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Crocin protects intestine tissue against carbon tetrachloride-mediated oxidative stress in rats

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Abstract. Saffron is used in traditional medicine for its hypolipidemic, anti-inflammatory and anti-carcinogenic properties as a natural remedy in treatment of diseases. The objective of the present study was to demonstrate the protective effect of crocin (one of the main ingredients of saffron) on carbon tetrachloride (CCl_4) damage in intestinal mucosa. MDA, GSH, SOD, CAT, TAS and TOS levels were measured in experimental animal tissue samples and these were compared with histologic lesions induced by CCl_4. CCl_4 caused an increase in MDA, SOD, CAT and TOS levels and a significant decrease in GSH and TAS levels in rat intestinal tracts. After crocin treatment, serious improvements were observed in histological lesions and biochemical results in the intestinal tract. In conclusion, crocin inhibited the toxic effects induced by CCl_4 in the intestine by its strong antioxidant properties.

Key words: CCl_4 — Crocin — Oxidative stress — MDA — Intestine

Introductıon

Carbon tetrachloride (CCl_4) is a colorless, transparent and volatile liquid xenobiotic that is used to induce liver damage extensively in empirical studies.

The cytochrome P450 (CYP) enzyme system is found in both the liver and digestive tract mucosa epithelium (Shimizu et al. 1990; Xie et al. 2011; Zhu and Zhang 2012). CYP2E1 is the major isoenzyme in CYP and metabolizes CCl_4 in addition to being the isoenzyme that plays an important role in the metabolism of xenobiotics and endogenous compounds (Nelson et al. 1996; Bolt et al. 2003), as well as initiating lipid peroxidation by production of reactive oxygen species (ROS) (Lieber 1997). Due to the cytotoxic effects of ROS and lipid peroxides (Lee et al. 1995) and the capacity of CYP2E1 for producing various intermediate products (Dai et al. 1993; Lu et al. 2008), this enzyme appears to play a key role in the pathogenesis of tissue damage.

The function of the CYP450 enzyme system includes the conversion of CCl_4 to a reactive toxic substance, namely the inter-metabolite trichloromethyl radical ($CCl_3^{\bullet}$), and then formation of trichloromethylperoxy radical ($CCl_3OO^{\bullet}$) in the presence of oxygen. Both free radicals could bind to proteins and lipids, causing tissue lipid peroxidation, inflammation, hepatotoxicity and malondialdehyde (MDA) accumulation (Recknagel et al. 1989). MDA is one of the final products of lipid peroxidation (Mohamed et al. 2008). Oxidative stress induced free oxygen radicals cause lipid peroxidation and may lead to cancer induced pathogenesis, liver diseases and toxic cellular damage (Muriel and Escobar 2003).

There is a balance between oxidants and antioxidants (Cornelli 2009). In case of excessive oxidant production or when antioxidants could not perform their tasks adequately, in other words, if an imbalance between the oxidants and

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antioxidants is present, oxidative stress occurs, and the oxidant molecules lead to protein, carbohydrate and nucleic acid damage (Halliwell 1991; Radi et al. 2014). To prevent oxidative stress-induced cellular damage, organisms have an endogenous enzymatic antioxidant defense systems such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and non-enzymatic antioxidants such as glutathione (GSH), uric acid, bilirubin, and vitamins C and E (Dunning et al. 2013). Endogenous antioxidant enzymes form an effective defense mechanism to neutralize and protect against the damage induced by free radicals (Bansal et al. 2005). SOD and CAT, which are both antioxidant enzyme systems, play significant roles in protecting the body from the adverse effects of lipid peroxidation and hydrogen peroxide *via* oxidative stress (Zhu et al. 2012). GSH, a non-enzymatic antioxidant, binds with free radicals, preserving the integration of cellular membranes, which have a significant role in the antioxidant defense mechanism equilibrium in the body (He et al. 2012).

Saffron (*Crocus sativus L.*) is an iridaceous plant, indigenous to Iran, Spain, Kashmir (India and Pakistan), Greece, Azerbaijan, China, Morocco, Mexico, Libya, Turkey and Austria. In addition to its antioxidant properties, saffron is used as a remedy in traditional medicine for its hypolipidemic, anti-inflammatory and anti-carcinogenic properties in various diseases (Rios et al. 1996). Saffron basically contains crocin, crocetin and safranal. It also includes proteins, sugar, vitamins, flavonoids, amino acids, vital minerals and other chemical components (Liakopoulou-Kyriakides and Kyriakidis 2002). Saffron contains over 150 volatile and aromatic compounds such as zeaxanthin, lycopene and α- and β-carotene, however crocin is the source of its golden and orange colors. Crocin is the glucosyl ester of 8,8'-diapocarotene-8,8'-dioic acid (crocetin), one of the many carotenoid families that are easily soluble in water.

Recent studies demonstrated that phytochemical therapy has been one of the most effective remedies for tissue injury, particularly *via* regulation of free radicals (Hassan et al. 2012).

The most important function of the small intestine is to absorb the nutrients and transfer these nutrients to the circulation. The intestinal mucosa could experience oxidative stress damage due to exposure to ROS produced by ingredients such as bacterial metabolites, oxidants, oxidized food residues in the lumen, metal ions (iron, copper etc.) (Halliwell et al. 2000). Although small intestine injuries caused by ROS were manifested with diseases such as ischemia-reperfusion (Halliwell and Gutteridge 2015), surgical stress (Prabhu et al. 2000), radiation enteritis (Mutlu-Türkoğlu et al. 2000), iron supplementation (Srigiridhar and Nair 1998), and zinc deficiency (Virgili et al. 1999), no information is available on ROS-related intestinal damage induced by CCl_4.

In the present study, we aimed to demonstrate oxidative damage caused by CCl_4-induced ROS products in intestinal tissues and the pathogenesis of the damage in tissues by examining the changes accompanied by antioxidant structures. One of the major objectives of our study was to demonstrate the protective effect of crocin against CCl_4 damage in the intestinal mucosa.

Material and Methods

Experimental animal

In the present study, 50 male Wistar albino rats with a mean weight of 225–250 g were procured from Inonu University, Faculty of Medicine, Experimental Animal Breeding and Research Center (INUTF-DEHUM). The study commenced after the experimental animal ethics committee approval was obtained. Fresh drinking water was provided and the cages were cleaned daily. The rats were kept under 21°C ambient conditions, and 55–60% ambient humidity with 12 hours light (08:00 to 20:00) and 12 hours darkness. Rats were fed *ad libitum* with standard pellet feed throughout the study.

Experimental Design

Fifty male Wistar rats obtained from the experimental animal unit were randomly divided into five groups: Control group (1 ml/kg/day physiological saline solution; $n = 10$), Corn oil group (1 ml/kg/day corn oil; $n = 10$), Crocin group (100 mg/kg/day crocin (Sigma Aldrich Co., USA, CAS Number; 42553-65-1); $n = 10$), CCl_4 group (1:1 carbon tetrachloride (Sigma Aldrich Co., USA) was dissolved in corn oil and a 0.5 ml/kg dose was applied every other day; $n = 10$), CCl_4+Crocin group (100 mg/kg/day crocin and 1:1 carbon tetrachloride were dissolved in corn oil and 0.5 ml/kg dose was applied every other day; $n = 10$).

All chemical applications were repeated regularly for 15 days at the same time every day orally (*via* gavage).

Samples

At the end of the experiment, all animals were decapitated under xylazine-ketamine anesthesia. Then, small and large intestine tissue samples were excised. Tissue samples were washed with physiological saline solution. A portion of the tissues were placed in 10% formaldehyde for histopathological examinations. The remaining portion was stored at –80°C until the biochemical analyzes were conducted.

Biochemical analysis

On the day of the experiment, small and large intestine tissues were removed from the deep freeze and weighed. Phos-

phate buffer was added to form a 10% homogenate and the product was homogenized at 12,000 rpm for 1–2 minutes in ice (IKA, Germany). The homogenates were used to measure MDA levels. The homogenates were centrifuged at 5000 rpm at +4°C for 30 minutes to obtain supernatant. The supernatant was used to measure GSH, SOD, CAT, total antioxidant status (TAS), total oxidant status (TOS) and protein levels.

The Ohkawa et al. (1979) method was used for MDA analysis. The measurement was conducted on the supernatant extracted from the n-butanol phase of the pink colored product of the reaction between the sample MDA and the thiobarbituric acid at 95°C and at 535 nm using a spectrophotometer (T80 UV/VIS Spectrometer, PG Instruments Ltd.). n-butanol was used as a blank and tetramethoxypropane was used as a standard. The results were expressed as nanomoles *per* gram of wet tissue.

GSH analysis was conducted based on the method developed by Ellman (1959). In this method, the reaction between the glutathione in the medium and 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) after these chemicals were added to the test tubes produce a yellow-green color. GSH content could be determined by the light intensity of the yellow-green color at a wavelength of 410 nm in a spectrophotometer. Distilled water was used as a blank. The results were expressed as nanomoles *per* gram of wet tissue.

SOD enzyme activity was determined with the method developed by Sun et al. (1988). The method utilizes the superoxide radicals produced by xanthine-xanthine oxidase. The superoxide radical reduces NBT (nitro blue tetrazolium), producing a blue-colored formazan. The SOD activity was measured using the absorbance of this formazan at 560 nm. Distilled water was used as a blank. SOD activity was expressed as U/g protein.

CAT enzyme activity was measured based on the method described by Aebi et al. (1974). Hydrogen peroxide (H_2O_2) causes absorption in the ultraviolet spectrum. The maximum absorbance is observed at 240 nm. Catalase decomposes the added hydrogen peroxidase into water and oxygen and the process is characterized by the reduction in absorbance at 240 nm. The enzyme activity could be measured by recording the decrease in absorbance for one minute. CAT activity was presented as K/g protein.

The Lowry method (Lowry et al. 1951) was used to determine the protein content of tissue homogenate samples.

Tissue TOS levels were determined with Erel's method (Erel 2005). In the study, total oxidant status kit (Rel Assay Diagnostics) was adapted for use on Synergy HT Biotek biochemical auto-analyzer. The ferrous ion chelator complexes were converted to ferric ions by the oxidants in the sample. The ferric ions and the chromogenic solution form a colored complex that is measured spectrophotometrically at 530 nm, revealing the sample oxidant content. Standard 20 μmol/l H_2O_2 solution was used in the procedure. The results were expressed in micro-molar hydrogen peroxide equivalent *per* liter (μmol H_2O_2 Equiv./l).

TAS levels were determined with Erel's method (Erel 2005). Rel Assay brand commercial kit was used for the measurement of TAS levels. The measurement is conducted by the reduction of colored ABTS cationic radical by the antioxidant molecules in the sample, resulting in decolorization of the colored radical in proportion to the total concentration of the antioxidant molecules it contains (Hu et al. 1993). A water-soluble analogue of vitamin E, Trolox was used as a calibrator. The results were presented in mmol Trolox Equiv./l.

Histological determination

Tissue samples obtained from small and large intestines were fixed using 10% formaldehyde for 48 hours at ambient temperature. The tissue samples were washed, dehydrated through increasing ethanol series (50%–99%) and rendered permeable through xylene and infiltration though melted paraffin series at 62°C, and embedded in paraffin blocks. 5–6 μm thick paraffin block sections were then excised with a microtome and placed on slides. All were stained with hematoxylin-eosin and then examined under a light microscope (Nikon Eclipse Ni), photographed with a camera (Nikon DS-Fi2) and analyzed with an image analysis system (Nikon NIS-Elements Documentation; Nikon Corporation, Tokyo, Japan).

Injury parameters for each section obtained from the small intestine (villus blunting, epithelial damage, villus damage, Lieberkühn crypt damage and inflammation) and the large intestine (Goblet cell decrease-loss, epithelial damage, mucosal fibrosis, Lieberkühn crypt damage and inflammation) were scored with 0–3 points (Normal histology = 0, Mild = 1, Moderate = 2, Severe = 3). The maximum score was 15 (Erben et al. 2014).

Statistical analysis

Statistical analysis was conducted with SPSS v. 21 software. Shapiro Wilk test was used to determine normal distribution of the data and descriptive analysis was conducted with mean and standard deviations. Homogeneity of the variances were tested by the Levene test. Groups with homogeneous variances were compared with one-way ANOVA followed by Tukey HSD paired comparison methods. For non-homogenous variances, the Welch test and the Tamhane T2 paired comparison method were used. Data were summarized using median, minimum and maximum values for histological scoring. The groups were compared with the Kruskal-Wallis test and paired comparisons were conducted with the Conover method. The significance level was accepted as 0.05 for all tests.

Results

Biochemical results

All biochemical parameters for small and large intestine tissues are presented in Table 1. CCl_4 administration caused a significant decrease ($p < 0.05$) in the small and large intestine GSH levels, whereas when treated with crocin, GSH levels returned to values close to that of the control group.

While intestinal tissue MDA levels demonstrated a statistically significant increase ($p < 0.05$) with CCl_4 application, there was a statistically significant decrease ($p < 0.05$) in control group values when CCl_4 was administered in conjunction with crocin.

Tissue SOD activity demonstrated a statistically significant increase ($p < 0.05$) with CCl_4 administration, while crocin treatment decreased the enzyme activity ($p < 0.05$) to a level that was close to the control group levels.

While administration of CCl_4 resulted in a significant decrease in CAT enzyme activity in intestine tissues ($p < 0.05$), its application with crocin caused a significant decrease in small intestine tissue ($p < 0.05$), and an insignificant decrease in large intestine tissue.

CCl_4 administration resulted in a significant decrease in tissue TAS levels ($p < 0.05$), whereas when CCl_4 was administered with crocin, a non-significant increase in tissue TAS levels was observed.

The tissue TOS levels increased significantly ($p < 0.05$) with CCl_4 application, while the crocin treatment resulted in a non-significant decrease in small intestine TOS levels and in a significant decrease in the large intestine TOS levels ($p < 0.05$) and approached control group levels.

Histological results

In small intestine sections of control, crocin and corn oil groups, the mucosa was smooth and included thin and long villi and *Lieberkühn crypts* on the villus bases. The surface of the villi was covered with monolayer *columnar epithelium* with microvilli containing Goblet cells and was observed in normal histological appearance. *Lamina propria* connective tissue and smooth muscle layers of *lamina muscularis mucosa* and *tunica muscularis* were assessed to be in normal histological structure (Fig. 1A–I).

Mucosal degeneration was observed in some areas in the small intestine sections in the CCl_4 group stained with hematoxylin-eosin that contained villi and *Lieberkühn crypts*. Hemorrhage, intense inflammatory cell infiltration and fibrosis were observed in the areas of degeneration. In certain areas, total villus degeneration and *Lieberkühn crypt* damage were detected. The pyknotic-heterochromatic nucleus structure was noted extensively in the *Lieberkühn crypt* epithelial cells. Numerous short, blunt-shaped villi were identified (Figure 1J–L).

Epithelial damage was observed in the apical regions of the villi in the small intestine sections stained with hematoxylin-eosin in the CCl_4+Crocin group. In some villi, epithelial layer separation was identified due to subepithelial edema. The heterochromatic appearance in *Lieberkühn crypt* epithelial cells was apparent. The collapse

Table 1. Small and large intestine tissues oxidant-antioxidant parameters of all groups

Group	MDA (nmol/gwt)	GSH (nmol/gwt)	SOD (U/g protein)	CAT (K/g protein)	TAS (mmol/l)	TOS (μmol/l)
Small intestine tissue						
Control	$481.40 \pm 53.88^{\&,+}$	$728.18 \pm 124.04^{\star}$	$394.86 \pm 63.50^{\&}$	$36.23 \pm 5.55^{\star,\&}$	$1.70 \pm 0.30^{\&}$	$6.76 \pm 1.76^{\&}$
Corn Oil	$472.69 \pm 45.18^{\&,+}$	$844.84 \pm 134.99^{\&}$	$400.34 \pm 96.02^{\&}$	$33.71 \pm 6.84^{\star,\&}$	$1.84 \pm 0.37^{\&}$	$7.76 \pm 1.19^{\&}$
Crocin	$423.11 \pm 59.68^{\&,+}$	$1008.93 \pm 200.58^{\S,\&,+}$	$329.84 \pm 88.99^{\&,+}$	$17.22 \pm 4.21^{\S,\#,\&,+}$	$2.26 \pm 0.28^{\&,+}$	$5.61 \pm 2.23^{\&}$
CCl_4	$695.12 \pm 98.42^{\S,\#,\star,+}$	$632.03 \pm 90.71^{\#,\star}$	$513.73 \pm 93.64^{\S,\#,\star}$	$55.34 \pm 14.67^{\S,\#,\star,+}$	$0.82 \pm 0.47^{\S,\#,\star}$	$10.60 \pm 1.25^{\S,\#,\star,+}$
CCl_4+Crocin	$578.46 \pm 48.46^{\S,\#,\star,\&}$	$701.25 \pm 75.01^{\star}$	$442.76 \pm 60.36^{\star}$	$35.96 \pm 8.68^{\star,\&}$	$1.30 \pm 0.59^{\star}$	$7.04 \pm 1.53^{\&}$
Large intestine tissue						
Control	$374.20 \pm 58.15^{\&}$	$530.75 \pm 18.08^{\star}$	285.04 ± 56.84	$26.42 \pm 2.13^{\#,\&,+}$	$2.54 \pm 0.43^{\star}$	$5.50 \pm 1.71^{\&}$
Corn Oil	$423.78 \pm 79.64^{\&}$	$547.41 \pm 15.76^{\star,\&}$	$252.01 \pm 71.47^{\&}$	$35.85 \pm 6.05^{\S,\&,+}$	2.95 ± 0.25	$6.33 \pm 1.87^{\&}$
Crocin	$338.69 \pm 22.13^{\&,+}$	$598.05 \pm 30.53^{\S,\#,\&,+}$	$240.43 \pm 49.70^{\&}$	$34.38 \pm 5.86^{\&,+}$	$3.42 \pm 0.27^{\S,\&}$	$4.19 \pm 1.56^{\&}$
CCl_4	$592.62 \pm 101.81^{\S,\#,\star,+}$	$518.57 \pm 22.09^{\#,\star,+}$	$340.91 \pm 39.81^{\#,\&}$	$49.05 \pm 11.71^{\S,\#,\star}$	$2.69 \pm 0.52^{\star}$	$12.51 \pm 4.92^{\S,\#,\star,+}$
CCl_4+Crocin	$425.12 \pm 63.20^{\star,\&}$	$545.49 \pm 12.98^{\star,\&}$	278.06 ± 59.35	$45.17 \pm 7.44^{\S,\#,\star}$	3.05 ± 0.29	$7.11 \pm 1.67^{\&}$

Data are expressed 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. Superscripts represents the statistically significant difference: § $p < 0.05$ when compared to Control group, $^{\#}$ $p < 0.05$ when compared to Corn oil group, * $p < 0.05$ when compared to Crocin group, $^{\&}$ $p < 0.05$ when compared to CCI_4 group, $^{+}$ $p < 0.05$ when compared to CCI_4+Crocin group.

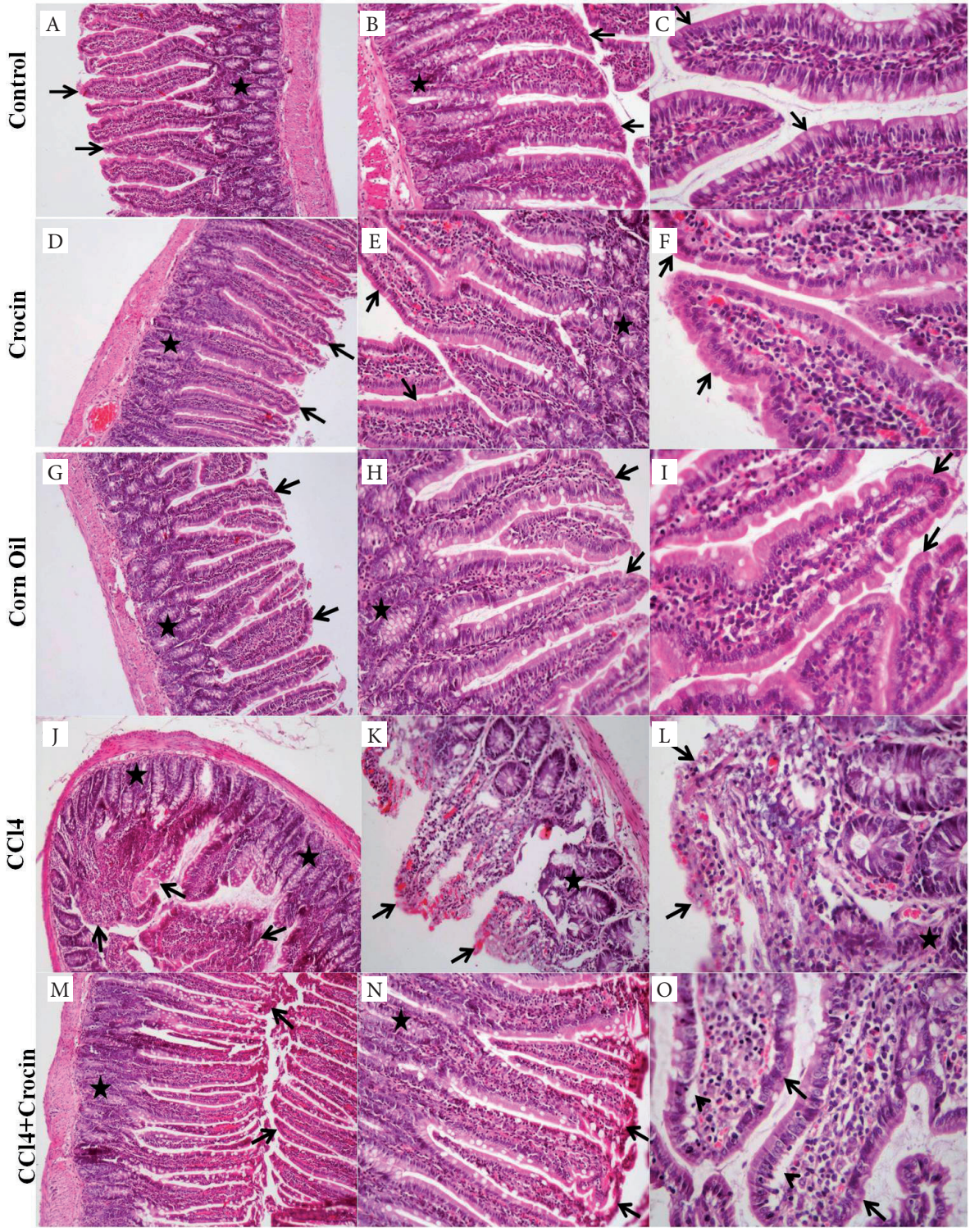

Figure 1. Histological microphotographs of small intestine tissue. Control group (**A–C**): Villus (arrow), *Lieberkühn crypt* (asterisk), absorptive epithelium on villus surface (arrow); Crocin group (**D–F**): Villus (arrow), *Lieberkühn crypt* (asterisk), absorptive epithelium on villus surface (arrow); Corn Oil group (**G–I**): Villus (arrow), *Lieberkühn crypt* (asterisk), absorptive epithelium on villus surface (arrow); CCl_4 group (**J–L**): Villus degeneration (arrow), *Lieberkühn crypt* (asterisk); CCl_4+Crocin group (**M–O**): (M): Villus (arrow), *Lieberkühn crypt* (asterisk), (N): epithelium damage in Villus apical (arrow), *Lieberkühn crypt* (asterisk). (O): absorptive epithelium on villus surface (arrow), sub-epithelial edema in villi (arrow head). Magnification: ×10 (A, D, G, M); ×20 (B, E, H, N); ×40 (C, F, I, L, O).

in *Lieberkühn crypts* was also notable. Moderate inflammatory cell infiltration was observed in the *lamina propria* connective tissue (Figure 1M–O).

Monolayer *columnar epithelium* rich in goblet cells on the mucosa surface and glands in the large intestine sections of control, crocin and corn oil groups was observed in normal histological appearance. *Lamina propria* was assessed to have the usual structure and mucosal lymphoid tissue groups were observed. The smooth muscle layers in *lamina muscularis mucosa* and the *tunica muscularis* were evaluated to have normal histological structure (Figure 2A–I).

Total mucosa degeneration regions were observed in some areas of the large intestine sections stained with hematoxylin-eosin in the CCl_4 group. Intensive inflammatory cell infiltration was present in the degeneration areas. In some areas, metaplasia, degeneration, flattening of epithelial cells and reduction of goblet cells were observed in the glandular epithelium of the large intestine. Furthermore, capillary congestion of the *lamina propria* connective tissue, increased eosinophil infiltration, occasional intraepithelial lymphocyte infiltration and lymphocyte clustering on the epithelial surface were noted. Collapse in *Lieberkühn crypts*, Goblet cell depletion and inflammatory cell infiltration in *lamina propria* were observed (Figure 2J–L).

The findings of degeneration and damage observed in the CCl_4 group in the large intestine sections stained with hematoxylin-eosin regressed and some were not even observed in the CCl_4+Crocin group. In sections, local degeneration was observed in the large intestine surface epithelial cells, however, it was noted that this degeneration was limited to the mucosa surface. Congestion was present in the capillaries near the mucosa surface. Inflammatory cell infiltration was observed in focal areas around the intestinal glands within the *lamina propria* connective tissue. The large intestine gland structures were observed in normal histological appearance (Figure 2M–O). Table 2 presents the small and large intestine tissues damage scores.

Table 2. Small and large intestine tissues damage scores

Group	Median	Minimum	Maximum
Small intestine tissue			
Control$^{\&,+}$	0	0	1
Corn Oil$^{\&,+}$	0	0	1
Crocin$^{\&,+}$	0	0	1
$CCl_4$$^{\S,\#,\star,+}$	10	8	11
CCl_4+Crocin$^{\S,\#,\star,\&}$	4	3	6
Large intestine tissue			
Control$^{\&,+}$	0	0	1
Corn Oil$^{\&,+}$	0	0	1
Crocin$^{\&,+}$	0	0	1
$CCl_4$$^{\S,\#,\star,+}$	8	7	10
CCl_4+Crocin$^{\S,\#,\star,\&}$	4	3	6

Data are summarized using median, minimum and maximum values for histological scoring. Superscripts represents the statistically significant difference: § $p < 0.05$ when compared to Control group, $^{\#}$ $p < 0.05$ when compared to Corn oil group, * $p < 0.05$ when compared to Crocin group, $^{\&}$ $p < 0.05$ when compared to CCI_4 group, $^{+}$ $p < 0.05$ when compared to CCI_4+Crocin group.

Discussion

Carbon tetrachloride (CCl_4) is one of the most used chemical in numerous empirical research to demonstrate liver damage that is associated with oxidative stress and free radicals (Dahiru et al. 2010). Previous empirical studies demonstrated that CCl_4 causes tissue damage in several organs, especially in liver and kidneys, leading to disorders similar to classical cirrhosis such as coagulative necrosis, severe fibrosis, mononuclear cell infiltration, hemorrhage, fat degeneration, and degenerative nodules especially in liver tissue (Kus et al. 2005).

Oxidative damage is caused by the imbalance between oxidants and antioxidants favoring the oxidants. In the organism, free radical formation is controlled by antioxidant defense system (Halliwell and Gutteridge 2015). However, the antioxidant defense system could not prevent the damages caused by free radicals in all cases, leading to oxidative damage, and antioxidants are introduced to remove the oxidative damage (Makhlouf et al. 2011).

Although previous research demonstrated that free radicals induced by CCl_4 impaired cellular processes in several tissues including liver (Selvakumar and Natarajan 2008) and kidneys (Chávez-Morales et al. 2017) as a result of their interaction with and oxidation of the lipids in the cell membrane (lipid peroxidation) and raised MDA levels due to lipid peroxidation and caused cellular damage, the information about their effects on the intestinal tissue is almost non-existent. Therefore, in our study, the degree of CCl_4-induced damage to the intestinal tissue and the protective effect of crocin on this damage were investigated.

Antioxidant enzyme (SOD and CAT) activities in small and large intestine tissues were measured and GSH and TAS levels were determined to demonstrate the antioxidant potential of crocin. It was considered that lipid peroxidation was an indicator of CCl_4 application-induced tissue damage. We determined a significant increase in SOD and CAT activities and MDA (an end product of lipid peroxidation) and TOS levels in CCl_4 administered rat small and large intestine tissues, while GSH and TAS content decreased as a result of the damage in the intestines induced by oxidative stress. Elevated MDA and TOS levels and SOD and CAT activities in intestine tissues further demonstrated the increased lipid peroxidation and reduced antioxidant defense mechanism

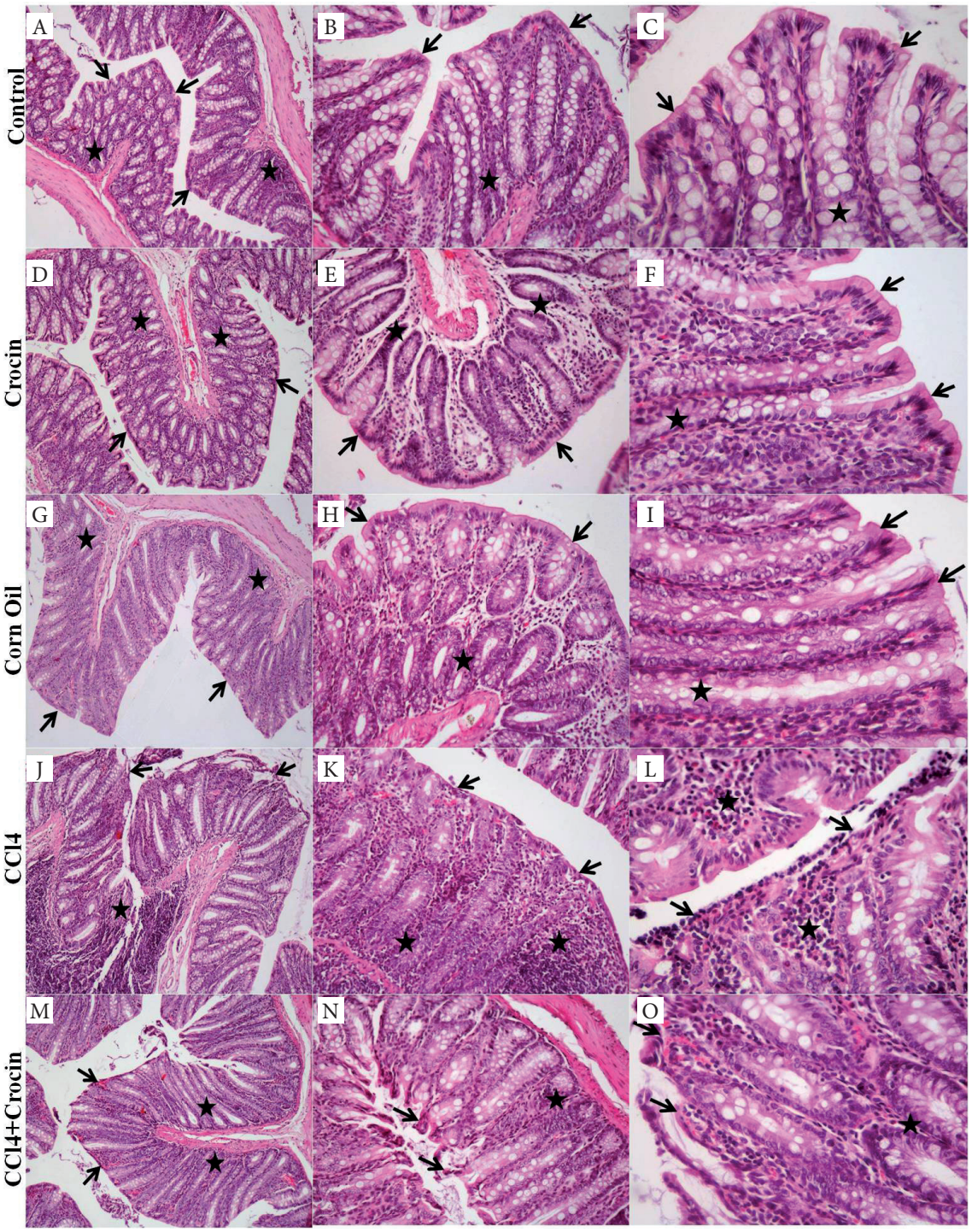

Figure 2. Histological microphotographs of large intestine tissue. Control group (**A–C**): surface epithelium (arrow), *Lieberkühn crypt* (asterisk); Crocin group (**D–F**): surface epithelium (arrow), *Lieberkühn crypt* (asterisk); Corn Oil group (**G–I**): surface epithelium (arrow), *Lieberkühn crypt* (asterisk); CCl_4 group (**J–L**): (J): surface epithelium damage (arrow), *Lieberkühn crypt* degeneration (asterisk), (K): surface epithelium metaplasia and Goblet cell loss (arrow), collapse in *Lieberkühn crypts* and Goblet cell loss, inflammatory cellular infiltration in *lamina propria* (asterisk), (L): surface epithelium metaplasia and degeneration (arrow), eosinophil infiltration in *lamina propria* (asterisk); CCl_4+Crocin group (**M–O**): (M): local metaplasia regions on surface epithelium (arrow), *Lieberkühn crypt* (asterisk), (N): degeneration in surface epithelium (arrow), *Lieberkühn crypt* (asterisk), (O): degeneration in surface epithelium and congestion in superficial capillaries (arrow), *Lieberkühn crypt* (asterisk). Magnification: ×10 (A, D, G, M); ×20 (B, E, H, N); ×40 (C, F, I, L, O).

that is charge of preventing increased free radical production, followed by the tissue damage. These findings were consistent with those reported by Ozturk et al. (2003), Gangarapu et al. (2013) and Ranjbar et al. (2014).

GSH constitutes a vital factor in the non-enzymatic antioxidant system with significant roles in the antioxidant defense of the body. GSH could neutralize free radicals, reduce hydrogen peroxide and stabilize sulfhydryl groups as an antioxidant. Hosseinzadeh et al. (2009) stated that ischemia/reperfusion-induced oxidative damage in rats was significantly inhibited by saffron extract and crocin. In the same study, it was reported that saffron extract and crocin decreased MDA levels and free radical induced lipid peroxidation thanks to their antioxidant activities. Altinoz et al. (2015) demonstrated that crocin administration reduced the elevated MDA levels and increased GSH levels in diabetes induced rat renal tissue.

In our experiment, we demonstrated that crocin protects small and large intestinal mucosa cell membranes from lipid peroxidation induced by CCl_4 and reverses the tissue damage. The tissue toxicity produced by CCl_4 was removed by other natural plant products, such as rosemary (Botsoglou et al. 2010), *Cnestis ferruginea* extracts (Rahmat et al. 2014). Similar to their mode of action, crocin remarkably reduced CCl_4-induced MDA levels. This impact of crocin was due to its strong antioxidant and radical scavenging effects (Assimopoulou et al. 2005).

The results of the abovementioned studies were consistent with the findings of the present study. This was due to the fact that elevated enzymatic antioxidant levels such as SOD and CAT and reduced non-enzymatic antioxidants GSH and TAS levels in CCl_4 administered animals returned to normal levels after crocin administration. Furthermore, the conjoint decrease in enzymatic antioxidants and MDA levels and increase in GSH levels was the evidence for the connection between the antioxidant regulation mechanisms. It was reported in previous studies that there was a correlation between antioxidant enzyme activities and enzyme mRNA expressions (Huang et al. 1999). These studies reported that examination of the brain tissue of diabetes-induced rats demonstrated elevated SOD enzyme activity and SOD enzyme mRNA expressions. It is possible that one of the most significant results of ROS-induced brain damage could be the increase in gene expression of these antioxidant enzymes. Variations in neuronal antioxidant enzyme levels such as SOD and CAT were parallel to the elevated oxidative stress (Bonnefont-Rousselot et al. 2000). Ghadrdoost et al. (2011) reported that saffron and crocin had a regulatory impact on hippocampal oxidative markers. The results of the abovementioned studies were consistent with the findings of the present study and it was reported in the latter that CCl_4 application significantly increased SOD and CAT activities in rats, however, administration of crocin reduced these enzyme activities.

In previous studies, it was further demonstrated that crocin administration increased GSH content and significantly decreased the elevated MDA levels in liver tissues of cisplatin (Sun et al. 2014), cyclophosphamide (Jnaneshwari et al. 2013), and diazinon (Lari et al. 2015) toxicity-induced rats.

The findings of recent studies established the protective effect of saffron and its active ingredients against several toxic factors in different tissues including the liver tissue (Boussabbeh et al. 2016; Chen et al. 2016). In a study by Sun et al. (2014), the effect of crocin on cisplatin induced liver damage was examined. In this study, it was demonstrated that crocin decreased the cisplatin induced hepatic focal necrosis. We have demonstrated using biochemical and histopathologic methods that crocin treatment could remove CCl_4 induced intestine tissue damage. Akbari et al. (2017) demonstrated that ischemia/reperfusion-induced liver damage was significantly improved by crocin administration at a dose of 200 mg/kg for 7 days (Huang et al. 1999). Our finding was consistent with other studies in the literature and the action of crocin might be due to its anti-inflammatory and antioxidant properties (El-Beshbishy et al. 2012; Omidi et al. 2014).

Ahmad et al. (2016) reported that saffron and crocin have antioxidant properties in reducing intercellular oxidant. Also, both chemical exhibit increament in eNOS expression suggesting its plant antioxidant effects and potential as athheroprotective agents in stimulated human coronary artery endothelial cells. Although clinical studies shown that saffron is effective in a short-term treatment of mild to improve Alzheimer's disease conditions, there is not any clinical evidence for crocin in this regard (Akhondzadeh et al. 2010a, 2010b).

Paolini et al. (2001) indicated that beta carotene supplement stimulated the activity of certain isoforms of CYP, called CYP1A1, CYP3A1, CYP2E1, CYP2B1. Other carotenoids with stimulating effects on enzymes metabolizing xenobiotics are canthaxanthin, astaxanthin and beta apo-8'-carotenal. On the other hand, lycopene has an *in vitro* inhibitory effect on CYP2E1, which is responsible for converting certain xenobiotics into carcinogens (Paolini et al. 2001). We considered that the crocin used in this study is a carotenoid such as lycopene and might had an inhibitory effect on CYP2E1 which is responsible for CCl_4 metabolism. Thus, another mechanism of the protective effect of crocin on intestinal tissues might be described as the inhibition of isoenzyme activities, including bioactivation of CYP2E1 and other isoenzymes (Tsujimoto et al. 2009). Thus, inhibition of the CYP2E1 enzyme prevents the transformation of CCl_4 into the reactive products, namely $CCl_3^{\bullet}$ and $CCl_3OO^{\bullet}$, in the intestinal tissue, preventing oxidative damage.

Biological and histological findings were consistent in the present study, adding to the significance of the current work. In the control, crocin and corn oil groups observed in normal

histological appearance. However, with CCl_4 administration, extensive degeneration in the small intestine mucosa and hemorrhage, intense inflammatory cell infiltration and fibrosis were observed in these areas. Furthermore, total villus degeneration and *Lieberkühn crypt* damage were detected in certain areas. There were similar extensive degeneration areas in the large intestine tissue and intense inflammatory cell infiltration in these degeneration areas. After crocin treatment, significant improvements were observed in histological results.

In conclusion, CCl_4 induced intestinal tissue damage and the therapeutic effect of crocin on the said damage was demonstrated in the current study for the first time and it was considered that this effect could be caused by the antioxidant action of crocin. When crocin, which has antioxidant properties, was administered for treatment of the abovementioned tissue damage, it was observed that MDA and TOS levels and SOD and CAT activities decreased, while GSH and TAS levels increased. We considered that this effect of crocin was due to its strong antioxidant properties. In the present study, we demonstrated using biochemical and histopathologic methods that crocin treatment could remove the CCl_4-induced intestine damage.

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

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