



## Assessment of Anti-inflammatory and Antioxidant Properties of Safranal on CCl<sub>4</sub>-Induced Oxidative Stress and Inflammation in Rats

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**Abstract:** The present study aimed to determine the antioxidative and anti-inflammatory effects of safranal on damage induced by CCl<sub>4</sub>. Experimental animals were divided into five groups. The first group was determined as the control group and no treatment was conducted. Second group rats were administered 1 mL/kg-day CCl<sub>4</sub> during the experiment. Rats in Groups 3, 4 and 5 were administered 1 mL/kg-day CCl<sub>4</sub> and 25 mg/kg, 50 mg/kg; 100 mg/kg safranal, respectively via gavage. Oxidative-antioxidant parameters, liver function enzymes and inflammatory cytokine levels were determined in liver samples obtained from the rats. Data analysis demonstrated that oxidative stress and inflammation markers were significantly higher in CCl<sub>4</sub> administered groups ( $p < 0.05$ ). Antioxidant parameters in high-dose safranal administered groups were not different when compared to the control group. Safranal had ameliorating effects on the increased liver function enzymes activities in CCl<sub>4</sub> administered groups. In conclusion, it was observed that CCl<sub>4</sub> administration led to hepatic damage and increased oxidative stress and inflammatory cytokine levels. It was observed that particularly high-dose administration of safranal promoted the antioxidant system. Safranal administration was not effective on IL-1 $\beta$  levels. However, high-dose (100 mg/kg) safranal was found to be inflammatory against TNF- $\alpha$  and IL-6 cytokines. In conclusion, it can be said that safranal has an anti-inflammatory potential and has a strong antioxidative effect.

**Key words:** Antioxidant, CCl<sub>4</sub>, inflammation, safranal.

### INTRODUCTION

*Crocus sativus* L. is a species in the genus *Crocus* and the family Iridaceae. The species is a perennial and bulbous plant with flowers, used in traditional

medicine and indigenous in Southwest Asia, Spain, France, Italy, Turkey and Iran. Saffron is the name given to the dried stigma of the plant, and in many areas the plant itself is called by this name. Saffron is used as a spice and food colorant due to its specific color, taste and smell. Saffron contains about 150 volatile/non-volatile phytochemicals. Terpenes and their esters and alcohols are the

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volatile substances in saffron. The basic non-volatile components in saffron include crocin, picrocrocin and safranal. Crocin is a coloring pigment, while picrocrocin is responsible for the bitter taste of saffron. Safranal is a monoterpene aldehyde in the form of de-glycolyzed picrocrocin (Alavizadeh and Hosseinzadeh 2014, Erfanparast et al. 2015, Ozkececi et al. 2016). Non-volatile components in the saffron are also responsible for the pharmacological effects of the plant. Studies demonstrated that saffron and its components have antioxidant, antidepressant, anxiolytic, antinociceptive, anti-inflammatory, hypotensive, and anticancer effects (Razavi and Hosseinzadeh 2015).

Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde-C<sub>10</sub>H<sub>14</sub>O) is a cyclic terpenic aldehyde produced by hydrolyzation of picrocrocin. Safranal is a compound found in several species in Centaurea family in addition to *Crocus sativus* L. Several studies reported dermatological, sexual, metabolic, antihypertensive, and antioxidative effects of safranal. Safranal is the most common compound in saffron essential oil. It constitutes 60-70% of the volatile fraction. It is the main factor behind the unique odor and characteristic aroma of saffron. This aroma is not found in the collected stigmas. It is produced by enzymatic and thermal degradation during storage (Rezaee and Hosseinzadeh 2013).

CCl<sub>4</sub> is a xenobiotic used to induce acute and chronic hepatotoxicity. It exhibits toxicity at the biochemical and cellular organelle level (Recknagel et al. 1989, Basu 2003). CCl<sub>4</sub> is transformed into the trichloromethyl free radical by cytochrome P450 in the liver. The trichloromethyl radical is highly active and is responsible for the CCl<sub>4</sub> induced necrosis in the liver, especially in the centrilobular region. This radical is a potent lipid peroxidation inducer. Oxidative stress and following free radicals and their derivatives induce lipid peroxidation by affecting the unsaturated fatty

acids in cellular membrane and destroy hepatocyte cell membranes in liver (Manibusan et al. 2007). However, it was suggested that liver damage may also be due to the activation of other cells in the liver (endothelial cells and hepatocytes) due to the action of proinflammatory mediators released from the activated kupffer cells. Lipid peroxidation in CCl<sub>4</sub>-induced liver injury is very important. Due to the damage induced by lipid peroxidation, fibrosis and cirrhosis may occur in the liver. Liver fibrosis is characterized by the increase in extracellular matrix components. The balance between the production and destruction of the extracellular matrix tends continuously towards the matrix production due to the activation of profibrogenic mediators induced by the formation of toxic oxygen radicals (Cabre et al. 2000, Zhang et al. 1996).

It is quite important to develop alternative and safe natural products against liver damage caused by hepatotoxins such as CCl<sub>4</sub>. The hepatoprotective agent should restore the normal structure of the liver and protect the normal physiological mechanisms destroyed by hepatotoxins. Several studies were conducted to determine the biological and pharmacological activities of safranal. In the present study, the hepatoprotective, antioxidative and anti-inflammatory properties of safranal against oxidative stress and inflammation in CCl<sub>4</sub> induced liver damage were investigated.

## MATERIALS AND METHODS

### MATERIALS

Prior to the study, Fırat University Animal Experiments Local Ethics Committee approval was obtained (Approval Date: 26/10/2016, No: 184, Protocol no: 2016/121). Thirty-five male Wistar Albino rats weighing 300-380 g were procured from the Fırat University Experimental Research Center (FÜDAM). The rats were kept in polypropylene special cages designed for rat care under 12 hours ideal light conditions and adequate temperatures.

The rats were fed ad libitum with standard rat feed and tap water. After the rats were adapted to the environment, experimental phase was initiated.

Rats were randomly divided into five experimental groups as follows: 1) Control group; Nothing was administered to the rats during the experiments. 2) Carbon tetrachloride (CCl<sub>4</sub>) group; 1 mL/kg-day CCl<sub>4</sub> was dissolved in corn oil [1: 1 (v/v)] and administered to the rats intraperitoneally. 3) Carbon tetrachloride+25 mg/kg safranal (CCl<sub>4</sub>+25 SAF) group; 1 mL/kg-day CCl<sub>4</sub> dissolved in corn oil [1: 1 (v/v)] was administered to the rats intraperitoneally in addition to 25 mg/kg-day safranal that was administered via gavage. 4) Carbon tetrachloride+50 mg/kg safranal (CCl<sub>4</sub> + 50 SAF) group; 1mL/kg-day CCl<sub>4</sub> was dissolved in corn oil [1: 1 (v/v)] and administered intraperitoneally and 50 mg/kg-day safranal was administered via gavage. 5) Carbon tetrachloride+100 mg/kg safranal (CCl<sub>4</sub> + 100 SAF) group; 1 mL/kg-day CCl<sub>4</sub> was dissolved in corn oil [1: 1 (v/v)] and administered intraperitoneally and 100 mg/kg-day safranal was administered via gavage.

All rats were sacrificed on the 14<sup>th</sup> day of the experiment and blood samples were obtained. Ketamine and xylazine were used to anesthetize the rats. 50 mg/kg-im ketamine (alpha, Egevet, Istanbul, Turkey) and 7 mg/kg-im Xylazine (Ramp, Bayer AG, Germany) were administered. A further dose of anesthesia was administered to the rats when needed. The abdomen and thorax were incised on the midline and the blood was drawn from the heart using an injector.

#### PREPARATION OF SERUM SAMPLES

Blood samples were taken from each animal and transferred into clean and dry test tubes. The serum was separated by centrifuge for 10 minutes at 3000 rpm at +4 °C. The serum was used to determine oxidant-antioxidant parameters, liver function enzyme and inflammatory cytokine levels.

#### BIOCHEMICAL ANALYSES

Experimental group MDA, NO, GSH, GPx, TAS, TOS, liver functional enzyme (AST, ALT, ALP) and inflammation marker (IL-1 $\beta$ , IL-6, TNF- $\alpha$ ) levels were measured in serum.

Serum MDA content was determined with HPLC. C18 column with a diameter of 250x4.6 mm 5  $\mu$ M and as the mobile phase, methanol that contained KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub> was used. The measurements were conducted with a fluorescence detector at 30 °C oven temperature and 0.8 mL/min mobile phase flow rate (Hong et al. 2000). Serum NO concentrations were determined with HPLC. Mobile phase (Na<sub>2</sub>CO<sub>3</sub> / NaHCO<sub>3</sub>) was added to the samples which was deproteinized with acetonitrile. NO levels were measured using centrifuged samples (Yılmaz et al. 2013).

Inflammation markers were determined with tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$ , IL-6 (e-Bioscience, Vienna, Austria) in ELISA (BIOTEK ELx800) device in accordance with the rat-specific kit protocols. The oxidative stress / antioxidant parameters total antioxidant status (TAS) and total oxidant status (TOS) levels were measured using kits (Rel Assay, Gaziantep, Turkey) that work with spectrophotometric methods (Erel 2004, 2005). The glutathione peroxidase (GPx) enzyme activity, directly correlated with selenium levels, was determined with commercial kits (Randox/Ransel, Crumlin, United Kingdom). In determining the glutathione (GSH) concentration, commercial kits that measure glutathione reductase enzyme were used (Cayman, Michagen, USA). Cytokine was expressed as pg/mL, GSH concentration was expressed as  $\mu$ g/mL, TAS was expressed as  $\mu$ mol Trolox Equiv./L and TOS was expressed as  $\mu$ mol H<sub>2</sub>O<sub>2</sub> Equiv./L.

Liver function enzymes, Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) were measured with an autoanalyzer (ADVIA 2400, Siemens) at

Fırat University, Faculty of Medicine, Medical Biochemistry Laboratory and the enzyme activities were expressed as U/L.

#### STATISTICAL ANALYSIS

The experiment findings are presented as mean  $\pm$  standard deviation. The significance of the difference between the groups was determined with Post Hoc Multiple Comparative Duncan test in One-Way ANOVA test.  $p < 0.05$  was considered statistically significant. Each different letter indicates a statistical difference between the groups.

#### RESULTS

Serum oxidant / antioxidant parameters (MDA, NO, GSH, GPx, TAS, TOS), liver function enzymes (AST, ALT, ALP) and inflammation markers (IL-1 $\beta$ , IL-6, TNF- $\alpha$ ) obtained with the analyses conducted on the laboratory data obtained from the laboratory were evaluated.

Serum oxidant/antioxidant parameter findings are presented in Table I. It was determined that CCl<sub>4</sub> group MDA level was higher when compared

to other groups ( $p < 0.05$ ). The control group MDA level was statistically different when compared to all other groups ( $p < 0.05$ ). Analysis of NO levels demonstrated that the CCl<sub>4</sub> group NO level was statistically higher when compared to other groups ( $p < 0.05$ ) and control group MDA level was statistically different when compared to other groups ( $p < 0.05$ ). Control group TOS was lower when compared to other groups, and CCl<sub>4</sub> group TOS was higher when compared to other groups. It was found that the GSH concentration in CCl<sub>4</sub> group was statistically lower when compared to other groups ( $p < 0.05$ ). GSH levels in high-dose safranal administration groups (50 mg/kg, 100 mg/kg) were close to the control levels. CCl<sub>4</sub> group GPx levels were statistically lower when compared to all other groups ( $p < 0.05$ ). GPx levels in levels in 100 mg/kg safranal administered group were close to the control. CCl<sub>4</sub> group TAS was statistically different when compared to all other groups ( $p < 0.05$ ). TAS in the 100 mg/kg safranal administered group was not different when compared to the control group.

Serum liver function enzyme (AST, ALT, ALP) findings are presented in Table II. It was

**TABLE I**  
Serum Oxidant/Antioxidant Parameters (MDA, NO, GSH, GPx, TAS, TOS).

	Control	CCl <sub>4</sub>	CCl <sub>4</sub> + 25 SAF	CCl <sub>4</sub> + 50 SAF	CCl <sub>4</sub> + 100 SAF
<b>MDA</b> ( $\mu\text{mol/L}$ )	0.118 $\pm$ 0.022 <sup>a</sup>	0.496 $\pm$ 0.034 <sup>d</sup>	0.348 $\pm$ 0.04 <sup>c</sup>	0.308 $\pm$ 0.011 <sup>c</sup>	0.208 $\pm$ 0.024 <sup>b</sup>
<b>NO</b> ( $\mu\text{mol/L}$ )	22.11 $\pm$ 1.9 <sup>a</sup>	60.51 $\pm$ 6.09 <sup>c</sup>	50.37 $\pm$ 3.61 <sup>d</sup>	37.24 $\pm$ 3.85 <sup>c</sup>	29.47 $\pm$ 1.96 <sup>b</sup>
<b>TOS</b> ( $\mu\text{mol H}_2\text{O}_2$ Equiv./L)	18.03 $\pm$ 0.26 <sup>a</sup>	31.0 $\pm$ 0.56 <sup>d</sup>	25.35 $\pm$ 0.7 <sup>c</sup>	24.1 $\pm$ 0.65 <sup>c</sup>	21.18 $\pm$ 0.67 <sup>b</sup>
<b>GSH</b> ( $\mu\text{g/mL}$ )	127.86 $\pm$ 1.68 <sup>c</sup>	107.66 $\pm$ 8.52 <sup>a</sup>	119.25 $\pm$ 0.81 <sup>b</sup>	126.24 $\pm$ 0.8 <sup>c,b</sup>	130.22 $\pm$ 3.97 <sup>c</sup>
<b>GPx</b> (U/L)	21.42 $\pm$ 0.32 <sup>a</sup>	5.59 $\pm$ 0.31 <sup>d</sup>	8.81 $\pm$ 0.19 <sup>c</sup>	12.23 $\pm$ 0.31 <sup>b</sup>	19.93 $\pm$ 0.34 <sup>a</sup>
<b>TAS</b> ( $\mu\text{mol Trolox Equiv./L}$ )	1.76 $\pm$ 0.03 <sup>a</sup>	1.05 $\pm$ 0.06 <sup>d</sup>	1.30 $\pm$ 0.03 <sup>c</sup>	1.67 $\pm$ 0.02 <sup>b</sup>	1.72 $\pm$ 0.03 <sup>ab</sup>

Data are presented as mean  $\pm$  standard deviation (n = 7). Statistical differences between the experimental groups are expressed with superscript letters (a-e). CCl<sub>4</sub>+25 SAF: CCl<sub>4</sub> and 25 mg/kg safranal administered group; CCl<sub>4</sub>+ 50 SAF: CCl<sub>4</sub> and 50 mg/kg safranal administered group; CCl<sub>4</sub>+ 100 SAF: CCl<sub>4</sub> and 100 mg/kg safranal administered group.

observed that CCl<sub>4</sub> and low dose (25 mg/kg) safranal administered group AST enzyme activities were found statistically higher when compared to other groups ( $p < 0.05$ ). Administration of high-dose (50 mg/kg, 100 mg/kg) safranal decreased AST levels in these groups, however, the AST levels in these groups were different when compared to the control. It was observed that ALP enzyme activities were similar to AST enzyme activities in these groups. It was found that CCl<sub>4</sub> administered group ALT enzyme activities were statistically different when compared to other groups ( $p < 0.05$ ). It was observed that ALT enzyme activities in all safranal administered groups were statistically different when compared to control.

Serum inflammation marker (IL-1 $\beta$ , IL-6, TNF- $\alpha$ ) findings are presented in Table III. It was

determined that CCl<sub>4</sub> and low-dose (25 mg/kg) safranal group TNF- $\alpha$  level was statistically higher when compared to other groups ( $p < 0.05$ ). There was no statistically significant difference between control group and high-dose (100 mg/kg) safranal group TNF- $\alpha$  level. IL-1 $\beta$  levels were different among all groups. It was observed that the IL-6 concentrations in CCl<sub>4</sub> administered groups were statistically higher when compared to other groups ( $p < 0.05$ ). No statistically significant difference was observed between control group and high-dose (50 mg/kg and 100 mg/kg) safranal group IL-6 concentrations.

#### DISCUSSION AND CONCLUSION

CCl<sub>4</sub> is one of the chemicals commonly used in liver damage induction models. CCl<sub>4</sub> is also a

**TABLE II**  
Serum liver function enzymes (AST, ALT, ALP).

	Control	CCl <sub>4</sub>	CCl <sub>4</sub> +25 SAF	CCl <sub>4</sub> +50 SAF	CCl <sub>4</sub> +100 SAF
<b>AST</b> (U/L)	17315.4±1555.8 <sup>a</sup>	134540±14021.4 <sup>c</sup>	127623.2±5264.8 <sup>c</sup>	92735.2±5987.1 <sup>b</sup>	82344.6±6499.5 <sup>b</sup>
<b>ALT</b> (U/L)	7471±1172.05 <sup>a</sup>	84851.4±6735.7 <sup>d</sup>	73430±5219.02 <sup>c</sup>	70048.4±5088.8 <sup>c</sup>	55534±4382.9 <sup>b</sup>
<b>ALP</b> (U/L)	208.4±19.6 <sup>a</sup>	548.6±44.16 <sup>c</sup>	515.6±18.77 <sup>c</sup>	451.0±16.19 <sup>b</sup>	410.6±16.21 <sup>b</sup>

Data are presented as mean  $\pm$  standard deviation ( $n = 7$ ). Statistical differences between the experimental groups are expressed with superscript letters (a-d). CCl<sub>4</sub>+25 SAF: CCl<sub>4</sub> and 25 mg/kg safranal administered group; CCl<sub>4</sub>+ 50 SAF: CCl<sub>4</sub> and 50 mg/kg safranal administered group; CCl<sub>4</sub>+ 100 SAF: CCl<sub>4</sub> and 100 mg/kg safranal administered group.

**TABLE III**  
Serum inflammation markers (IL-1 $\beta$ , IL-6, TNF- $\alpha$ ).

	Control	CCl <sub>4</sub>	CCl <sub>4</sub> +25 SAF	CCl <sub>4</sub> +50 SAF	CCl <sub>4</sub> +100 SAF
<b>TNF-<math>\alpha</math></b> (pg/mL)	272.28±5.17 <sup>a</sup>	396.79±13.63 <sup>c</sup>	372.08±9.31 <sup>c</sup>	327.76±14.98 <sup>b</sup>	296.16±26.92 <sup>a</sup>
<b>IL-1<math>\beta</math></b> (pg/mL)	170.86±1.26 <sup>a</sup>	1715.24±23.1 <sup>c</sup>	1328.9±27.45 <sup>d</sup>	1041.62±39.95 <sup>c</sup>	838.63±39.7 <sup>b</sup>
<b>IL-6</b> (pg/mL)	40.01±2.82 <sup>a</sup>	84.88±5.29 <sup>c</sup>	60.88±6.55 <sup>b</sup>	49.34±4.91 <sup>ab</sup>	44.67±5.19 <sup>a</sup>

Data are presented as mean  $\pm$  standard deviation ( $n = 7$ ). Statistical differences between the experimental groups are expressed with superscript letters (a-e). CCl<sub>4</sub>+25 SAF: CCl<sub>4</sub> and 25 mg/kg safranal administered group; CCl<sub>4</sub>+ 50 SAF: CCl<sub>4</sub> and 50 mg/kg safranal administered group; CCl<sub>4</sub>+ 100 SAF: CCl<sub>4</sub> and 100 mg/kg safranal administered group.



xenobiotic used to screen for hepatoprotective and liver therapy drugs. The CCl<sub>4</sub> metabolism is initiated with the formation of trichloromethyl free radicals through the activity of cytochrome P450 oxygenase system in endoplasmic reticulum. The trichloromethyl radical reacts with biomolecules such as fatty acids, proteins, lipids, nucleic acids and amino acids (Bahashwan et al. 2015). Lipid peroxidation is the most important indicator of liver cell damage. Lipid peroxidation reflects the deterioration of the balance between free radicals and antioxidants. If antioxidant level is lower than oxidant levels, it could bind to the critical physical compounds including the membrane double bonds and lead to damages (Poli et al. 1981). Malondialdehyde (MDA) is a secondary product of peroxidation of polyunsaturated fatty acids and serves as a marker to determine lipid peroxidation levels (Vajdovich et al. 1995). In the present study, it was observed that CCl<sub>4</sub> administration led to lipid peroxidation. Furthermore, it was observed that safranal administration had positive effects on lipid peroxidation. It was reported that safranal stabilized bio-membranes in biological systems and reduced unsaturated membrane lipids and reactive oxygen species. Thus, the positive effects of safranal could have been observed on lipid peroxidation. Similar to the present study, Hosseinzadeh and Sadeghnia (2005) created oxidative damage induced by ischemia-reperfusion injury (IRI) in the rat hippocampus. They reported that safranal reduced the damage-induced MDA due to its antioxidant capacity (Hosseinzadeh and Sadeghnia 2005).

NO is a non-macrobiotic free radical synthesized from L-arginine by nitric oxide synthase (NOS) due to the unpaired electron. The NOS enzyme is found in endothelial cells. It is known that a large amount of NO is produced in macrophages due to the induction of nitric oxide synthase by inducible forms of iNOS lipopolysaccharides, interferon- $\gamma$  and TNF to create a defense system against pathogens (Cendan et al. 1996). Excessive

NO production inhibits the activation of mast cells and the adhesion of leukocytes. Furthermore, it is known that it promotes the production of peroxynitrite, a particularly strong oxidant (Radi 2013). In the present study, it was observed that CCl<sub>4</sub> administration increased NO concentrations, and particularly high-dose safranal administration decreased NO concentrations. In a previous study (Farahmand et al. 2013), the effects of increasing free radical production with age on liver were investigated. Similar to our study, it was reported that safranal was effective on lipid peroxidation and serum NO levels and safranal even had anti-aging effects.

There are active defense mechanisms in the organism to prevent/neutralize free radicals. The defense is possible thanks to endogenous antioxidants such as glutathione peroxidase (GPx). Endogenous antioxidant enzymes act jointly against the ROS. It was demonstrated that the depletion of hepatic GPx was associated with improved toxicity induced by chemicals such as CCl<sub>4</sub>. In the current study, it was observed that liver tissue GPx levels decreased in CCl<sub>4</sub> administered groups. Concurrently, it was observed that GPx activity in the group where the highest safranal dose was administered similar to the control levels and high safranal dose increased antioxidant enzyme levels. It was suggested that hepatic GPx is a liver enzyme reserve responsible for reducing hepatotoxicity induced by the active metabolites of CCl<sub>4</sub>. GPx is also an important determinant in oxidative damage (Thanh et al. 2015). GPx could have been used to prevent the emergence of active metabolites of CCl<sub>4</sub> and to scavenge free radicals involved in lipid peroxidation. GPx are both intracellular and extracellular antioxidants with various enzymatic processes that reduce hydrogen peroxide and hydroperoxides (Ng et al. 2007). The increase in GPx levels in safranal administered groups could be due to GPx synthesis or GPx regeneration. It could also occur due to the ability of safranal to

stimulate the antioxidant enzymes against ROS induced by CCl<sub>4</sub>. Glutathione (GSH) is the first line of defense in antioxidant defense. It affects reactive oxygen species (ROS) (Engelmann et al. 2005). In the present study, it was observed that GSH concentration was low in the CCl<sub>4</sub> group and 50 mg/kg and 100 mg/kg safranal administration increased the GSH levels ( $p < 0.05$ ). GSH-dependent enzymes offer a second line of defense since they primarily detoxify harmful by-products of ROS and help prevent the proliferation of free radicals. The decrease in the GSH concentration in the CCl<sub>4</sub> group could be due to GSH utilization during the reduction of NADPH-dependent peroxides. The administration of 50 mg and 100 mg safranal increased GSH levels, promoting the antioxidant defense. GSH level in the 25 mg/kg group was lower than the control group. This may be due to inadequate safranal concentration. Because high-dose safranal (50 mg/kg and 100 mg/kg) positive effects are observed. It was reported that safranal modulates antioxidant gene expression and regulates mitochondrial antioxidant genes, creating a lower mitochondrial oxygen radical potential (Kanakis et al. 2007, Assimopoulou et al. 2005). In another study, it was observed that safranal promoted the antioxidant system by increasing gastric mucosal damage, increasing GSH levels and reducing lipid peroxidation in rat gastric mucosa (Kianbakht and Mozaffari 2009).

Chemical agents such as CCl<sub>4</sub> could lead to immune system, oxidative and inflammatory damages due to their physical and biological effects. Close interactions between oxidative stress, inflammatory response, and immune system (such as macrophage, neutrophil and epithelial cell and IL-8, IL-1 $\beta$ , IL-6, TNF- $\alpha$  activation and increase) were reported (Hajjar and Gotto 2013). Separate measurement of antioxidant/oxidant parameters requires time-consuming, expensive and complex techniques. The measurement of total antioxidant status (TAS) and total oxidant status (TOS) is

preferred and widely used to obtain information about basic status (Aksoy et al. 2013). It was found that CCl<sub>4</sub> group TAS level was lower when compared to other groups ( $p < 0.05$ ). It was found that 100 mg / kg safranal administered group TAS level was close to control group level. Analysis of the TOS levels demonstrated that CCl<sub>4</sub> administered group TOS level was quite high. In all groups treated with safranal, TOS levels were reduced, albeit partially. Total oxidant status (TOS) is a general marker used in the determination of oxidative stress. Oxidative stress-lowering effects of all application doses of safranal are observed. However, these effects are not statistically significant. Animals treated with CCl<sub>4</sub> develop a reversible acute centrilobular liver necrosis. Hepatotoxicity is thought to be in two stages. The first step is to produce a highly reactive trichloromethyl radical, which leads to cytochrome P450 and CCl<sub>4</sub> metabasolization, lipid peroxidation and membrane damage in hepatocytes. The second step is the inflammatory response of kupffer cells. Kupffer cells are activated with free radicals and secrete cytokines that attract and activate neutrophils. Neutrophils release reactive oxygen species to increase liver damage (Louis et al. 1998). It has been observed that, in relation to the limited anti-inflammatory effect of safranal, it has decreased relatively in oxidative stress. In a study, Hazman and Ovalı (2015) investigated the effects of safranal with experimental type 2 diabetes induced rats using streptozotocin and high-fat diet. They concluded that safranal had antioxidative properties and increased TAS levels and decreased TOS levels.

CCl<sub>4</sub> produces an experimental damage that appears histologically as viral hepatitis. Hepatotoxicity commences with a change in the endoplasmic reticulum, which leads to the release of metabolic enzymes in intracellular structures. Liver damage induced elevated serum levels are in circulation since these enzymes are located in cellular cytoplasm and are released

(Kang and Koppula 2014). Several studies demonstrated that CCl<sub>4</sub> induced an increase in serum ALT, AST and ALP levels due to cellular membrane and mitochondrial damage in liver cells (Maheshwari et al. 2015). In the present study, it was determined that ALT, AST and ALP enzyme activities in CCl<sub>4</sub> administered group were quite high. Safranal administration had positive effects on hepatic enzymes, however, the decrease was not statistically significant. The membrane stabilizer effect of safranal on increased serum enzymes in CCl<sub>4</sub>-induced liver damage could prevent intracellular enzyme infiltration. This is consistent with the widely accepted view that serum levels of transaminases return to normal with the recovery of the hepatic parenchyma and regeneration of the hepatocytes (Thabrew et al. 1987). This could be explained by the prevention of the production of free radicals, as well as the protective potential of safranal against hepatotoxins.

Due to the biological effects of phytochemicals, *Crocus sativus* L. have positive health effects. Its components such as the plant and safranal could be effective on inflammatory and immune system disorders by modulating the proinflammatory cytokines, oxidative stress markers and immune factors. Inflammation control is among the main biomedical and health management studies since inflammation is known to play an important role in the pathogenesis of several diseases. Discovery of a natural agent with therapeutic value has been the objective of several studies conducted for the prevention and treatment of inflammatory diseases (Aggarwal and Harikumar 2009).

Excessive administration of xenobiotics induces immunologic reactions including liver cell dysfunction, necrosis, and natural and adaptive immune responses. Kupffer cells release various factors, including prostaglandins, IL, TNF- $\alpha$  and various cytokines. TNF- $\alpha$  secreted from kupffer cells stimulates a number of cellular responses including cell proliferation, production of other

inflammatory mediators, and programmed necrosis. Factors that increase the synthesis of TNF- $\alpha$  include parasites, viruses, micro-bacteria, enterotoxins, endotoxins and TNF itself. TNF- $\alpha$  secreted from kupffer cells directly leads to liver cell necrosis. It was demonstrated that various inflammatory cytokines such as TNF- $\alpha$ , Inf- $\gamma$ , IL- $\beta$  produced in drug-induced liver damage contributed to the progression of tissue damage. It is known that inflammation plays an important role in classical toxicological process and TNF- $\alpha$ , an inflammatory cytokine, is one of the most important mediators in this process. TNF- $\alpha$  is a proinflammatory cytokine produced mainly in liver macrophages. It is the primary mediator in systemic toxicity and liver damage (Bonzo et al 2015, Ganey and Roth 2001). Hammam et al. (2013) reported that increased TNF- $\alpha$  levels play an important role in liver damage and increased TNF- $\alpha$  levels are an indirect indicator of liver damage. Zhang et al. (2015) investigated the effects of safranal on neuronal apoptosis associated with inflammation in spinal cord injury (SCI). They reported that safranal exhibited anti-inflammatory effects by increasing the expression of IL-1 $\beta$ , TNF- $\alpha$  and p38 MAPK inflammatory cytokines after SCI. In the present study, it was determined that high-dose safranal administration statistically significantly reduced TNF- $\alpha$  levels against CCl<sub>4</sub>-induced inflammation ( $p < 0.05$ ).

Interleukins are structures that are released by leukocytes and perform immune intercellular stimulatory or inhibitory stimuli. Interleukin-1 (IL-1) is an important cytokine produced in inflammation sites. IL-1 is primarily synthesized by all nucleated cells, especially monocytes and macrophages, T lymphocytes, and natural killer cells (NK) (Delaleu and Bickel 2004). Although it is synthesized continuously in certain cells, it is usually released by stimulated macrophages and monocytes. T lymphocyte stimulating agents also stimulate the IL-1 production by stimulating the macrophages. The biological effects of IL-1



are similar to TNF and depend on the freed cytokine levels. It mediates low-intensity regional inflammatory events. IL-1 enhances the subsequent IL-1 synthesis through mononuclear phagocytes and its impact on vascular endothelium and induces IL-6 synthesis. Several cytokines (such as TNF- $\alpha$ , IL-6, IL-10) are indicated to be expressed in human cytokine diseases and experimental liver injury. They have emerged as a major endogenous agent of hepatotoxicity in various experimental model injuries by direct cytotoxicity and triggering of the inflammatory cascade. However, the involvement of these cytokines in the pathogenesis of CCl<sub>4</sub> liver inflammation remains controversial. Because some of the studies, the cytokine receptors given to the experimental animals and then CCl<sub>4</sub> hepatotoxicity was created. As a result, it has been determined that cytokines do not provide a neutralization and protective effect against hepatotoxicity with CCl<sub>4</sub> (Bruccoleri et al. 1997, Czaja et al. 1995, Louis et al. 1998). In a previous study, it was discussed that safranal reduced renal damage and had curative effects in diabetic nephropathy. It was reported that TNF- $\alpha$  and IL-1 $\beta$  induced nephropathy by secretion from the renal glomerular membrane, and safranal exhibited anti-inflammatory effects by relatively decreasing the cytokine levels (Hazman and Ovalı 2015). Similar findings were observed in the present study. It was determined that the IL-1 $\beta$  concentration in CCl<sub>4</sub> administered group was relatively reduced by the action of safranal.

CCl<sub>4</sub> also induces the activation of immune systems through infiltration of inflammatory cells into the injury site. Thus, the immune cells could be responsible for further development of hepatotoxicity through the repeated cycle of inflammation by the release of proinflammatory cytokines such as TNF- $\alpha$  and IL-6. Interleukin-6 (IL-6) is a cytokine released by macrophages, monocytes, T and B lymphocytes and several tumor cells. Like other cytokines, it plays an important role in the pathogenesis of inflammation. The best described

effects of IL-6 are associated with B lymphocytes and hepatocytes (Michalopoulos 2007, Cressman et al. 1996). IL-6 stimulates differentiation and antibody secretion in B lymphocytes and acts as a growth factor. In hepatocytes, it activates NK cells and macrophages and stimulates the formation of acute phase protein. It is of great importance in protecting the liver from toxic damage (Debonera et al. 2001). Amin et al. (2014) investigated the anti-inflammatory properties of the *Crocus sativa* ethanol extract in chronic constriction injury model in rats. They concluded that *Crocus sativa* ethanol extract led to a decrease in TNF- $\alpha$ , IL-1 $\beta$  and IL-6 concentrations, thereby improving neuropathic pain. Similarly, IL-6 findings demonstrated that 50 mg/kg and 100 mg/kg safranal administration in CCl<sub>4</sub> induced inflammation decreased IL-6 levels statistically in the present study (p <0.05).

In conclusion, safranal is a phytochemical known for its antioxidative and anti-inflammatory properties. Thus, similar effects of safranal were observed in the present study, which was planned based on the premise that safranal could inhibit CCl<sub>4</sub>-induced liver damage and oxidative stress and reduce inflammation levels. In the present study, it was observed that CCl<sub>4</sub> led to the formation of or increased lipid peroxidation, free radicals, resulting in hepatic damage and inflammation. Safranal has positive effects on induced hepatotoxicity. Although safranal reduces oxidative stress, it was observed that it primarily promotes the antioxidant system. It could be argued that especially high-dose safranal administration had anti-inflammatory as well as antioxidant effects.

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## AUTHOR CONTRIBUTIONS

Laçine Aksoy and Ömer Naci Alayunt contributed to the study by conducting the study, experimental design, project design and data analysis. Yasemin Sunucu Karafakioğlu, Sevcan Sevimli and Ömer Naci Alayunt have contributed to experimental animal practices, laboratory studies and data analysis.

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