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**Abstract**
The objective of the present study was to demonstrate the protective effect of crocin on the adverse effects of tartrazine on liver. Crocin is a carotenoid and a strong free radical scavenger. Forty rats were randomly divided into 4 groups (n = 10). The first group was the control group (C) and saline solution was administered to this group. The second group (Cr) was administered 50 mg/kg crocin. The third group (T) was administered 500mg/kg tartrazine. The fourth group (T+Cr) was administered the same doses of both crocin and tartrazine as the previous groups for 21 days. It was determined that tartrazine increased liver superoxide dismutase (SOD) activity, malondialdehyde (MDA) and total oxidant status (TOS) levels and catalase (CAT) activity, decreased glutathione (GSH), and total antioxidant status (TAS) levels. Furthermore, tartrazine administration resulted in significant increases in plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities and pathological changes in the liver. When tartrazine administered rats were treated with crocin for 21 days, the biochemical parameters improved, and liver tissues were restored. Thus, it was demonstrated that crocin had protective effects on the adverse effects caused by tartrazine administration.


Keywords: tartrazine; crocin; oxidative stress; hepatotoxicity

**Changelog**
We have revised our manuscript according to the reviewers' comments. Please find the all changes made on the manuscript as a red color.

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# Protective effect of crocin on food azo dye tartrazine induced hepatic damage by improving biochemical parameters and oxidative stress biomarkers in rats

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**Running Title:** Crocin protects against tartrazine toxicity

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**Abstract**

The objective of the present study was to demonstrate the protective effect of crocin on the adverse effects of tartrazine on liver. Crocin is a carotenoid and a strong free radical scavenger. Forty rats were randomly divided into 4 groups (n = 10). The first group was the control group (C) and saline solution was administered to this group. The second group (Cr) was administered 50 mg/kg crocin. The third group (T) was administered 500mg/kg tartrazine. The fourth group (T+Cr) was administered the same doses of both crocin and tartrazine as the previous groups for 21 days. It was determined that tartrazine increased liver superoxide dismutase (SOD) activity, malondialdehyde (MDA) and total oxidant status (TOS) levels and catalase (CAT) activity, decreased glutathione (GSH), and total antioxidant status (TAS) levels. Furthermore, tartrazine administration resulted in significant increases in plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities and pathological changes in the liver. When tartrazine administered rats were treated with crocin for 21 days, the biochemical parameters improved, and liver tissues were restored. Thus, it was demonstrated that crocin had protective effects on the adverse effects caused by tartrazine administration.

**Keywords;** tartrazine; crocin; oxidative stress; hepatotoxicity

## Introduction

Tartrazine [trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-[(E)-(4-sulfonatophenyl) diazenyl]-1H-pyrazole-3-carboxylate] is a synthetic monoazo dye that contains pyrazoline ring. Tartrazine (E-102), also known as azo dye since it contains a -N=N- group, is an orange-yellow powder dye readily soluble in water and forms golden colored solutions (Deshpande, 2002). Tartrazine is frequently used as a food additive in aromatic beverages, canned foods, ice creams, confectionery, and in many human drugs and cosmetic products (Mahfouz and Al-Shammrani, 2013; Mpountoukas et al., 2010). Used in several countries around the world, tartrazine is the second most commonly used food dye in the United States since 1916. The Food Drug administration (FDA) established acceptable daily intake (ADI) for tartrazine of 0-7.5 mg/kg/day (Walton et al., 1999).

The maximum daily intake of tartrazine was determined as 5 mg /kg/day by the US Food and Drug Administration (FDA).

The primary mechanism in rat, rabbit and human metabolisms of tartrazine taken with foods is the formation of aromatic amines by azo-reductases from intestinal flora. The presence of electron carriers released by bacteria and the anaerobic conditions in the colon cause the reduction of tartrazine to two metabolites as sulfanilic acid (an aromatic amine) and aminopyrazolone (Chung et al., 1978; Watabe et al., 1980). It was suggested that the mutagenic, carcinogenic and toxic effects of azo dyes such as tartrazine may be the result of the reductive biotransformation of the azo bond during the metabolism of the dye (Demirkol et al., 2012; Soares et al., 2015). Although intestinal microbial flora plays an important role in tartrazine metabolism, other enzymes in the liver may also break the azo bonds and reduce the nitro groups (Soares et al., 2015). As a result of the reactions both in the intestines and liver, if azo dyes are reduced to fully aromatic amines, these aromatic amines are then oxidized to N-hydroxy derivatives with cytochrome P450 enzymes (Demirkol et al., 2012). These metabolites have the potential to alter the cell cycle through the interphase and in the regenerative hyperplasia process. Therefore, they can have a significant contribution to the development of cancer (Bezerra et al., 2016).

Reactive oxygen species (ROS) occur continuously as a product of aerobic metabolism in living organisms, but they may be over-produced in pathophysiological conditions. Oxidative stress is the degradation of the balance between ROS production and

cellular antioxidant defense systems in favor of oxidants. (Persson et al., 2014). Detoxification of ROS is one of the prerequisites for aerobic life and there are antioxidant defense systems in living organisms. These defense systems are non-enzymatic, such as reduced glutathione (GSH) and enzymatic systems such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) (Droge, 2002).

Saffron (*Crocus sativus L.*) is an iridaceous plant indigenous to and planted in Iran, Kashmir (India and Pakistan), Greece, Azerbaijan, China, Morocco, Mexico, Libya, Turkey and Austria. The most important components of the chemical structure of saffron are crocin, crocetine and safranal. Crocin is a carotenoid pigment and has the structure of crocetin di-gentiobiose ester (Bathaie and Mousavi, 2010). In recent studies, it has been shown that saffron and its main components (crocin, crocetin and safranal) have several beneficial effects in mice such as hypolipidemic, hypoglycemic, antiatherosclerotic, nephroprotective and antitumor effects (Kianbakht and Hajiaghaee, 2011; Bathaie and Mousavi, 2013). It is also known that crocin has a strong free radical scavenger and antioxidant properties. (Hosseinzadeh et al., 2009b). Recently, phytochemical treatments have been one of the best solutions for overcoming hepatotoxicity through free radical regulation mechanisms (Hassan et al., 2012).

We considered in the present study that the hepatotoxicity induced by tartrazine and its metabolites was a consequence of the increase in ROS. The most important objective of the present study was to provide evidence for the therapeutic potential of crocin in tartrazine induced liver toxicity.

## Material and methods

### *Animals*

The study was conducted with 40 female Wistar albino rats that weighed 225-250 g and procured from Inonu University, Faculty of Medicine, Experimental Animals Breeding and Research Center (INUTF-DEHUM). The study was approved by the Inonu University experimental animals ethics committee (2016 / A-96). Rats were kept under 21°C, 55-60% humidity, 12 hours light (08: 00-20: 00): 12 hours dark conditions. Rats were fed *ad libitum* with standard pellet feed in the study. Drinking water was supplied daily and rat cages were cleaned every day.

*Experimental Design*

Rats were divided into 4 groups of 10 rats in each group: control (C) group was administered only physiological saline solution; crocin (Cr) group was administered 50 mg/kg /day crocin (Hosseinzadeh et al., 2009a) (Sigma Aldrich Co., USA, CAS no: 42553-65-1); tartrazine (T) group was administered 500 mg/kg/day tartrazine (Amin et al., 2010) (Sigma Aldrich Co., USA, CAS no: 1934-21-0); tartrazine+crocin (T+Cr) group was administered 50 mg/kg/day crocin+500 mg/kg tartrazine. The applied chemicals were dissolved in saline solution and 1 ml/kg solution was administered with gavage for 21 days.

*Samples*

At the end of the study, laparotomy was conducted on rats under xylazine ketamine anesthesia and blood was drawn from the heart into heparinized tubes. Then, the liver tissues of the decapitated rats were incised and washed with physiological saline to remove the excess blood. One part of the tissue was fixed in 10% formaldehyde and the other part was stored at -80 °C for biochemical analyzes.

*Biochemistry*

On the day of the analysis, the tissues were removed from the deepfreeze and weighed. Phosphate buffer was added to obtain 10% homogenates and the tissues were homogenized for 1-2 minutes in ice at 12,000 rpm (IKA Ultra Turrax T 25 basic, IKA Labotechnik, Staufen, Germany). Homogenates were tested for malondialdehyde (MDA) levels. The supernatants were obtained by centrifuging the remaining homogenates at 600 g for 30 minutes at +4$^{o}$C. GSH, SOD, CAT, total antioxidant status (TAS), total oxidant status (TOS) and protein levels were studied on the supernatant.

MDA analysis was conducted with the method described by Ohkawa et al. (1979). Tissue homogenate was mixed with 1% H3PO4 and 0.6% thiobarbituric acid. The mixture was heated in water bath for 45 minutes and then extracted in n-butanol; n-butanol was used as the blind and tetramethoxypropane was used as the standard. MDA level was measured with a spectrophotometer (T80 UV / VIS Spectrometer, PG Instruments Ltd., Leicestershire, UK) at 535 nm. The results are expressed in nanomol/g wet tissue.

GSH level was measured based on the method described by Ellman (1959). After 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) (Sigma Aldrich Co., USA, CAS no: 69-78-3) was

added in the tubes, a yellow-green color is formed as a result of the reaction between DTNB and the glutathione in the medium. The reduced glutathione amount was determined by measuring the color density at 410 nm with a spectrophotometer. The results are expressed in nanomol/g wet tissue.

SOD activity was measured by the method reported by Sun et al. (1988). Superoxide radicals are produced by xanthine-xanthine oxidase. The superoxide radical forms a color by reducing NBT (nitro blue tetrazolium) (Sigma Aldrich Co., USA, CAS no: 298-93-9) to blue colored formazan. The absorbance of the produced formazan at 560 nm is used to calculate the SOD activity. Distilled water was used as the blind. SOD activity was expressed in U/g protein.

CAT activity was measured using the method reported by Aebi et al. (1984). Hydrogen peroxide ($H_2O_2$) absorbs ultraviolet radiation; the maximum absorption wavelength is 240 nm. The separation of $H_2O_2$ from water and oxygen by the catalase enzyme in the supernatant leads to a decrease in the absorbance at 240 nm. The reduction observed in the absorption was recorded for 1 minute to measure the enzyme activity. CAT activity was expressed in K/g protein.

The tissue homogenate protein content was calculated with the Lowry method (Lowry et al., 1951).

TOS level was measured with the method developed by Erel (2005). It was measured using a total oxidant status kit (Rel Assay Diagnostics, Gaziantep, Turkey). The results are expressed in μmol $H_2O_2$ equiv./l.

The TAS level was studied with the Erel method (2004). TAS was measured with a Rel Assay brand kit (Rel Assay Diagnostics, Gaziantep, Turkey). Results are expressed in mmol trolox equiv./l.

Plasma AST (Architect / Aeroset Aspartate Aminotransferase Reagent Kit), ALT (Architect / Aeroset Alanine Aminotransferase Reagent Kit) and ALP (Architect / Aeroset Alkaline Phosphatase Reagent Kit) levels were determined with the respective kits and the enzymatic colorimetric method in an auto-analyzer (Architect C8000).

*Histology*

For the histopathological examination, liver tissue samples were fixed with 10% formaldehyde containing phosphate buffer in the room temperature for 48 hours. After they were fixed, liver tissue samples were passed through ethanol series with increasing degrees (50%, 70%, 96%, 99.9%) for dehydration. They were then passed through xylene series for transparency and molten paraffin series at 62°C for infiltration, and then embedded in paraffin blocks. Five-six μm thick sections were prepared with the paraffin blocks using a microtome. Hematoxylen-eosin (H-E) and periodic acid-Schiff (PAS) staining were performed on the sections transferred into slides. The sections were examined with Nikon Eclipse Ni-U light microscope, photographed with a Nikon DS-Fi2 camera and analyzed with a Nikon NIS-Elements Documentation image analysis software (Nikon Corporation, Tokyo, Japan).

Histopathologic deviations (focal necrosis, inflammatory cell infiltration, hydropic degeneration in hepatocytes, vascular and sinusoidal congestion) in liver sections that were stained with hematoxylin-eosin were scored between 0 and 3 (0; N/A, 1; mild-rare, 2; moderate, 3; severe-prevalent), so that the maximum total score would be 12. In sections stained with periodic acid-Schiff, intracytoplasmic glycogen content in hepatocytes was scored between 0 and 3 (0; no positive staining, 1; weak-minimal staining, 2; moderate staining, 3; strong-prevalent staining).

*Statistical Analysis*

Statistical analysis was conducted with SPSS 21 software. Shapiro-Wilk test was used to determine the normal distribution of the data, and Levene test was used to determine the homogeneity of the variances. Data with a normal distribution were summarized with mean and standard deviation values, and data with homogeneous variances were analyzed with one-way analysis of variance and Tukey HSD paired comparison method. Since the group variances were not homogeneous, the Welch test and the Games-Howell paired comparison method were used to compare the GSH levels. Median, minimum and maximum values were used to summarize data without normal distribution, and Kruskal-Wallis test and Conover paired comparison method were used for group comparisons. Significance level was accepted as $p < 0,05$ in all tests.

## Results

*Biochemistry*

Liver tissue oxidant–antioxidant parameters are presented in Table 1 for all groups. Tartrazine administration resulted in a significant increase ($p < 0.05$) in liver MDA levels when compared to C (65.8%) and Cr groups, while MDA levels in T+Cr group decreased significantly ($p < 0.05$) when compared to and T group.

It was observed that GSH levels increased significantly ($p < 0.05$) in the Cr group (71.4%) when compared to the C group, while the GSH levels in the T group decreased significantly ($p < 0.05$) when compared to the C (%21.5) and Cr groups. Compared to T group, the decreased GSH levels demonstrated a significant increase in the T+Cr group ($p < 0.05$) and even approached Cr group values.

Tartrazine administration resulted in a significant increase in SOD enzyme activity in liver tissue when compared to C (53.0%) and Cr groups ($P < 0.05$), while SOD activity significantly decreased in T+Cr group when compared to the T group. Crocin administration resulted in a significant increase ($P < 0.05$) in CAT activity in Cr group when compared to the C group (106.8%) Conversely, when tartrazine administration led to a significant decrease in liver CAT enzyme activity (36.5%) ($p < 0.05$), the same enzyme activity increased significantly ($p < 0.05$) in the T+Cr group when compared to the T group and approached control group levels.

It was determined that tartrazine administration significantly decreased liver TAS levels ($P < 0.05$) when compared to C (25.7%) and Cr groups, while the decreased TAS levels in the T group significantly increased in T+Cr group ($P < 0.05$). Conversely, tartrazine administration led to significant increases ($P < 0.05$) in TOS levels when compared to C (83.3%) and Cr groups, TOS levels were significantly lower ($p < 0.05$) in the T+Cr group when compared to the T group.

Plasma AST, ALT and ALP values significantly increased ($p < 0.05$) when compared to the C and Cr groups after tartrazine administration due to the liver damage, while the same values decreased significantly in the T+Cr group ($p < 0.05$) when compared to the T group. Plasma AST, ALT and ALP levels of all groups are presented in Table 2.

*Histology*

Hepatocyte cords, sinusoids, central venous and portal areas in the C (Figure 1A-C) and Cr group (Figure 1D-F) hepatic parenchyma sections after hematoxylin-eosin staining

were in normal hepatic lobular appearance. Radially organized hepatocyte cords were observed in hepatic lobules, around the central vein. The sinusoid lumen located between the hepatocyte cords reflected clear and normal histological appearance. The hepatocyte cytoplasm was homogeneous and eosinophilic stained in mild to moderate intensity. Hepatocyte nuclei were centrally located with smooth contours and euchromatic structure. Central veins, portal triad connective tissues and vascular structures and bile ducts were open and in normal histological appearance. Kupffer cells were observed at normal density and with normal microscopic structure. Intracytoplasmic glycogen accumulation was prevalent and at intense-medium dense PAS + staining level in periodic acid-Schiff-stained sections (Figure 2A-B).

Hepatic lobulation was normal in the T group (Figure 1G-I) liver sections stained with hematoxylin-eosin. However, hydropic degeneration was prevalent in moderate to severe levels in hepatocytes, and focal necrosis regions and apoptotic bodies in different diameters were identified. Inflammatory cell infiltration in periportal regions and dense congestion in central veins, sinusoids and vascular structures in portal areas were noted. Slightly-minimal PAS + staining observed in periodic acid-Schiff-stained sections was considered as a significant reduction in intracytoplasmic glycogen content (Figure 2C).

Rare focal necrosis areas and hepatocyte groups that exhibited limited regions of mild hydropic degeneration symptoms were determined in the parenchyma of the hepatocyte-eosin-stained T+Cr group (Figure 1J-L) liver sections. In some portal regions, minimal inflammatory cell infiltration was observed. Minimal congestion was observed in vascular structures in central veins, sinusoids and vascular structures in the portal triads. However, in general, there was a significant decrease in all damage symptoms, an increase in PAS + staining prevalence and intensity when compared to the T group (Figure 2D). Hematoxylin-eosin-stained liver tissue damage scores are presented in Table 3 and periodic acid-Schiff (PAS) stained liver tissue scores are presented in Table 4.

## Discussion

The aim of this study was to establish liver damage caused by a food dye, tartrazin, and to determine the protective effect of crocin against this disorder by biochemical tests and histological examinations.

The synthetic food dyes are widely used in the food industry since they are relatively durable and inexpensive (Nayak and Nath, 2010). The first catabolic step in the reduction of azo dyes is the reduction of the azo bond to produce aromatic amines accompanied by a reduction in visible light absorbance and subsequent degradation of the dye color (Soares et al., 2015). The aromatic amines, some of which are known as carcinogens, were found in urine of dye manufacturing workers and experimental animals after administration of azo dyes (Cerniglia et al., 1986). Furthermore, Siraki et al.(2002) found that incubation of hepatocytes with aromatic amines caused a reduction in mitochondrial membrane potential before cytotoxicity was observed. Tartrazine causes changes in the biochemical profiles of kidney and liver tissues, and concurrently, leads to a risk at higher doses and results in oxidative stress in the tissues via free radical formation (Himri et al., 2011). The antioxidant system is involved in the free radical-mediated tissue or cellular damage defense system (Mourad and Noor, 2011). In the present study, daily oral administration of tartrazine to rats for twenty-one days resulted in suppression of the antioxidant system. It was found that tartrazine administration significantly increased the levels of SOD, the primary free radical scavenging antioxidant enzyme that detoxifies superoxide (O2-) (Arjuman et al., 2007). Tartrazine and its metabolites may cause the formation of ROS in the tissue and the increased ROS leads to an increase in cellular SOD activity and $H_2O_2$ levels. However, due to the serious decreases observed in CAT activity after tartrazine administration, increased cellular $H_2O_2$ could not be sufficiently detoxified, resulting in oxidative stress in the liver. Recent studies on the administration of food dyes demonstrated that food dyes increase the production of ROS, leading to oxidative stress (Himri et al., 2011; Visweswaran and Krishnamoorthy, 2012). The increase in ROS production such as superoxide anion, hydroxyl radical and $H_2O_2$ in an organism is correlated with the decrease in antioxidant defense mechanism of cell including SOD, CAT and GSH to prevent the cell death by the these toxic radicals and leads to oxidative stress (El-Tohamy, 2012). Oxidative stress is caused by the production of free radicals and the destruction of the oxidant-antioxidant balance, where the free radicals are scavenged by the antioxidant system (Friederich et al., 2009; Kaneto et al., 2010). On the other hand the MDA levels were increased as an indicator of lipid peroxidation by the ROS effect on the lipids of the cellular membrane. Interestingly, crocin treatment resulted in significant improvement in SOD and CAT activities when compared to the tartrazine group, and even resulted in close to control levels CAT activity. Gao et al. (2011) reported that tartrazine administration resulted in a decrease in CAT, SOD and glutathione reductase (GR) antioxidant enzyme activities. The findings of the present study were

297 consistent with recent reports that showed reduced antioxidant enzyme activities in the liver
298 (Amin et al., 2010) and brain (Gao et al., 2011) tissues of tartrazine administered rats. The
299 difference between our study and the study of Amin et al., in this study SOD activity
300 increased after tartrazine treatment, while previously Amin et al. described decrease of SOD
301 activity after administration of tartrazine to rats. Similar to previous studies (Zheng et al.,
302 2007), the present study revealed that crocin can protect tissues via the mechanisms that
303 regulate the antioxidant defense system.

304 GSH is a non-enzymatic antioxidant that plays an active role in endogenous
305 antioxidant defense, neutralizing free radicals and reducing hydrogen peroxide levels (Meister
306 and Anderson, 1983). In the present study, we observed that the GSH level significantly
307 decreased with tartrazine administration. GSH depletion caused oxidative damage in the liver
308 due to the imbalance in the oxidant/antioxidant system. Conversely, it was observed that the
309 tartrazine administration significantly increased MDA levels. On the other hand, it was found
310 that when treated with crocin, there was a statistically significant decrease in MDA levels
311 accompanied by a significant increase in GSH levels when compared to the tartrazine group.
312 Amin et al. (2010) demonstrated that GSH, SOD, and CAT were significantly decreased and
313 MDA increased in liver tissue homogenats in rats consumed high dose tartrazine (500 mg/kg)
314 and both doses of carmoisine (8 and 100 mg/kg) for 30 days. Also, Erdemli et al.(2017)
315 reported that there was a significant increase in MDA, TOS, SOD, CAT, BUN (blood urea
316 nitrogen) and creatinine levels, while a significant decrease in GSH and TAS levels in
317 tartrazine administered rat kidney tissues. Although the results of our study are appropriate for
318 the study of Amin et al., the differences between Erdemli et al. and our study may be due to
319 exposure of the liver to tartrazine and its metabolites in high amounts, resulting in excessive
320 ROS production and disruption of antioxidant enzyme activities. Since tartrazine is primarily
321 transported to the liver after the digestive tract and is metabolized by cytochrome P450
322 enzymes. Previous studies demonstrated that crocin increased intracellular GSH levels, and
323 thus preventing necrosis in hypoxic PC12 cells (Ochiai et al., 2004). Furthermore, Ochiai et
324 al. (2007) reported that saffron extract and crocin reduced free radical induced lipid
325 peroxidation and MDA levels due to their antioxidant properties. Hosseinzadeh et al. (2009a,
326 2005) reported that saffron extract and crocin significantly reduced ischemia reperfusion
327 induced oxidative stress in rat tissues.

328 Biological membranes are particularly prone to ROS attack, and toxic radicals cause
329 peroxidation of unsaturated fatty acids in membranes. This leads to reduced membrane

fluidity and degradation of membrane integrity and function, leading to severe pathological changes (Halliwell, 1987). Increased ROS or free radical production may lead to autoxidation in hepatic cells, resulting in explicit liver lesions (Suzuki et al., 2001). In a study, administration of high doses of tartrazine, a food coloring agent, to rats lead to elevated plasma enzyme activities (AST, ALT and ALP), increased hepatocyte permeability, damages and injuries (Stryer, 1995). Since ALT enzymes are normally localized in the cytoplasm, AST is mainly localized in organelles such as the mitochondria (Rajagopal et al., 2003). Increased AST and ALT levels suggested that food azo dyes administered to rats led to hepatic cellular and mitochondrial membrane damage. Mekkawy et al. (1998) found that serum ALT, AST and ALP levels were significantly increased when they administered tartrazine and carmoisine synthetic dyes. The present study findings were consistent with those of the previous studies, and tartrazine administration caused liver damage and an increase in plasma AST, ALT and ALP levels. Khayyat et al. (2017) observed an increase in plasma AST, ALP, and ALT after tartrazine administration. In a different study, tartrazine-administered rats exhibited elevated AST, ALP and ALT levels (Al-Seeni et al., 2017). When tartrazine was administered with crocin in the present study, it was observed that the activities of these liver enzymes were significantly reduced when compared to the tartrazine group. The results of the present study were consistent with previous studies where the AST, ALT, and ALP levels, which were elevated by hepatotoxicity induced by morphine (Salahshoor, 2016), amiodarone (Riaz et al., 2016), patulin (Boussabbeh et al., 2016) and acetaminophen (Omidi et al., 2014) administration in animal models, significantly decreased with crocin treatment.

Consistent with our biochemical findings, histological results demonstrated that the use of tartrazine led to moderate to severe degeneration in the liver, focal necrosis and intense congestion and inflammatory cell infiltration. It was demonstrated that the tartrazine induced damage was minimized when treated with crocin. In similar histopathologic studies, brown pigment accumulation in kidney and hepatic portal tracts and Van Kupffer cells induced by the coloring carmoisine was demonstrated (Aboel-Zahab et al., 1997).

In conclusion, the present study demonstrated that the tartrazine induced rat liver damage was due to oxidative stress, and this damage was eliminated by crocin treatment based on the biochemical and histological findings. Crocin has antioxidant properties due to its ROS scavenging effect. Thus, elevated plasma ALT, AST and ALP levels and liver MDA, TOS and SOD levels after tartrazine administration decreased with crocin treatment, while decreased GSH and TAS levels and CAT activity increased after the treatment. Thus, it is

363 required to raise consumer awareness on the adverse effects of these azo dyes, and to indicate
364 the type and concentration of each ingredient included in food products.

365 Based on results we suggest to "rise of consumer awareness". However, dose used in
366 this study 500mg/kg/day is 100 higher than ADI determined as 5mg/kg/day by FDA.

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

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**Figur and Table Legends**

**Figure 1.** Hematoxylin-eosin-stained liver tissue photomicrographs. A) C group. Central vein (arrowheads), portal triad (arrows). H&E. 10x. B) C Group, Portal triad (Arrows). H&E. 20x. C) C group. Portal triad (Arrows). H&E. 40x. D) Cr group. Central vein (Arrowheads); portal triad (arrows). H&E. 10x. E) Cr group. Portal triad (Arrows). H&E. 20x. F) Cr group. Central vein (arrowheads). H&E. 40x. (G); T group. Congestion in central vein (arrowheads / tips); portal triad and vascular congestion (arrows). H&E. 10x. H) T group, hydropic degeneration in hepatocytes (asterisk); focal necrosis (black arrow); apoptotic particle (white arrow). H&E. 20x, 100x. I) T group. Hydropic degeneration in hepatocytes (asterisk); apoptotic particle (arrow). H&E. 40x, 100x. J) T+Cr group. Central vein (arrowheads); portal triad (black arrows); focal necrosis (white arrow). H&E. 10x. K) T+Cr group. Central vein (arrowheads);

portal triad (arrows). H&E. 20x. L) T+Cr group. Central vein (arrowheads); congestion (arrow). H&E. 40x.

**Figure 2.** Periodic acid-Schiff (PAS) stained liver tissue photomicrographs A) C group. Central vein (arrowheads); portal triad (arrows). PAS. 20x. B) Cr group. Central vein (arrowheads); portal triad (arrows). PAS. 20x. C) T group. Weak intracytoplasmic PAS + staining in hepatocytes (asterisk); periportal area cell inflammation (arrows). PAS. 20x. D) T+Cr group. Central vein (arrowheads); portal triad (arrows). PAS. 20x.

**Table 1**. Liver tissue oxidant–antioxidant parameters of all groups.

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. Groups: Control (C); received normal saline solution, Crocin (Cr); received crocin, Tartrazine (T); received tartrazine, Tartrazine+Crocin (T+Cr); received tartrazine with crocin.
Superscripts represents the statistically significant difference.
§; $p<0.05$ when compared to C group
#; $p<0.05$ when compared to Cr group
*; $p<0.05$ when compared to T group
&; $p<0.05$ when compared to T+Cr group

**Table 2.** Plasma AST, ALT and ALP levels of all groups.

Data are summarized with median, minimum and maximum values (n=10). AST; aspartate aminotransferase, ALT; alanine aminotransferase, ALP; alkaline phosphatase. Groups: Control (C); Crocin (Cr); Tartrazine (T); Tartrazine+Crocin (T+Cr).
Superscripts represents the statistically significant difference.
§; $p<0.05$ when compared to C group
#; $p<0.05$ when compared to Cr group
*; $p<0.05$ when compared to T group
&; $p<0.05$ when compared to T+Cr group

**Table 3.** Hematoxylin-eosin-stained liver tissue damage scores.

Data are summarized with median, minimum and maximum values for histological scoring. Groups: Control (C); Crocin (Cr); Tartrazine (T); Tartrazine+Crocin (T+Cr). Superscripts represents the statistically significant difference.

§; $p<0.05$ when compared to C group

#; $p<0.05$ when compared to Cr group

*; $p<0.05$ when compared to T group

&; $p<0.05$ when compared to T+Cr group

**Table 4.** Periodic acid-Schiff (PAS) stained liver tissue scores.

Data are summarized with median, minimum and maximum values for histological scoring. Groups: Control (C); Crocin (Cr); Tartrazine (T); Tartrazine+Crocin (T+Cr). Superscripts represents the statistically significant difference.

§; $p<0.05$ when compared to C group

#; $p<0.05$ when compared to Cr group

*; $p<0.05$ when compared to T group

&; $p<0.05$ when compared to T+Cr group

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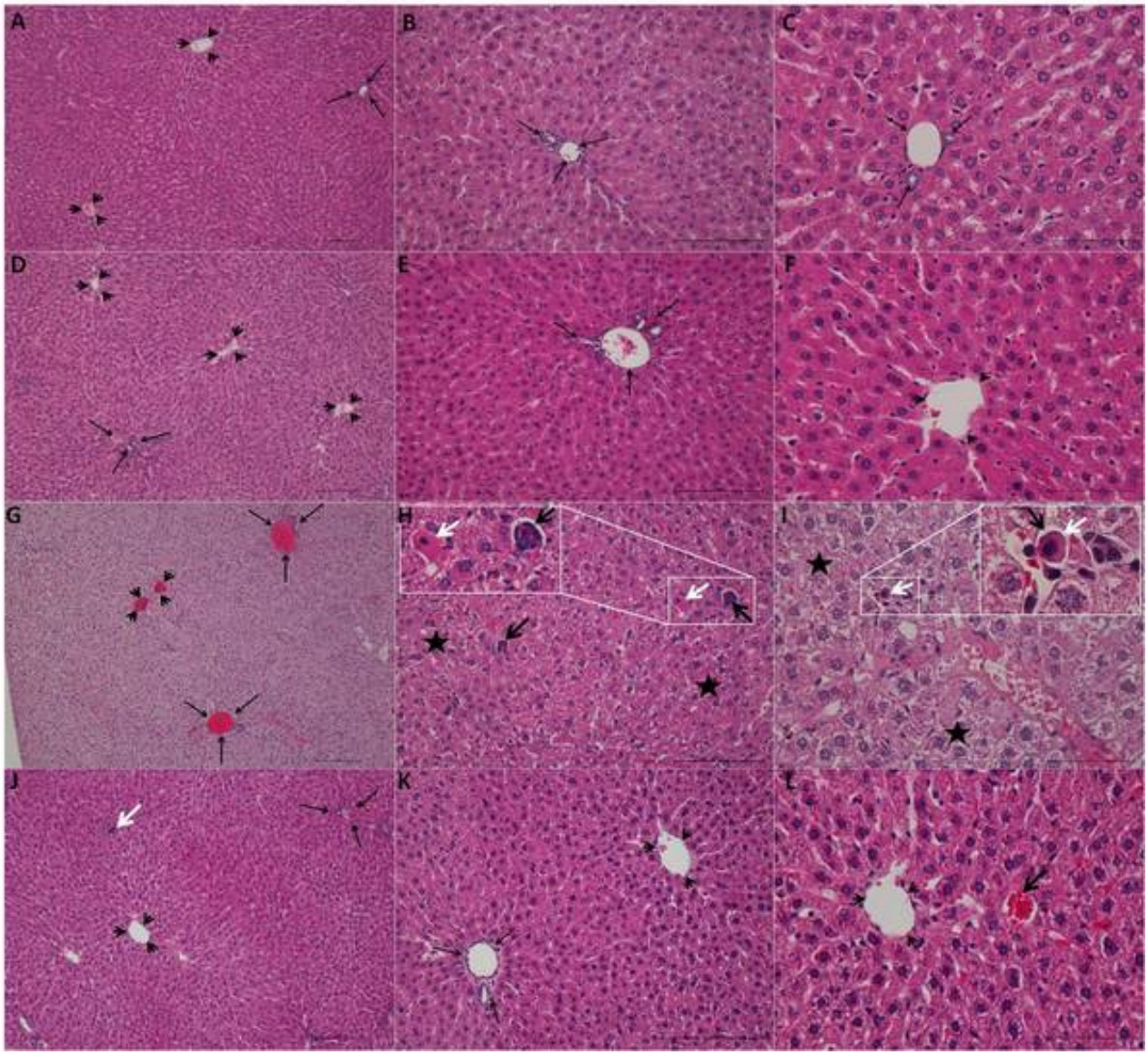

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