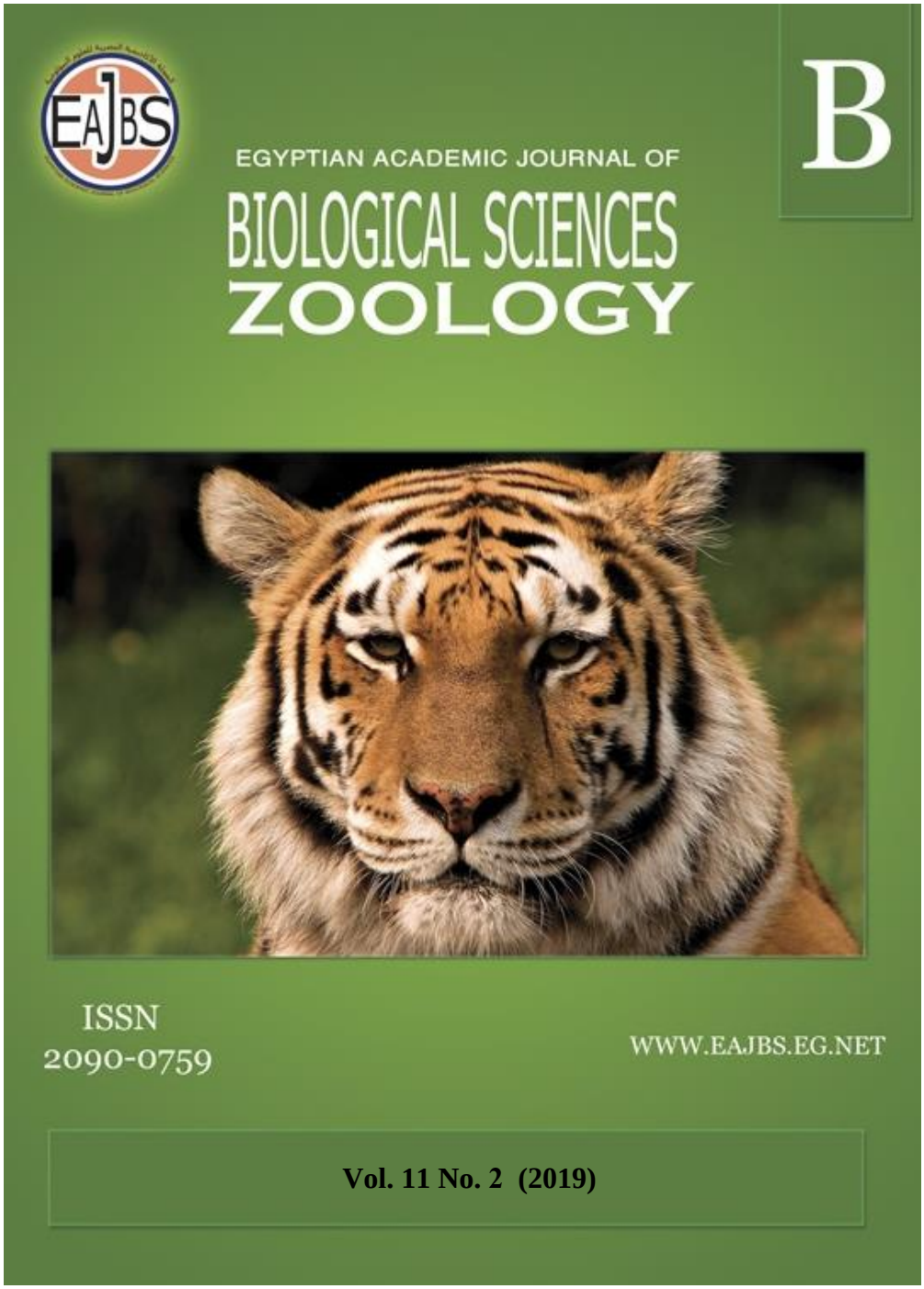


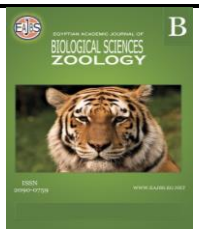
Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society of Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

The Journal publishes original research papers and reviews from any zoological discipline or from directly allied fields in ecology, behavioral biology, physiology & biochemistry.

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 11(2): 1- 18 (2019)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
www.eajbsz.journals.ekb.eg



The Protective Effect of Saffron (*Crocus sativus l.*) against Carbon Tetrachloride Induced Toxicity in Some Organs of Albino Rats

Gamal H. El-Sokkary[1] and Eatemad A. Awadalla[*2]
1-Department of Zoology Faculty of Science Assiut University,Assiut, 71516 Egypt
2-Department of Zoology Faculty of Science Aswan University, Aswan, 81528 Egypt.
E. Mail.: Eatemad2000@Aswu.edu.eg

ARTICLE INFO
Article History
Received:15 /2/2019
Accepted:10/4/2019

***Keywords*:**
Saffron; Carbon tetrachloride; Antioxidant enzymes; Lipid peroxidation; Morphological changes.

ABSTRACT

Saffron (*Crocus sativus L.*) has been extensively exploited in folk medicine for the treatment of a number of ailments. Free radicals propagation has been implicated in carbon tetrachloride (CCl_4)-induced toxicity, which is involved in lipid peroxidation (LPO). The present study was planned to evaluate the efficacy of saffron on CCl_4-induced injuries in some organs of rats. Thirty male albino rats were used in the current study and divided into three groups; control, CCl_4 group (0.5 ml/kg body wt.) and CCl_4 + saffron (20 mg/kg body wt.). Specimens from livers, kidneys and lungs were taken for biochemical and histopathological studies. The results showed that the activities of superoxide dismutase (SOD) and catalase (CAT), in addition to the concentration of glutathione (GSH) were decreased while malondialdehyde (MDA) level was increased after CCl_4 administration. Also, different morphological changes and marked expression of Bax protein were detected. Treatment with saffron extract was effectively alleviated the alterations in the biochemical markers and morphological structure of liver, kidney and lung. The present study confirms the restoration of normalcy and accredits the ameliorative role of saffron against CCl_4-induced toxicity.

INTRODUCTION

Toxicity is the capacity of a substance to cause destructive consequences for a solitary cell, a group of cells, an organ system, or the whole body. Numerous chemical compounds found in the environment are harmful and require exact identification of their potential risks to both human and animal health. CCl_4 is a widely utilized chemical dissolvable in different industrial procedures. Among different chemical substances that harm the liver, CCl_4 is observed to be the most hepatotoxic (Assayed et *al.*, 2010). In animal cells, the metabolism of CCl_4 initiates reactive oxygen species (ROS) development through trichloromethyl (CCl_3) radical and chloride (Cl) by means of the cytochrome P-450 enzyme system *(*Adewole et al., 2007). In the presence of oxygen, CCl_3 free radical further combines with cellular lipids and proteins to form a

trichloromethylperoxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than CCl_3 free radical. Thus, lipid peroxidation, the destruction of Ca^{2+} homeostasis, and finally cell death can elicit by trichloromethylperoxyl free radical *(*Recknagel *et al.,* 1989*)*. One of the main causes of CCl_4-induced liver injury is lipid peroxidation by its free radical derivatives. It has been hypothesized that one of the principal causes of oxidative stress is the destruction of the cells by lipid peroxidation *(*Recknagel *et al.,* 1989*)*. Also, induction of renal dysfunction through the pathogenesis of CCl_4 may be due to the functional case of liver or may develop independently to a hepatic case *(*Rincón *et al.,* 1999). El-Shorbagy (2017) found that administration of CCl_4 induced alteration in the histological architecture of kidney tissues, down-regulated mRNA expression of Bcl-2 gene and reduced the concentrations of some antioxidant enzymes. Ganie *et al.* (2011) showed that CCl_4 caused a marked increase in the levels of thiobarbituric acid reactive substances (TBARS) in kidney and lung tissue homogenates whereas the levels of Glutathione (GSH), Superoxide dismutase (SOD), Glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) levels were reduced. In addition, Mizuguchi *et al.* (2006) reported that CCl_4 exposure has been exhibited to cause damage to the lungs.

So many plant species are being used to treat or inhibit the progress of diseases. Saffron, Crocus sativus, is identified as Zaa'fran and used in the production of Arabic c offee as an essential spice agent. Saffron has been widely utilized as a flavor and nourishment colorant because of its color and taste (Winterhalter and Straubinger, 2000). Saffron has numerous chemical constituent including crocin-1, picrocrocin, starry, vitamins, Bl and B2, fixed oils, carotenoids, colchicine, quercitin, proteins, wax and mucilage (Tarantilis *et al.,* 1995*)*. Safran has appeared to have many properties in popular medicine, such as antispasmodic, eupeptic anticatarrhal, nerve soothing, carminative, diaphoretic, expectorant, stimulant, stomachic and aphrodisiac *(*Moghaddasi, 2010). Modern investigations demonstrated that saffron extracts have numerous therapeutic activities including hypolipemic, anti-inflammatory, anti-oxidant, anti-diabetic and anti-carcinogenic *(*Mohajeri *et al.,* 2011). Also, the saffron extract was found to have a hepatoprotective impact (Iranshahi *et al.,* 2011*)*. Saffron and its derivatives have so many flavonoids and anthocyanin compounds. Flavonoids can inhibit the fatty acids enzymatic peroxidation and have free radical scavenging property. Therefore, it possesses the potency to act as an antioxidant agent.

Crocin has antioxidant properties and there is a great amount of it in the stigma of saffron. Crocin and crocetin are carotenoid glycosides and soluble in ethanol and water. It was proposed that the effectiveness of saffron stigmas in healing liver damages induced by CCl_4 is due to these glycosides. It is well known that the protective effect of saffron has a significant correlation with its antioxidant activities *(*Chen *et al.,* 2008*;* Lin and Huang, 2000).

Based on the above-mentioned literatures, the present study was planned to investigate the possible protective effects of saffron as an antioxidant against CCl_4-induced oxidative stress in the liver, kidney and lung of rats.

MATERIALS AND METHODS

Preparation of Saffron Extract:

Saffron, the dried stigmas of Crocus sativus flower were obtained from the local market, Aswan, Egypt. In 100 ml of distilled water, one gram of saffron was soaked. After 2 hours it was homogenized in the same distilled water, stirred for 1 hour and

filtered. The residue was re-extracted with fresh distilled water. This aqueous extract was lyophilized and stored for further use at 4°C (Premkumar *et al.,* 2003).

Chemicals:

Carbon tetrachloride (CAS no. 0.025mol) was obtained from El-Gomhorya Pharmaceutical Company, Cairo, Egypt. Chemicals for biochemical analysis were obtained from Biodiagnostic Co., Cairo, Egypt. All other chemicals were commercially available of the highest purity.

Animals and Experimental Design:

Thirty adult male albino rats (4 months old) weighing 120–140g were purchased from the Animal House, The Egyptian Co. for Vaccines Production at Helwan. The rats were housed in well-ventilated cages at 25±3°C under daylight and healthy condition. Diet and fresh water were supplied ad-libitum. The animal ethical committee of Aswan University, Egypt in accordance with the guide for the care and use of laboratory animals approved conservation of animals and experimental procedures. After one week of adaptation, rats were divided into 3 main groups (10 rats each):

-The first group was served as a control group and received distilled water.

-The second group was administered intraperitoneally with 0.5 ml/kg body wt. of CCl_4 diluted in liquid olive oil (1:1) twice a week for four weeks (Eidi *et al.,* 2012).

-The third group was given CCL_4 followed by oral administration with saffron extract at a dose of 20 mg/kg body wt., daily for four weeks (Sakr *et al.,* 2014). Twenty four hours after the end of the experiment, animals were sacrificed and dissected to evaluate the biochemical and histopathological alterations.

Biochemical Studies:

Portions of the selected organs (liver, kidney and lung) were frozen at -80°C to determine the oxidized lipids (LPO) as indicated by malondialdehyde (MDA) levels, CAT, SOD activities and GSH concentration in all groups. MDA level was assayed according to (Ohkawa *et al.,* 1979), CAT activity was analysis by the method of *Aebi* (1983), the activity of SOD was estimated according to (Paoletti and Mocali, 1990) and GSH concentration was determined by the method of Maral *et al.* (1977).

Histological Study:

After scarification, specimen from the liver, kidney and lung were taken from the three groups. They were fixed in 10% neutral formalin buffer, embedded in paraffin, cut at 5 microns and prepared for the Harris′s hematoxylin and eosin stain, (Gabe, *1976*). Microscopic fields in all examined sections were randomly selected and magnified using high-power light microscope (Olympus BX43F Tokyo163-0914 Japan). Image analysis was done using a personal computer, camera software (Olympus DP74 Tokyo 163-0914 Japan) and an optical microscope.

Bax Immunohistochemistry:

Five microns thick, paraffin sections were floated onto coated slides (Sigma). Slides were deparaffinized with xylene and dehydrated in graded series of ethanol. Endogenous peroxidase was quenched with 3%H_2O_2: Methanol (1:1) for 30 min at room temperature. Staining required boiling in 10 mM citrate buffer, pH 6.0 for 20 min followed by cooling at room temperature for 20 min. Sections were rinsed in Phosphate Buffer Saline (PBS) and then blocked with 6% horse serum and 4% Bovine Serum Albumin (BSA) in PBS for 1h at room temperature. Primary antibody was mice polyclonal anti-Bax (1:150; Santa Cruz Biotechnology). It was diluted in 1% horse serum and 4% BSA in PBS and left one hour at room temperature. Sections were washed twice for 5 min in PBS. Immune-histochemical staining was performed using an avidin-biotin-peroxidase complex (ABC). Bax antibody location was determined

with the addition of 3,3´-Diaminobenzidine (DAB) chromogen (Dako Denmark): 3% H_2O_2 for 3 min and washing with water stopped color development. Sections were counterstained with hematoxylin, dehydrated and mounted in Canada balsam (DPX, Poole, UK). As the negative control, non-specific mice and goat IgG was used instead of the primary antibody.

Statistical Analysis:

Results were expressed as means ± SD. Statistical differences were analyzed using a one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls t-test. Statistical significance was accepted at $p<0.05$.

RESULTS

Biochemical Results:

The quantitative data of MDA, SOD, CAT and GSH in the liver, kidney and lung tissues are summarized in Table 1. The results confirmed that MDA level was increased in the liver, kidney and lung tissues of CCl_4-administered rats. Statistically, the increase was highly significant ($P<0.01$) in the liver and significant ($P<0.05$) in both kidney and lung versus to those of the control group. Also, CCl_4 administration was significantly inhibited ($P< 0.05$) the activities of both SOD and CAT while it was highly significant decreased ($P< 0.01$) the concentration of GSH in the studied organs. Marked reduction in the level of MDA in the liver, kidney and lung was detected when saffron gave to CCl_4-administered group. Statistically, this reduction was highly significant ($P< 0.01$) in the liver and significant ($P< 0.05$) in both kidney and lung. Also, saffron treatment stimulated the activities of SOD and CAT and increased the concentration of GSH in the liver, kidney and lung tissues. The statistical analysis of this data revealed that the stimulation of SOD and CAT activities was significant ($P< 0.05$) while the increment of GSH concentration was highly significant ($P< 0.01$).

Table 1. The effects of saffron extract on peroxidation and tissue antioxidants level in carbon tetrachloride induced tissue toxicity.

Parameters	Tissue Type	Control	CCl_4	Saffron+ CCl_4
MDA (nmol/g Tissue)	Liver	4.832 ± 0.4147	12.896 ± 0.5005**	4.986 ±0.356**
	Kidney	5.498±0.797	8.998±4.052*	5.85±0.593*
	Lung	5.080± 0.409	9.971 ±0.304*	5.205± 0.301*
SOD (Units/g Tissue)	Liver	10.728± 0.423	4.60±0.572*	8.541±0.443*
	Kidney	10.128 ±0.493	4.08±0.463*	8.048± 0.220*
	Lung	7.729 ± 0.778	4.06± 0.513*	5.538±0.798*
CAT (Units/g Tissue)	Liver	66.728 ±3.523	27.105 ± 2.737*	60.652 ±1.928*
	Kidney	56.96 ± 2.013	23.294±3.376*	46.094 ±4.346*
	Lung	88.61 ±4.337	46.58 ± 3.203*	74.192 ± 2.715*
GSH (µgm/g Tissue)	Liver	10.598 ± 0.627	5.52 ± 0.749**	8.05 ± 0.2871**
	Kidney	10.128 ± 0.493	4.080 ± 0.464**	8. 248 ± 0.430**
	Lung	56.964 ± 2.013	23.284 ± 3.317**	46.094± 4.347**

Values are means ± S. D. of 10 animals in each group. ** highly significant ($P< 0.01$), * significant ($P<0.05$).

Histopathological Examination:

Microscopic examination to the liver sections of CCl_4-administered rats showed severe changes in the liver architecture (Figs. 1b, c) in comparing with the control group (Fig. 1a). The administration of CCl_4 induced several alterations as centrilobular necrosis, congested and dilated blood vessels, vacuolated hepatocytes with pyknotic nuclei and portal area with invading infiltrative inflammatory cells (Figs.1b, c). Saffron treatment to the rats administered with CCl_4 kept nearly the normal appearance of liver tissue and the majority of alterations caused by CCl_4 were disappeared (Fig.1d).

The examination of kidney sections of CCl_4-administered rats showed marked deleterious morphological changes (Figs. 2b, c) versus those of the control group (Fig. 2a). The changes include varying distinct glomerular and tubular degeneration forms; glomerular atrophy with dilatation of Bowman's space and some tubular lumina contained a considerable amount of debris and some renal tubular cells underwent necrotic changes (Fig. 2b, c). Comparing with CCl_4 group, the kidneys of saffron-treated rats preserved normal morphology and appear with normal architecture nearly like those of controls (Fig. 2d).

Administration of CCl_4 to the rat induced different changes in the morphology of the lungs (Figs 3b, c) comparing with those of controls (Fig. 3a). The changes include loss of normal alveolar architecture and deterioration of lung tissues accompanied by intense inflammatory infiltrates, some debris, intra-alveolar hemorrhage, prominent disorganized thickening of the alveolar septa and collapse of the alveolar space (Figs. 3b, c). Co-treatment with saffron to CCl_4-treated rats showed well-preserved lung parenchyma with normal alveoli and normal bronchioles (Fig. 3d).

Immunohistochemical Investigation:

Sections of the control group of the liver (Fig. 4a), kidney (Fig.5a) and lung (Fig. 6a) showed weak stain of proapoptotic Bax protein. Contrarily, sections of the liver (Fig. 4b), kidney (Fig.5b) and lung (Fig. 6b) of rats administered with CCl_4 exhibited marked staining of proapoptotic Bax protein comparing with control sections. Co-treatment with saffron resulted in negative and/or slight staining of Bax in the liver (Fig. 4c), kidney (Fig.5c) and lung (Fig. 6c) and tissues appear nearly like those of controls.

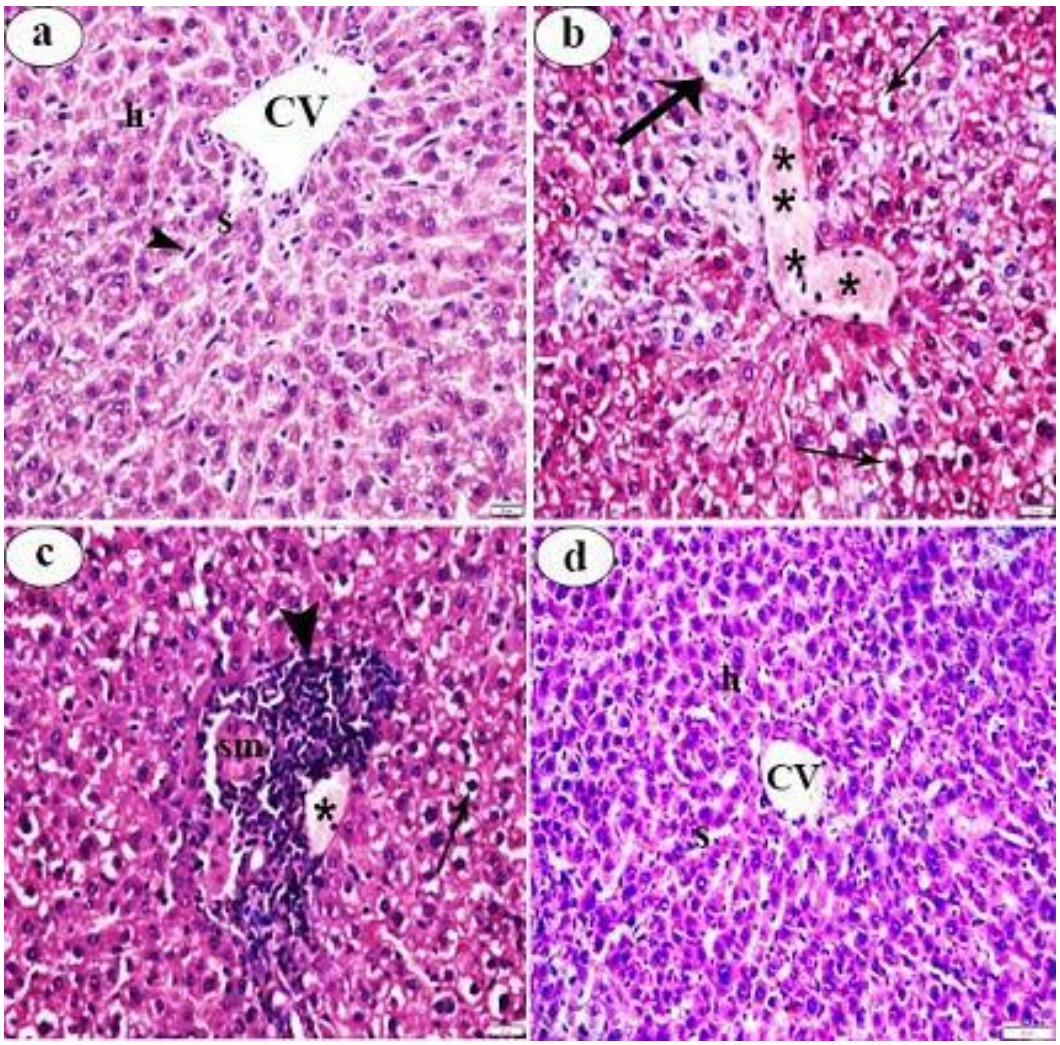


Fig.1. Sections of liver from the control and experimental groups. (**a**): Control rats showing the normal hepatocytes (h) arranged in cords around the central vein (CV), sinusoidal space (s) contain Kupffer cells (arrowhead). (**b, c**): CCl_4-given group showing vacuolated hepatocytes with pyknotic nuclei (thin arrows), necrosis (thick arrow), congested blood vessels (stars), infiltrative inflammatory cells (arrowhead), and degenerated hepatocytes fused together forming eosinophilic syncytial masses (sm). (**d**): Saffron+CCl_4 group showing the normal appearance of the hepatocytes (h), blood sinusoids (S) and central vein (CV). Scale bar =50 µm, H&E stain.

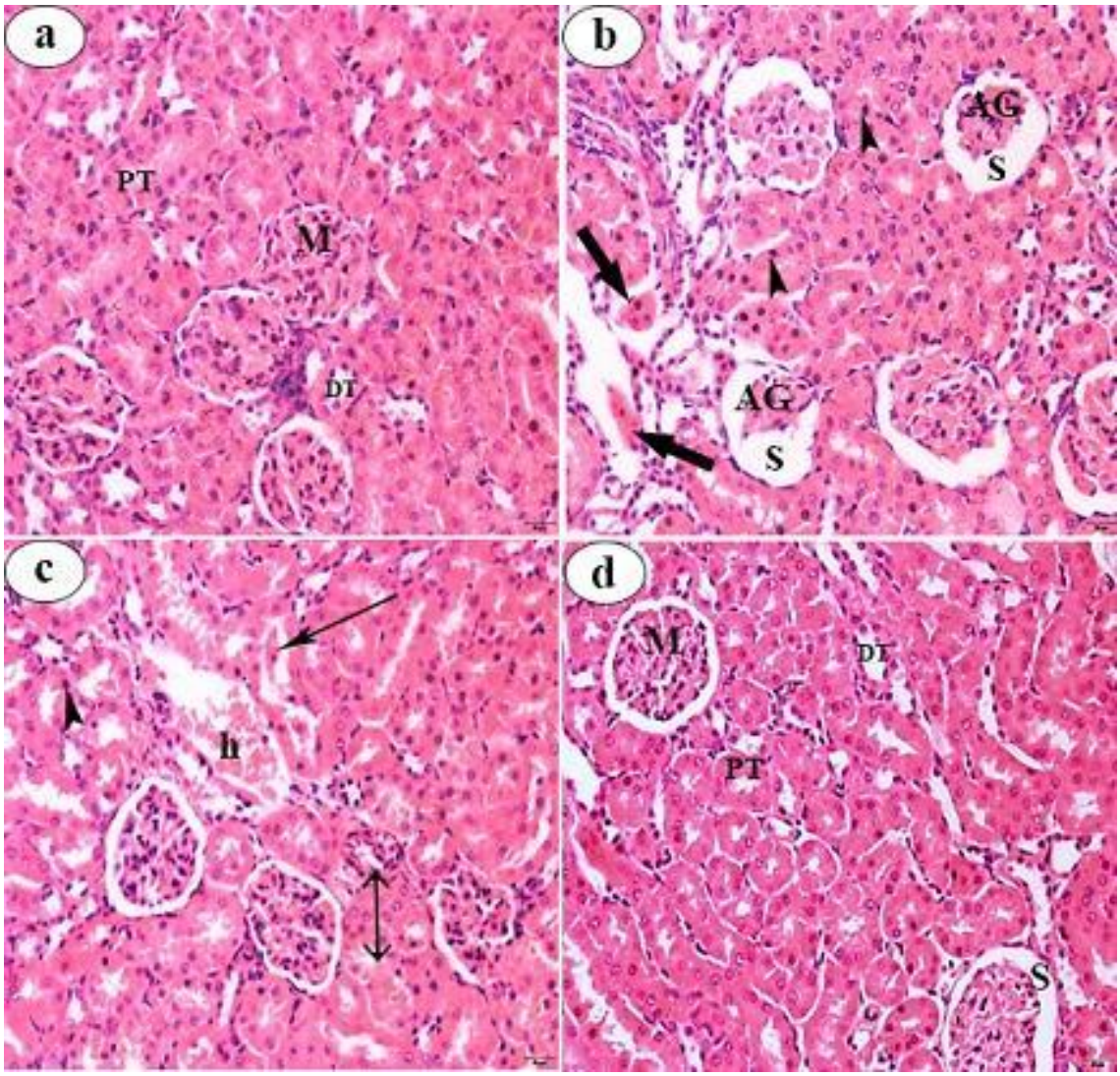


Fig.2. T.S. of the kidney from the control and experimental groups. (**a**): Control rat showing normal Malpighian corpuscles (M) and renal tubules; distal (DT) and proximal (PT) convoluted tubules. (**b, c**): CCl_4 -rat showing atrophy of glomeruli (AG) with dilated Bowman's space (S), pyknotic nuclei (arrowhead), necrosis of tubular cells (thick arrow), debris present in some tubular lumina (thin arrows), inflammatory cells infiltration in the interstitium (double head arrow) and hemorrhage interstitium (h). (**d**): Saffron+CCl_4 rat displaying a nearly normal appearance of renal structure; Malpighian corpuscles (M) and renal tubules; distal (DT) and proximal (PT) convoluted tubules. Note: the Bowman's space (S) is somewhat dilated. Scale bar = 50µm, H&E stain.

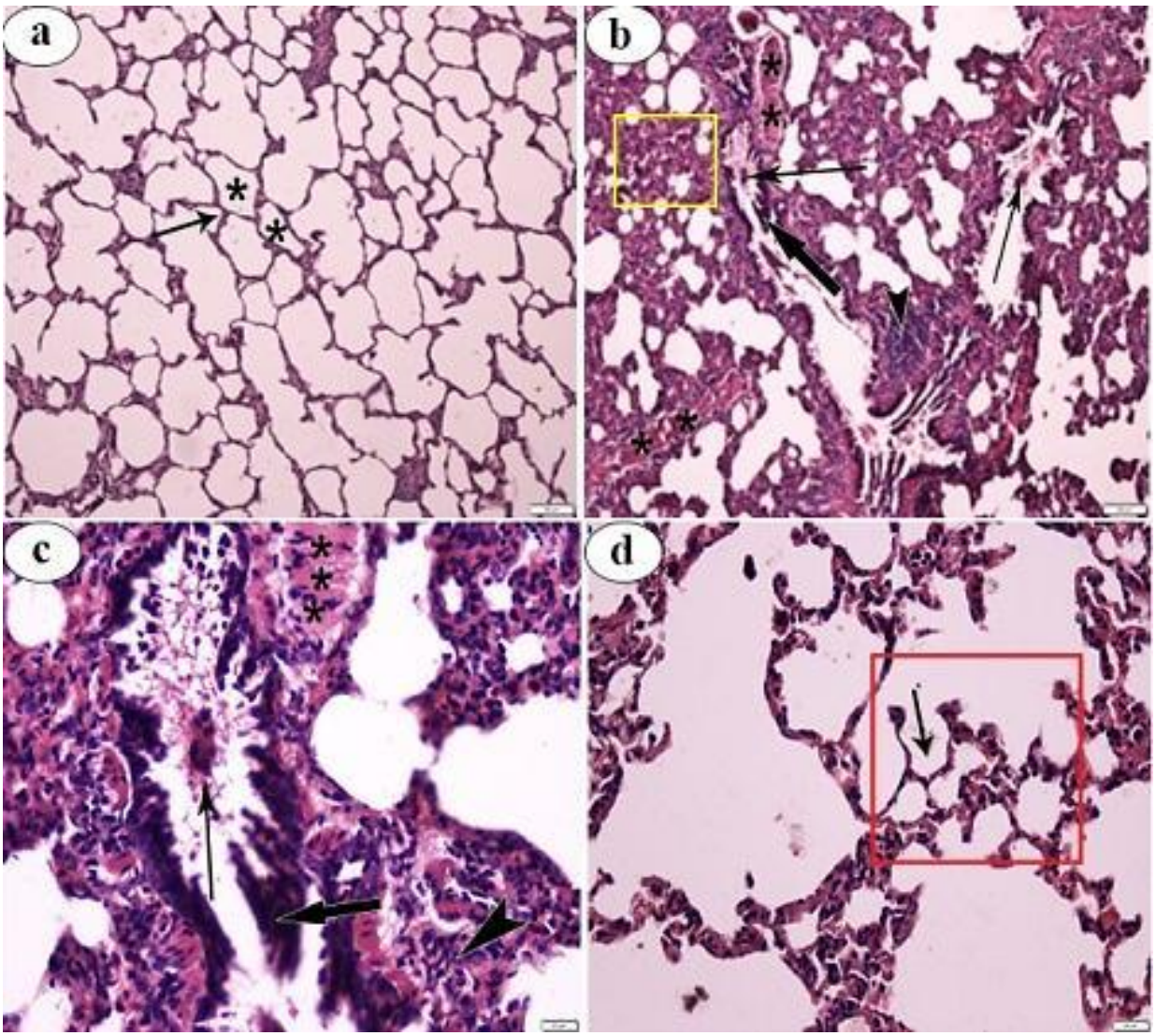


Fig. 3. T.S. of the lung from the control and experimental groups. (**a**): Control animal showing normal lung tissue with normal alveoli (arrow) and alveolar sacs (stars). (**b)**: CCl_4 given rat showing prominent disorganized thickening of the alveolar septa and collapse of the alveolar space (yellow square), infiltration of inflammatory cells (arrowhead), some debris (thin arrow), epithelium damage (thick arrow), intra-alveolar hemorrhage (star). **(c**): Magnification of the previous section. (**d**): Saffron+CCl_4 group showing the nearly normal appearance of the lung tissue (red square) with normal alveoli (arrow). Scale bar =50 µm, H&E stain.

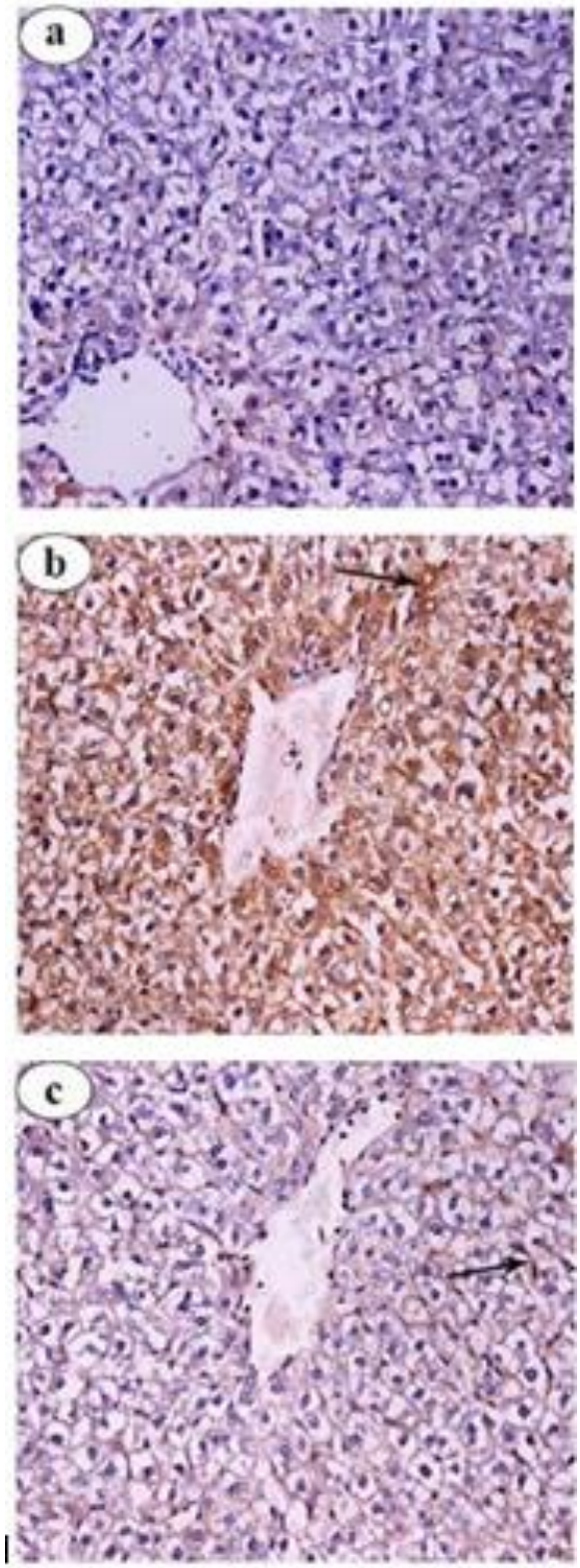


Fig.4. Photomicrograph showing the expression of Pro-apoptotic Bax in the liver. (**a**): Control rat shows weak expression of Bax protein. (**b**): CCl4 given rat shows strong expression of Bax (arrow). (**c**): Saffron+CCl4 rat shows slight immune-staining of Bax (arrow) as similar as the control group. Scale bar =50 µm

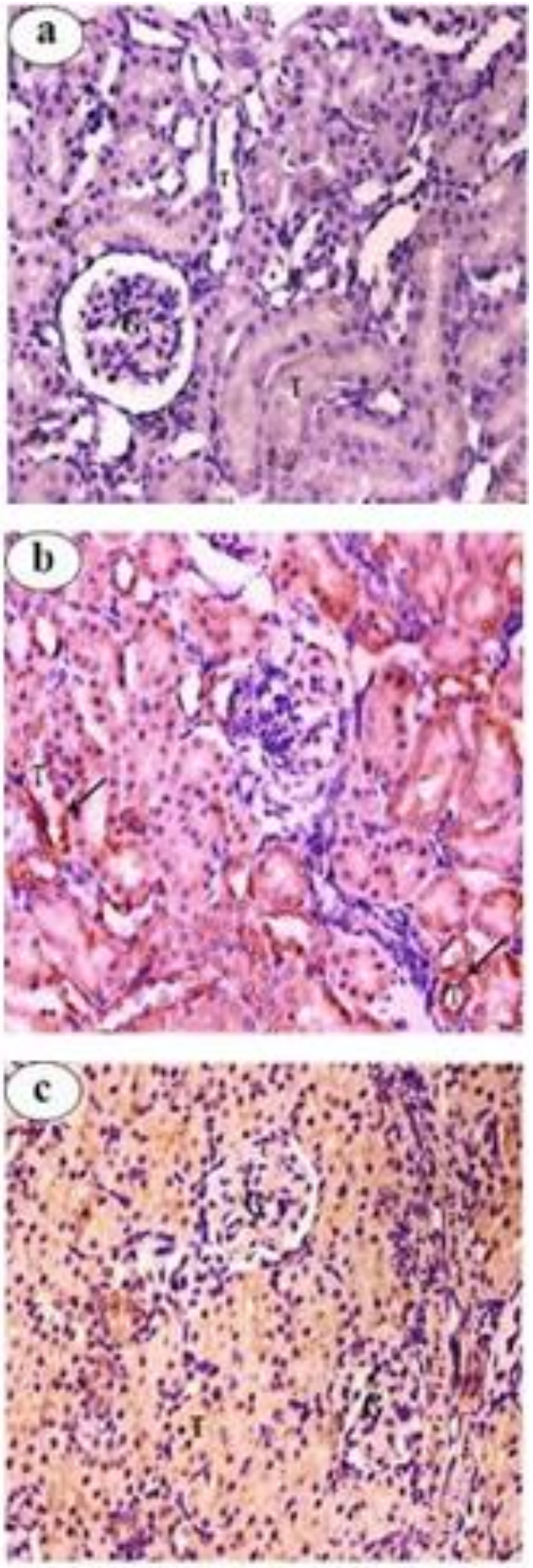


Fig.5. Photomicrograph showing the expression of Pro-apoptotic Bax in the kidney. (**a**): Control rat shows negative immunostaining of Bax. (**b**): CCl4 administered rat shows strong immunostaining of Bax (arrow) in the renal tubules (T). (**c**): Saffron+CCl4 shows negative staining of Bax similar to control group. Scale bar =50 µm

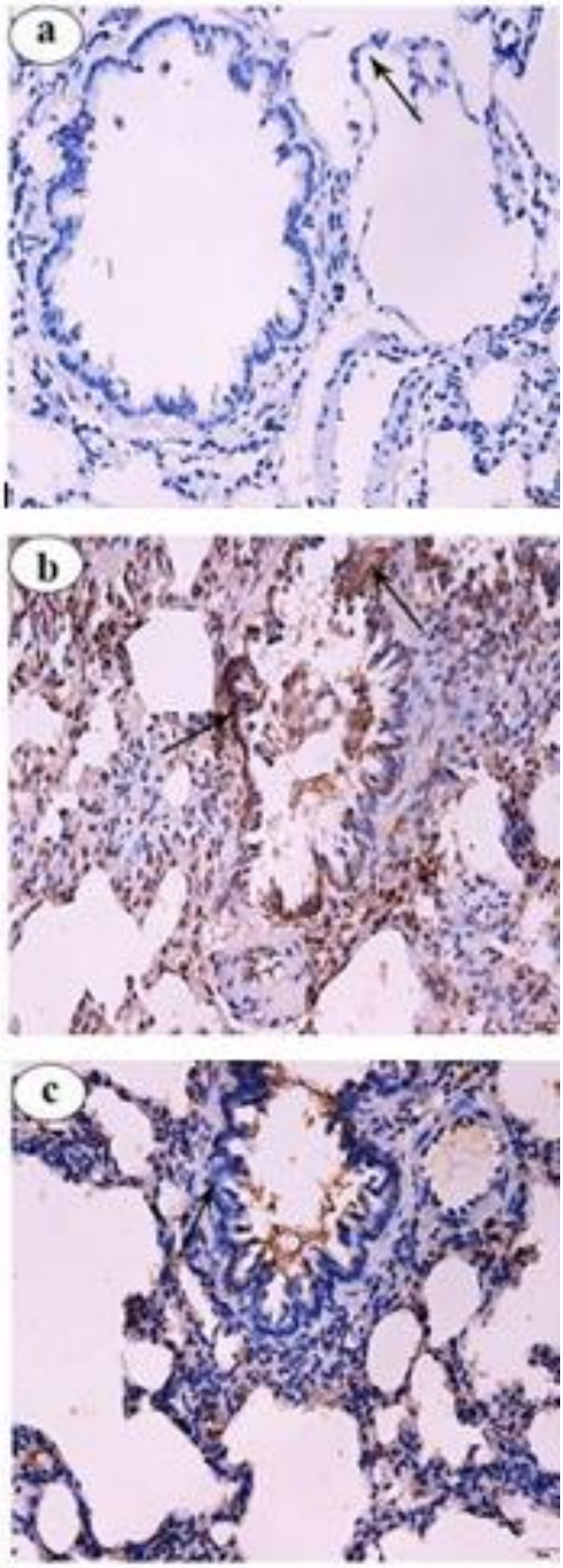


Fig.6. Photomicrograph showing the expression of Pro-apoptotic Bax in the lung. (**a**): Control rat shows weak immunostaining of Bax. (**b**): CCl4 given rat shows strong immunostaining of Bax (arrow). (**c**): Saffron+CCl4 rat displays moderate immunostaining of Bax (arrow). Scale bar =50 µm.

DISCUSSION

It is well established that CCl_4 is a lipid-soluble potent agent that when bound to lipid and protein produces a degeneration in many tissues (Cabr*e et al., 2000*). It has been hypothesized that one of the principal causes of CCl_4-induced tissue injury is lipid peroxidation by its free radical derivatives. This hypothesis is in the same line with the current study which confirms that CCl_4 increased MDA level, inhibited the activities of SOD and CAT and decreased the concentration of GSH in the liver, kidney and lung tissues. It has been observed that CCl_4 administration significantly increased the release of hepatic enzymes, destruction of cytochrome P-450, hepatocellular necrosis, and lipid peroxidation products such as malondialdehyde and 4-hydroxynonenol (Melin *et al.*, 2000). The data obtained by Escobar *et al.* (1996) indicate that the loss of enzymatic activity can cause by enhanced free radical concentration resulting from oxidative stress conditions. The oxidative stress caused by CCl_4 intoxication would lead to impairment of antioxidant enzymes; for example, SOD and CAT activities, or reactive intermediates formed throughout bioactivation of CCl_4 may bind to those compounds that are responsible for their inactivation *(*Ahmad *et al.*, 1987). Also, the liver of mice treated with CCl_4 exhibited the distinct characteristics of acute liver disease such as increased level of GOT, GPT, lipid peroxidation, apoptosis and decreasing of antioxidant enzymes in the liver (Kang *et al.*, 2013).

The present results were consistent with the several studies that reported that administration of CCl_4 induced a significant increase in oxidative stress and LPO of renal tissue (Adewole *et al.*, 2007; Khan *et al.*, 2009; Ogeturk *et al.*, 2005*)*. The declined activity of SOD, CAT and GSH in renal tissue of rats treated with CCl_4 may be due to an accumulation of free radicals, which would further stimulate LPO and inactivation of the antioxidative enzymes.

The main administration means of CCl_4 is achieved via intratracheal inhalation. Although the major target organ of CCl_4 toxicity is the liver, intraperitoneal injection of CCl_4 caused diffuse alveolar damage in rat lungs. In this study, an increase of LPO, inhibition in the activities of both SOD and CAT and a decrease in the concentration of GSH in the lungs after exposure to CCl_4 were recorded. Our results were supported by those of Khan *et al.* (2009) who postulated that CCl_3 radical and Cl respond with polyunsaturated fats of lung membranes and stimulate LPO and DNA fragmentation. These radicals deplete activities of antioxidant enzymes such as CAT, SOD and GPx *(*Khan *et al.*, 2010).

At the level of tissue, carbon tetrachloride was commonly marked by its toxicity leading to the liver lesion and liver fibrosis (Dong *et al.*, 2016; *Huang et al.*, 1998). Oxidative stress resulting from increased free radical production after CCl_4 intoxication may play an important role in the degenerative processes in the hepatic tissue. In the present study, we found marked changes in the liver as dilation and congestion of the hepatic vascularity, vacuolated hepatocytes with pyknotic nuclei, inflammation, and necrosis. The present results were in harmony with the opinion of Naji *et al.* (2017) who confirmed that the changes noted in hepatic cells treated with CCl_4 are due to a cellular injury occurring by alteration in membrane permeability resulting from free radicals which in turn resulting from its toxicity. Also, Dong *et al.* (2018) found that microscopic examination of the CCl_4-given rat sections exhibited fatty changes along with the increase of inflammatory collections, the loss of normal hepatocytes, obvious collagen deposition and fiber segmentation formation. Moreover, Sahreen *et al.* (2014) reported that CCl_4 induction in rats caused DNA fragmentation and histopathological abnormalities. Also, Khan *et al.* (2012) reported that free radicals

result in endothelial cell damage, increased vascular permeability, progressive degenerative action of intracellular enzymes, metabolic disturbances and protein synthesis inhibition for the growth and maturation of the liver and ultimately hepatocyte necrosis. Moreover, the outcomes of products of peroxidation (MDA) and changes of structures of the endoplasmic reticulum and other membranous organelles leading to liver damage *(*Azri *et al.,* 1992).

Also, CCl_4 administration mediated lipid peroxidation of kidney lipid structures, leading to subcellular damages as detected by histopathological demonstration. In the present study, the kidneys of CCl_4-administered rats have shown deleterious morphological findings such as glomerular atrophy, dilatation of Bowman's space and necrotic changes of renal tubular cells with pyknotic nuclei. Similar histopathological alterations were recorded by Ogeturk *et al.* (2005) and Khan *et al.* (2009) who observed tubular epithelial cells alterations including vacuolization, atrophy and finally detachment of epithelial cells, indicated tubular necrosis in kidneys of rats treated with CCl_4.

As well, the morphological investigations of the current study revealed different changes in the lungs induced by CCl_4 administration. These changes were loss of normal alveolar architecture, intense inflammatory infiltrates, some debris, intra-alveolar hemorrhage, prominent disorganized thickening of the alveolar septa and collapse of the alveolar space.

These observations received strong support from the findings of Sharma and Chauhan (2017) who elucidated that the CCl_4 treated mice showed a loss of normal alveolar architecture, severe destruction of alveolar ducts which resulted in cytoplasmic vacuolization, parenchymal congestion accompanied by intense inflammatory infiltrates, reduced air spaces with thickened alveolar wall were observed. Also, Mizuguchi *et al.* (2006) found that CCl_4-treated lung showed marked interstitial infiltration by inflammatory cells, and extensive thickening of interalveolar septa.

In modern medicine, plants occupy a significant berth as raw materials for some important drug preparations *(*Khare, *2008).* Rajesh and Latha (2004) showed that various herbal extracts could protect organs against CCl_4-induced oxidative stress. In the present study, saffron extract treatment significantly decreased LPO level, regained the decreased GSH and stimulated the activities of both SOD and CAT toward normal values in all selected organs. These results indicate that saffron confirming its antioxidant role in CCl_4 toxicity. This finding was in agreement with Sakr *et al.* (2017) who reported that treatment with saffron extract modulates the activities of antioxidant enzymes by increasing the levels of SOD and CAT and reducing the level of MDA. Also, Premkumar *et al.* (2003) observed an increase in the levels of GSH concentration as well as the activities of GST, GPx, CAT and SOD.

Our result may be attributed to the finding of Wang *et al. (2008)* who mentioned that phenolic and flavonoid compounds, which are widely found as secondary metabolites in plants, are important due to their ability to serve as antioxidants. Many phenolic compounds have been reported to possess potent antioxidant activity, anticarcinogenic, antibacterial, antiviral and anti-inflammatory activities in a greater or lesser extent *(*Tapiero *et al.,* 2002). Also, Bravo (1998) reported that the most important function of flavonoids is their antioxidant activity, as they have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals.

Again, we found an improvement of the above parameters in lung tissue when the saffron extract was given to the CCl_4-administered rats. Crocetin derivatives from saffron are mainly responsible for these pharmacological properties. The use of

crocetin in lung cancer was found to decrease LPO, glutathione metabolizing enzymes and also revert the histopathological changes relevant to tumor incidence proving it to be a potential anti-tumor agent *(*Nair *et al.*, 1991; Negbi, 2003).

Interestingly, our results showed that at the level of tissue, saffron extract treatment to CCl_4- administered rats retrieved their organs architecture toward normal appearance. This finding was in agreement with Iranshahi *et al.* (2011) who revealed that the aqueous and ethanolic extracts of saffron displayed hepatoprotective effects against liver injuries stimulated by CCl_4 in mice. Similarly, pretreatment with saffron extract only reduced DNA damage in liver, lung, kidney, and spleen *(*Hosseinzadeh and Sadeghnia, 2007). Also, Asadi *et al.* (2014) and Sakr *et al.* (2014) reported that saffron extract ameliorates the testicular damage, sperm count and abnormalities induced by sodium valproate and cadmium in albino rats.

Moreover, our results showed that saffron extract treatment to CCl_4-given rats leads to approximately normal kidney structure and appear like those of controls. This finding was in the same line with Sakr *et al.* (2017*)* who reported that there was a marked improvement of the histological structure of the kidney of mice bearing solid tumors treated with saffron. Also, Bahmani *et al.* (2014) reported that the histopathological examination of the kidney and liver tissue sections of newborn mice which their mothers received different doses of saffron showed no pathological changes. In addition, Ajami *et al.* (2010) documented that saffron extract can reduce gentamicin-induced nephrotoxicity and preserve renal function and histology.

Also, our results revealed that saffron treatment preserved lung parenchyma without inflammatory cells infiltration. These observations are confirmed by Saxena *et al.* (1984) who reported that the anti-inflammatory efficiency of saffron is certainly related to its strong antioxidant and radical scavenging virtues which seem to chiefly attribute to crocetin and crocins derivatives. Also, Crocus sativa L. compounds derived from stigmas, and petals of saffron were reported to exhibit antioxidant, antigenotoxic and important anti-inflammatory activities. In addition, Ma *et al.* (1998) confirmed that the anti-inflammatory responses might also result from the saffron content of flavonoids, tannins, anthocyanins, alkaloids and saponins.

Finally, we evaluated the effect of CCl_4 on cellular apoptosis. In our study, the expression levels of Bax were investigated by the immunohistochemical stain. These results showed that the administration of CCl_4 increased Bax expression in all studied organs and this change in expression could be reversed by saffron treatment. These findings reinforced by Wei *et al.* (2018) who confirmed that CCl_4 increased Bax and down-regulated Bcl-2 expression. Several properties of crocin derivative such as antiapoptotic properties counteracting membrane lipid peroxidation and caspase 3-activation, while increasing GSH levels enables to prevent or suppress apoptotic cell death *(*Ochiai *et al.*, 2004).

Conclusion

Thanks, Saffron extract that is able present protective activity against CCl_4-intoxicity. Therefore, it is concluded that saffron extract can be used to treat CCl_4-induced hepatic, renal and lung toxicity. The protective effect of saffron extract demonstrated in the present study may enhance its therapeutic benefits as a strong antioxidant.

Acknowledgments

The authors acknowledge Aswan University for providing the infrastructure to conduct the research.

REFERENCES

Adewole, S.; Salako, A.; Doherty, O. and Naicker, T. (2007): Effect of melatonin on carbon tetrachloride-induced kidney injury in Wistar rats. African J. Biomed. Res., 10.

Aebi, H.E. (1983): Methods of enzymatic analysis (Bergmeyer H. U., ed), 2nd Ed., Vol. 2, pp. 673-78, Verlag Chemie, Weinheim.

Ahmad, F.F.; Cowan, D.L.; Sun, A.Y. (1987): Detection of free radical formation in various tissues after acute carbon tetrachloride administration in gerbil. Life Sci., 41: 2469–2475.

Ajami, M.; Eghtesadi, S.; Pazoki-Toroudi, H.; Habibey, R. and Ebrahimi, S.A. (2010): Effect of crocus sativus on gentamicin induced nephrotoxicity. Biol. Res., 43: 83–90.

Asadi, M.H.; Zafari, F.; Sarveazad, A.; Abbasi, M.; Safa, M.; Koruji, M.; Yari, A. and Miran, R.A. (2014): Saffron improves epididymal sperm parameters in rats exposed to cadmium. Nephro-urology monthly, 6(1).

Assayed, M.E.; Khalaf, A.A. and Salem, H.A. (2010): Protective effects of garlic extract and vitamin C against in vivo cypermethrin-induced teratogenic effects in rat offspring. Food Chem. Toxicol., 48: 3153–3158.

Azri, S.; Mata, H.P.; Reid, L.L.; Gandolfi, A.J. and Brendel, K. (1992): Further examination of the selective toxicity of CCl4 in rat liver slices. Toxicol. Appl. Pharmacol., 112: 81–86.

Bahmani, M.; Rafieian, M.; Baradaran, A.; Rafieian, S. and Rafieian-kopaei, M. (2014): Nephrotoxicity and hepatotoxicity evaluation of Crocus sativus stigmas in neonates of nursing mice. J. Nephropathol., 3: 81.

Bravo, L. (1998): Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev., 56: 317–333.

Cabre, M.; Camps, J.; Paternain, J.L.; Ferre, N. and Joven, J. (2000): Time-course of changes in hepatic lipid peroxidation and glutathione metabolism in rats with carbon tetrachloride-induced cirrhosis. Clin. Exp. Pharmacol. Physiol., 27: 694–699.

Chen, Y.; Zhang, H.; Tian, X.; Zhao, C.; Cai, L.; Liu, Y.; Jia, L.; Yin, H.-X. and Chen, C. (2008): Antioxidant potential of crocins and ethanol extracts of Gardenia jasminoides ELLIS and Crocus sativus L.: A relationship investigation between antioxidant activity and crocin contents. Food Chem., 109: 484–492.

Dong, S.; Cai, F.; Chen, Q.; Song, Y.; Sun, Y.; Wei, B.; Li, X.; Hu, Y.; Liu, P. and Su, S. (2018): Chinese herbal formula Fuzheng Huayu alleviates CCl 4-induced liver fibrosis in rats: a transcriptomic and proteomic analysis. Acta Pharmacol. Sin.. 39: 930.

Dong, S.; Chen, Q.-L.; Song, Y.-N.; Sun, Y.; Wei, B.; Li, X.-Y.; Hu, Y.-Y.; Liu, P. and Su, S.-B.(2016): Mechanisms of CCl4-induced liver fibrosis with combined transcriptomic and proteomic analysis. J. Toxicol. Sci., 41: 561–572.

Eidi, A.; Mortazavi, P.; Bazargan, M. and Zaringhalam, J.(2012): Hepatoprotective activity of cinnamon ethanolic extract against CCI4-induced liver injury in rats. EXCLI J., 11: 495.

El-Shorbagy, H.M. (2017): Molecular and Anti-oxidant Effects of Wheat Germ Oil on CCl4-Induced Renal Injury in Mice. J. Appl. Pharm. Sci., 7: 94–102.

Escobar, J.A.; Rubio, M.A. and Lissi, E.A. (1996): SOD and catalase inactivation by singlet oxygen and peroxyl radicals. Free Radic. Biol. Med., 20: 285–290.

Gabe, M. (1976): Histological Techniques. New York Heidelberg Berlin/Paris New York Barcelone Milan.

Ganie, S.A.; Haq, E.; Hamid, A.; Qurishi, Y.; Mahmood, Z.; Zargar, B.A.; Masood, A. and Zargar, M.A. (2011): Carbon tetrachloride induced kidney and lung tissue damages and antioxidant activities of the aqueous rhizome extract of Podophyllum hexandrum. BMC Complement. Altern. Med., 11: 17.

Hosseinzadeh, H. and Sadeghnia, H.R. (2007): Effect of safranal, a constituent of Crocus sativus (Saffron), on methyl methanesulfonate (MMS)–induced DNA damage in mouse organs: an alkaline single-cell gel electrophoresis (Comet) assay. DNA Cell Biol., 26: 841–846.

Huang, Z.-G.; Zhai, W.-R.; Zhang, Y.-E. and Zhang, X.-R. (1998): Study of heteroserum-induced rat liver fibrosis model and its mechanism. World J. Gastroenterol., 4: 206.

Iranshahi, M.; Khoshangosht, M.; Mohammadkhani, Z. and Karimi, G. (2011): Protective effects of aqueous and ethanolic extracts of saffron stigma and petal on liver toxicity induced by carbon tetrachloride in mice. Pharmacologyonline, 1: 203–212.

Kang, M.-C.; Kang, S.-M.; Ahn, G.; Kim, K.-N.; Kang, N.; Samarakoon, K.W.; Oh, M.-C.; Lee, J.-S. and Jeon, Y.-J. (2013): Protective effect of a marine polyphenol, dieckol against carbon tetrachloride-induced acute liver damage in mouse. Environ. Toxicol. Pharmacol., 35: 517–523.

Khan, M.R.; Marium, A.; Shabbir, M.; Saeed, N. and Bokhari, J. (2012): Antioxidant and hepatoprotective effects of Oxalis corniculata against carbon tetrachloride (CCl4) induced injuries in rat. African J. Pharm. Pharmacol., 6: 2255–2267.

Khan, M.R.; Rizvi, W.; Khan, G.N.; Khan, R.A. and Shaheen, S. (2009): Carbon tetrachloride-induced nephrotoxicity in rats: Protective role of Digera muricata. J. Ethnopharmacol., 122: 91–99.

Khan, R.A.; Khan, M.R.; Sahreen, S. and Bokhari, J. (2010): Prevention of CCl4-induced nephrotoxicity with Sonchus asper in rat. Food Chem. Toxicol., 48: 2469–2476.

Khare, C.P. (2008): Indian medicinal plants: an illustrated dictionary. Springer Science & Business Media.

Lin, C. and Huang, P. (2000): Antioxidant and hepatoprotective effects of Acathopanax senticosus. Phyther. Res. An Int. J. Devoted to Pharmacol. Toxicol. Eval. Nat. Prod. Deriv., 14: 489–494.

Ma, S.; Zhou, S. and Shu, B. (1998): Pharmacological studies on Crocus glycosides I. Effects on anti-inflammatory and immune function. Chinese Tradit. Herb. Drugs, 29: 536–538.

Maral, J.; Puget, K. and Michelson, A.M. (1977): Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals. Biochem. Biophys. Res. Commun., 77: 1525–1535.

Melin, A.; Perromat, A. and Déléris, G. (2000): Pharmacologic application of Fourier transform IR spectroscopy: in vivo toxicity of carbon tetrachloride on rat liver. Biopolym. Orig. Res. Biomol., 57: 160–168.

Mizuguchi, S.; Takemura, S.; Minamiyama, Y.; Kodai, S.; Tsukioka, T.; Inoue, K.; Okada, S. and Suehiro, S. (2006): S-allyl cysteine attenuated CCl4-induced oxidative stress and pulmonary fibrosis in rats. Biofactors., 26: 81–92.

Moghaddasi, M.S. (2010): Saffron chemicals and medicine usage. J. Med. plants Res., 4: 427–430.

Mohajeri, D.; Doustar, Y.; Rezaei, A. and Mesgari-Abbasi, M. (2011):

Hepatoprotective effect of ethanolic extract of Crocus sativus L.(Saffron) stigma in comparison with silymarin against rifampin induced hepatotoxicity in rats. Zahedan J. Res. Med. Sci., 12: 53–59.

Nair, S.C.; Pannikar, B. and Panikkar, K.R. (1991): Antitumour activity of saffron (Crocus sativus). Cancer Lett., 57: 109–114.

Naji, K.M.; Al-Shaibani, E.S.; Alhadi, F.A. and D'souza, M.R. (2017): Hepatoprotective and antioxidant effects of single clove garlic against CCl 4-induced hepatic damage in rabbits. BMC Complement. Altern. Med., 17: 411.

Negbi, M.(2003): Saffron: Crocus Sativus L. CRC Press.

Ochiai, T., Ohno, S.; Soeda, S.; Tanaka, H.; Shoyama, Y. and Shimeno, H. (2004): Crocin prevents the death of rat pheochromyctoma (PC-12) cells by its antioxidant effects stronger than those of α-tocopherol. Neurosci. Lett., 362: 61–64.

Ogeturk, M.; Kus, I.; Colakoglu, N.; Zararsiz, I.; Ilhan, N. and Sarsilmaz, M. (2005): Caffeic acid phenethyl ester protects kidneys against carbon tetrachloride toxicity in rats. J. Ethnopharmacol., 97: 273–280.

Ohkawa, H.; Ohishi, N. and Yagi, K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351–358.

Paoletti, F. and Mocali, A. (1990): [18] Determination of superoxide dismutase activity by purely chemical system based on NAD (P) H oOxidation, in: Methods in Enzymology, 186, 209-220.

Premkumar, K.; Abraham, S.K.; Santhiya, S.T. and Ramesh, A. (2003): Protective effects of saffron (Crocus sativus Linn.) on genotoxins-induced oxidative stress in Swiss albino mice. Phyther. Res., 17: 614–617.

Premkumar, K.; Abraham, S.K.; Santhiya, S.T. and Ramesh, A. (2003): Inhibitory effects of aqueous crude extract of Saffron (Crocus sativus L.) on chemical-induced genotoxicity in mice. Asia Pac. J. Clin. Nutr. 12.

Rajesh, M.G. and Latha, M.S. (2004): Protective activity of Glycyrrhiza glabra Linn. on carbon tetrachloride-induced peroxidative damage. indian J. Pharmacol., 36: 284.

Recknagel, R.O.; Glende Jr, E.A.; Dolak, J.A. and Waller, R.L. (1989): Mechanisms of carbon tetrachloride toxicity. Pharmacol. Ther., 43: 139–154.

Rincón, A.R.; Covarrubias, A.; Pedraza-Chaverrí, J.; Poo, J.L.; Armendáriz-Borunda, J. and Panduro, A. (1999): Differential effect of CCl4 on renal function in cirrhotic and non-cirrhotic rats. Exp. Toxicol. Pathol., 51: 199–205.

Sakr, S.A.; Ferial A. and El-mesady, A.M.H. (2017): Aluminum Induced Reproductive Dysfunction in Male Rats : The Ameliorative Effect of Saffron Extract. Ijppr. Human, 10 (3): 180-195. .

Sahreen, S.; Khan, M.R.; Khan, R.A. and Alkreathy, H.M. (2014): Cardioprotective role of leaves extracts of Carissa opaca against CCl 4 induced toxicity in rats. BMC Res. Notes, 7: 224.

Sakr, S.A.; Zowail, M.E. and Marzouk, A.M. (2014): Effect of saffron (Crocus sativus L.) on sodium valporate induced cytogenetic and testicular alterations in albino rats. Anat. Cell Biol., 47: 171–179.

Saxena, R.S.; Gupta, B.; Saxena, K.K.; Singh, R.C. and Prasad, D.M. (1984): Study of anti-inflammatory activity in the leaves of Nyctanthes arbor tristis Linn.—an Indian medicinal plant. J. Ethnopharmacol., 11: 319–330.

Sharma, S. and Chauhan, S. (2017): Protective Effect of Vitamin C on Carbon Tetrachloride Administered Toxicity in Lungs of Mice. Int. J. Sci. Res., 6: 1050–1056.

Tapiero, H.; Tew, K.D.; Ba, G.N. and Mathe, G. (2002): Polyphenols: do they play a

role in the prevention of human pathologies? Biomed. Pharmacother., 56: 200–207.
Tarantilis, P.A.; Tsoupras, G. and Polissiou, M. (1995): Determination of saffron (Crocus sativus L.) components in crude plant extract using high-performance liquid chromatography-UV-visible photodiode-array detection-mass spectrometry. J. Chromatogr., A 699: 107–118.
Wang, Y.-C.; Chuang, Y.-C. and Hsu, H.-W. (2008): The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. Food Chem., 106: 277–284.
Wei, L.; Chen, Q.; Guo, A.; Fan, J.; Wang, R. and Zhang, H. (2018): Asiatic acid attenuates CCl 4-induced liver fibrosis in rats by regulating the PI3K/AKT/mTOR and Bcl-2/Bax signaling pathways. Int. Immunopharmacol., 60: 1–8.
Winterhalter, P. and Straubinger, M. (2000): Saffron—renewed interest in an ancient spice. Food Rev. Int., 16: 39–59.

ARABIC SUMMARY

التأثير الوقائي للزعفران ضد سمية رابع كلوريد الكربون في بعض أعضاء الفئران البيضاء

جمال حسن السكرى[1] – اعتماد أحمد عوض الله[2*]
[1] قسم علم الحيوان- كلية العلوم – جامعة أسيوط
[2*]قسم علم الحيوان – كلية العلوم – جامعة أسوان

لقد تم استغلال الزعفران (*L sativus Crocus*) على نطاق واسع في الطب الشعبي لعلاج عدد من الأمراض. و تسبب رابع كلوريد الكربون المحدث للسمية فى إنتشار الجذور الحرة ، والتي تشارك في أكسدة الدهون. تم التخطيط لهذه الدراسة لتقييم فعالية الزعفران في علاج الإصابات الناجمة عن رابع كلوريد الكربون في بعض أجهزة الفئران. في الدراسة الحالية ، تم استخدام ثلاثين فأرا من ذكور الفئران البيضاء و قد تم تقسيمها إلى ثلاث مجموعات ؛ المجموعة الضابطة ، و مجموعة رابع كلوريد الكربون (5 مل / كغ وزن الجسم) ، و مجموعة رابع كلوريد الكربون (5 مل / كغ وزن الجسم.) + الزعفران (20 ملغ / كغ وزن الجسم). تم أخذ عينات من الكبد والكلى والرئتين لإجراء دراسات كيميائية حيوية ونسيجية . أوضحت النتائج أن النشاطات الخاصة بإزالة سوبر أكسيد ديسموتاز (SOD) والكاتالاز (CAT) ، بالإضافة إلى تركيز الجلوتاثيون (GSH) قد انخفضت بينما تم زيادة مستوى مالون دا ألدهيد (MDA) بعد المعالجة برابع كلوريد الكربون . أيضا ، تم الكشف عن التغيرات المورفولوجية المختلفة والتعبير الملحوظ من البروتين Bax . المعالجة بمستخلص الزعفران خففت بشكل فعال التغيرات في العلامات البيوكيميائية والبنية المورفولوجية للكبد والكلى والرئة. تؤكد هذه الدراسة على استعادة الحياة الطبيعية وأجازت الدور التحسينى للزعفران ضد السمية التي يسببها رابع كلوريد الكربون.