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PROPHYLACTIC EFFECT OF VITAMIN E ON CARMOISINE FOOD DYE INDUCED KIDNEY DAMAGE IN MALE MICE: HISTOLOGICAL, PHYSIOLOGICAL AND IMMUNOLOGICAL STUDIES

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ABSTRACT

The study evaluates the immunomodulatory effect of vitamin E against the side effect of synthetic food dye-induced oxidative damage and nephrotoxicity in male mice. Mice were divided into 4 groups *viz.*, group 1 as control mice, group 2 received vitamin E (100 mg kg^{-1} bw), group 3 administered with carmoisine (250 mg kg^{-1} bw), and group 4 received carmoisine and vitamin E for 28 days. Nephrotoxicity induce in mice was assessed by observing the changes in levels of renal markers, and antioxidant status. Administration of vitamin E significantly restored the levels of renal functions (urea and creatinine), antioxidants status and immunological parameters (TNF-a, Cox-2, Bcl-2, CD95 and caspase 3). The prophylactic role of vitamin E against food dye-induced toxicity may be attributed to their antioxidant effects and free radical scavenging properties.

Keywords: Antioxidant, carmoisine, mice, nephrotoxicity, vitamin E.

INTRODUCTION

In recent years the consumption of processed food has increased manifold, simultaneously leading to the increase in the production and consumption of food dyes (Amin and Al-Sherhi, 2018). Synthetic colours are often added to the food to make them visually acceptable. Processed food like ice-cream, soft drinks, confections, candies, snacks, jelly, jam and cereals aimed at children are typically brightly coloured. The consumption of processed food is more in children than adults (Ibrahim and El-Sherbeny, 2016). E numbers are used as codes for food colour additives in the European Union (Ibrahim and El-Sherbeny, 2016). Not only the natural additives originating from sources like vegetables, insects, etc., but also the artificial food colours derived from petroleum or mixtures made from natural foodstuffs take E numbers which range from 100 to 199 (Ibrahim and El-Sherbeny, 2016). Some E number food dyes are banned in European Union and USA, yet these are illegally used in food products (Ibrahim and El-Sherbeny, 2016).

Synthetic food colours are artificially produced by use of chemicals or by modification of their precursor compounds (König, 2015). They are classified as azo-dyes (carmoisine, azorubine, tartrazine, sunset yellow FCF, amaranth, ponceau 4R, allura red AC, brilliant black BN, brown HT, etc.) and triarylmethane dyes (König, 2015). Synthetic food dyes are widely used due to their low cost, colouring properties, uniformity and stability (Ashida *et al.*, 2000). Food additive are consumed by children many times a day through gums, chips, chocolates, soft drinks, etc. (Ibrahim and El-

Sherbeny, 2016). However, many of these become toxic after prolonged use and induce many health problems such as indigestion, anemia, allergic reactions, pathological lesions in brain, kidney, spleen and liver, tumours, paralysis, organ dysfunction, mental retardation, abnormalities in offspring, etc. (Moutinho *et al.*, 2007; Ibrahim and El-Sherbeny, 2016).

Carmoisine (E122), also known as azorubine, is a synthetic red to maroon azo dye ($C_{20}H_{12}N_2Na_2O_7S_2$) which is added to food products to make them appealing (Amin *et al.*, 2010). Carmoisine dyes have widely been used to make the foods tastier and more attractive with glowing bright colours (Amin and Al-Sherhi, 2018). The dye mainly affects youth and children who usually are attracted towards colour and do not regulate their feeding habits as compared to the adult. However, carmoisine-use reportedly has many side effects and adversely affects kidney, liver, brain and nervous system (Moutinho *et al.*, 2007). The excessive use of dye (> 4.0 mg kg^{-1} body weight) induces many harmful effects, including carcinogenic ones (Aboel-Zahab *et al.*, 1997; Helal, 2001; Mehidi *et al.*, 2013). Amin *et al.* (2010) reported that carmoisine when oxidized produces reactive oxygen species (ROS) and free radicals which induce organ damage, lesions in liver, kidney and chromosomal depletion in young male rats (Shafi Khan *et al.*, 2020). Elekima (2016) reported that carmoisine administration enhances triglycerides, total cholesterol and low-density lipoprotein cholesterol contents and decreases high density lipoprotein cholesterol content; this alteration in lipid profile is usually associated with developing cardiovascular disease.

Vitamins are known scavengers of free radicals and improve the immunological status of organisms. Vitamin E is a fat-soluble vitamin and is a powerful antioxidant. Vitamin E reduces oxidative stress and protects the body against reactive oxygen species (Herrera and Barbas, 2001; Traber and Atkinson, 2007). Eight fat-soluble compounds are grouped under "Vitamin E", including tocopherols and tocotrienols. α-tocopherol is an important lipid-soluble antioxidant and the most biologically active form of vitamin E. Vitamin E is a powerful antioxidant that reduces ROS. ROS can cause a chronic inflammatory response (Herrera and Barbas, 2001). Its mechanism of function through glutathione peroxidase pathway protects cell membranes from oxidation by reacting with lipid radicals produced during lipid peroxidation chain reaction (Wefers and Sies, 1988; Traber and Atkinson, 2007). The perusal of literature has revealed that no information is presently available on the immunomodulatory effect of vitamin E against carmoisine administration; so, the present study was aimed to evaluate the prophylactic effect of vitamin E on renal tissue structure, function and antioxidant status as a result of food dyes administration in mice. Also, the immunological state of kidney was assessed by evaluating the COX-2, Bcl-2, caspase3 and tumor necrosis factor (TNF-α) expression in renal tissue and the role of vitamin E in inhibiting free radical activity and improving kidney function was explored.

MATERIALS AND METHODS

Experimental animals

Male mice strain BALB/c were procured from Medical Experimental Research Centre (MERC), Mansoura University (Egypt) after the approval from Ethical Committee of the Department in accordance with the ethical guidelines for proper care and use of laboratory animals (Canadian Council on Animal Care, 1993). Twenty-eight male mice, weighing 25 ± 5 g (8 weeks old) were used in the present study. Prior to experimentation, the animals were acclimatized for one week in an acclimatization room having conventional environmental conditions controlled for temperature and humidity and a photoperiod with timer, respecting the daily cycle of rodents i.e., 12 h day^{-1} and 12 h $night^{-1}$. Drinking water was provided in graduated polyethylene bottles placed in metal grids in the upper part of cages. Food (standard diet) was provided to animals throughout the study period. Mice were divided into 4 groups of 7 mice each. Group 1 mice received distilled water and represented control group. Group 2 received vitamin E suspended in normal saline solution (100 mg kg^{-1} bw)

subcutaneously while group 3 received carmoisine dissolved in distilled water (250 mg kg^{-1} bw) orally, and group 4 received carmoisine (orally) and vitamin E (subcutaneously) for 28 days. At the end of study, all animals were anaesthetized with diethyl ether to minimize suffering and scarified.

Biochemical analysis

Blood samples were collected from the heart and left to clot for separating the serum after centrifugation. The separated serum was frozen immediately (-10°C) until used in analysis. Serum level of kidney function: creatinine, urea and electrolyte Na and K were determined (Tietz, 1994).

Antioxidant status

The antioxidant status was determined in renal tissue by enzyme-linked immunosorbent assay (ELISA) technique via measurement of reduced glutathione (GSH), glutathione S-transferase (GST), malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT). The assay employs the double-sandwich ELISA technique. The enzyme reaction yields a blue product that turns yellow when the stop solution is added. The intensity of colour measured is in proportion to the amount of GSH, GST, MDA, SOD and CAT bound in the initial step. The sample values were read from the standard curve. The optical density was determined with a microplate reader at a wavelength of 450 nm. All the analyses were conducted as per the manufacture's guidelines according to the datasheet from Cusabio Biotech Co., Ltd.

Histopathological investigations

Renal tissue samples were prepared according to routine histological technique (Bancroft and Gamble, 2002). Kidney samples were rinsed with normal saline, and cut into many small specimens. The specimens were fixed in 10% formalin, dehydrated with ascending ethanol concentrations (70 to 100%), cleared in xylene and finally embedded in wax paraffin. Histological blocks of 4 µm thickness were sectioned using a microtome. For evaluating the pathological damage, the sections were stained with hematoxylin and eosin (H-E) and observed under a light microscope (Basic Biological Microscope, model: BA210 with 5 mega pixel digital camera) using X400 magnification power.

Immunohistochemical studies

The expression of tumour necrosis factor-alpha (2-TU037-07, Quartett, Berlin, Germany) and cyclooxygenase-2 (COX-2) (RB-9072-P0, Thermo Scientific, UK) in kidney sections was determined immunohistochemically in formalin-fixed, paraffin-embedded tissue according to the manufacture's guidelines. Blocks were cut into 4 µm thick sections, mounted on glass slides, deparaffinized with xylene, rehydrated with graded ethanol and washed with water. These sections were heated in a microwave for 10-15 min in 10 mM citrate buffer with pH 6.0 for antigen retrieval. They were then treated with 3% H_2O_2 for 10 min at room temperature for blocking of endogenous peroxidases. Slides were probed with either monoclonal anti-tumour necrosis factor-alpha (1:100, rabbit Ab), or polyclonal anti-COX-2 (1:100, rabbit Ab). Sections were washed, incubated with biotin-labeled anti-mouse Ig G and stained with a streptavidin-peroxidase as per the manufacturer's guidelines. In negative control slides the primary antibody was omitted.

Determination of anti-apoptotic and apoptotic markers

For anti-apoptotic and apoptotic marker determination, the expression of Bcl-2, CD95 and caspase 3, were carried out by using anti-Bcl-2 antibody, FITC anti-CD95 and FITC anti-active caspase 3, respectively, according to the manufacture's guidelines. Antibodies were used at the indicated dilutions from the following sources. Data were analyzed on a flow cytometer (Coulter EPICS XL) using 488 nm argon laser beam source and a 515 nm band pass filter for FITC-fluorescence.

Statistical analysis

The average means of different groups were analyzed as mean ± standard deviation (SD) and compared using one-way analysis of variance on SPSS (Statistical Processor System Support for Windows, version 16.0 (SPSS, Chicago, IL), for comparing all the experimental groups. Values of $P < 0.05$ were considered to be significant by using post hoc-least significant difference analysis (LSD).

RESULTS AND DISCUSSION

Kidney function analysis

Carmoisine administration for 28 days induced significant ($p<0.05$) elevations in the serum levels of creatinine and urea as evidences of renal damage (Table 1). On the other hand, present study revealed significant increase in Na^+ and non-significant increase in K^+ serum levels. Kidney injuries were attributed to the fact that the glomeruli are the primary site of action of several chemicals and it may be injured by any toxic, metabolic and immunologic mechanisms. The toxic irritant substances brought to the kidney by circulatory blood cause degenerative changes in kidney tissues.

Table 1: Biochemical analysis of kidney function and electrolytes in control and experimental groups of mice

Parameters	Control (C)	Vitamin E	Carmoisine	Carmoisine + vitamin E
Creatinine (mg dL^{-1})	1.26 ± 0.31	1.06 ± 0.19	2.62 ± 0.32^{abd}	1.26 ± 0.19^{c}
Urea (mg dL^{-1})	37.21 ± 2.54	31.39 ± 3.12	82.11 ± 3.41^{abd}	45.09 ± 2.73^{c}
Na+ (mEq L^{-1})	141.37 ± 8.80	144.29 ± 9.45	162.06 ± 12.49^{a}	150.26 ± 5.00
K+ (mmol L^{-1})	15.10 ± 2.56	13.01 ± 4.47	15.89 ± 3.64	14.93 ± 2.36

The values are mean ± SD. Superscript letters denote significance at $p<0.05$; *a*: significant to C, *b*: significance to vitamin E, *c*: significant to carmoisine, and *d*: significant to carmoisine + vitamin E.

The present study revealed changes in kidney function, antioxidant status and immunological parameters as a result of carmoisine administration which lead to remarkable histopathological effects in renal tissue. This is in agreement with many studies conducted by Amin *et al.* (2010) and El-Wahab and Moram (2013) who administered carmoisine to rats for 30 and 42 days, respectively, and observed significant increase in urea, creatinine, total protein and albumin in serum as compared to the control rats. These significant changes altered biochemical markers adversely in vital organ such as kidney. Ashour and Abdelaziz (2009) showed that the administration of fast green and tartrazine (synthetic organic azo dyes) caused augmentation in serum creatinine and urea (Himri *et al.*, 2011); which resulted in renal dyfunction. Cemek *et al.* (2014) showed that carmoisine administration resulted in the reduction of zinc and iron content in renal tissue that have a part in renal dysfunction also. In present study the elevations in kidney function parameters were significantly inhibited in carmoisine + vitamin E group; which showed decrease levels as compared to the carrmoisine group.

Antioxidant status

The antioxidant status in mice, determined by measuring reduced glutathione (GSH), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT), showed significant decrease in carmoisine-administered mice when compared to control and vitamin E-injected mice (Table 2). Amin *et al.* (2010) reported that oral administration of two carmoisine doses [one low and other high dose] for 30 days decreased GSH, SOD and CAT but increased MDA in tissue homogenate of rats thereby altered biochemical markers in vital organs like kidney.

Table 2: Antioxidant status in control and experimental groups of mice

Parameters	Control (C)	Vitamin E	Carmoisine	Carmoisine + vitamin E
GSH (μg mL^{-1})	6.12 ± 0.30	7.00 ± 0.52	4.54 ± 0.38^{ab}	5.87 ± 0.19^{bc}
GST (ng mL^{-1})	0.37 ± 0.02	0.42 ± 0.03	0.32 ± 0.02^{abd}	0.44 ± 0.02^{ac}
SOD (U mL^{-1})	30.42 ± 1.71	33.21 ± 2.73	21.78 ± 1.82^{abd}	27.77 ± 1.81^{bc}
CAT (ng mL^{-1})	48.14 ± 1.43	51.64 ± 2.65	43.34 ± 2.19^{ab}	48.21 ± 1.87^{bc}
MDA (ng mL^{-1})	42.31 ± 2.03	29.07 ± 2.45	81.35 ± 2.59^{abd}	66.21 ± 3.42^{abc}

The values are mean ± SD. Superscript letters denote significance at $p<0.05$; *a*: significant to C, *b*: significance to vitamin E, *c*: significant to carmoisine, and *d*: significant to carmoisine + vitamin E.

Fijer and Al-Mashhedy (2016) found that the administration of 250 mg carmoisine kg^{-1} bw significantly decreased SOD, GST, glutathione peroxidase (GPx) and nitric oxide levels, while MDA level showed significant increase. Similarly, Moeen *et al.* (2018) reported a decrease in total antioxidant capacity, glutathione and CAT activity, beside an elevation in oxidized glutathione in the serum of rat administrated with carmoisine. The decrease in GSH levels inflicted damage due to the increased cellular oxidative stress. Oxidative stress observed in present study may perhaps be due to carmoisine metabolism (Fijer and Al-Mashhedy 2016), where carmoisine may be reduced through the intestinal flora forming semiquinone radicals and aromatic amines or via azoreductase in liver (Amin *et al.*, 2010). The resulting compounds from carmoisine metabolism can be oxidized by P450 enzyme oxidase forming superoxide and hydroxyl radicals and hydrogen peroxide (H_2O_2) that can alter antioxidant defense enzymes such as SOD, GST and non-enzyme antioxidant such as GSH (Maekawa *et al.*, 1987) leading to the weakness of cellular defense against free radicals (Sivaramakrishnan, *et al.*, 2008; Demirkol *et al.*, 2012).

Antioxidant levels in carmoisine + vitamin E group delineated significant increase in GSH, GST, SOD and CAT levels as compared to carmoisine group. On the other hand, malondialdehyde (MDA) level increased in carmoisine group as compared to control and vitamin E groups. The amelioration of antioxidant status due to vitamin E injection can be related to vitamin antioxidant capacity in reducing free radical formation by carmoisine toxicity (Zaidi and Bau, 2004). Vitamin E emends antioxidants status by permitting free radicals to extract a hydrogen atom from antioxidant molecule, and not from polyunsaturated fatty acids, thus leading to the interruption in free radical chain reactions that causing unreactive antioxidant radical species that alleviating kidney toxicity (Pascoe *et al.*, 1987).

Histological investigations

Kidney is a critical organ for filtering the plasma and constitutively reabsorbs the selected parts of glomerular filtrate which is an energy consuming process. Kidney sections from control mice illustrated normal architecture of renal tissue; rounded glomeruli, proximal convoluted tubules with narrow lumen lined by cuboidal epithelium in renal cortex and distal convoluted tubules with wide lumen (Fig. 1). The renal medulla showed normal architecture of collecting tubules from mice treated with vitamin E (Fig. 2).

Sections of the kidney from the mice administered with carmoisine for 28 days revealed dissolution in renal cortex with segmented renal corpuscle, hyaline cast deposited in the cavities of

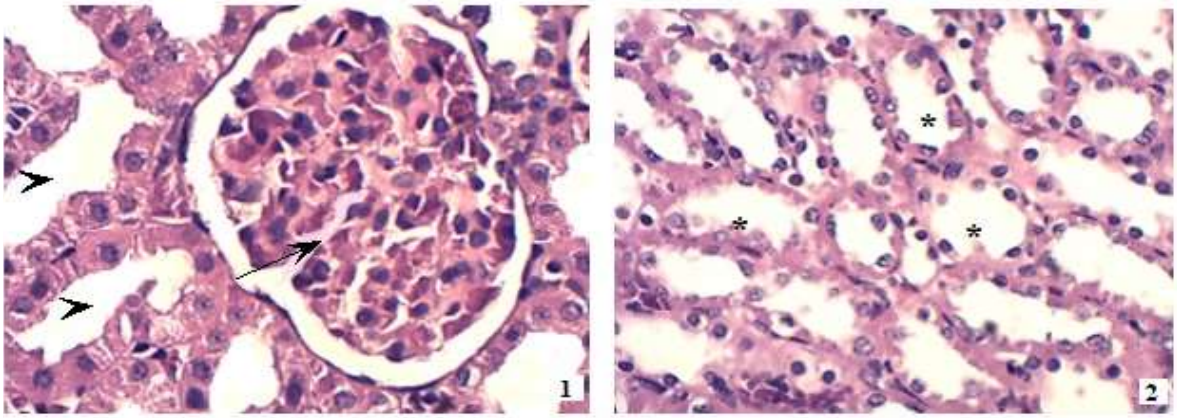

Fig. 1: Photomicrograph of kidney section from control mouse showing normal architecture of renal tissue, rounded glomeruli (arrow), distal convoluted tubules with wide lumen (head arrow) and proximal convoluted tubules with narrow lumen lined by cuboidal epithelium in renal cortex (*); (H-E, X 400)

Fig. 2: Photomicrograph of kidney section from vitamin E-injected mouse showing normal architecture of renal medulla with normal collecting tubules (arrows), (H-E, X 400)

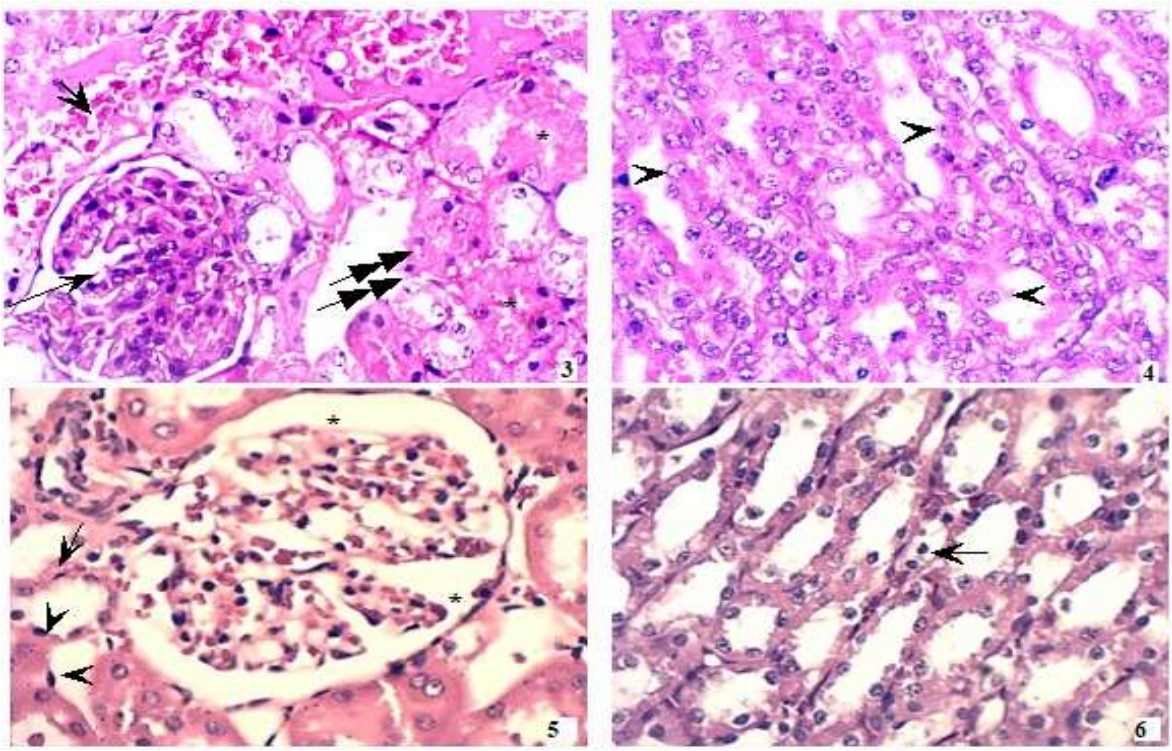

Fig. 3: Photomicrograph of kidney sections from mouse which received carmoisine, showing dissolution in renal cortex with segmented renal corpuscle (thin arrow), hyaline cast (*) deposited in the cavities of renal tubules, hydropic change in epithelial lining with pyknotic nuclei (double head arrows), and dilated congested blood vessel stuffed with RBCs (thick arrow); (H-E, X 400).

Fig. 4: Photomicrograph of kidney sections from mouse which received carmoisine, showing degenerative nuclei (head arrows) of epithelial cell of medulla renal tubules; (H-E, X 400).

Fig. 5: Photomicrograph of kidney sections from mouse which received carmoisine + vitamin E, showing slightly segmented glomerulus with widened capsule (*) and normal renal tubules (thin arrow); that still have some degenerative and pyknotic nuclei (head arrows); (H-E, X 400).

Fig. 6: Photomicrograph of kidney sections from mouse which received carmoisine + vitamin E, showing inflammatory cells like monocyte (arrow) invading medulla side by side along the normal medullary tubules; (H-E, X 400).

renal tubules, hydropic change in the epithelial lining with pyknotic nuclei, dilated congested-blood vessel stuffed with RBCs (Fig. 3) and degenerative nuclei of epithelial cell of medulla renal tubules (Fig. 4).

The adverse effects observed in kidney histopathology may be attributed to the toxic effects of reductive transformation of carmoisine during its metabolism in liver (Chequer *et al.*, 2011; Fijer and Al-Mashhedy, 2016). The concomitant renal distortions noticed in this study authenticated with elevation in concentration of urea, creatinine and electrolytes in serum of rats treated with carmoisine resulted in renal function impairment.

Himri *et al.* (2011) reported that histopathological damage in kidney of rats appeared as atrophy of glomeruli, thickened basement membrane, glomerular capillaries dilatation, hydronephrosis congenital cystic, tubular degeneration, intercapillary sclerosis, and tuberculosis, a condition leading to calcium deposition (hypervitaminosis D) due to the treatment of tartrazine (5, 7.5 and 10 mg kg^{-1}). Rus *et al.* (2009) and Elbanna *et al.* (2017) showed that perivascular edema, renal tubular apoptosis, and vascular congestion as a result of carmoisine administration for 3 weeks. Khayyat *et al.* (2018)

demonstrated renal and hepatic alterations in histological picture of albino rats administered with sunset yellow and allura red food dyes.

Despite short duration of treatment with vitamin E, it depicted protective effect in improving the renal architecture. Lesions that appeared as a result of carmoisine administration were remarkably reduced in renal tissue sections of vitamin E-treated rats. Kidney sections from mice which received carmoisine + vitamin E delineated slightly segmented glomerulus with widened capsule and normal renal tubules; that had some degenerative and pyknotic nuclei (Fig. 5), inflammatory cells like monocytes invading medulla side by side along the normal medullary tubules (Fig. 6). The administration of vitamin E along with carmoisine restored histological picture of renal tissue, as a result of its free radical scavenge activity (Pascoe *et al.*,1987; Zaidi and Bau, 2004).

Immunohistochemical studies

Tumour necrosis factor-alpha (TNF-alpha): TNF-alpha, an inflammatory cytokine produced by macrophages/ monocytes during acute inflammation, is responsible for diverse signaling events within cells, leading to necrosis or apoptosis. Cross sections of kidney from control and vitamin E-injected mice stained immunohistochemically for TNF-alpha delineated the normal TNF-alpha expression in renal tubules (Figs. 7 & 8). Carmoisine administration caused an increment in TNF-alpha expression in dilated congested blood vessels and in the cytoplasm of degenerative and pyknotic cells of renal tubules (Fig. 9). On the other hand, kidney section from mice which received carmoisine + vitamin E showed inhibition in TNF-alpha expression as compared to carmoisine group (Fig. 10).

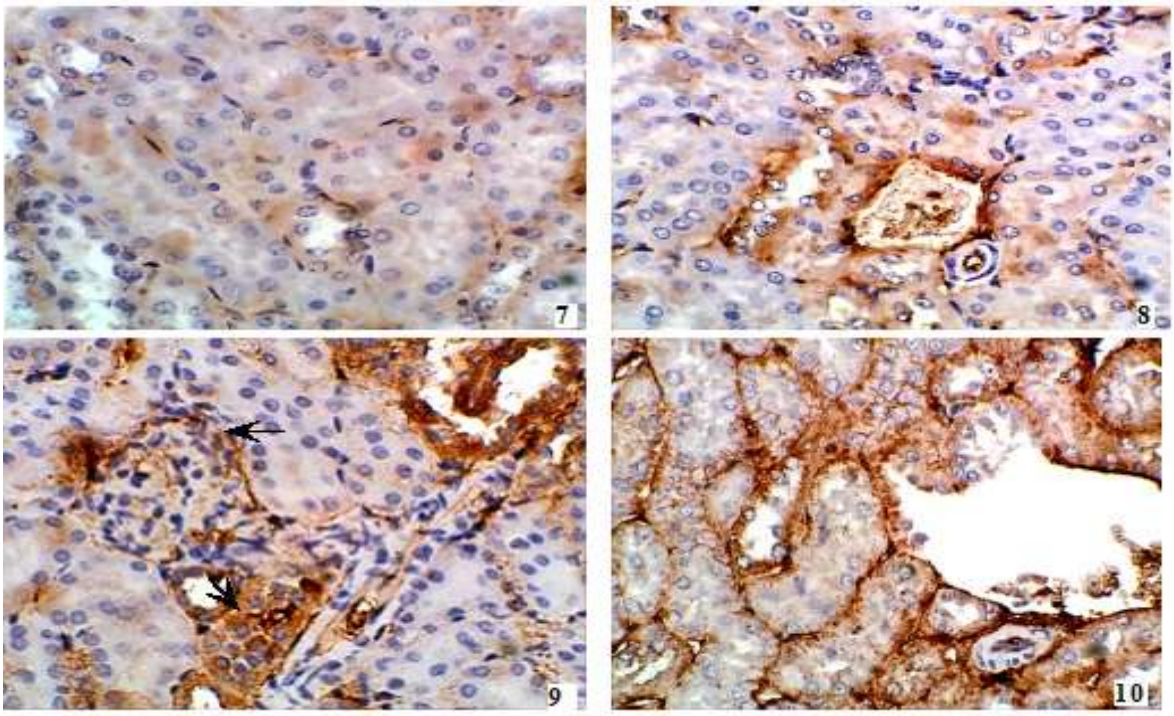

Fig. 7: Photomicrograph of kidney sections from control mouse showing TNF-alpha expression in proximal convoluted tubules;

Fig. 8: Photomicrograph of kidney sections from vitamin E-injected mouse, showing TNF-alpha expression renal tubules;

Fig. 9: Photomicrograph of kidney sections from mouse which received carmoisine showing increased expression of TNF-alpha in dilated congested blood vessels (thick arrow) and in the cytoplasm of degenerative and pyknotic cells of renal tubules;

Fig. 10: Photomicrograph of kidney sections from mouse which received carmoisine + vitamin E, showing decreased expression of TNF-alpha in renal tubules in comparison to carmoisine group (immunostaining, X400).

The increase in TNF-alpha levels lead to renal dysfunction. Prospect sources of TNF in inflamed kidney include infiltrating leukocytes, podocytes, mesangial cells, proximal tubules, ascending limbs and collecting ducts (Zager *et al.*, 2005; Rosa *et al.*, 2012).

The administration of carmoisine resulted in the formation of reactive oxygen species due to decline in the antioxidant status as authenticated in present study. These oxygen-free radicals could promote the production of inflammatory cytokines and cellular apoptosis. H_2O_2 stimulates NFκB (transcription factor) that induces TNF-α gene expression. H_2O_2 also stimulates p38 MAP kinase (p38 mitogen-activated protein kinase), which plays major role for NFκB induction and TNF-alpha formation (Yin *et al.*, 1997). TNF-alpha increment is involved in many inflammatory diseases of renal tissue such as diabetic nephropathy, acute renal failure, glomerulonephritis, and autoimmune lupus nephritis (Bertani *et al.*, 1989; Azuma *et al.*, 1997; Boswell *et al.*, 1998), resulting in pro-inflammatory mediator formation (Donnahoo *et al.*, 2001).

The mechanism of TNF-alpha-induced renal injury is multiple. TNF-alpha stimulates cellular dysfunction and apoptosis in renal tissues and inhibits glomerular filtration rate, glomerular permeability and glomerular blood flow via inducing vasoactive mediators formation (i.e. ndothelin-1, nitric oxide, platelet-activating factor, prostaglandins, IL-1) [Baud and Ardaillou, 1995; Gomez-Chiarri *et al.*, 1994; Soler *et al.*, 1996). Thus, it could be concluded that TNF-alpha affects both autocrine and the paracrine that attributes to kidney damage (Radeke *et al.*, 1990; Mullin *et al.*, 1992).

Cyclooxygenase-2 (COX-2) expression: COX-2 is an enzyme responsible for inflammation and pain. Kidney cross sections from control and vitamin E-injected mice showed normal COX-2 expression as observed in proximal and distal convoluted tubules (Fig. 11 & 12). The administration of carmoisine for 28 days up-regulated COX-2 expression in epithelial cells of renal tubules (Fig. 13). These observations are in agreement with Khayyat *et al.* (2018) who reported that the administration

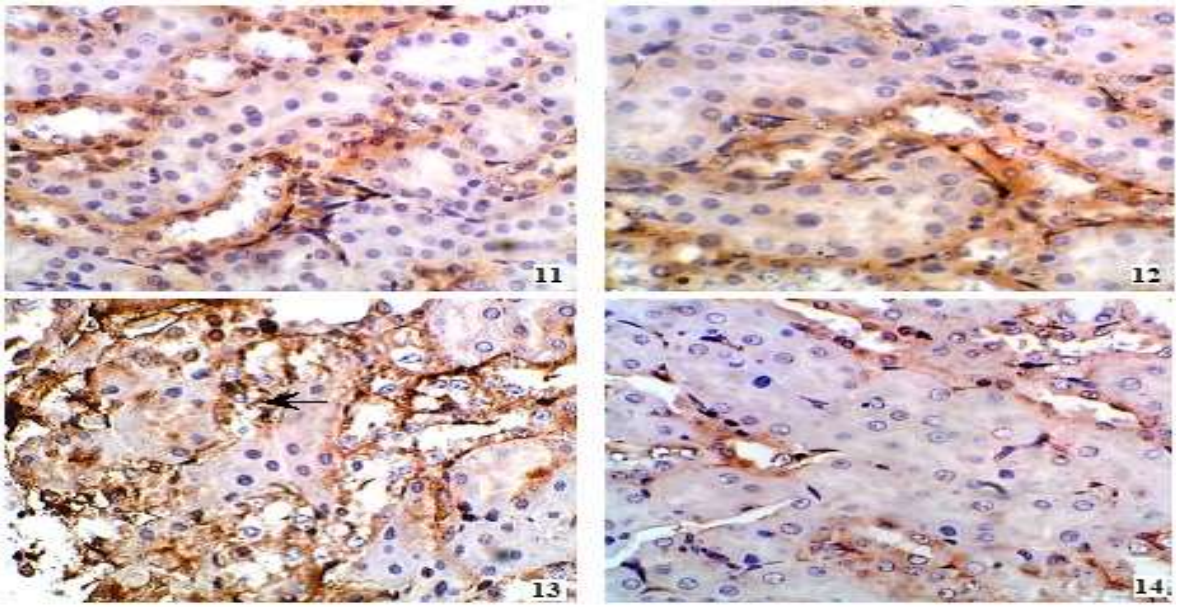

Fig. 11: Photomicrograph of a kidney section from control mouse showing normal COX-2 expression in proximal and distal convoluted tubules; (Immunostaining, X400).

Fig. 12: Photomicrograph of a kidney section from vitamin E-injected mouse showing normal COX-2 expression in renal tubules; (Immunostaining, X400).

Fig. 13: Photomicrograph of a kidney section from mouse which received carmoisine for 28 days showing increased expression of COX-2 in the epithelial cells of renal tubules; (Immunostaining, X400).

Fig. 14: Photomicrograph of a kidney section from mouse which received carmoisine + vitamin E, showing highly decreased expression of COX-2 in renal tubules in comparison to carmoisine group (Immunostaining, X400).

of Allura Red and Sunset Yellow food dyes caused increase of COX2 expression. On the other hand, the kidney sections from mice received carmoisine + vitamin E showed highly decreased expression of COX-2 in renal tubules when compared with carmoisine group (Fig. 14).

Flow-cytometric detection of apoptotic and anti-apoptotic marker activities

Carmoisine administration caused 60.2 and 58.0%, enhancement in the concentrations of caspase 3 (indicator for apoptosis) and CD95 (called Fas or apoptosis antigen 1), respectively. Decrease in Bcl-2 (anti-apoptotic protein) levels (32.3%) relative to control group has been depicted in Table 3. This elevation in caspase 3 and CD95 and inhibition in Bcl-2 is an obvious evident for renal inflammation that occurred in the damaged tubular cells.

Table 3: Caspase 3, CD95 and Bcl-2 levels in control and experimental groups (%) mice

Parameters	Control (C)	Vitamin E	Carmoisine	Carmoisine + vitamin E
Caspase 3	26.45 ± 1.87	27.07 ± 3.28	60.22 ± 2.86^{abd}	47.31 ± 1.93^{abc}
CD95	35.30 ± 1.56	33.14 ± 5.35	58.02 ± 14.69^{abd}	44.61 ± 3.12^{c}
Bcl-2	48.12 ± 0.96	49.41 ± 2.39	32.35 ± 1.89^{abd}	50.71 ± 11.5^{c}

The values are mean ± SD. Superscript letters denote the significance at ($p<0.05$); *a*: significant to C, *b*: significance to Vitamin E, *c*: significant to carmoisine, and *d*: significant to carmoisine + vitamin E.

The mechanism of Fas augmentation is very complicated, as IL-1α and tumor necrosis factor (TNF) induce Fas expression in cultured renal tubular cells, apoptosis and Fas up-regulation along with renal tubular atrophy. It was concluded that tubular cells are targeted for Fas-dependent apoptotic deletion, which is attributed to the tubular atrophy. Many pro-inflammatory markers such as IL-1 and TNF indirectly stimulate tubular atrophy via Fas-dependent renal tubular cells apoptosis (Schelling and Cleveland, 1999). Increase in apoptosis in remnant renal tissue is likely to be Caspase-3-dependent because its association increases in Caspase-3 levels causing renal cell apoptosis and fibrosis (Yang *et al.*, 2001). Decrease in Bcl-2 expression in present study is in agreement with Khayyat *et al.* (2018) who showed that there is a down-regulation of Bcl-2 as a result of the administration of Sunset Yellow and Allura Red food dyes.

Vitamin E injection counteracted the side effects of carmoisine on apoptotic marker production where these recorded 47.3, 44.6 and 50.7% for caspase 3, CD95, and Bcl-2, respectively. These results suggest the protective effect of vitamin E in preventing renal damages.

Conclusion: This is the first study concerned the therapeutic effect of vitamin E on immunological status of renal tissue; against deleterious effects of administering synthetic food dye carmoisine. Vitamin E exhibited modulatory effect in ameliorating the immunological parameters tested in this study. The application of these methods will improve human health and decrease the cost of treatment of diseases. The **c**onsumption of carmoisine as a synthetic food additive adversely affects the health so its use should be restricted especially in children-related food stuffs. Safety updates about food dyes affecting health especially with respect to behavioral changes, nervous disorders, genotoxicity, reproductive toxicity and carcinogenicity should be announced to public. Since, consumers do not know the kind of synthetic food dyes or their doses in food stuffs; hence, it must be made mandatory for industrial food companies to label complete information about their products including the concentration and name of food dyes used in their products.

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Conflict of interest: The authors declare that they have no competing conflict of interest.

***Ethical statement*:** This work carried out at the Medical Experimental Research Centre (MERC), Mansoura University (Egypt) after the approval from Ethical Committee of the Department in accordance with the ethical guidelines for proper care and use of laboratory animals (Canadian Council on Animal Care, 1993).

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