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ABSTRACT

Owing to its lipophilic property, carbon tetrachloride (CCl$_4$) is rapidly absorbed by both the liver and brain. We investigated the protective effects of crocin against brain damage caused by CCl$_4$. Fifty rats were divided into five groups of ten: control, corn oil, crocin, CCl$_4$ and CCl$_4$ + crocin. CCl$_4$ administration decreased glutathione (GSH) and total antioxidant status (TAS) levels, and catalase (CAT) activity, while significant increases were observed in malondialdehyde (MDA) and total oxidant status (TOS) levels and superoxide dismutase (SOD) activity. The cerebral cortex nuclear lamina developed a spongy appearance, neuronal degeneration was observed in the hippocampus, and heterochromatic and pyknotic neurons with increased cytoplasmic eosinophilia were observed in the hippocampus after CCl$_4$ treatment. Because crocin exhibits strong antioxidant properties, crocin treatment increased GSH and TAS levels and CAT activities, and decreased MDA and TOS levels and SOD activity; significant improvements also were observed in histologic architecture. We found that crocin administration nearly eliminated CCl$_4$ induced brain damage by preventing oxidative stress.

Reactive oxygen species (ROS) are normal products of aerobic metabolism in all living tissues; however, under pathophysiological conditions, they may be produced in large quantities. Oxidative stress is caused by imbalance between ROS production and cellular antioxidant defenses in favor of the oxidants (Sies 1997). Oxidative stress participates in the etiology of cardiovascular diseases, neurodegenerative disorders, cancer and senescence (Halliwell and Gutteridge 1984; Lin and Beal 2006; Nathan and Cunningham-Bussel 2013). The main source of free radicals in living organisms is the auto-oxidation of flavin thiols, activity in the electron transport chain, oxidases and cyclo-oxygenases, and peroxidases (Forman et al. 2010). Environmental sources of oxidative stress include xenobiotics, organic solvents, pesticides, tobacco smoke, anesthesia, drugs and radiation (Forman et al. 2010).

Carbon tetrachloride (CCl$_4$) commonly is used to induce hepatotoxicity in experimental animals. CCl$_4$ hepatotoxicity is characterized by hepatocellular necrosis due to fatty deposits. Acute toxic CCl$_4$ doses cause hepatocellular necrosis and fatal hepatic failure frequently occurs when the regenerative capacity of the liver is exceeded. High doses of CCl$_4$ cause nonspecific toxicity such as central nervous system depression and respiratory failure, which result in death (Recknagel et al. 1989). Free radicals formed during CCl$_4$ metabolism cause endoplasmic reticulum (ER) damage, accumulation of lipids, reduced protein synthesis and mixed-function oxidase activity (Weber et al. 2003).

CCl$_4$ becomes toxic after metabolic activation. CCl$_4$ is metabolized to the highly reactive trichloromethyl radical (CCl$_3$•) by cytochrome P450 (CYP) (mostly CYP2E1) in the ER of liver cells. Cytochrome P450 (CYP) enzyme systems are found in both liver hepatocytes and digestive system epithelium (Shimizu et al. 1990). CYP2E1 is the basic isoenzyme of CYP and it participates in xenobiotic metabolism (Nelson et al. 1993). The CYP2E1 enzyme, however, may participate in tissue pathogenesis by causing harmful intermediate metabolites while metabolizing xenobiotics, which cause ROS production and lipid oxidation (Lee et al. 1995). CCl$_3$• reacts rapidly with oxygen to form the highly reactive trichloromethyl peroxyl radical (CCl$_3$OO•) and with lipids to form lipid oxidation products (Risal et al. 2012). Polyunsaturated fatty acids (PUFA) of the mitochondria and ER are particularly susceptible to oxidation by free radicals. Lipid oxidation caused by free radicals is a significant mechanism of hepatic injury caused by CCl$_4$ (Weber et al. 2003).

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The brain is vulnerable to oxidative stress owing to its high oxygen consumption and high PUFA content that can be metabolized by oxygen free radicals. In addition, the brain contains large amounts of iron, which is associated with free radical injury. Because iron can transfer single electrons between ferrous and ferric states, it is a powerful catalyst for free radical reactions. Fe^{+2} converts hydrogen peroxide (H_2O_2) to the hydroxyl radical ($\bullet OH$), which may be the most reactive free radical in vivo (Halliwell and Gutteridge 1984). Brain tissue also lacks a defense system sufficient to cope with oxidative stress (Somani et al. 1996; Chong et al. 2005). Neurotoxic compounds cause oxidative stress by inducing lipid oxidation and decreasing antioxidant defenses in the brain (Verma and Srivastava 2001; Latini et al. 2003; Srivastava and Shivanandappa 2005). Therefore, the brain is vulnerable to the effects of CCl_4. Although CCl_4 hepatotoxicity is well known, we have found only a few reports concerning the effects of CCl_4 on the brain (Szymonik-Lesiuk et al. 2003).

The protective effects of natural products on CCl_4 induced damage have been reported. Water extract of *Persea Americana*, ginseng extract (Brai et al. 2014) and Red Sea *Suberea mollis* sponge extract (Abbas et al. 2014) have been reported to exhibit hepatoprotective effects. *Cucurbita pepo* rind (Zaib and Khan 2014) and purple grape juice (Dani et al. 2008) exhibit neuroprotective properties. The protective effects of the these natural products were attributed to their content of phytochemicals, such as polyphenols, that have been shown to possess antioxidant properties (Wang et al. 1996; Kaur and Kapoor 2002).

Saffron consists of the dried stigmas of *Crocus sativus L.* (Iridaceae) flowers; it is one of the most valuable and expensive spices in the world (Mousavi et al. 2010). The plant contains orange pigments and typically is used as a colorant and aromatic spice in a wide variety of cuisines, confectionery preparations and perfumes. Saffron extract includes several carotenoids including crocetin, crocetin di-glucose ester, crocetin gentiobiose glucose ester and crocetin di-gentiobiose ester (crocin). These carotenoids scavenge free radicals, especially superoxide anions (Erben-Russ et al. 1987), which protect cells against oxidative stress. Saffron extract and its active components exhibit anticonvulsant, antidepressant, anti-inflammatory and anti-tumor effects (Hosseinzadeh and Younesi 2002).

It appears that free radical induced cellular stress is responsible for neurodegenerative effects of central nervous system (CNS) disorders such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis, and pathological conditions such as ischemia and excitotoxicity (Halliwell 1992; Olanow 1993). Antioxidant treatment is important for preventing oxidative stress related neuronal damage (Yamamoto et al. 1997; Bastianetto et al. 1999). Owing to its strong antioxidant activity, saffron extract, crocetin or crocin may be beneficial for treating neurodegenerative disorders of the brain.

We investigated the neuroprotective effects of crocin, one of the active ingredients of saffron, for ameliorating in brain tissue damage caused by CCl_4 induced oxidative stress using biochemical and histopathological methods.

Material and methods

Animals

We used 50 225–250 g male Wistar albino rats obtained from Inonu University Faculty of Medicine Experimental Animal Breeding and Research Center (INUTF-DEHUM). Our study was approved by the Inonu University experimental animal ethics committee (2016/A-60). Rats were housed at 21 °C, 55–60% humidity and a 12 h (08:00–20:00) light:12 h dark cycle. Rats had access to standard pellet food and water *ad libitum* throughout the study. Drinking water was renewed and cages were cleaned daily.

Experimental design

The rats were divided randomly into five groups of 10. The control (C) group was given saline solution. The corn oil (Co) group was treated with corn oil. The crocin (Cr) group was treated with 100 mg/kg/day crocin (42,553–65-1; Sigma Aldrich, St. Louis, MO). The CCl_4 group was treated with 1:1 CCl_4 (Sigma Aldrich) every other day. The CCl_4 + Cr group was treated with 1:1 CCl_4 and 100 mg/kg crocin every other day.

Crocin and CCl_4 were dissolved in saline and corn oil, respectively. All applications were 1 ml/kg by gavage and repeated for 15 days at the same hour.

After 15 days, rats were decapitated under xylazine-ketamine anesthesia. The brain was removed carefully and washed with saline. One hemisphere of the brain was stored for biochemistry at −80 °C, the other hemisphere was fixed in 10% formaldehyde for histological examination.

Biochemistry

On the day of the analysis, the tissues were removed from the freezer and weighed. Phosphate buffer was added to make a 10% homogenate and the tissues were homogenized for 1−2 min on ice at 12,000 rpm (IKA Ultra Turrax

T 25 basic; IKA Labotechnik, Staufen, Germany). Homogenates were used to measure malondialdehyde (MDA) levels. The supernatant was obtained by centrifuging the remaining homogenate at 600 x g for 30 min at 4 °C. The supernatant was used to measure superoxide dismutase (SOD) and catalase (CAT) activities and reduced glutathione (GSH), total antioxidant status (TAS), total oxidant status (TOS) and protein levels.

MDA analysis was performed using the method described by Ochawa et al. (1979). Tissue homogenate, 0.5 ml, was mixed with 3 ml 1% H_3PO_4 and 1 ml 0.6% thiobarbituric acid. The mixture was heated on a boiling water bath for 45 min, then extracted in 4 ml n-butanol; n-butanol was used as a blank and tetramethoxypropane was considered the standard. The MDA level was measured using a spectrophotometer (T80 UV/VIS Spectrometer; PG Instruments Ltd., Leicestershire, UK) at 535 nm. The results are presented as nmol/g wet tissue.

GSH was measured using the method described by Ellman (1959). After adding 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) to the sample, a yellow-green color develops due to the reaction between the DTNB and glutathione in the medium. The amount of reduced glutathione was determined by measuring the absorbance at 410 nm using a spectrophotometer. Distilled water was used as a blank. The results are presented as nanomol/g wet tissue.

SOD activity was measured using the method reported by Sun et al. (1988). Superoxide radicals are produced by xanthine-xanthine oxidase. The superoxide radical generates a color by reducing NBT (nitroblue tetrazolium) to a blue colored formazan. The absorbance of the formazan at 560 nm was used to calculate the SOD activity. Distilled water was used as a blank. SOD activity is presented as U/g protein.

CAT activity was measured using the method reported by Aebi et al. (1974). H_2O_2 absorbs ultraviolet light; the wavelength of maximum absorption is 240 nm. CAT catalyzed decomposition of H_2O_2 into water (H_2O) and oxygen (O_2) in the supernatant decreases absorbance at 240 nm. The decreased absorbance was recorded for 1 min to measure the enzyme activity. CAT activity is presented as K/g protein. The K constant is calculated by the following equation (Polat et al. 2018):

$$K = \frac{1}{\Delta t} \frac{E_{initial}}{E_{final}} \frac{2.3}{\Delta t} \times \ln \frac{E_{initial}}{E_{final}} = X \log$$

where Δt is the measured reaction time, E is optical density at 240 nm, and 2.3 is the factor to convert from In to log

The TOS level was determined using Erel's method (Erel 2005). A total oxidant status kit (Rel Assay Diagnostics, Gaziantep, Turkey) was used. The oxidants in the sample transform the ferrous ion chelator complexes into ferric ions. Ferric ions form a colored complex with the chromogenic solution. The absorbance of this complex was measured with an ELISA reader at 25 °C to determine the TOS level at 530 nm, which is directly proportional to the sample oxidant levels. The results are presented as μmol H_2O_2 equiv/l.

TAS level was measured using the method described by Erel (2004). Measurements were conducted using the TAS Rel Assay brand kit (Rel Assay Diagnostics). The measurement is based on the decoloring of the antioxidant molecules. Based on the kit instructions, 500 μl reagent 1 (measurement buffer) and 30 μl supernatant were combined and absorbance was measured at 660 nm by an ELISA at 25 °C to determine TAS levels. Subsequently, 75 μl reagent 2 (colored ABTS solution) was added to the mixture and the product was incubated for 10 min. TAS levels were determined by reading the absorbance at 660 nm after incubation. Trolox, a water-soluble vitamin E compound, was used as a calibrator. Results are expressed as mmol trolox equiv/l.

Histology

Brain tissue samples were fixed at 4 °C for 48 h in 10% neutral buffered formalin. Tissue samples were embedded in paraffin blocks after they were passed through graded ethanols and xylene. Cross sections were cut at 6 μm using a microtome, placed on slides and stained with hematoxylin and eosin (H & E) (Bancroft et al. 2013). The stained sections were examined and photographed with Nikon Eclipse Ni-U light microscope, Nikon DS-Fi2 camera and Nikon NIS-Elements Documentation image analysis system (Nikon Corp., Tokyo, Japan). We scored semiquantitatively necrosis of the neurons, pyknotic nuclei, irregular nuclear contours and increased cytoplasmic eosinophilia as 0, normal; 1, 1−10%; 2, 11−20%; 3, 21−40%; 4, 41−100% in the cerebral cortex and hippocampus with a maximum total score = 16.

Statistical analysis

The normal distribution of biochemical data was assessed by Shapiro-Wilk test and the homogeneity of the variances was examined using the Levene test. The data were recorded as means ± SD. Group comparisons were conducted using one-way analysis of variance and Tukey HSD paired comparison method. Histopathologic scores were recorded as medians with minimum and maximum values. The Kruskal-Wallis test and the Conover pairwise comparison method was used for group comparisons. The significance level was accepted as $p \leq 0.05$ for all tests.

Results

Biochemistry

Biochemistry results are presented in Table 1. Whereas CCl_4 administration caused significant increases in MDA, SOD and TOS levels in the CCl_4 group compared to all other groups, the CCl_4 + Cr group exhibited significant decreases in these levels and even approached control group levels. We also found that MDA, SOD and TOS levels in the Cr group were significantly lower than for the C and Co groups. GSH, CAT and TAS levels in the Cr group were significantly higher than those for the C and Co groups. We found that GSH, CAT and TAS levels decreased significantly in the CCl_4 group compared to all other groups, while values observed for the CCl_4 + Cr group approached control group levels. We also found that GSH, CAT and TAS levels in the CCl_4 + Cr group approached control group levels.

Histology

The histology of the cerebral cortex and hippocampus appeared normal in the C (Figure 1a-d), Co (Figure 1e-h) and Cr (Figure 1i-l) groups. The cytoplasm of neurons exhibited normal eosinophilia and neuronal nuclei were euchromatic and exhibited a normal contour in both the cerebral cortex and hippocampus.

In the CCl_4 group, neurons of the cerebral cortex exhibited heterochromatic nuclei, irregular nuclear

**Table 1.** Brain tissue oxidant-antioxidant parameters of all groups

Groups	MDA (nmol/g)	GSH (nmol/g)	SOD (U/g protein)	CAT (K/g protein)	TAS (mmol/l)	TOS (µmol/l)
C	1259.09 ± 45.66[a]	794.30 ± 38.59[a]	73.00 ± 3.58[a]	0.82 ± 0.07[a]	0.78 ± 0.10[a]	26.13 ± 0.95[a]
Co	1224.80 ± 56.2[a]	796.52 ± 63.38[a]	73.14 ± 4.14[a]	0.79 ± 0.11[a]	0.78 ± 0.08[a]	24.92 ± 2.14[a.d]
Cr	779.65 ± 38.03[b]	1229.57 ± 79.14[b]	52.60 ± 2.66[b]	1.34 ± 0.11[b]	1.68 ± 0.10[b]	14.87 ± 1.34[b]
CCl_4	1656.88 ± 83.59[c]	553.37 ± 37.56[c]	90.76 ± 3.78[c]	0.57 ± 0.06[c]	0.31 ± 0.06[c]	35.79 ± 2.34[c]
CCl_4 + Cr	1046.45 ± 104.30[d]	772.03 ± 65.99[a]	65.86 ± 2.99[d]	0.77 ± 0.07[a]	0.85 ± 0.09[a]	23.30 ± 2.28[d]

Data are means ± SD (n = 10). MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; TAS, total antioxidant status; TOS, total oxidant status. Groups: C, control; Co, corn oil; Cr, crocin; CCl_4, carbon tetrachloride; CCl_4 + Cr, carbon tetrachloride + crocin. Groups with different superscripts indicate statistical significance at $p < 0.05$.
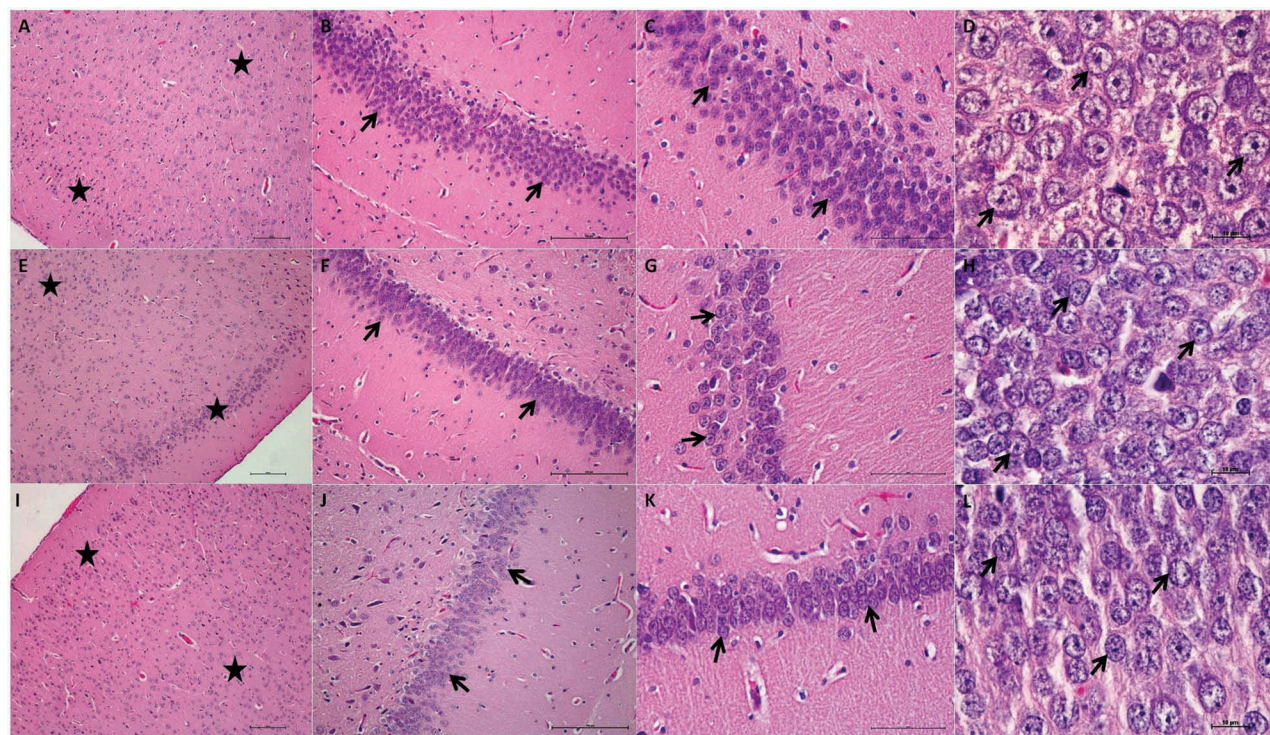
Figure 1. Brain tissue for groups C, Co and Cr. a) Group C, cerebral cortex (asterisk). H & E. x 10. b) Group C, hippocampus neuron nuclei (arrows). H & E. x 20. c) Group C, hippocampus neuron nuclei (arrows). H & E. x 40. d) Group Co, cerebral cortex (asterisk). H & E. x 10. e) Group Co, hippocampus neuron nuclei (arrows). H & E. x 20. f) Group Co, hippocampus neuron nuclei (arrows). H & E. x 40. g) Group Cr, cerebral cortex (asterisk). H & E. x 10. h) Group Cr, hippocampus neuron nuclei (arrows). H & E. x 20. i) Group Cr, hippocampus neuron nuclei (arrows). H & E. x 40. All magnifications are objective lens only.
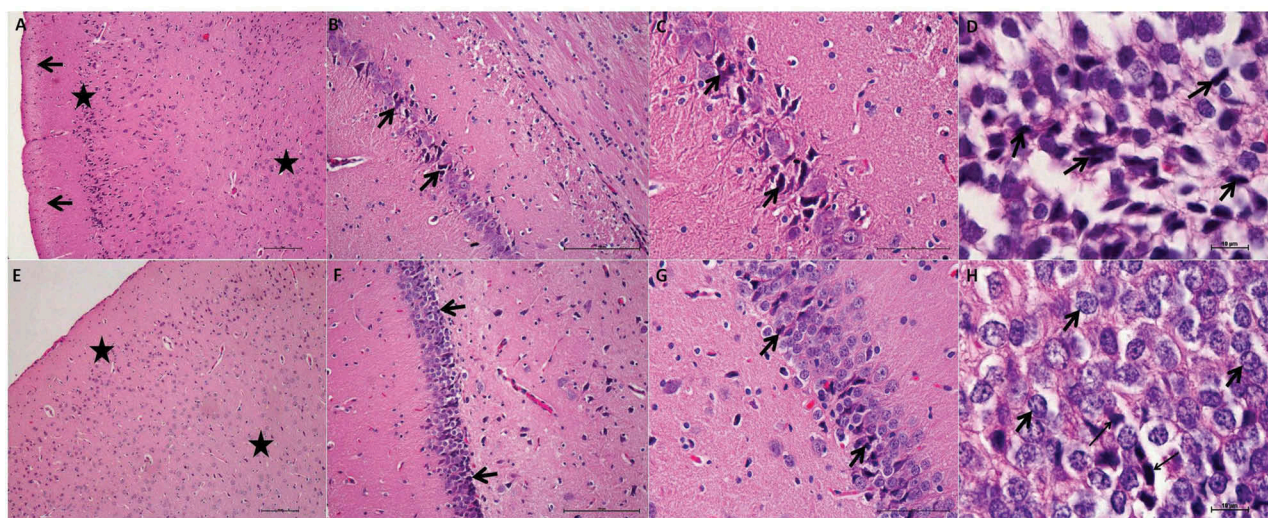
Figure 2. Brain tissues for groups CCl$_4$ and CCl$_4$ + Cr. a) CCl$_4$ group, cerebral cortex (asterisk), cancellous appearance in molecular layer (arrow). H & E. x 10. b) CCl$_4$ group, neurons with pyknotic nucleus in hippocampus (arrows). H & E, x 20. c) CCl$_4$ group, neuron degeneration and neurons with pyknotic nucleus in hippocampus (arrows). H & E. x 40. d) CCl$_4$ + Cr group, cerebral cortex (asterisk). H & E. x 10. e) CCl$_4$ + Cr group, neurons with pyknotic nucleus in hippocampus (arrows). H & E. x 20. f) CCl$_4$ + Cr group, neurons with pyknotic nucleus in hippocampus (arrows). H & E. x 40. All magnifications are objective lens only.

contours and occasionally pyknotic nuclei. Mild to moderate degrees of spongy tissue were observed in molecular layer of the cerebral cortex (Figure 2a). Neuronal degeneration, irregular nuclear contours, heterochromatic and pyknotic nuclei, and increased cytoplasmic eosinophilia were observed in the neurons of the hippocampus (Figure 2b,c).

In the CCl$_4$ + Cr group, the molecular layer of the cerebral cortex exhibited nearly normal histologic appearance (Figure 2d). Although heterochromatic and pyknotic neuronal nuclei were observed in the cerebral cortex and hippocampus, the neuronal damage score was significantly reduced compared to the CCl$_4$ group (Figure 2d-f). Cerebral cortical damage scores are presented in Table 2; hippocampal damage scores are presented in Table 3.

Table 2. Cerebral cortex damage scores

Groups	Mean	Minimum	Maximum
C[a]	0	0	1
Co[a]	0	0	0
Cr[a]	0	0	1
CCl$_4$[b]	1	0	2
CCl$_4$ + Cr[a]	0.5	0	1

Groups: C, control; Co, corn oil; Cr, crocin; CCl$_4$, carbon tetrachloride; CCl$_4$ + Cr, carbon tetrachloride + crocin. Groups with different superscripts indicate statistical significance at $p < 0.05$.

Table 3. Brain hippocampus damage scores

Groups	Mean	Minimum	Maximum
C[a]	0	0	1
Co[a]	0	0	1
Cr[a]	0	0	1
CCl$_4$[b]	4,5	2	5
CCl$_4$ + Cr[c]	1	0	3

Groups: C, control; Co, corn oil; Cr, crocin; CCl$_4$, carbon tetrachloride; CCl$_4$ + Cr, carbon tetrachloride + crocin. Groups with different superscripts indicate statistical significance at $p < 0.05$.

Discussion

CCl$_4$ is lipophilic and readily penetrates cell membranes. CCl$_4$ is absorbed rapidly by both the liver and the brain, but toxic effects on the brain are less well known (Szymonik-Lesiuk et al. 2003). CCl$_4$ exerts its toxic effects by producing the free radical, CCl$_3$•, which causes membrane lipid oxidation (Recknagel et al. 1989). Hepatic damage caused by a single 1 ml/kg dose of CCl$_4$ is due to increased oxidative stress (Srivastava and Shivanandappa 2010; Ritesh et al. 2015). Ritesh et al. (2015) demonstrated that the same dose of CCl$_4$ that causes hepatotoxicity also causes oxidative stress in the brain; 2-thiobarbituric acid

reactive substances (TBARS), an index of lipid oxidation, have been shown to be higher in the brain than in the liver (Ritesh et al. 2015).

Free radical lipid oxidation is an important mechanism for the pathogenesis of hepatic injury caused by CCl$_4$; the mechanism for neurotoxicity in the brain may be similar. The brain is especially sensitive to oxidative stress. Furthermore, neurons are rich in PUFA, which are particularly prone to ROS induced lipid oxidation (Lavrentiadou et al. 2013).

Tissue toxicity caused by xenobiotics can be prevented by various plant species including quercetin (Uthra et al. 2017), rosemary (Botsoglou et al. 2010)

and *Cnestis ferrugina* extract (Rahmat et al. 2014). We investigated the effects of crocin therapy on CCl_4 induced brain injury in a rat model. We found that crocin treatment significantly improved CCl_4 induced damage. The neuroprotective activity of crocin is due largely to its antioxidant property (Ahmad et al. 2005; Ochiai et al. 2007; Zheng et al. 2007). Radical scavenging activities of crocin have been reported in addition to its anti-inflammatory effect (Nam et al. 2010). Also, even at high experimental doses, crocin exhibits low toxicity in rats (Wang et al. 1984). Genotoxicity tests in vitro have demonstrated that crocin and crocetin do not present genotoxic risks (Ozaki et al. 2002).

Earlier reports have indicated that CCl_4 causes oxidative stress in kidney and liver (Ma et al. 2014a, 2014b). Ma et al. (2016) reported that severe oxidative stress in the hippocampus of CCl_4 treated mouse is caused by the induction of CYP2E1 and that lipid oxidation is characterized by marked increases in ROS and MDA levels. Our findings are consistent with earlier studies and demonstrate that the MDA level, an end product of lipid oxidation, and the TOS level were significantly elevated in the CCl_4 treated group. We also found that crocin significantly reduced MDA and TOS levels, which are lipid oxidation products, and protected the brain from oxidative stress caused by CCl_4.

ROS are produced constantly during normal cell metabolism and they are scavenged by cellular enzymatic antioxidants (Martinez-Cayuela 1995). Oxidative stress is reduced by increasing cellular antioxidants to protect the tissues against oxidative injury (Nomura and Yamaoka 1999; Cao and Li 2002). Primary antioxidant enzymes involved in direct elimination of free radicals include SOD, which removes superoxide radicals (O_2 •); CAT, which catalyzes decomposition of H_2O_2 into H_2O and O_2; and glutathione peroxidase (GPx), which converts H_2O_2 into H_2O and prevents the formation of the hydroxyl radical (•OH) (Halliwell 1994; Martinez-Cayuela 1995). A delicate balance is required between the formation of H_2O_2 by the dismutation of O_2• by SOD and removal by CAT and GPx; any imbalance affects other enzyme activities (Sinet and Garber 1981; Kono and Fridovich 1982). Reduced activity of SOD, CAT and GPx is associated with excess ROS, which can cause deterioration of cell membrane integrity and functions (Reddy and Lokesh 1992; Jayakumar et al. 2008; Annadurai et al. 2011).

Generally, lipid oxidation is caused by free radical attacks on the unsaturated fatty acids of cell membranes, because the double bonds in the membranes allow the hydrogen atoms to be removed easily by ROS such as •OH (Halliwell 1989). Under aerobic conditions, lipid oxidation proceeds by O_2 combining with conjugated dienes to form additional organic peroxy

radicals. Peroxy radicals separate hydrogens from adjacent fatty acid chains, thus extending the lipid oxidation process. In addition, the peroxy radicals could be conjugated with an extracted hydrogen atom to generate lipid hydroperoxides that dissociate into alkoxy radicals and aldehydes in the presence of Fe^{2+}. Therefore, the movement of a single •OH may initiate a chain reaction that produces a large number of toxic reactants that disrupt membrane integrity and damage membrane proteins. Under circumstances of increased ROS production, the antioxidant defense system cannot cope with these radicals and tissue damage results.

We found increased TOS and MDA levels and decreased TAS levels in CCl_4 treated brain tissue, which indicates increased lipid oxidation. Therefore, we suggest that brain damage is caused by the inability of endogenous antioxidant defense mechanisms to prevent excessive free radical formation. We also found that CCl_4 exposure increased enzyme SOD activity and decreased CAT activity, which decreased the antioxidant capacity of the brain by increasing H_2O_2 formation. Consequently, we believe that increased H_2O_2 causes formation of OH•, which causes tissue damage by lipid oxidation. Most neurotoxic chemicals, including ethanol, cause oxidative stress in the brain (Houze et al. 1991). The activities of antioxidant enzymes returned to approximately control group levels after crocin treatment by reducing SOD activity and increasing CAT activity in CCl_4 treated rats.

Our findings are consistent with those of Ma et al. (2016), who used quercetin to protect against CCl_4 induced cerebral damage and by Coballase-Urrutia et al. (2017), who used *Tilia* extract. In both studies, antioxidant enzymes reached normal levels after treatment. To the contrary, Safhi et al. (2018) reported that kidney TBARS levels were elevated and antioxidant enzyme activities were decreased following CCl_4 induced nephrotoxicity. When zingerone was administered as an antioxidant, these investigators observed that TBARS levels decreased and antioxidant enzyme activities increased. Zheng et al. (2007) recommended crocin application to mice with transient global cerebral ischemia, because it decreases oxidative stress by increasing SOD and GPx activities, which protects brain capillaries. Our findings were consistent with the report by Zheng et al. (2007) SOD and CAT activities returned to approximately control group values after crocin treatment of CCl_4 treated rats.

GSH is a cytosolic tripeptide found in millimolar concentrations in all cell types; it is a non-enzymatic regulator of intracellular redox homeostasis (Meister and Anderson 1983). We found that brain GSH levels were decreased significantly by CCl_4 administration. GSH depletion increases the susceptibility of the brain to oxidative stress

due to impaired redox balance. GSH also functions as a substrate for GPx and glutathione S-tranferase (GST), which participate in detoxification of electrophilic metabolites by forming the GSH conjugate (Hayes et al. 2005). During the enzymatic reaction catalyzed by GPx, GSH is oxidized to GSSG, which is converted to reduced GSH by glutathione reductase (GR). Therefore, GR participates in balancing the cellular GSH level. We found significant reductions in reduced GSH levels, which resulted in depletion of GSH and a CCl$_4$-induced decrease in brain GR activity, which possibly affected GSH regeneration. (Martinez-Cayuela 1995). GSH levels in CCl$_4$ treated animals were decreased significantly after crocin treatment and approached control values. Ochiai et al. (2007) reported that crocin increased GSH synthesis by promoting γ-glutamyl cysteinyl synthase (γ-GCS) mRNA expression, which catalyzes GSH synthesis as a rate limiting enzyme, and that crocin can significantly reduce infarcted areas caused by occlusion of the middle cerebral artery (MCA) in mice.

Saffron and its active components have been shown to exhibit neuroprotective effects in brain disease in vivo and in vitro (Ochiai et al. 2007). Initial small scale clinical trials of saffron treatment of depression and mild Alzheimer's disease have produced promising results (Akhondzadeh et al. 2010). Shati et al. (2011) reported that saffron extract administration counteracted aluminum chloride (AlCl$_3$) induced neurotoxicity in Balb/c and C57BL/6 mice. These investigators determined oxidative stress and antioxidant status by measuring SOD, CAT, GPx, TBARS and TAS, scanning the up-down regulated genes such as B-cell lymphoma 2 (Bcl-2), R-spondin and the inositol polyphosphate 4-phosphatase genes (INPP4B) in brain homogenate and measuring serum tumor markers such as arginase and a-l-fucosidase.

Consistent with our biochemical findings, we found a significant increase in tissue damage scores in the cerebral cortex and hippocampus following CCl$_4$ application due to oxidative stress; we also found significantly decreased damage scores following crocin treatment. Crocin exhibits antioxidant effects owing to its free oxygen radical scavenging properties. Therefore, brain MDA, TOS and SOD levels that were increased following CCl$_4$ administration decreased after crocin treatment, while decreased GSH and TAS levels and CAT activity increased after crocin treatment.

Disclosure statement

No potential conflict of interest was reported by the authors.

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