Research Article

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Crocin (active constituent of saffron) improves CCl_4-induced liver damage by modulating oxidative stress in rats

Crocin (safranın aktif bileşeni) sıçanlarda oksidatif stresi düzeneyerek CCl_4 kaynaklı karaciğer hasarını iyileştirir

https://doi.org/10.1515/tjb-2017-0173
Received June 29, 2017; accepted October 8, 2018; previously published online June 27, 2019

Abstract

Background: CCl_4 is a widely used xenobiotic for the purpose of causing liver damage in experimental studies. In this study, we aimed to reveal the effects of crocin on liver injury caused by CCl_4 via free radical scavenging properties.

Materials and methods: Animals were divided into five groups of 10: control; corn oil; crocin; CCl_4; CCl_4 + crocin. Tissue samples were carefully removed and separated for biochemical and histological investigations.

Results: CCl_4 administration led to significant increases in MDA, SOD, CAT and TOS in liver tissue, and AST, ALT and ALP levels in plasma ($p < 0.05$). In addition, CCl_4 caused significant decreases in GSH and TAS ($p < 0.05$). When animals were treated with crocin, high MDA, SOD, CAT, TOS levels, and AST and ALP activities decreased and GSH and TAS levels increased. Control group exhibited normal histological appearance; however extensive necrosis areas were detected in the CCl_4 group. In the CCl_4 + crocin group, pathological changes were markedly decreased and the appearance of liver tissue was almost similar to the control groups.

Conclusion: Our results showed that crocin suppresses oxidative stress with antioxidant properties and has a protective effect on tissue damage caused by CCl_4.

Keywords: CCl_4; Crocin; Oxidative stress; MDA; Hepatotoxicity.

Öz

Amaç: CCl_4 deneysel çalışmalarda karaciğer hasarına neden olmak için yaygın olarak kullanılan bir ksenobiyotiktir. Bu çalışmada CCl_4'ün yol açtığı karaciğer hasarı üzerine, serbest radikal süpürücü özellikleri olan krosin'in etkilerini ortaya koymayı amaçladık.

Gereç ve Yöntemler: Hayvanlar beş gruba ayrıldı: kontrol; mısır yağı; krosin; CCl_4; CCl_4 + krosin. Doku örnekleri dikkatli bir şekilde çıkarılarak, biyokimyasal ve histolojik incelemeler için ayrıldı.

Bulgular: CCl_4 uygulaması, karaciğer dokusunda MDA, SOD, CAT ve TOS ile plazmada AST, ALT ve ALP düzeylerinde anlamlı artışlara yol açmıştır ($p < 0.05$). Ayrıca CCl_4, GSH ve TAS'de anlamlı düşüşe neden olmuştur ($p < 0.05$). Hayvanlar krosin ile tedavi edildiğinde yüksek olan MDA, SOD, CAT, TOS düzeyleri ve AST ve ALP aktiviteleri azalırken GSH ve TAS seviyeleri ise artış gösterdi. Kontrol grubu normal histolojik görünüm sergiledi; ancak CCl_4 grubunda geniş nekroz alanları tespit edildi. CCl_4 + krosin

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grubunda patolojik değişiklikler belirgin olarak azaldı ve karaciğer dokusunun görünümü kontrol grupları ile neredeyse benzerdi.

Sonuç: Elde ettiğimiz sonuçlara göre, krosin oksidatif stresi antioksidan özellikler ile baskıladığı ve CCl_4'ün neden olduğu doku hasarı üzerinde koruyucu bir etkiye sahip olduğu ortaya konmuştur.

Anahtar Kelimeler: CCl_4; Krosin; Oksidatif stres; MDA; Hepatotoksisite.

Introduction

The liver tissue has a key role in regulating a wide range of physiological processes in the organism, including metabolic, secretory and storage activities [1]. The liver is responsible for minimizing the harmful effects caused by exogenous compounds. The liver, which also plays a role in drug and xenobiotic mechanisms, may be exposed to degradative damage by the emergence of detoxification products [2]. Carbon tetrachloride (CCl_4), a xenobiotic, is a chemical commonly used to generate experimental liver damage. CCl_4 is an uncolored, transparent, volatile liquid that is used in experiments to induce liver damage. Cytochrome P450 (CYP) enzyme system in the liver converts CCl_4 into a reactive toxicant, the inter-metabolite trichloromethyl radical ($CCl_3^{\cdot}$), which leads to the formation of trichloromethylperoxy ($CCl_3OO^{\cdot}$) in the presence of oxygen. Reaction of $CCl_3OO^{\cdot}$, a reactive toxic substance, with polyunsaturated fatty acids initiates lipid peroxidation or leads to the degradation of the cell membrane by covalent bonding of lipids with proteins, thereby causing liver damage [3]. Liver damage activates Kupffer cells, resulting in the release of early inflammation mediators such as superoxide anions, which cause the formation of reactive oxygen species (ROS), and especially peroxynitrites and hydrogen peroxides (H_2O_2), leading to oxidative stress. There is a very delicate balance between the oxidants and the antioxidants in the organism [4]. If oxidants are over-produced or antioxidants perform their tasks at lower than normal levels, in other words, when the balance between oxidant – antioxidant quantities is impaired, the oxidant molecules in the organism start to exhibit harmful effects on the proteins, carbohydrates and nucleic acids [5]. Therefore, the balance between ROS and the antioxidant defense system becomes an important requirement for the prevention of cellular damage induced by oxidative stress. The antioxidant systems include non-enzymatic [reduced glutathione (GSH), uric acid, and vitamin E] as well as enzymatic antioxidants [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] [6]. Thus, antioxidants play a key role in preventing CCl_4-induced liver injury.

Saffron (Crocus sativus L.) is grown in countries such as Iran, Spain, Kashmir (India and Pakistan), Greece, Azerbaijan, China, Morocco, Mexico, Libya, Turkey and Austria and has a very high economic value. Besides the antioxidant properties of saffron, it is used for its hypolipidemic, anti-inflammatory and anti-carcinogenic properties as a medicine in the treatment of many diseases in traditional medicine [7]. The chemical contents of saffron are mostly crocin, crocetin and safranal. In addition to its major ingredients, saffron also has nearly 150 chemical ingredients, including protein, sugar, vitamins, flavonoids, amino acids, vital minerals and other chemical components [8].

Recently, phytochemical therapy has been one of the most effective ways of overcoming hepatotoxicity, especially through mechanisms of regulation of free radicals [9].

In this study, CCl_4-induced hepatotoxicity was related to ROS production and crocin is a radical scavenging chemical with strong antioxidant properties. The aim of this study was to demonstrate the protective effect of crocin on hepatotoxicity caused by CCl_4 via biochemical and histopathological evidence.

Materials and methods

Animals

In our study, 50 male Wistar rats weighing 225–250 g were purchased from Inonu University, Faculty of Medicine, Experimental Animal Breeding and Research Center (INUTF-DEHUM). The experimental applications on rats began after the approval of the Ethics Committee (2015/A-96). The drinking water of rats was changed daily and the cages were cleaned every day. The climate of the room was at the maximum level for rats (temperature 21°C, humidity 55–60%) and the rats were kept under 12 h light (08:00–20:00) and 12 h darkness (20:00–08:00). Animals were fed ad libitum with pellet feed prepared for rats during the study period.

Experimental design

Fifty animals were divided randomly into five groups, each group containing 10 rats as follows:

Control (C) group: 1 mL/kg/day normal saline solution was given.
Corn oil (Co) group: 1 mL/kg/day corn oil was given.
Crocin (Cr) group: 100 mg/kg/day crocin (Sigma Chemical Co., St Louis, MO, USA) was given [10].
Carbon tetrachloride (CCl_4) group: 1:1 CCl_4 (Sigma Chemical Co., St Louis, MO, USA) was given 0.5 mL/kg every other day.
Carbon tetrachloride + Crocin (CCl_4 + Cr) group: 100 mg/kg crocin and 1:1 CCl_4 were given every other day at the dose of 0.5 mL/kg.

CCl_4 and crocin were dissolved in corn oil and physiological saline solution respectively, and prepared freshly before application. Chemicals were administered to the rats by gavage, repeating at the same hour each day for 15 days.

Samples

At the end of 15 days, the experiment was terminated and blood was taken from the heart to heparinized tubes for biochemical analysis under xylazine-ketamine anesthesia. After that, all rats were decapitated and liver tissue was carefully removed. The liver tissues were washed with physiological saline solution in a sterile cup to remove blood. The liver tissues were divided into two equal parts and one of them was placed in 10% formaldehyde for histological examination. The other part was separated for biochemical analysis and kept at −80°C until the day determined for analysis.

Biochemistry

For analysis, tissues were taken from the freezer and weighed. Phosphate buffer was added to tissue samples to obtain a 10% mixture and homogenized in ice at 12,000 rpm for 1–2 min (IKA, Germany).

MDA was measured in tissue homogenate. The remaining homogenate was centrifuged at 5000 rpm for 30 min at +4°C to obtain the supernatant. SOD and CAT enzyme activities of the tissues and GSH, total antioxidant status (TAS) and total oxidant status (TOS) levels were determined in supernatant.

MDA levels were determined based on the method of Ohkawa et al. [11]. The principle of the method is based on the reaction of MDA in the tissue homogenate with thiobarbituric acid at 95°C to form a pink colored product and then extraction with n-butanol for 5 min. The resulting mixture was centrifuged for 20 min at 5000 rpm and the color of the supernatant was measured using a spectrophotometer at 535 nm.

GSH levels were determined based on the method of Ellman [12]. After addition of the chemicals according to the method, GSH of the supernatant reacts with 5,5′-dithiobis 2-nitrobenzoic acid (DTNB) to form a yellow color. The amount of GSH was determined by measuring the yellow color using a spectrophotometer at 410 nm.

The activity of the SOD enzyme was measured based on the method of Sun et al. [13]. After the addition of the chemicals according to the method, xanthine is converted to uric acid by xanthine oxidase, causing the formation of superoxide radicals and H_2O_2. The formation of superoxide radicals leads the reduction of NBT (nitro blue tetrazolium) in the medium and the formation of a blue color. The activity of the SOD enzyme was calculated by measuring the absorbance of the blue color in a spectrophotometer at 560 nm.

The activity of the CAT enzyme was measured based on the method of Aebi [14]. As H_2O_2 exhibits absorption in the ultraviolet spectrum, the maximum absorbance is measured at 240 nm. The addition of H_2O_2 to the test medium causes the decomposition of H_2O_2 into water and oxygen by the CAT and a decrease in absorbance at 240 nm. The reduction in absorbance was recorded for 1 min to calculate the CAT activity.

TOS levels were measured based on the method of Erel [15]. TOS kit (Rel Assay Diagnostics, Gaziantep, Turkey) was used and TOS measurement was determined by measuring the resulting color after the addition of reagents and supernatant according to the kit principles at 530 nm with ELISA.

TAS levels were measured based on the method of Erel [16]. Rel Assay brand kit (Rel Assay Diagnostics) was used and TAS measurement was determined by reading the resulting color after the addition of reagents and supernatants based on the kit procedure at 660 nm via ELISA.

Blood samples were centrifuged at 4000 rpm for a few minutes to obtain plasma. Plasma AST, ALT and ALP levels were measured by using commercial Architect C8000 automatic analyzer kids (Architect/Aeroset Aspartate Aminotransferase Reagent Kit, Alanine Aminotransferase Reagent Kit, Alkaline Phosphatase Reagent Kit, respectively).

Histology

Tissue samples were fixed with 10% formaldehyde for 48 h under ambient temperature. Tissue specimens were

then embedded in paraffin blocks following the lavage and dehydration process by passing through incremental ethanol series (50%–99%), permeabilization process by passing through xylene, and infiltration process by passing though melted paraffin series at 62°C. 5–6 μm thick sections were excised from the paraffin blocks using a microtome and placed on the slides. All sections were stained with hematoxylin-eosin and then examined under light microscopy (Nikon Eclipse Ni) and photographed using a camera (Nikon DS-Fi2) and analyzed with the image analysis system (Nikon NIS-Elements Documentation) (Nikon Corporation, Tokyo, Japan).

In liver sections; focal necrosis, apoptosis and focal inflammation scores (1 focus/×10 magnification = 1, 2–4 focus/×10 magnification = 2, 5–10 focus/×10 magnification = 3, focus >10/×10 magnification = 4) were determined. Bridging necrosis score (focal bridging necrosis = 1, Zone3 necrosis (limited) = 2, Zone3 necrosis (plenty) = 3, Zone3 necrosis and fewer portal-central bridging necrosis = 4, Zone3 necrosis and multiple portal-necrosis = 5, panacea or multi-acinar necrosis = 6) was determined.

Portal inflammation score (mild, in some or all portal areas = 1, medium, in some or all portal areas = 2, medium/severe, in all portal areas = 3, severe, in all portal areas = 4) was identified.

Fibrosis score (fibrous portal expansion ± short septum (focal) = 1, fibrous portal expansion ± short septum (extensive) = 2, rare portal-portal bridging = 3, frequent portal-portal bridging = 4, frequent bridging fibrosis and rare nodule formation = 5, cirrhosis = 6) was determined [17].

Hepatocyte vacuolization (no vacuolization = 0, mild and low vacuolization prevalence = 1, moderate and extensive vacuolization = 2, severe and extensive vacuolization = 3) was determined [18]. Liver damage scores are presented in Table 1.

Table 1: Liver tissue damage scores.

Groups	Mean	Minimum	Maximum
C[a]	0	0	0
Co[a]	0	0	0
Cr[a]	0	0	0
CCl_4[b]	15	10	17
CCl_4 + Cr[c]	5	4	7

Data are summarized using median, minimum and maximum values for histological scoring (n = 10). Groups: control (C), received normal saline solution; corn oil (Co), received corn oil; crocin (Cr), received crocin; CCl_4, received CCl_4; CCl_4 + Cr, received CCl_4 with crocin. Different superscripts in the groups represent the statistical significance ($p < 0.05$).

Statistical analysis

The distributional properties of data were examined by Shapiro-Wilk test, mean and standard deviations were obtained for descriptive analysis. The homogeneity of the variances was examined by the Levene test. In order to compare groups with homogeneous variance, one-way ANOVA and then Tukey HSD paired comparison methods were used. The Welch test and Tamhane T2 matched comparison method were used for the non-homogeneous variances. Histopathological scores were summarized using median, minimum and maximum values. Conover pairwise comparison method and the Kruskal-Wallis test were used to compare groups. p-Values smaller than 0.05 were accepted statistically significant ($p < 0.05$) in all tests.

Results

Biochemistry

Oxidant–antioxidant parameters of liver tissue are presented in Table 2 for all groups. When the liver tissue was examined based on GSH levels, it was determined that crocin application caused a significant increase ($p < 0.05$) in GSH levels when compared to the C group. While CCl_4 administration resulted in a decrease in GSH levels, an increase in GSH levels was observed when CCl_4 was co-administered with crocin.

We demonstrated that CCl_4 administration caused to increase significantly ($p < 0.05$) in MDA levels compared to the C group, but MDA levels were decreased significantly ($p < 0.05$) in the CCl_4 + Cr group compared to the CCl_4 group. While CCl_4 administration resulted in a statistically significant increase ($p < 0.05$) in SOD activity compared to all other groups, SOD activity was decreased significantly ($p < 0.05$) in the CCl_4 + Cr group compared to the CCl_4 group. On the other hand, we found that CAT activity was increased statistically significantly ($p < 0.05$) in the CCl_4 group compared to the C group, but co-administration of crocin with CCl_4 induced an insignificant decrease in the increase in the abovementioned activity. We found that crocin application increased in TAS values, whereas CCl_4 application decreased in TAS values but these differences were not statistically significant. On the contrary, CCl_4 administration caused an increase in TOS values, whereas CCl_4 administration with crocin resulted in a decrease in TOS values.

AST, ALT and ALP levels are presented in Table 3 for all groups. Plasma AST, ALT and ALP values exhibited a

Table 2: Liver tissue oxidant–antioxidant parameters for all groups.

Groups	MDA (nmol/gwt)	GSH (nmol/gwt)	SOD (U/g protein)	CAT (K/g protein)	TAS (mmol/L)	TOS (μmol/L)
C	561.80±64.36^{a}	1649.29±213.33^{a}	37.54±6.23^{a,c}	47.98±13.58^{a}	2.41±0.11	11.25±1.14^{a,b}
Co	482.07±79.92^{a,b}	1876.85±213.77^{a}	43.46±7.22^{a,b}	55.69±13.21^{a,b}	2.37±0.15	10.92±0.66^{a}
Cr	455.60±76.93^{b}	2319.14±270.00^{b}	33.65±9.38^{c}	47.29±12.04^{a}	2.49±0.21	10.14±0.92^{a}
CCl_4	1461.61±413.58^{d}	1588.40±192.37^{a}	56.46±5.45^{b}	67.32±3.93^{b}	2.31±0.17	16.47±4.24^{a,b}
CCl_4+Cr	911.20±170.17^{e}	1833.26±417.60^{a}	46.39±4.87^{d}	61.08±5.66	2.45±0.15	12.87±1.06^{b}

Data are presented as mean±standard deviation (n=10). MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; TAS, total antioxidant status; TOS, total oxidant status; gwt, gram wet tissue. Groups: control (C), received normal saline solution; corn oil (Co), received corn oil; Crocin (Cr), received crocin; CCl_4, received CCl_4; CCl_4+Cr, received CCl_4 with crocin. Different superscripts in the groups represent the statistical significance ($p<0.05$).

Table 3: Plasma AST, ALT and ALP levels for all groups.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
C	102.30±14.31^{a}	90.00±80.47^{a}	414.60±95.22^{a,c}
Co	107.80±32.88^{a}	77.20±31.69^{a}	357.90±108.39^{a}
Cr	161.00±132.43^{a}	53.30±7.96^{a}	361.40±99.78^{a}
CCl_4	1453.10±586.04^{b}	1575.70±596.21^{b}	948.90±192.38^{b}
CCl_4+Cr	627.50±383.77^{c}	545.30±254.67^{c}	713.40±277.74^{b,c}

Data are presented as mean±standard deviation (n=10). AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase. Different superscripts in the groups represent the statistical significance ($p<0.05$).

significant increase ($p<0.05$) in the CCl_4 group, whereas CCl_4 administration with crocin resulted in a significant decrease ($p<0.05$) in elevated AST, ALT and ALP values.

Histology

Hematoxylin-eosin-stained sections in control (Figure 1A–C), crocin (Figure 1D–F) and corn oil (Figure 1G–I) groups demonstrated normal liver lobular structures and central vein and hepatocyte cords located at the center of liver

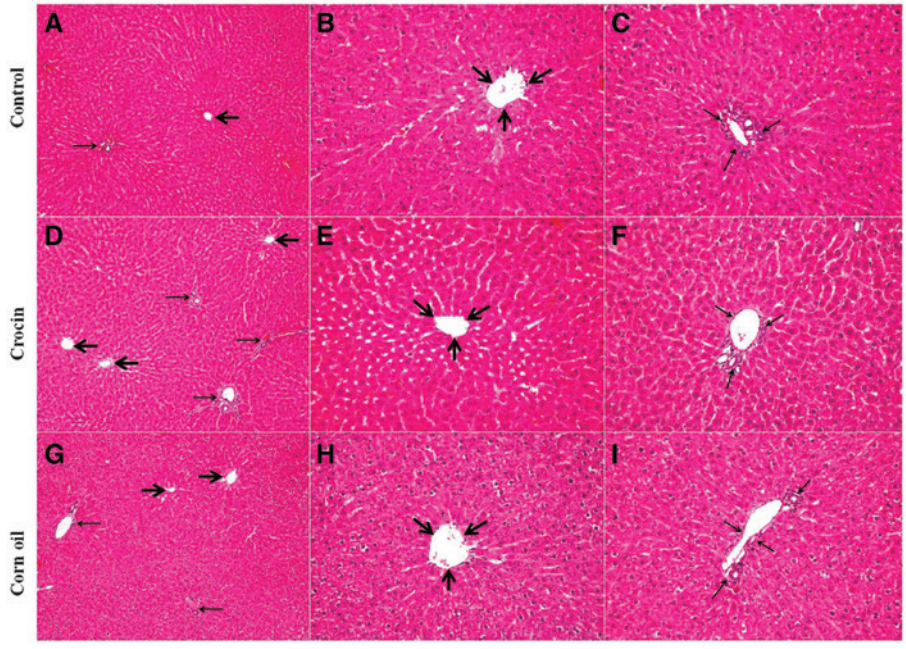

Figure 1: Liver histological microphotographs for C, Cr and Co groups.
(A–C); C group, (A); central vein (thick arrow), portal area (fine arrow). H–E, ×10, (B); central vein (arrow). H–E, ×20, (C); portal area (arrow). H–E, ×20, (D–F); Cr group, (D); Dcentral vein (thick arrow), portal area (fine arrow). H–E, ×10, (E); central vein (arrow). H–E, ×20, (F); portal area (arrow). H–E, ×20, (G–I); Co group, (G); central vein (thick arrow), portal area (fine arrow). H–E, ×10, (H); central vein (arrow). H–E, ×20, (I); portal area (arrow). H–E, ×20.

lobules and sinusoidal capillaries were observed in normal histological structure. Eosinophilic cytoplasm and centrally located, euchromatic, uniform circular-shaped nucleus structures were observed in hepatocytes and assessed to be in normal histological appearance. Kupffer cells were observed at normal density and with normal microscopic structure. Lumens of arteries, veins and bile ducts in portal triads that were observed around the liver lobes were clear and evaluated to be in normal histological appearance.

Extensive necrosis areas were observed in all liver sections stained with hematoxylin-eosin in the CCl_4 group. Bridging was noticed between the necrosis areas in the liver parenchyma. Inflammation in varying degrees was present in necrosis areas. Intracytoplasmic vacuolization was observed between the hepatocytes in the parenchymal areas that were observed in necrotic areas. Among hepatocytes in the liver parenchyma, numerous mitotic figures and apoptotic particles were detected. Fibrosis was observed in necrosis areas at different levels, around the central vein and in portal areas (Figure 2A–E).

It was observed that the hepatic histological damage parameters observed in the CCl_4 group were significantly reduced in liver sections stained with hematoxylin-eosin in the CCl_4 + Cr group. Sporadic focal necrosis areas were detected in liver sections and lytic necrosis were detected in hepatocytes. Minimal inflammation and fibrosis were observed around the central vein and portal areas. Intracytoplasmic vacuolization was observed in certain hepatocytes. Mitotic figures were found in certain areas in hepatocytes (Figure 2F–K).

Discussion

The current study aimed to demonstrate the therapeutic effect of crocin on CCl_4 induced hepatotoxicity by inhibiting oxidative stress.

Previous studies have reported that CCl_4 causes oxidative stress-induced tissue damage in many organs, particularly in the liver and kidneys, and leads to classical cirrhosis-like disorders such as coagulative necrosis, severe fibrosis, leukocyte infiltration, hemorrhage, fat degeneration, and degenerative nodules especially in liver tissue [19]. The CCl_4 tissue damage mechanism begins with the formation of $CCl_3^{\cdot}$ by CYP enzyme system. The formation of $CCl_3OO^{\cdot}$, a highly reactive product, results from the reaction of $CCl_3^{\cdot}$ with oxygen [20]. These radicals resulting from the metabolization of CCl_4 attack the proteins and/or lipids of the tissues and therefore initiate lipid peroxidation, inflammation, hepatotoxicity and MDA formation in the tissues [3]. MDA is one of the end products of lipid peroxidation and is considered as an indicator of lipid peroxidation [21]. Lipid peroxidation, which is induced by free oxygen radicals produced by oxidative stress, is responsible for the pathogenesis due to cancer, liver diseases and toxic cellular damage [22].

Immediately after exposure to CCl_4, transfusion of the transaminases into circulation reflects the increases in serum and hepatic injury enzymatically. Therefore, crocin, a hepatoprotective agent, was chosen to correct the hepatic injury induced by CCl_4. In liver damage, intracellular AST, ALT, and ALP enzymes are excreted outside the cell and plasma levels increase. Therefore,

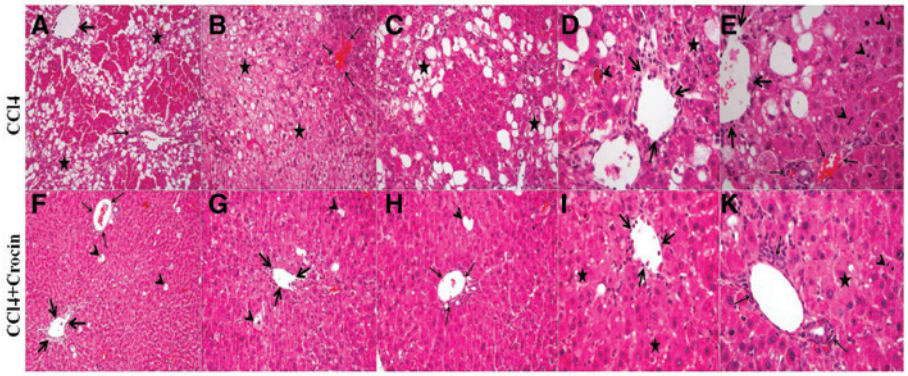

Figure 2: Liver histological photographs for CCl_4 and CCl_4 + Cr groups.
(A–E); CCl_4 group, (A); central vein (thick arrow), portal area (fine arrow), bridge necrosis (asterisk). H–E, ×10, (B); vascular congestion in portal area (arrow), vacuolization in hepatocytes (asterisk). H–E, ×20, (C); bridge necrosis and vacuolization in hepatocytes (asterisk). H–E, ×20, (E); thickening of the central venous wall (thick arrow), vascular congestion in portal area (fine arrow), mitotic figure (arrowhead). H–E, ×40, (F–K); CCl_4 + Cr group, (F); central vein (thick arrow), portal area (fine arrow), lytic necrosis in hepatocytes (arrowhead). H–E, ×10, (G); central vein (arrow), lytic necrosis in hepatocytes (arrowhead). H–E, ×20, (H); portal area (arrow), lytic necrosis in hepatocytes (arrowhead). H–E, ×20, (I); central vein (arrow), vacuolization in hepatocytes (asterisk). H–E, ×40, (K); portal area (arrow), mitotic figure (arrowhead), vacuolysis in hepatocytes (asterisk). H–E, ×40.

these enzymes are considered as indicators of liver damage [23].

In the current study, we demonstrated that CCl_4 administration caused to increase significantly ($p < 0.05$) in plasma AST, ALT and ALP levels. This may be due to elevated levels of cytosolic enzymes in serum levels such as ALT, AST and ALP, owing to the disturbance of the liver cell membrane caused by oxidative stress. The results of previous studies were consistent with the results of the present study, demonstrating that the serum levels of these enzymes were elevated after the hepatic injury induced by CCl_4 [24, 25].

The action of hepatoprotective drugs is through the reduction of liver damage caused by CCl_4 or other hepatotoxicants or restoration of normal liver physiology [26]. In the present study, we demonstrated that crocin administration improves subsequent elevations in the levels of AST, ALT and ALP by CCl_4 administration. The decrease in ALT, AST and ALP levels was a result of the crocin regulating the integrity of the hepatic cellular membrane owing to its strong antioxidant properties, which was a clear evidence of the hepatoprotective effect observed after its administration. Our study showed compatibility with previous studies, in which the researchers demonstrated that increased levels of AST, ALT and ALP were significantly reduced by crocin in experimental liver damage caused by morphine [27], amiodarone [28], patulin [29] and acetaminophen [30] in animal models.

The organism contains endogenous antioxidant enzymes as a defense mechanism for scavenging and protecting against tissue damages caused by free radicals [31]. SOD and CAT enzymes are involved in the enzymatic antioxidant system and play a key role in protecting the body against the harmful effects of lipid peroxidation and H_2O_2 caused by oxidative stress [32].

GSH is a member of the non-enzymatic antioxidant system and combines with free radicals to protect the integration of cellular membranes, which play an important role in the balance of the antioxidant defense mechanisms of tissues [33]. Therefore, antioxidant enzyme (SOD and CAT) activities and GSH and TAS levels were measured in liver tissue to determine the antioxidant activity of crocin in the current study. It is considered that lipid peroxidation demonstrates the destructive liver damage process induced by CCl_4 administration. In the present study, we found a remarkable increase in SOD and CAT activities and MDA and TOS levels, while GSH and TAS levels decreased due to oxidative stress induced liver damage caused by CCl_4 administration in rat liver tissues. Elevation of MDA and TOS levels and SOD and CAT activities in liver tissue demonstrated the tissue damage proceeded by increased lipid peroxidation and reduced antioxidant defense mechanism that is responsible for the prevention of increased free radical production. Our results showed compatibility with Gangarapu et al. [24], Ozturk et al. [34] and Ranjbar et al. [35]. The researchers proved that CCl_4 administration in these studies caused a significant increase in MDA levels and SOD and CAT activities and a decrease in GSH content in rat liver tissues.

Based on our previous [36] and current studies, it could be argued that crocin exerts its protective effect through the antioxidant regulatory mechanism. Typically, oxidative stress is modulated by the antioxidant defense system [37].

GSH has a vital role in the antioxidant defense systems of tissues. In order to protect tissues from oxidant attacks, GSH neutralizes free radicals, stabilizes sulfhydryl groups, acts as the co-enzyme of the GPx enzyme, which converts H_2O_2 into water.

In one study, it was reported that saffron extract and crocin significantly reduced ischemia-reperfusion-induced oxidative damage in rat kidneys [38]. The researchers found that saffron extract and crocin decreased MDA levels and lipid peroxidation induced by free radicals due to its radical scavenging properties. Furthermore, Altinoz et al. [39] showed that elevated MDA levels in renal tissues of diabetes induced rats were lowered and GSH levels were increased with crocin administration. These findings were consistent with the present study results, which was due to the fact that elevated levels of enzymatic antioxidants, including SOD and CAT, in animals exposed to CCl_4 intoxication, and reduced levels of GSH and TAS were back to normal levels after crocin treatment. Furthermore, the concurrent decrease in SOD and CAT activities and MDA levels and the increase in GSH levels confirm the association between the antioxidant regulation mechanisms. Certain studies reported that the antioxidant enzyme activities were correlated with the enzyme mRNA expressions [40]. These studies have shown that SOD enzyme activity and SOD enzyme mRNA expression increased in various regions of the brain tissue of diabetes induced rats. The elevation in gene expression of these antioxidant enzymes is likely to be one of the most important causes of ROS-induced damage that occurs in the brain or in diabetes. In another study, it was reported that changes in antioxidant enzyme activities, including SOD and CAT, in neurons were due to increased oxidative stress [41]. Ghadrdoost et al. [42] suggested that saffron extract and crocin improved oxidative stress markers in the hippocampus. Our study was supported by previous studies and the present study resulted in increases in SOD and

CAT activities after CCl_4 application, while we observed that these enzyme activities approach normal values due to decrease in markers which caused oxidative stress after crocin treatment. Furthermore, previous studies have shown that crocin increases liver GSH content in animal models, while significantly reducing high MDA levels by induction of cisplatin [43], cyclophosphamide [44] and diazinone [45] toxicity in animal models.

Another interesting finding in our study was the consistency of the biochemical and histological results. We examined the disorders including focal necrosis, apoptosis, inflammation, bridge necrosis and fibrosis in the liver tissue. Control, crocin and corn oil application groups exhibited normal liver appearance. When CCl_4 was administered, extensive bridge necrosis and extensive areas of inflammation were observed in the liver parenchyma. Furthermore, extensive parenchymal vacuolization and apoptotic particles were observed in the parenchymal areas and different degrees of fibrosis were observed around the central vein and portal regions. When crocin was applied, significant improvement was observed in liver histological damage parameters. Akbari et al. [46] demonstrated that ischemia-reperfusion induced liver damage significantly improved with crocin administration at a dose of 200 mg/kg for 7 days.

Recent studies have shown that saffron and its active ingredients have a protective effect against several toxic substances in various tissues including the liver tissue [47, 48]. Sun et al. [43] investigated the treatment effect of crocin on hepatoxicity caused by cisplatin induction. They showed that crocin reduced cisplatin induced hepatic focal necrosis. Consistent with the literature, we demonstrated that we were able to remove the hepatic damage induced by CCl_4 with crocin treatment by using biochemical parameters and histologic examinations. We demonstrated that crocin inhibited apoptosis and parenchymal tissue damage caused by CCl_4 in the liver, and we considered that the hepatoprotective effect of crocin might be due to its anti-inflammatory and antioxidant action.

In conclusion, we demonstrated that crocin inhibits oxidative stress and has protective effects on CCl_4-induced liver damage. Crocin scavenges ROS and has an antioxidant effect. While MDA and TOS levels and AST, ALT, ALP, SOD and CAT levels decreased due to the said antioxidant effect, GSH and TAS levels increased after the crocin treatment.

Acknowledgement: This study was supported financially by Karabük University scientific research fund (KBU-BAP-15/2-YL-009).

Conflict of interest: The authors declared no conflict of interest.

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