACCGGGTGCGGTAGAGTA
BCL2	Sense	CAGCATGCGACCTCTGTTTG
	Antisense	CTCACTTGTGGCCCAGGTAT

to sit in PBS. Subsequently, tissues were treated with 3-amino-9-ethylcarbazole solution (AEC) Substrate + AEC Chromogen (AEC Substrate, TA-015 and HAS, AEC Chromogen, TA-002-HAC, Lab Vision Corporation, USA) and, after the image signal was received, it was washed simultaneously with PBS. Tissues were then subjected to hematoxylin counter staining and rinsed with PBS and dH2O and mounted with mounting solution (Large Volume Vision Mount, TA-125-UG, Lab Vision Corporation, USA). In the evaluation of the immunohistochemical staining; a histological score was generated based on the prevalence (0.1: <25%, 0.4: 26-50%, 0.6: 51-75%, and 0.9: 76-100%) and severity (0: no, +0.5: very less, +1: less, +2: moderate, and +3: severe) of immunoreactivity, (histological score = prevalence x severity).

TUNEL method

Tissue sections (5–6 µm) were subjected to TUNEL staining and apoptotic cells identification using ApopTag Plus Peroxidase *In Situ* Apoptosis Detection Kit (Chemicon, cat no: S7101, USA) in accordance with the manufacturer's instructions. Apoptotic cells were visualized using diaminobenzidine (DAB) substrate. Sections were counter stained with Harris hematoxylin and mounted with the appropriate mounting solution. In the evolution of TUNEL assay, nuclei stained blue with Harris hematoxylin was evaluated as normal, whereas nuclei stained brown were evaluated as apoptotic. Under 10X magnification, at least 500 normal and apoptotic cells were counted in sections. The apoptotic index (AI) was calculated by the ratio of apoptotic cells to total (normal + apoptotic) cells.

Isolation of total RNA from tissues and preparation of cDNA samples

Isolation of total RNA was achieved by using GeneJET RNA Purification Kit (Thermo Fisher Scientific) and manufacturer's recommended protocols were followed. Briefly, tissue samples were maintained at room temperature prior to isolation and sections of tissues were collected into sterile tubes. For the homogenization of samples, the Heidolph Silent Crusher-S homogenizator instrument was used. The quantity and quality of the obtained RNAs was determined by using NanoDrop 2000 (Thermo Fisher Scientific) instrument. Later, RNA samples were converted to cDNA by using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). cDNA synthesis reactions were performed with the Sensquest Thermoblock 96 PCR instrument. Primers pairs used for the expression analysis of genes are presented in Table 1.

Analysis of gene expression levels by quantitative PCR (Real-Time PCR) method

Gene expression analysis was achieved by using Maxima SYBR Green/ROX qPCR Master Mix (2X) (Thermo Fisher Scientific) according to the manufacturer's instructions and the reactions were held in QIAGEN Rotor-Gene Q Real-time PCR (QIAGEN Sample & Assay Technologies, Germany).

Determination of malondialdehyde level

The levels of malondialdehyde (MDA) were measured spectrophotometrically using a UV-VIS spectrophotometer (Hitachi, Japan) at 532 nm according to previously published method (22). MDA levels are expressed as mmol/mg protein concentrations using calibrating curves.

Statistical analysis

The obtained data were determined as mean ± standard deviation. For the statistical analysis SPSS (version 22) program was used. Analysis was performed using a One-way ANOVA and posthoc tukey test. A p-value of less than 0.05 was considered statistically significant.

Results

Bax, Caspase3, and TUNEL immunoreactivity were observed in hepatocellular tissues (red arrow) under a light microscope. Bax Immunoreactivity in the control liver (Fig. 1a) and olive oil applied liver group (Fig.1b) were similar to each other, while Bax immunoreactivity was significantly increased in the CCl4 (Fig. 1c) group as compared with the control group ($p<0.05$). Also, a statistically significant decrease was observed in the CCl4 + *S. cryptantha* 200 (Fig. 1d) and CCl4 + *S. cryptantha* 400 (Fig. 1e, the most reduction in Bax expression was observed with *S. cryptantha* 400 mg/kg supplementation) groups as compared to CCl4 group ($p<0.05$). When Caspase3 Immunoreactivity of liver was evaluated, the control (Fig 1f) and olive oil (Fig.1g) groups were not significantly different.

Caspase3 immunoreactivity of liver tissues was significantly increased in the CCl4 group (Fig. 1h) as

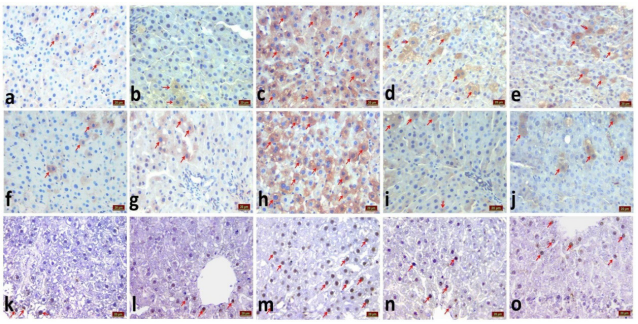

Figure 1. Results of Bax, Caspase3, and TUNEL staining in the liver after CCl4 and *S. cryptantha* extract application. Bax, caspase, and TUNEL staining positivity was observed in hepatocytes (red arrow) in the liver. Bax (a: control; b: Olive oil; c: CCl4; d: 200 mg/kg *S. cryptantha* extract + CCl4 and e: 400 mg/kg *S. cryptantha* extract + CCl4); Caspase 3 (f: control; g: olive oil; h: CCl4; i: 200 mg/kg *S. cryptantha* extract + CCl4 and j: 400 mg/kg *S. cryptantha* extract + CCl4), and TUNEL (k: control; l: olive oil; m:CCl4; n: 200 mg/kg *S. cryptantha* extract + CCl4, and o: 400 mg/kg *S. cryptantha* extract + CCl4).

compared with the control group (Fig. 1f; $p<0.05$). Additionally, a statistically significant decrease was determined in Caspase3 Immunoreactivity of liver tissue of the CCl4 + *S. cryptantha* 200 (Fig. 1i) and CCl4 + *S. cryptantha* 400 (Fig.1j) groups as compared to CCl4 group ($p<0.05$). Caspase3 Immunoreactivity of liver tissue was most significant in terms of reduction with the *S. cryptantha* 400 mg/kg supplementation after the CCl4 induced hepatic injury. TUNEL assay was also used to determine cells undergoing apoptosis. As a result, TUNEL positivity was observed in hepatocytes (red arrow) in the liver and TUNEL positivity of liver tissues was also found to be similar in the control (Fig. 1k) and the olive oil (Fig. 1l) groups. Furthermore, TUNEL positivity of liver tissues was found to be increased with the CCl4 group (Fig. 1m) as compared to control group (Fig.1k; $p<0.05$) and with extract supplementation. When *S. cryptantha* 200 mg/kg (Fig. 1n) and *S. cryptantha* 400 mg/kg (Fig.1o) extract were given to the rats after CCl4 liver injury, it was observed that expression of TUNEL in the liver tissues was statistically and significantly decreased. The highest rate of decrease in the TUNEL expression of liver tissues were observed with *S. cryptantha* 400 mg/kg supplementations as compared to CCl4 group ($p<0.05$) and *S. cryptantha* 200 mg/kg supplementations group. The histological scoring of Bax and Caspase3 immunoreactivity and apoptotic index (%) of all groups was also summarized in Table 2.

mRNA expression levels of apoptotic Caspase3 and anti-apoptotic Bcl 2 proteins were also evaluated by Real-time PCR method after CCl4 and *S. cryptantha*

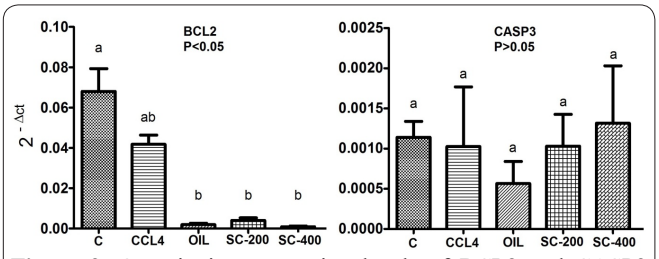

Figure 2. Quantitative expression levels of BCL2 and CASP3 genes after CCl4 and *S. cryptantha* extract application. C: control, CCl: carbon tetrachloride; Oil: olive oil, SC-200: *S. cryptantha* 200 mg/kg, and SC-400: *S. cryptantha* 400 mg/kg; [a, b, ab]As compared to control group, [a]As compared to CCl4 group, ($p<0.05$).

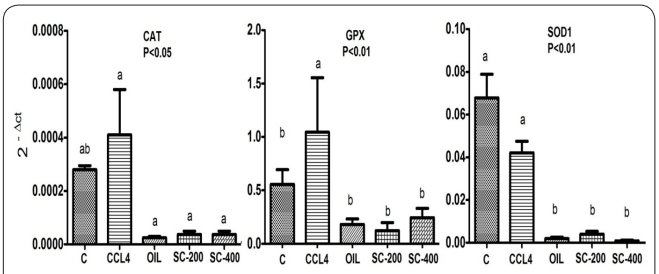

Figure 3. Quantitative mRNA transcription levels of antioxidant genes after CCl4 and *S. cryptantha* extract application. C: Control, CCl: carbon tetrachloride; Oil: Olive Oil, SC-200: *S. cryptantha* 200 mg/kg, and SC-400: *S. cryptantha* 400 mg/kg; [a, ab]As compared to control group, [a,b]As compared to CCl4 group, ($p<0.05$).

extract addition. Particularly, expression levels of anti-apoptotic Bcl2 were found to be decreased in the CCl4 group compared to the control (Fig. 2). In extract treated groups, the rate of this decrease was prominently increased. Although expression levels of Caspase3 in 200 and 400 mg / kg doses of extract-treated groups were increased, this increase was not statistically significant (Fig. 2).

In this work, real-time PCR was used to determine gene expression changes of antioxidant CAT, SOD and GPX1 genes in liver tissues of rats after CCl4 and plant extract treatments (Fig. 3). In particular, while expression levels of CAT and SOD1 was found to be diminished, GPX1 was increased in the CCl4 group as compared to control group. Additionally, CAT and GPX1 expression levels decreased significantly in both control and CCl4 control groups. Only the decrease in SOD1 expression was not statistically significant (Fig. 3).

In this study, similar results were also obtained for the control and olive oil groups in the spectrophotometric analysis of tissue MDA levels. MDA levels of CCl4 group were significantly increased when compared with control group and olive oil group ($p<0.05$). MDA levels of CCl4 group were significantly increased when com-

Table 2. Histological scores of Bax; Caspase3 immunoreactivity; apoptotic index (%) and MDA values of experimental groups.

Groups	Bax	Caspase3	Apoptotic index (%)	MDA (mmol/mg protein)
I	0.32±0.15	0.31±0.11	2.66±1.81	93.53±6.35
II	2.25±0.49[a]	2.40±0.46[a]	19.20±2.48[a]	251.31±27.75[a]
III	0.25±0.12	0.34±0.12	2.33±1.50	103.03±13.22
IV	0.96±0.18[b]	0.98±0.17[b]	10.33±1.21[b]	124.33±6.86[b]
V	1.03±0.18[b]	0.91±0.14[b]	8.75±0.50[b]	131.16±12.89[b]

Values were given as mean ± standard deviation. Group I: control; group II CCl4; group III: Olive oil group IV: CCl4+*S. cryptantha* 200 mg/kg group; group V: CCl4+ *S. cryptantha* 400 mg/kg group; [a,b] As compared to control group, [a]As compared to CCl4 group, ($p<0.05$).

pared with control group and olive oil group ($p<0.05$). MDA levels in liver tissues of CCl4+*Salvia cryptantha* 200 mg/kg group and CCl4+ *Salvia cryptantha* 400 mg/kg group were significantly lower than that of CCl4 group ($p<0.05$). There was no difference between the CCl4+*Salvia cryptantha* 200 mg/kg group and CCl4+ *Salvia cryptantha* 400 mg/kg group. All results of MDA level are summarized in Table 2 after CCl4 and *S. cryptantha* extract addition.

Discussion

Liver is an organ where many toxic substances are cleared and detoxified. In many studies, various chemicals such as Co-amoxiclav (23), D-galactosamine (24), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (25), acetaminophen (26), cisplatin (27), halo alkane xenobiotic (28), paracetamol (29) and CCl4 (30) have been reported to cause liver damage. In particular, CCl4 has been shown to cause damage in the liver resulting from oxidative stress. CCl4 has been shown to be converted to trichloromethyl radicals in the cytochrome P450 system and these radicals cause direct damage to hepatocytes (31). This damage has been shown to be eliminated by antioxidant molecules (32). In animal studies, it has been reported that may antioxidant compounds and antioxidant-rich plants inhibit oxidative stress generated as a result of chemicals that cause liver damage (33-36). Lee et al (2003) reported that *S. miltiorrhiza* plant extracts were reduced CCl4-induced acute hepatotoxicity by inhibiting P450 E1 protein, which plays key role in the exudative conversion (37). Another study also revealed that Co-amoxiclav-induced liver toxicity was eliminated by essential oil of *S. officinalis* by reducing oxidative stress (23). It has also been found that *S. officinalis* reduces paracetamol-induced liver damage (38). Moreover, Lamiaceae extracts have long been used for the treatment of various diseases due their low side effects. In particular, the Salvia species have been used in the treatment of several types of diseases including cancer (39), cardiovascular diseases (40), neurological diseases (41), and rheumatoid diseases (42). Also, some phytochemicals of *S. cryptantha* have been previously determined. Baser et al (1995) identified 1,8-Cineole, camphor, α-pinene, and camphene as the main compounds in the essential oil of *S. cryptantha,* which was obtained via water distillation method (43). Also, Ulubelen et al (1987) was isolated horminone, 7-acetylhorminone, 2β-hydroxyroyleanone and cryptanol compounds form *S. cryptantha* (44). In another study, in the essential oils of this plant α-pinene, eucalyptol, camphor, camphene and borneol were identified (10). However, no compound was identified in the extracts of *S. cryptantha*.

All the above reports demonstrate liver tissues protection from natural compounds and medicinal plant extracts. However, limited information is available on the hepato-protective effects of the Salvia species. In Turkey, the rate of endemism of the Salvia genus is very high, and there are several studies showing their bioactivities of these species. In this study, BAX and CASP3 protein expression levels were found to be decreased as compared to the CCl4 administration group after administration of *S. cryptantha* extract. Also, mRNA expression levels of CASP3 were not significantly altered. These results indicate that *S. cryptantha* might have important hepato-protective potential by decreasing apoptosis through Bax and Caspase3 reduction.

Apoptotic cells are morphologically characterized by membrane shrinkage, plasma and nuclear membrane blebbing, relocalization of organelles, and compaction as well as vesicles containing intracellular material (45). Furthermore, apoptosis and necrosis have been widely observed in many studies upon CCl4 administration (46,47). Using these features, apoptotic cells were determined by TUNEL staining and found to be reduced in rats treated with *S. cryptantha* extract as compared to the control only treated with CCl4. Consistent with these findings, Jin et al. (2014) reported that while the number of apoptotic cells and expression levels of CASP3 were increased in D-galactosamine/lipopolysaccharide induced fulminant liver damage, the levels of apoptosis and inflammatory mediators of IL-1alfa and IL-1beta were reduced after cryptotanshinone administration, which was obtained from *S. multiorrhiza* (48). In our study, protein levels of BAX and CASP3 were also found to be diminished as a result of histopathological staining. Diminished BAX and CASP3 with *S. cryptantha* extract supplementation might have apoptotic control in the liver tissues.

Furthermore, antioxidant enzymes have key roles in detoxifying toxic molecules. In our study, we also therefore evaluated mRNA expression levels of antioxidant enzymes. It was found that the *S. cryptantha* extract significantly reduced the expression of antioxidant CAT and GPX enzymes and did not significantly affect SOD1 expression. These findings indicate that, due to high amounts of antioxidant phytochemicals of Salvia species, enzymes might be reduced on a cellular basis to provide antioxidant balance in the cell. Also, Kandimalla et al (2016) showed that SOD and CAT activity increased after the application of different extracts from *A. reticulata* plant (36). El-hosseiny et al (2016) reported that GPX levels significantly increased after application of *S. officinalis* volatile oil in Co-amoxiclave-induced liver injury (23). Additionally, Lee et al (2003) established that expression levels of SOD were diminished after Silmarin and *S. miltiorrhiza* applications in CCl4-induced liver injury as compared to control group, and it was slightly increased compared to CCl4 control (37). Taken together, there were no consistent results based on our evaluation of previously published data and present antioxidant enzymes data with and without plants extract supplementation effects. The expression levels of some of these antioxidant enzymes were previously shown to be increased, and some of researchers were shown to be decreased in treatment groups. Our decreased MDA levels after *S. cryptantha* supplementation supported that this plant has a substantial antioxidant properties. Therefore, our results suggest that *S. cryptantha* extract reduces the CCl4 induced oxidative stress and inhibits lipid peroxidation. It has been also reported that antioxidant compounds or extracts containing antioxidant compounds decreased MDA levels in tissues.

S. cryptantha, which is an endemic species in Turkey, was found to reduce apoptotic cell death, Bax and Caspase3 levels, improve SOD, glutathione peroxi-

dase (GPx), and catalase expression and to reduce the serum levels of MDA in CCl4-induced liver damage. Therefore, the extract of *S. cryptantha* may be used as a hepato-protective agent against toxic effects caused by CCl4 and other chemicals.

Conflict of Interest

There is no conflict of interest.

Acknowledgments

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