Journal of
Fisheries and Aquatic Science

ISSN 1816-4927

Academic
Journals Inc.

www.academicjournals.com

Journal of Fisheries and Aquatic Science 7 (4): 240-265, 2012
ISSN 1816-4927 / DOI: 10.3923/jfas.2012.240.265

Protective Roles of Tomato Paste and Vitamin E on Cadmium-induced Histological and Histochemical Changes of Liver of *Oreochromis niloticus* (Linnaeus, 1758)

[1,2]I.A.A. Mekkawy, [1]U.M. Mahmoud, [1]E.T. Wassif and [1]M. Naguib
[1]Department of Zoology, Faculty of Science, Assiut University, Egypt
[2]Department of Biology, Faculty of Science, Taif University, Saudi Arabia

Corresponding Author: I.A.A. Mekkawy, Department of Biology, Faculty of Science, Taif University, Taif, Saudi Arabia

ABSTRACT

The present study was carried out to investigate the potential protective effects of tomato paste (9 mg lycopene kg^{-1} b.wt.) in comparison with vitamin E (50 mg kg^{-1} b.wt.) against the impacts of cadmium (Cd) toxicity (4.64 mg L^{-1}, ¼ of 96 h- LC_{50}) Oreochromis niloticus exposed for 15 and 30 days. Cd impacts were evaluated in terms of histopathological and histochemical characteristics of the liver and hepatopancreas. Various changes have been observed in the liver tissues of the fish exposure to Cd in the two periods. These changes included liver cord disarray, enlargement of ballooning degeneration, dilatation and congestion of some blood vessels with blood cells. A considerable number of hepatocytes showed cytoplasmic vacuolation. Küpffer cells were evident in the dilated hepatic sinusoids. A gradual increase in the damage was noticed by longer period of exposure. The pathologic effect of cadmium on hepatopancreas included a decrease in the size of the acinar cells with an increase in their number. Histochemically, glycogen content decreased in the liver and hepatopancreas with the increase of the period of exposure. These Cd-induced parameters were significantly improved with supplementation of vitamin E and/or tomato paste. These findings were emphasized by liver enzymes ones demonstrating the beneficial supplementation of vitamin E and/or tomato paste in reducing the harmful effects of Cd on the normal enzyme levels and structures of the liver and hepatopancreas.

Key words: Fishes, liver, hepatopancreas, cadmium, tomato paste, vitamin E

INTRODUCTION

The health of aquatic ecosystems is best evaluated by fish physiological and biochemical characteristics which serve as biomarkers of environmental pollution (Kock *et al*., 1996). In this concern *Oreochromis niloticus*, is a common appropriate model in toxicological research (Figueiredo-Fernandes *et al*., 2006a, b; Garcia-Santos *et al*., 2006) and in biomonitoring programmers (Gadagbui *et al*., 1996).

Heavy metals were recorded naturally in varying levels in all ground and surface waters (Martin and Coughtrey, 1982). According to Mason (1991), these metals represent one of the five major types of toxic pollutants commonly present in surface waters. Some heavy metals are nonessential and play no significant biological roles whereas others are essential elements for the normal metabolism of organisms (Cross and Sunda, 1985; Rainbow, 1985; Rainbow and White, 1989; Sanders, 1997). Anthropogenic activities cause an increased discharge of various

concentrations of both essential and nonessential metals into natural aquatic ecosystems. Before significant changes can be identified in fish behavior or external appearance, the initial effects of heavy metal pollution may be evident at cellular or tissue levels (Bais and Lokhande, 2012; Van Dyk *et al*., 2007).

The nonessential heavy metal, Cadmium (Cd) is has an accumulative toxicity effect to aquatic organisms even in minute concentrations (Cicik and Engin, 2005). It has been demonstrated to stimulate free radical production in concern with the lipid, protein and DNA oxidative deterioration and initiating various pathological conditions in humans and animals (Hussein and Mekkawy, 2001; Manca *et al*., 1991; Sarkar *et al*., 1997; Shaikh *et al*., 1999; Shen and Sangiah, 1995).

Variability in the Cd-induced toxic effects on fish is evident especially in interrupting development and growth (Lemaire-Gony and Lemaire, 1992; Mekkawy and Lashein, 2003), anemia (Abdel-Rahman, 1997; Houston *et al*., 1993; Larsson *et al*., 1985), preventing Ca^{+2} uptake through the gills (Verbost *et al*., 1987), disturbing liver functions (Soengas *et al*., 1996), skeletal deformations (Muramoto, 1981) and pathological changes in some tissues and organs (Karlsson-Norrgren *et al*., 1985). Cadmium fish toxicity and factors controlling such toxicity were discussed and reviewed by Alabaster and Lloyd (1982) and Lloyd (1992) and Heath (1995).

The study of pollutant effects on the histology of different organs of fish is an important basic task leading to understanding the impact of such pollutants on a given ecosystem (Meyers *et al*., 1992; Young *et al*., 1994). The liver of fish is an important organ sharing in many metabolic processes and is the major site of detoxification (Hogan and Knowles, 1968; Verity and Reigth, 1967). According to Hinton and Lauren (1990), the liver is essential for both the metabolism and the excretion of toxic substances and so it serves as a model for studying the interactions between environmental factors and hepatic structures and functions (Brusle and Anadon, 1996). Heavy metals-induced hepatic histological changes could be considered as biomarkers. The estimation of Cd-free radical generations and the antioxidant defense has become an important aspect of investigation in mammals and animals especially with the natural antioxidants like tomato carotenoids including β-carotene, γ-carotene and lycopene (Bramley, 2000; Clinton, 1998; Krinsky, 2001; Tapiero *et al*., 2004; Visioli *et al*., 2003) and vitamin E (α-tocopherol). In animal health, these naturally occurring antioxidants inactivate harmful free radicals produced through normal cellular activity and from various pollutants and stressors (El-Demerdash *et al*., 2004a). These micronutrients could, at least enhance immunity by maintaining the functional and structural integrity of important immune components (Chew, 1995; El-Demerdash *et al*., 2004b; Pregiosi *et al*., 1998; Yousef *et al*., 2003). Therefore, the assessment of the relative antioxidant potency of vitamin E and carotenoids has received particular attention (El-Demerdash *et al*., 2004a; Heber and Lu, 2002; Wertz *et al*., 2004) with emphasize on the conclusion that lycopene has a higher antioxidant potential than α-tocopherol and β-carotene (Di Mascio *et al*., 1989; Rao and Agarwal, 1999; Woodall *et al*., 1997). There is no interpretation of the mechanism controlling the antioxidant property of carotenoids (Aust *et al*., 2001; Woodall *et al*., 1997).

Human consumption of lycopene-rich foods was found to be related to the lower levels of some health problems including cancer and cardiovascular diseases (Mekkawy *et al*., 2010). According to some authors, it is difficult to determine what proportions of their health-protective effects are attributable to lycopene because lycopene-rich foods contain a number of other beneficial phytonutrients (Hadley *et al*., 2002). Since tomatoes and tomato products are the major dietary source of lycopene (Zhang *et al*., 2008), lycopene could be used as indicator of the additive or synergistic anti-carcinogenic effects of their phytonutrients (Mekkawy *et al*., 2010). Emphasizing

on such observation, Boileau *et al.* (2003) stated that consumption of tomato powder but not lycopene inhibited prostate carcinogenesis. Tomatoes develop sets of interacting compounds (carotenoids) to accomplish their antioxidant functions rather than relying on single compounds (Gann and Khachik, 2003). These compounds behave in similar way in animal body. The antioxidant role of these phytonutrients depends on the ability of animal species to achieve their biologically relevant tissue concentrations.

According to the aforementioned findings and low price of tomatoes in a definite season in Egypt, the present work was suggested as extension to previous work (Mekkawy *et al.*, 2010) and aimed to study the effect of cadmium on the light of diet supplementation of tomato paste and/or vitamin E on histological and histochemical characteristics of the Nile fish, *Oreochromis niloticus* (Linnaeus 1758). Do the current results have applicable role in human liver stressed by heavy metal toxicity is a question to be answered.

MATERIALS AND METHODS

Sample collection and treatment manipulation: One hundred and twelve healthy fish of the Nile tilapia, *Oreochromis niloticus* (120±17.8 g in weight, 19±1.04 cm in length) were caught from the fish farm of Faculty of Agriculture, Assiut University, Egypt. Fishes immediately were transported to the fish laboratory in the Department of Zoology, Faculty of Science, Assiut University. The experimental fishes were reared in aerated glass tanks (100 L capacity) and acclimatized for two weeks before being used in the experimental study. The experimental fish fed pellets at a rate of 4.5% of fish body weight twice daily. Feces and residual food were aspirated regularly. The water temperature, pH and Dissolved Oxygen (DO) concentrations were measured daily (24.2±.08 °C, 6.8±.11 pH and 6.5±.89 mg L^{-1} DO). Light cycle was 12 light and 12 h dark.

Preparation of tomato paste to adjust the lycopene dose: Tomatoes used for the experiment were obtained from the local market. Fresh peeled, deseeded tomatoes were pulped well to a smooth consistency in a warring blender. The lycopene content in tomato paste was estimated spectrophotometrically according to the methods of Ranganna (1976) and Choudhari and Ananthanarayan (2007). The lycopene concentration in the tomato paste was 30.028 mg/100 g (Okajima *et al.*, 1998). Based on the review of Xianquan *et al.* (2005), such concentration could not be affected by current conditions of diet preparation and storage of a short time (37°C for 4 weeks). In addition to lycopene, tomato paste composition include water, proteins, carbohydrates, fibers, calcium, potassium, zinc, copper, manganese, iron, vitamin C, vitamin E, β-carotenoids and other phytonutrients.

Experimental design: Fishes were weighed, measured and classified randomly into 8 groups (14 fish/tank) according to dose of cadmium, tomato paste in terms of lycopene, vitamin E and their combinations (Table 1). The diets (maize and soy bean, 15 mg kg^{-1} fish) were pellet after addition of vitamin E and tomato paste doses for the treated groups and the addition of suitable amounts of molasses and water. The diets were dried at room temperature and stored in small bags for fish feeding.

Stock solution (1000 ppm) of cadmium as cadmium chloride ($CdCl_2$ $2.5H_2O$) was prepared and stored in clean glass bottles and diluted to concentration of 4.64 mg L^{-1}. Such low sublethal cadmium concentration (¼ of 96 h- LC_{50}) was chosen according to levels monitored by Almeida *et al.* (2002). Cadmium doses were prepared and added constantly to the aquarium for four

Table 1: The fish groups exposed to cadmium (4.64 mg L^{-1}) and tomato paste (9 mg lycopene/kg body weight) and vitamin E (50 mg kg^{-1} body weight) and their combinations

Treatment	C	VE	Tp	VE+ Tp	Cd	Cd+VE	Cd+ Tp	Cd+VE+Tp
Cadmium (mg L^{-1})	0	0	0	0	4.64	4.64	4.64	4.64
Tomato paste (mg kg^{-1})	0	0	9	9	0	0	9	9
Vitamin E (mg kg^{-1})	0	50	0	50	0	50	0	50

C: Control, VE: Vitamin E, Tp: Tomato paste and Cd: Cadmium

weeks. The test water was replaced daily with the required amount of stock solution to prevent deterioration of water quality and replenish cadmium levels. Tomato paste was added to the diet in concentration of 30 mg kg^{-1} b.wt. (9 mg lycopene kg^{-1} b.wt.). Dose response of lycopene was described previously by Rodriguez *et al.* (2004). Also, vitamin E (α-tocopherol) was supplemented in 50 mg kg^{-1} b.wt. Such vitamin E concentration was chosen according to levels monitored by Ortuno *et al.* (2001). It is worthy to mention that vitamin E (α-tocopherol) in tomato paste was estimated to be 38.67±2.29 mg/100 g tomato paste dry weight with no effect by industrial processing (Capanoglu *et al.*, 2008).

Histological and histopathological examination: For microscopic preparations, after intervals of 15 and 30 days, 3 surviving fish of each group were removed and sacrificed. Small pieces of the liver were taken and immediately fixed in 10% neutral buffered formalin. Fixed tissues were processed routinely for paraffin embedding technique. Embedded tissues were sectioned at 7 μ in thickness and then stained by following stains: Harris's hematoxylin and eosin stain (HE) (Bancroft and Stevens, 1982) and Masson's Trichrome stain for the collagenous fibers (Humason, 1979). This method is preceded by hematoxylin staining for a brilliant permanent nuclear stain.

Histochemical preparation: Estimation of general carbohydrates represents the important parameter among the histochemical ones. For the demonstration of the polysaccharides status, periodic acid Schiff's (PAS) technique was applied (McManus, 1946). In this regard, carbohydrates were first oxidized with 0.1% periodic acid; aldehyde groups (-HCO-HCO), were liberated from the glycol reagent, producing a compound of magenta coloration. Alcian blue (AB-PH 2.5) and periodic acid-Schiff (PAS) method visualized by Mowry (1956) was indicated by appearance of blue color for acid mucin, magenta for neutral mucin and mixture of the two colors for carbohydrate. The nuclei colored pale blue.

RESULTS

Control liver: In control liver of *Oreochromis niloticus* the cords of hepatocytes tend to have a regular radial pattern enclosing the sinusoidal network for a short distance into the perivenular areas (Fig. 1a-c). These areas become less regular outside the perivenular zone. The individual hepatocyte is polygonal in shape and has a single spherical nucleus. The nuclei are mostly centrally located within the hepatocytes with some nuclei tending to occur closer to the cell periphery bordering the sinusoids. The endothelial cells that line these sinusoids as well as their nuclei are flattened and elongated (Fig. 1b-d).

The exocrine pancreatic tissue (hepatopancreas) is a pronounced feature in the liver of *Oreochromis niloticus*. It consists of a large number of acini (Fig. 1b). Each acinus is made up of conical glandular cells with an eccentric, deeply stained nucleus and prominent nucleolus. The

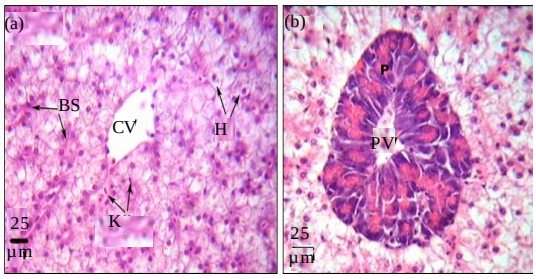

Fig. 1(a-b): Sections of control fish liver and hepatopancreas. (a) Fish liver showing the general structure, Blood sinusoids (BS), Central vein (CV), hepatocytes (H) and Küpffer cell (K) (H and E X 400). (b) Fish hepatopancreas (P) showing the portal vein (PV) and the basophilic portion with nucleus and the acidophilic cytoplasm of the acinar cells. (H and E X 400)

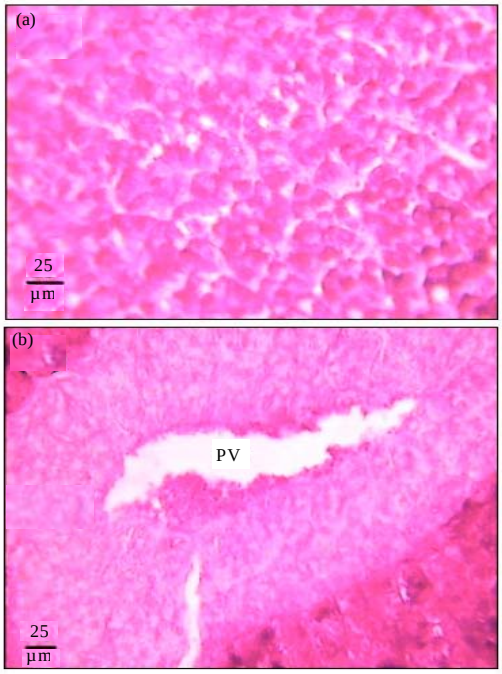

Fig. 2 (a-b): Sections of control fish liver and hepatopancreas. (PAS X 400). (a) Control fish liver showing the great amount of glycogen in the cytoplasm of hepatocytes. (b) Control fish hepatopancreas (P) showing glycogen content in hepatopancreatic tissue

cytoplasm is homogenous and basophilic in the nuclear portion of the cell; the remaining part of the cell is acidophil (Fig. 1b).

PAS-technique exhibited distinct distribution of the polysaccharide materials in the hepatocytes and pancreatic cells of the control fish for 15 and 30 days. The positively stained materials have been proved to be glycogen as verified by PAS-staining with and without pre-treatment with diastase. Such reaction appeared comparatively higher in the hepatocytes around the central vein areas than at the peripherals (Fig. 2a, b).

Treatment with tomato paste or vitamin E and their combinations: Histological examination revealed that the treatment of fish with tomato paste and/or vitamin E for 15 days had no effect on the appearance of the liver structure and hepatopancreas which had essentially normal appearance. An improvement in the histological structure of the hepatocytes and pancreatic tissues was noticed in fish administrated lycopene and/or vitamin E for 30 days as compared to the control ones. The boundary between cells was noticed more or less clearly and the nuclei of the hepatic cells were seen to be enlarged and hyperchromatic (Fig. 3a-c, Fig. 4a-c).

PAS-technique exhibited distinct distribution of the polysaccharide materials appeared as massive red colored patches located exclusively in the cytoplasm of the hepatocytes and pancreatic cells in the fish administrated lycopene and/or vitamin E (Fig. 5a-c, 6a-c) for 15 and 30 days. The positively stained materials have been proved to be glycogen as verified by PAS-staining with and without pre-treatment with diastase. Like the control, PAS-reaction appeared comparatively higher in the hepatocytes around the vein areas than at the peripherals.

Treatment with cadmium chloride: In comparison with the control, variable alterations had indicated in the liver sections of Cd-treated fish for 15 days. These alterations were mainly represented by liver nonhomogenous architecture (Fig. 7a-c). Sinusoidal lumen was collapsed and few Küpffer cells were observed. Hydropic and vacuolar degenerations were evident in some specimens. Various degrees of vacuolar degeneration, varying from mild to complete replacement of the hepatic cells by vacuoles (Fig. 7a) were noticed in some places with the rupture of hepatocyte membranes. Dilatation of some blood vessels congested with blood cells was revealed (Fig. 7a).

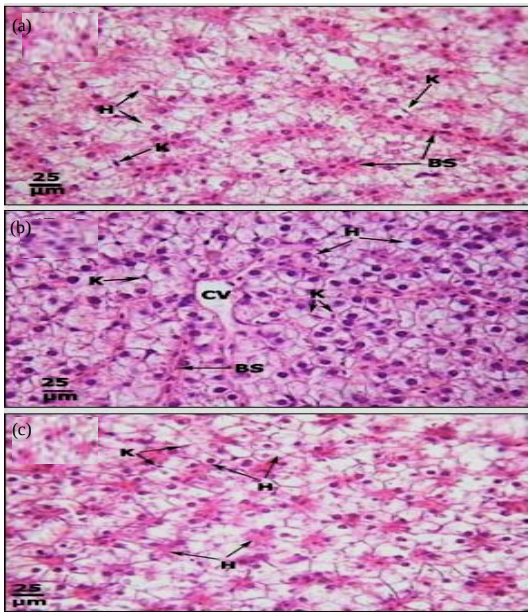

Fig. 3(a-c): Sections of treated fish liver for 30 days showing the general structure of the liver. Blood sinusoids (BS), central vein (CV), hepatocytes (H) and Küpffer cell (K). (H and E.X 400). (a) Tomato paste treatment, (b) Vitamin E treatment and (c) Vitamin E and tomato paste simultaneous treatment

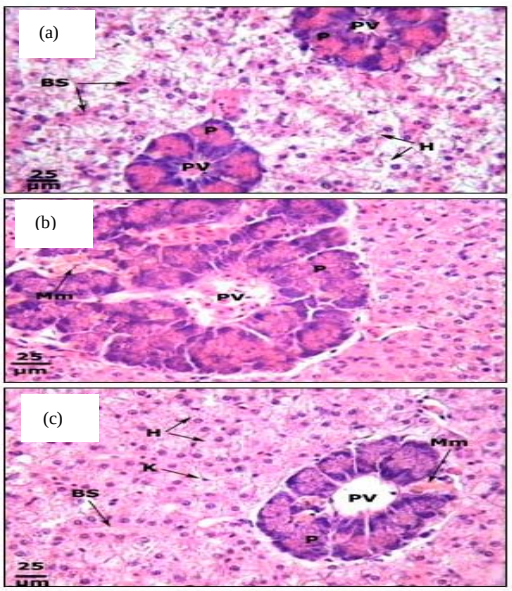

Fig. 4(a-c): Sections of treated fish hepatopancreas for 30 days showing small aggregation of melanomacrophage (Mm), portal vein (PV) and acinar cells with basophilic and acidophilic cytoplasm. (H and E. X 400). (a) Tomato paste treatment, (b) Vitamin E treatment and (c) Vitamin E and tomato paste simultaneous treatment

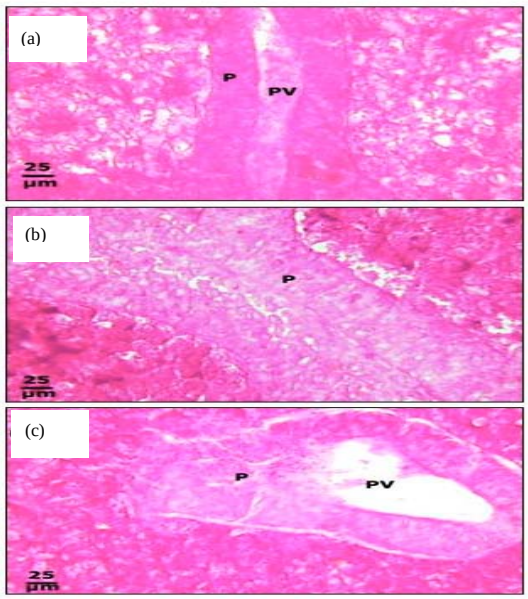

Fig. 5(a-c): Sections of treated fish liver for 30 days showing, great amount of glycogen in the cytoplasm of hepatocytes. (PAS X 400). (a) Tomato paste treatment, (b) Vitamin E treatment and (c) Vitamin E and tomato paste simultaneous treatment

Figure 2b-c show proliferation of the hepatic cells with a decrease in cell size. Accordingly, the hepatocytes lost their normal polygonal shape and boundary between cells became invisible. Also, infiltration of the inflammatory cells inside the central vein and between the hepatocytes and extravasated erythrocytes (haemorrhage) were observed (Fig. 7b). Areas of hepatic necrosis started to appear in some regions and rupture of the wall of the central vein were noticed (Fig. 7c).

In addition to preceding changes, more conspicuous signs of irregularly shaped advanced necrotic areas were recorded for Cd-treated fishes for 30 days. The hepatocytes are delimited by ruptured cell membranes in some areas and dispersion of cell contents and loss of stainability in others (Fig. 8a). Individual cells were necrotic with condensed granules, some of them were characterized by the absence of nuclei while the others were having pyknotic nuclei and extravasated erythrocytes (haemorrhage) were observed (Fig. 8a). Moreover, more hydropic and little vacuolar degeneration of the hepatocytes were observed more than the previous period with infiltration of inflammatory cells, dilatation in blood sinusoid and granulated cytoplasm (Fig. 8b).

Cd-exposed fish for 15 days showed an aggregation of melanomacrophage and inflammatory cells around the hepatopancreatic acini. Extravasated erythrocytes (haemorrhage) and dilatation in portal vein of hepatopancreas was revealed (Fig. 9a). In addition to the previous alterations, Cd-exposed fish for 30 days revealed proliferation of the acinar cells, Absence of the acidic portion of these cells, rupture and peripheral fibrosis in the portal vein and aggregation of melanomacrophage cells were observed (Fig. 9b).

Examination of liver sections stained with Masson's trichrome stain produced an accumulation of connective tissue fibers around the central vein and hepatic sinusoids and infiltration of inflammatory cell with necrotic area after 15 days (Fig. 10a). Such accumulation of connective tissue increases with an inflammatory area after 30 days of Cd-exposure (Fig. 10b). Moreover,

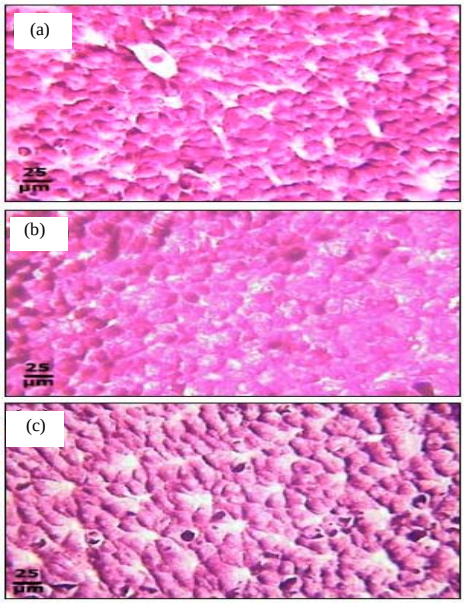

Fig. 6(a-c): Sections of treated fish hepatopancreas for 30 days showing, good PAS-reactivity in the acinar cell. (PAS X 400). (a) Tomato paste treatment, (b) Vitamin E treatment and (c) Vitamin E and tomato paste simultaneous treatment

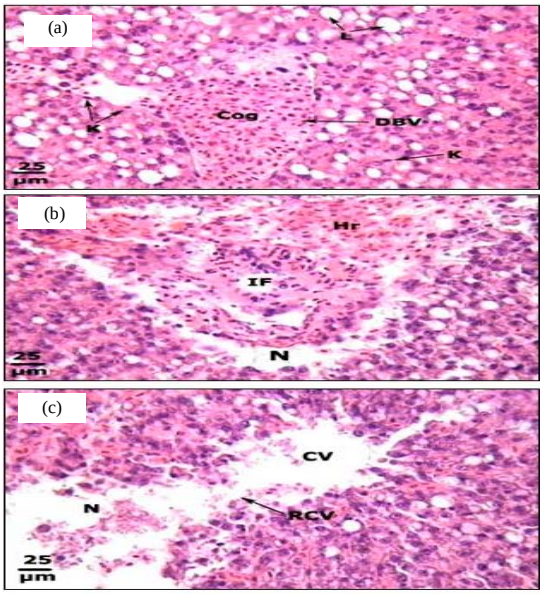

Fig. 7(a-c): Sections of treated fish liver exposed to (4.46 mg L^{-1}) cadmium chloride for 15 days showing histological altrations in liver tissues. (H andE X 400). (a) Showing dilatation of blood vessel (DBV), congested with blood (Cog), Küpffer cells (K) and fatty degeneration (lipid droplets) (L), (b) Showing proliferation of hepatocytes (crowded of nuclei), haemorrhage (Hr), infiltrations of inflammatory cell (IF) and large necrotic areas (N) and (c) Showing vaculation of hepatic cells and large necrotic areas (N). Rupture of wall of the central vein (RCV)

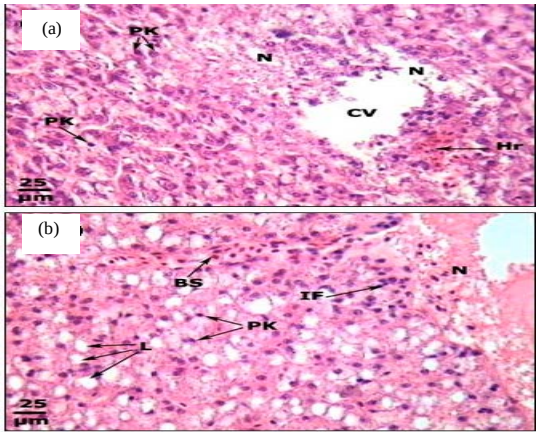

Fig. 8(a-b): Sections of treated fish liver exposed to (4.46 mg L^{-1}) cadmium chloride for 30 days. (H and E X 400), (a) Showing ruptured central vein lining (CV), haemorrhage (Hr), necrotic zone (N) with condensed granules and pyknotic nuclei (Pk) and (b) Showing congested blood sinusoids (BS), inflammatory area (IF), fatty degeneration (lipid droplets) (L) and necrosis (N)

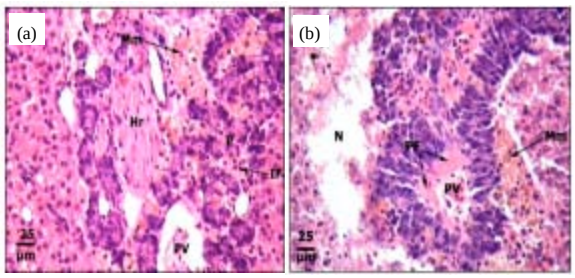

Fig. 9(a-b): Sections of treated fish hepatopancreas exposed to (4.46 mg L^{-1}) cadmium chloride, (a) for 15 and (b) for 30 days. (H and E. X 400). (a) Showing haemorrhage (Hr), infiltration of inflammatory cells (IF) between the hepatopancreatic tissues and aggregation of melanomacrophage cells (Mm) around the pancreatic acini. Dilatation in portal vein (PV) and (b) Showing proliferation of the pancreatic acini (crowded pancreatic cells), notice the absence of the acidophilic portion of the pancreatic cells, aggregation of melanomacrophage cells (Mm) between the acini, necrotic area (N), also, peripheral fibrosis (PF) of the portal vein

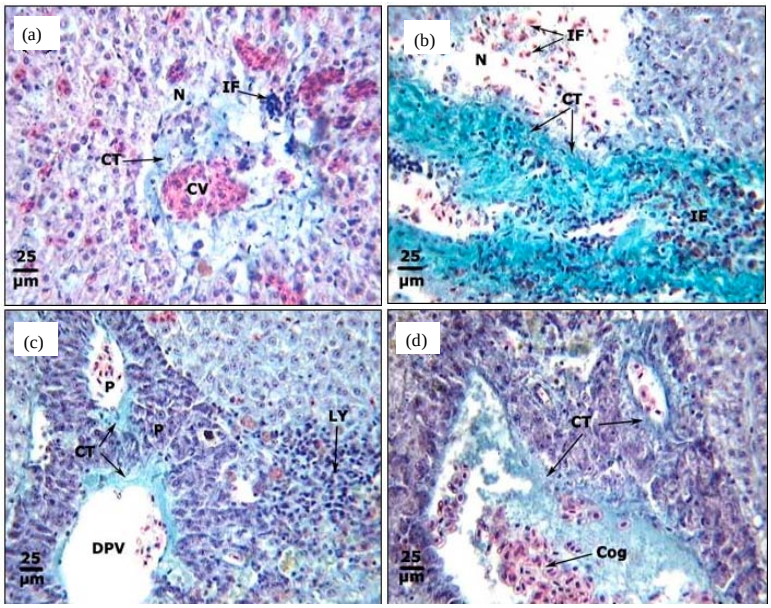

Fig. 10(a-d): Sections of treated fish liver (a and b) and hepatopancreas (c and d), exposed to (4.46 mg L^{-1}) cadmium chloride for 15 and 30 days. (Masson's trichrome X 400). (a) Showing an accumulation of connective tissue fiber (CT), inflammatory (IF) and necrotic (N) areas, (b) Showing a huge amount of connective tissue fiber (CT) around the blood vessel, inflammatory (IF) and necrotic (N) areas, (c) Showing a large amount of connective tissue fibers (CT) located around the portal vein (PV), an aggregation of lymphocytes (LY) between the hepatopancreatic tissues and dilatation of portal vein (DPV) and (d) Showing peripheral fibrosis (connective tissue) (CT) of the portal veins, congestion (Cog) of the portal vein

examination of hepatopancreas sections revealed a marked increase in the collagenous fibers in the wall of the portal vein (peripheral fibrosis) and between the tissues with an aggregation of lymphocytes between the hepatopancreatic tissues after 15 and 30 days (Fig. 10c-d). Also, dilatation of the portal vein and blood congestion inside the portal vein were noticed.

In the liver of Cd-administered fish, histochemical investigation revealed that there was a remarkable depletion in the glycogen content in hepatocyte and hepatopancreatic tissues after 15 day (Fig. 11a-b). The cytoplasm of the majority of hepatocytes exhibited a faint coloration with PAS reaction compared to those of control ones. After 30 days of exposure, more depletion in the glycogen content in both organs was observed (Fig. 11c-d).

Treatment with cadmium chloride plus tomato paste: Cd-exposed fish dietary supplemented with lycopene for 15 days retained their normal appearance of the hepatic tissues reflecting the protective potential effect of lycopene (Tomato paste). Each hepatocyte has its own nucleus. The number of Küpffer cells increased with a marked decrease in the number of the melanomacrophage cells. However, some cells still suffering from vacuolar degeneration in some areas and blood congestion inside the central vein (Fig. 12a). After 30 days of exposure and supplementation of tomato paste, the hepatic tissues retained its normal structure with tiny aggregations of melanomacrophage cells and infiltration of inflammatory cell (Fig. 12b).

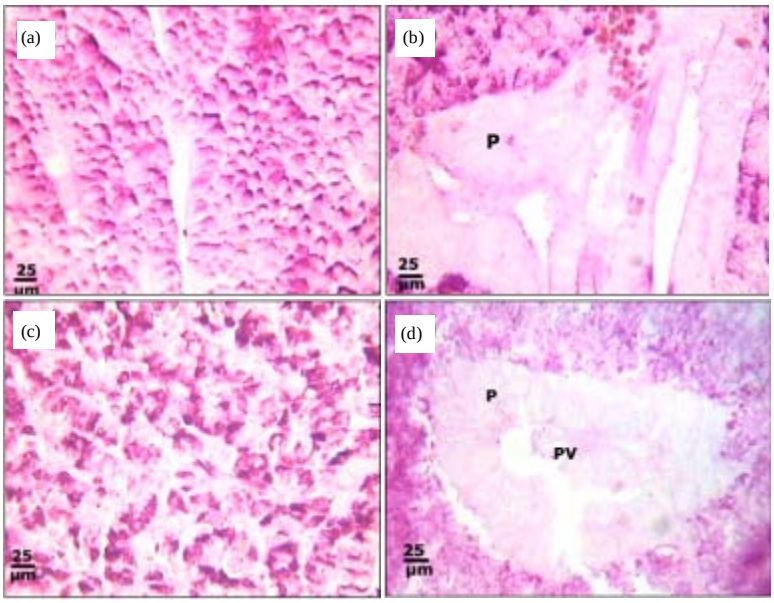

Fig. 11(a-d): Sections of fish liver (a) and hepatopancreas(b) exposed to (4.46 mg L^{-1}) cadmium chloride for 15 days, and fish liver (c) and hepatopancreas (d) exposed to the same dose for 30 days. (PAS, X 400). (a) Showing a remarkable depletion of glycogen in the liver cells, (b) Showing depletion in glycogen content of hepatopancreas, (c) Showing moderate amount of glycogen in the liver cells after 30 days and (d) Showing more depletion of glycogen content in the hepatopancreas after 30 days

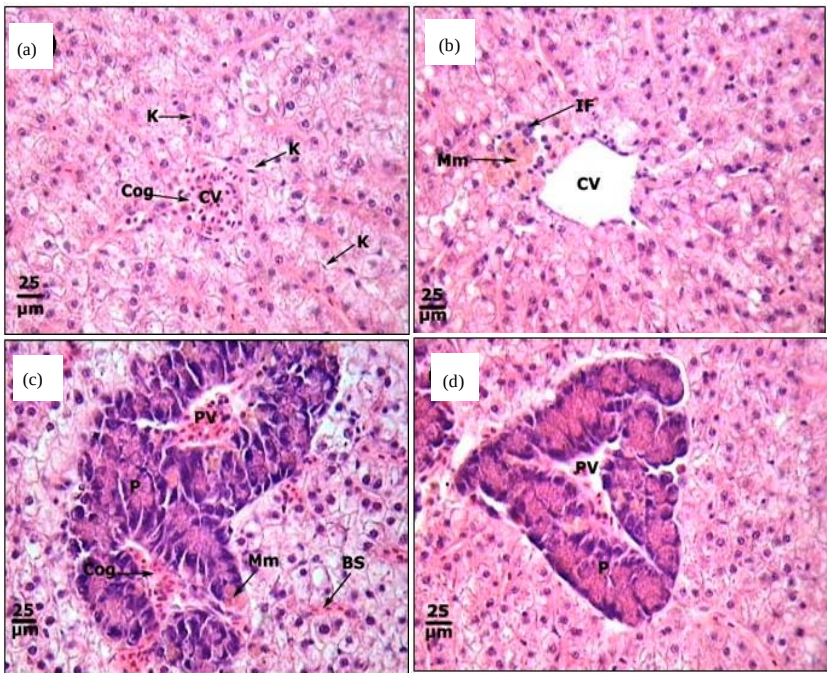

Fig. 12(a-d): Sections of treated fish liver (a and b) and hepatopancreas (c and d) exposed to lycopene+(4.46 mg L^{-1}) cadmium for 15 and 30 days. (H and E X 400), (a) Fish liver Showing normal structure of hepatic tissue with blood congestion (Cog) in central vein (CV) and in blood sinusoids and clear cytoplasmic vacuolation. The number of Küpffer cells increased (K), (b) Fish liver Showing the general structure and arrangement of the hepatocytes, central vein (CV) and infiltration of inflammatory cell (IF) and small aggregation of melanomacrophage (Mm) after 30 day of exposure, (c) Fish hepatopancreas showing proliferation of the pancreatic acini and blood congestion (Cog) in portal vein (PV) and (d) Fish hepatopancreas showing the normal structure of the hepatic and hepatopancreatic tissues after 30 day of exposure

The hepatopancreatic tissue shows normal structure with presence of its acidic portion, few aggregation of melanomacrophage (Mm) and blood congestion in portal vein were detected after 15 days (Fig. 12c). After 30 days of exposure and supplementation of tomato paste, the hepatopancreatic tissues retained its normal structure (Fig. 12d). So time of dietary supplementation of tomato paste may affect the process of repairing hepatocytes, sinusoids, blood vessels and hepatopancreatic tissue.

PAS-technique revealed distinct accumulation of carbohydrate materials in the hepatocytes and pancreatic acini in comparison to those of Cd-treated fish after 15 days (Fig. 13a-b). More accumulation of carbohydrate materials was observed in liver of fish treated for 30 days (Fig. 13c-d). These results referred to the role of tomato paste in preservation of carbohydrate materials.

Treatment with cadmium chloride plus vitamin E: Administration of vitamin E (α-tochopherol) plus cadmium to fishes for 15 days, produced histopathological alterations in the liver

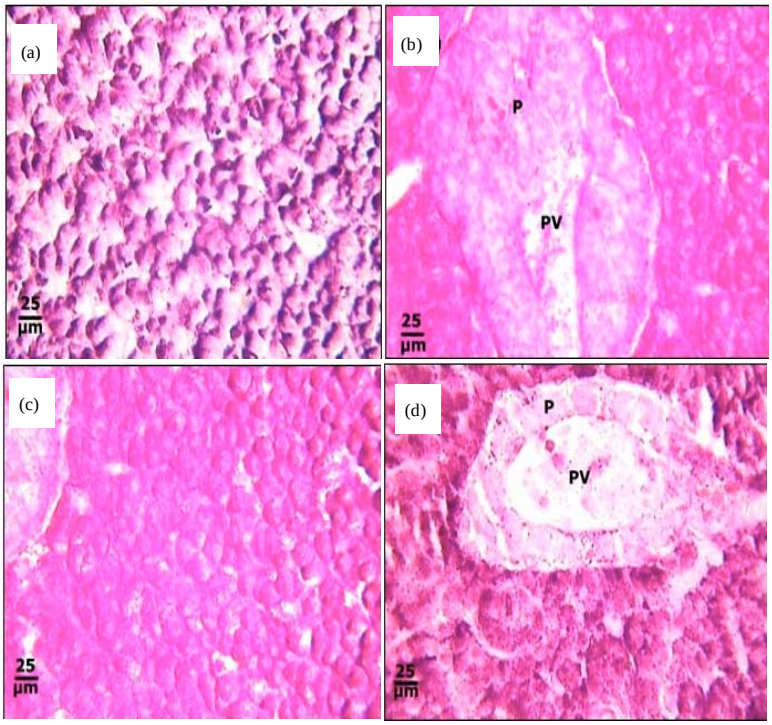

Fig. 13(a-d): Sections of treated fish liver (a) and hepatopancreas(b) exposed to tomato paste with (4.46 mg L^{-1}) cadmium for 15 days and fish liver (c) and hepatopancreas (d) exposed to the same dose for 30 days. (PAS, X 400). (a) Fish liver showing a remarkable good amount of glycogen in the liver cells. (b) Fish hepatopancreas showing moderate amount of glycogen content in the hepatopancreas. (c) Fish liver Showing moderate amount of glycogen in the liver cells after 30 days and (d) Fish hepatopancreas showing more depletion of glycogen content in the hepatopancreas after 30 days

tissue. These alterations included proliferation of hepatocytes in some fishes as indicated by the crowded of the nuclei and the limits between cells not clear. Some of hepatocytes showed vacuole degeneration (Fig. 14a). In others, the majority of the hepatocytes and sinusoids presented normal appearance.

After 30 days of exposure to the same dose of cadmium and vitamin E, improvement of the hepatic tissue was noticed when compared to those of Cd-treated fish. The majority of the hepatocytes and sinusoids showed normal appearance with small infiltration of the lymphocyte cells beside the central vein (Fig. 14b). However, some samples still suffering from a great reduction in the size of the hepatocytes. An aggregation of these cells in clumps was noticed as well the boundaries between cells are not clear. The nuclei of these aggregated cells were seen to be enlarged and hyperchromatic.

After 15 days of exposure, the hepatopancreatic tissue display peripheral fibrosis and dilatation in portal vein of hepatopancreas. Tiny aggregations of melanomacrophage cells were observed (Fig. 14c). After 30 days of exposure, the pancreatic cells of the examined sections retained its

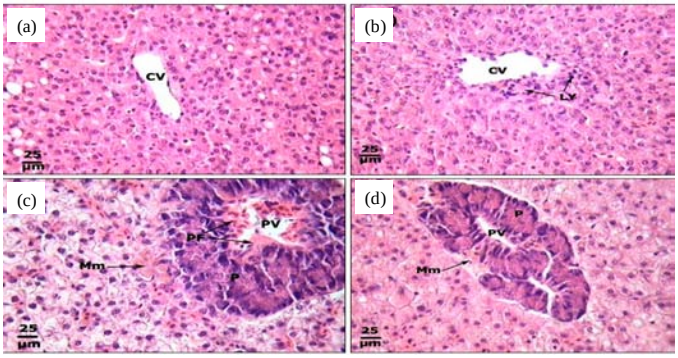

Fig. 14(a-d): Sections of treated fish liver (a and b) and hepatopancreas (c and d) exposed to vitamin E plus (4.46 mg L^{-1}) cadmium for 15 and 30 days. (H and E. X 400). (a) Fish liver showing proliferation of some hepatocytes and vacuole degeneration. (b) Fish liver showing the general structure and arrangement of the hepatocytes with lymphatic infiltration (LY) beside the central vein (CV) after 30 days (c) Fish hepatopancreas showing small aggregation of melanomacrophage cells (Mm), peripheral fibrosis (PF) and dilatation in portal vein (PV) of hepatopancreas and (d) Fish hepatopancreas showing normal structure of hepatopancreatic tissue with few number of melanomacrophage (Mm) cells after 30 days

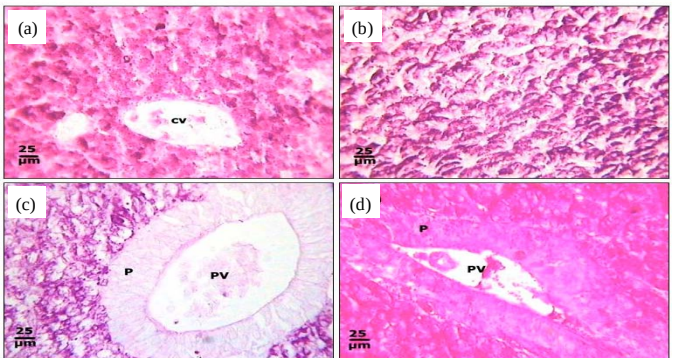

Fig. 15(a-d): Sections of treated fish liver (a and b) and hepatopancreas (c and d) exposed to vitamin E with (4.46 mg L^{-1}) cadmium for 15 and 30 days. (PAS X 400). (a) Fish liver Showing a moderate amount of glycogen in the liver cells. (b) Fish liver showing good amount of glycogen content in the liver cells after 30 days. (c) Fish hepatopancreas Showing depletion in glycogen content in the hepatopancreatic tissue and (d) Fish hepatopancreas showing increase in glycogen content in the hepatopancreas after 30 days

normal structure with a few number of melanomacrophage cells (Fig. 14d). One can conclude that tomato paste has a better protective role than vitamin E in this concern.

Histochemically, PAS-technique revealed a good preservation of carbohydrate materials (glycogen) in hepatocytes by vitamin E after 15 and 30 days (Fig. 15a-b). However, the

hepatopancreatic tissue revealed slight accumulation of glycogen in the acinar cells compared to those of Cd-treated fish after 15 days, such accumulation increased after 30 days (Fig. 15c-d). These results reflect the protective role of vitamin E in preservation of glycogen in a way similar to tomato paste.

Treatment with cadmium chloride, vitamin E and tomato paste: Apart from mild hydropic degeneration and proliferation of hepatic cells, an improvement in the hepatic tissue with slight cytoplasmic vacuolation was noticed when compared to those of Cd-treated fish or Cd- treated fish plus vitamin E for 15 days (Fig. 16a). After 30 days, beneficial improvements were noticed in the hepatic tissue of the exposed fishes. The majority of the hepatocytes and sinusoids had normal appearance with few proliferation of hepatic cell (Fig. 16b).

The hepatopancreatic tissue shows proliferation of the pancreatic acini, peripheral fibrosis and large aggregation of melanomacrophage cells around the pancreatic acini after 15 days of exposure (Fig. 16c). After 30 days of exposure to the same doses of cadmium, tomato paste and vitamin E, the pancreatic tissue of the examined sections retained its normal structure with small aggregation of melanomacrophage cells around the pancreatic acini (Fig. 16d).

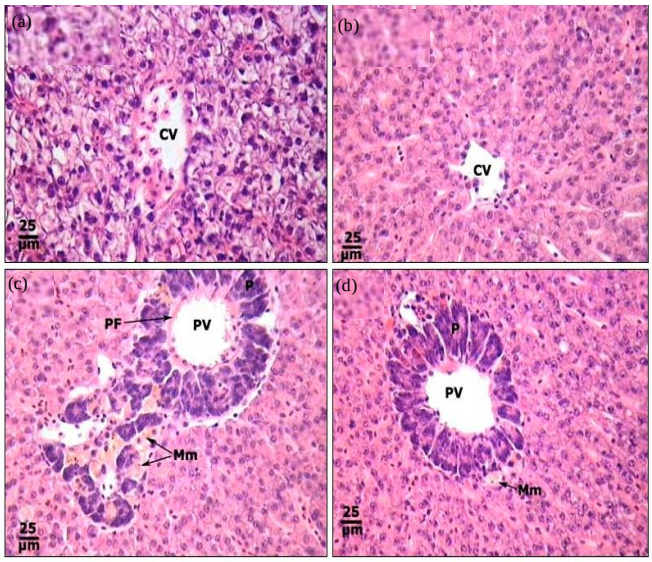

Fig. 16(a-d): Sections of treated fish liver (a and b) and hepatopancreas (c and d) exposed to tomato paste, vitamin E plus (4.46) cadmium for 15 and 30 days. (H and E. X 400). (a) Fish liver showing more or less normal structure of the liver (CV) central vein, (b) Fish liver showing proliferation and normal structure and arrangement of the hepatocytes, central vein (CV). after 30 days, (c) Fish hepatopancreas showing proliferation of the pancreatic acini, large aggregation of melanomacrophage (Mm) around the pancreatic acini and peripheral fibrosis (PF) in portal vein and (d) Fish hepatopancreas showing normal structure of pancreatic tissue with small aggregation of melanomacrophage cell (Mm) after 30 days

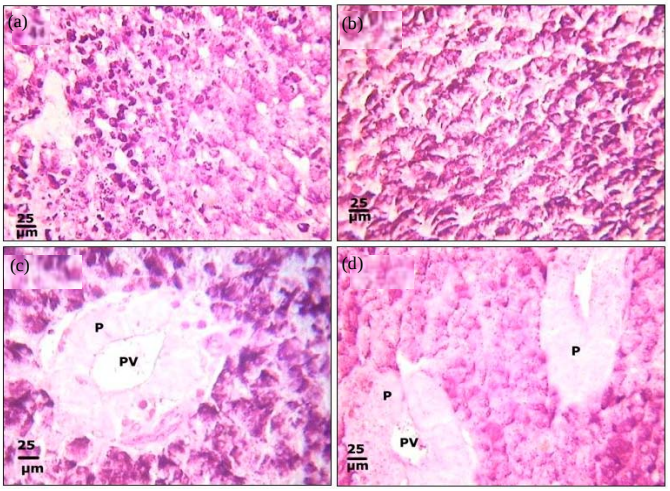

Fig. 17(a-d): Sections of treated fish liver (a and b) and hepatopancreas (c and d) exposed to tomato paste, vitamin E with (4.46 mg L^{-1}) cadmium for 15 and 30 days. (PAS.X 400). (a) Fish liver showing a moderate amount of glycogen in the liver cells, (b) Fish liver showing good amount of glycogen content in the liver cells after 30 days, (c) Fish hepatopancreas showing depletion in glycogen content in the hepatopancreatic tissue and (d) Fish hepatopancreas showing depletion in glycogen content in the hepatopancreatic tissue after 30 days

By using PAS- technique, a good restoration of glycogen content was noticed in the liver cells and hepatopancreatic tissue after 15 days with increased accumulation of glycogen after 30 days of exposure of fish to cadmium chloride, tomato paste and vitamin E synchronal (Fig. 17a-d).

DISCUSSION

Heavy metals disturb different living processes at the molecular and subcellular levels of biological organization leading to cell injury and in turn to degenerative and neoplastic diseases in target organs (Folmar, 1993; Cicik and Engin, 2005; Jacobson-Kram and Keller, 2001; Pacheco and Santos, 2002). Accordingly, changes in histopathological and biochemical characteristics as bioindicators are reliable evident of toxicity in fish organs (Schwaiger *et al*., 1996; Teh *et al*., 1997). There are several reports on the impact of environmental toxicants on fish revealed by histopathological, histochemical and biochemical studies of vital organs such as gills, liver and kidney (Barlas, 1999; Cengiz and Unlu, 2002; Cengiz *et al*., 2001; Erkmen *et al*., 2000; Gill *et al*., 1988; Richmonds and Dutta, 1989). To what extent this situation was revealed in Cd-stressed *O. niloticus* fed supplemented diet with tomato paste or vitamin E is considered.

Gill *et al*. (1990), Cengiz *et al*. (2001), Fanta *et al*. (2003), Cengiz and Unlu (2006), Velmurugan *et al*. (2007) and Benli *et al*. (2008) reported hepatic lesions including hypertrophy, vacuolization, nuclear pyknosis, karyolysis, karyohexis, fatty degeneration, hydropic degenerations, sinusoids enlargement, hemorrhage, infiltration of mononuclear lymphocyte, Cloudy swelling, focal necrosis, increase of Küpffer cells, circulatory disturbance, narrowing of sinusoids and congestion.

The miniaturization of histological changes in fish liver is a highly sensitive and accurate way to assess the effects of xenobiotic compounds in field and experimental studies

(Figueiredo-Fernandes *et al*., 2007). In the present investigation, some of pathological features noticed in the liver of fish exposed to 4.64 mg L^{-1} of cadmium chloride at both periods of exposure were the cytoplasmic vacuolation and fatty degeneration associated with lipid accumulation. Hinton and Lauren (1990) reported that vacuolation of hepatocytes are associated with the inhibition of protein synthesis, energy depletion, disaggregation of microtubules, or shifts in substrate utilization. A similar result was reported by Van Dyk *et al*. (2007) on *Oreochromis mossambicus* after treatment with cadmium and zinc. Such vacuolation was regarded by El-Banhawy *et al*. (1986) as a cellular defense mechanism against injurious substances to prevent their interfering with cellular metabolism. The cytoplasmic vacuolization may be also attributed to the increased number and activities of lysosomes and their contents El-Banhawy *et al*. (1993). Desai *et al*. (1984) and Gabr (1986) confirmed these conclusions on *Oreochromis mossambica* and *Oreochromis niloticus* after treatment with monocrotophos and diasinon insecticides, respectively.

Cellular degeneration followed by nuclear pyknosis and necrosis was observed in the present investigation. These alternations are in agreement with those of Wassif *et al*. (2000), Elezabi *et al*. (2001), Fathalla *et al*. (2001), Mohamed *et al*. (2005) and Van Dyk *et al*. (2007). Such cellular degeneration occurs directly by denaturation of volume-regulating ATPases or indirectly by disruption of the cellular energy transfer processes required for ionic regulation (Hinton and Lauren (1990). El-Banhawy *et al*. (1993) mentioned that there is a relationship between pathological alternations (liver damage) and reduction in activities of oxidative enzymes such as cytochrome oxidase, succinic dehydrogenase, coenzyme I, diphosphopyridine nucleotidase DPN, cytochrome p-450 and β-glucuronidase under various pathological conditions.

Desai *et al*. (1984) suggested that the necrosis could be either due to direct effect of the compound on the cells or to an accumulation of acetylcholine in the tissues.

Venous congestion was recorded in the present study may be due to the decline in the haematological parameters which observed in the present study. Such observation was reported by Mekkawy *et al*. (1996), since the haemoglobin contents are insufficient for the respiration of the tissue. This results was in agreement with Fathalla *et al*. (2001), Khidr *et al*. (2001) and Mohamed *et al*. (2005).

The remarkable abundance of the lymphocytic infiltration in the liver tissues was another pathological sign recorded in the present work. Such pathological sign was confirmed by many authors as a response of heavy metal stress (El-Banhawy *et al*., 1993; Khidr *et al*., 2001; Mohamed, 2009; Wassif *et al*., 2000). The Küpffer cells were increased in size and number as a defense mechanism against stress (Kadry, 1989; Mahmoud, 1999; Popper and Schaffner, 1957) and against any foreign material in the circulating blood (Mahmoud, 1999).

In the present investigation, a remarkable collection of inflammatory cells adjacent to some blood vessels and invasion of the parenchyma cells were observed. Such cellular infiltration might be due to the presence of necrotic cells which act as an irritant substance attracting the inflammatory cells (Walter and Israel, 1974). In general, the abundance of leukocytes and lymphocytes in particular is a prominent response of body tissues facing any injurious impacts (El-Banhawy *et al*., 1993).

The number, size and contents of melanomacrophages are highly variable, not only between species but also related to the health status of fish (Bunton *et al*., 1987; Matthiessen and Roberts, 1982). Migration of such melanomacrophages into the liver parenchyma after exposure to cadmium was observed in the present study. A similar result has been reported by Braunbeck *et al*. (1990) in Zebra fish (*Brachydanio rerio*) and trout (*Salmo gairdneri*) following exposure to 4-chloroaniline

(Braunbeck *et al.*, 1992) in *Oryzias latipes* after exposure to diethylnitrosamine and by Oulmi *et al.* (1995a) in rainbow trout (*Oncorhynchus mykiss*) exposed to sublethal concentration of linuron. The migration of macrophages and removal of damaged cell components as well as necrotic hepatocytes represented adaptive rather than degenerative features (Oulmi *et al.*, 1995a).

Chronic liver diseases are characterized by progressive accumulation of connective tissue undergoing fibrotic degeneration (Popper and Kent, 1975). Under Cd-stress, a remarkable collection of connective tissue fiber adjacent to some blood vessels in the liver tissue was observed in the present work. The common link between chronic liver damage and hepatic fibrosis representing severe oxidative stress, has been found to be associated with the activation of Hepatic Stellate Cells (HSC) (Svegtiati Baroni *et al.*, 1998). Such oxidative stress seems to occur at a prefibrotic stage suggesting a causative relation between oxidative events and liver fibrosis (Vendemiale *et al.*, 2001). These findings were confirmed by many authors (El-Sokkary *et al.*, 2005; Gualdi *et al.*, 1994; Hernandez-Munoz *et al.*, 1997; Muriel *et al.*, 1994; Niemela *et al.*, 1995).

The disruption or dissolution of normal arrangement of the hepatocytes observed in the present study has been frequently reported by Couch (1975), Smith and Piper (1975) and Hacking *et al.* (1978).

The aggregation of lymphocytes around the hepatopancreatic acini, proliferation of the hepatopancreatic acini, crowdness of cells and depletion in the acidity of the apical portion of pancreatic acinar cells were observed in the hepatopancreas of fish exposed to 4.64 mg L^{-1} of cadmium chloride at both periods of exposure. Wassif *et al.* (2000) and Hussein and Mekkawy (2001) recorded similar results when the first authors exposed *Oreochromis niloticus* to different concentrations of atrazine and the others exposed *Oreochromis zillii* to sublethal concentration of lead.

Depletion in the acidity of the apical portion of pancreatic acinar cells after exposure to cadmium was observed in the present study. A similar result has been reported by Hara *et al.* (1994) who found that bis (tributylin) oxide (TBTO) inhibited both the synthesis and secretion of zymogen granules in the rat exocrine pancreas. They attributed this inhibition to mitochondria dysfunction due to the toxic effects of TBTO. Oulmi *et al.* (1995b) reported that the exposure of rainbow trout to atrazine caused disorganization of Golgi fields in the distal segment cells of the renal tubules.

In the present investigation, histochemical results showed a remarkable decrease in glycogen amount in the liver of fish exposed to cadmium chloride. This finding is consistent with other studies, which showed a reduction in hepatic glycogen following exposure to xenobiotic compounds (Biagianti-Risbourg, 1996; Gluth and Hanke, 1985) and exposure to herbicides (Mekkawy *et al.*, 1996; Wassif *et al.*, 2000).

This observation might be due to increased glycolytic activity to meet the energy demands imposed by enhanced metabolic activity, hormone-mediated stress phenomenon (Gluth and Hanke, 1985; Hanke *et al.*, 1983). Heath (1995) explained the loss of glycogen as a depressed feeding and/or elevated levels of the stress hormones, cortisol and adrenaline. Glycogen can also be depleted in response to some physiological processes such as sexual maturation (Yamamoto and Egami, 1974), or nonchemical stresses, such as temperature (Braunbeck *et al.*, 1987) and hypoxia (De Zwaan and Zandee, 1972; Mekkawy *et al.*, 1996; Yuness, 2005).

In the present study, administration of antioxidants tomato paste (tomato paste) as dietary supplementation remarkable protective effects and restored the general structure and the carbohydrate contents of the liver. Matos *et al.* (2001) reported minimum histopathological and no macro- morphological changes in the liver treated with tomato paste against Ferric Nitrilotriacetate

toxicity in Rats. These results postulated that carotenoids (lycopene) are well known as highly efficient scavengers of singlet-oxygen ($^1O_2$) and other excited species. During $^1O_2$ quenching, energy is transferred from $^1O_2$ to the lycopene molecule, converting it to the energy-rich triplet state and thus preventing their damage (Atessahin *et al*., 2005).

In the present study, administration of antioxidant vitamin E to fish exposed to cadmium chloride display remarkable protective effects and restored the general structure and carbohydrate contents in the liver. The present results are in agreement with those observed in liver of *Clarias gariepinus* exposed to vitamin E against Lead -induced oxidative stress (Mohamed, 2006) and in liver of rats exposed to vitamin E against cisplatin -induced oxidative stress (Naziro *et al*., 2004).

The histopathological and histochemical changes resulted from cadmium oxidative stress was nearly disappeared in tissues of the studied organs by administration of lycopene and Vitamin E.

REFERENCES

Abdel-Rahman, M.A., 1997. Toxicological studies of heavy metals on *Siganus rivulatus*. M.Sc. Thesis, Alexandria University, Alexandria, Egypt.

Alabaster, J.S. and R. Lloyd, 1982. Water Quality Criteria for Freshwater Fish. 2nd Edn., Butterworths, London.

Almeida, J.A., Y.S. Diniz, S.F.G. Marques, L.A. Faine, B.O. Ribas, R.C. Burneiko and E.L.B. Novelli, 2002. The use of oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination. Environ. Int., 27: 673-679.

Atessahin, A., S. Yilmaz, I. Karahan, A.O. Ceribasi and A. Karaoglu, 2005. Effects of lycopene against cisplatin-induced nephrotoxicity and oxidative stress in rats. Toxicology, 212: 116-123.

Aust, O., H. Sies, W. Stahl and M.C. Polidori, 2001. Analysis of lipophilic antioxidants in human serum and tissues: Tocopherols and carotenoids. J. Chromatogr., 936: 83-93.

Bais, U.E. and M.V. Lokhande, 2012. Effect of cadmium chloride on histopathological changes in the freshwater fish *Ophiocephalus striatus* (*Channa*). Int. J. Zool. Res., 8: 23-32.

Bancroft, J.D. and A. Stevens, 1982. Theory and Practice of Histological Techniques. 3rd Edn., Churchill Livingstone, Edinburgh, London, ISBN: 9780443035593, Pages: 726.

Barlas, N., 1999. Histopathological examination of gill, liver and kidney tissues of carp (*Cyprinus carpio* L., 1758) fish in the upper Sakarya River Basin. Turk. J. Vet. Anim. Sci., 23: 277-284.

Benli, A.C.K., G. Koksal and A. Ozkul, 2008. Sublethal ammonia exposure of Nile tilapia (*Oreochromis niloticus* L.): Effects on gill, liver and kidney histology. Chemosphere, 72: 1355-1358.

Boileau, T.W., Z. Liao, S. Kim, S. Lemeshow, J.W. Erdman Jr. and S.K. Clinton, 2003. Prostate carcinogenesis in N-methyl-N-nitrosourea (NMU)-testosterone-treated rats fed tomato powder, lycopene, or energy-restricted diets. J. Natl. Cancer Inst., 95: 1578-1586.

Biagianti-Risbourg, S., 1996. Disturbances (Ultra) Structural Liver of Fish Supplies Used as Biomarkers of Quality Health of Aquatic Environments. In: Use of Biomarkers in Ecotoxicologie, Fundamentals, Lagadic, L., J.C. Amiard, T. Caqueta and F.S. Ramadi (Eds.). Masson Pub., Paris, pp: 355-391.

Bramley, P.M., 2000. Is lycopene beneficial to human health?. Phytochemistry, 54: 233-236.

Braunbeck, T., K. Gorgas, V. Storch and A. Volki, 1987. Ultrastructure of hepatocytes in golden ide (*Leuciscus idus melanotus* L.: Cyprinidae: Teleostei) during thermal adaptation. Anat. Embryol., 175: 303-313.

Braunbeck, T., V. Storch and H. Bresch, 1990. Species-specific reaction of liver ultrastructure in zebra fish (*Brachydanio rerio*) and trout (*Salmo gairdneri*) after prolonged exposure to 4-chloroaniline. Arch. Environ. Contam. Toxicol., 19: 405-418.

Braunbeck, T.A., S.J. The, S.M. Lester and D.E. Hinton, 1992. Ultrastructural alterations in liver of medaka (*Oryzias latipes*) exposed to diethylnitrosamine. Toxicol. Pathol., 20: 179-196.

Brusle, J. and G.G. Anadon, 1996. The Structure and Function of Fish Liver. In: Fish Morphology, Munshi, J.S.D. and H.M. Dutta, (Eds.). Science Publishers Inc., Enfield, NH USA., pp: 77-93.

Bunton, T.E., S.M. Baksi, S.G. George and J.M. Frazier, 1987. Abnormal hepatic copper storage in a teleost fish (*Morone americana*). Vet. Pathol., 24: 515-524.

Capanoglu, E., J. Beekwilder, D. Boyacioglu, R. Hall and R. de Vos, 2008. Changes in antioxidant and metabolite profiles during production of tomato paste. J. Agric. Food Chem., 56: 964-973.

Cengiz, E.I., E. Unlu and K. Balci, 2001. The histopathological effects of thiodan on the liver and gut of mosquitofish, *Gambusia affinis*. J. Environ. Sci. Health. B, 32: 75-85.

Cengiz, E.I. and E. Unlu, 2002. Histopathological changes in the gills of mosquitofish. *Gambusia affinis* exposed to endosulfan. Bull. Environ. Contam. Toxicol., 68: 290-296.

Cengiz, E.I. and E. Unlu, 2006. Sublethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquitofish, *Gambusia affinis*: A microscopic study. Environ. Toxicol. Pharmacol., 21: 246-253.

Chew, B.P., 1995. Antioxidant vitamins affect food animal immunity and health. J. Nutr., 125: 1804S-1808S.

Choudhari, S.M. and L. Ananthanarayan, 2007. Enzyme aided extraction of lycopene from tomato tissues. Food Chem., 102: 77-81.

Cicik, B. and K. Engin, 2005. The Effect of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio* (L. 1758). Turk. J. Vet. Anim. Sci., 29: 113-117.

Clinton, S., 1998. Lycopene: Chemistry, Biology and Implications for human health and disease. Nutr. Rev., 56: 35-51.

Couch, J.A., 1975. Histopathological Effects of Pesticides and Related Chemicals on the Liver of Fishes. In: The Pathology of Fishes, Ribelin, W.E. and G.S. Migaki (Eds.). University of Wisconsin Press, Madison, USA., pp: 559-584.

Cross, F.A. and W.G. Sunda, 1985. The Relationship Between Chemical Speciation and Bioavailability of Trace Metals to Marine Organisms: A Review. In: Utilization of Coastal Ecosystems: Planning, Pollution and Productivity, Chao, N.L. and W. Kirby-Smith, (Eds.). Rio Grande, Brazil, pp: 169-182.

De Zwaan, A. and D.I. Zandee, 1972. The utilization of glycogen and accumulation of some intermediates during anaerobiosis in *Mytilus edulis* L. Comp. Biochem. Physiol. Part B: Comp. Biochem., 43: 47-54.

Desai, A.K., U.M. Joshi and P.M. Ambadkar, 1984. Histological observations on the liver of Tilapia mossambica after exposure to monocrotophos, an organophosphorus insecticide. Toxicol. Lett., 21: 325-331.

Di Mascio, P., S. Kaiser and H. Sies, 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch. Biochem. Biophys., 274: 532-538.

El-Banhawy, M.A., A. Al-Zahaby and A. Shalaby, 1986. Histopathological studies on the effect of cyolane on the ileum of Clarias lazira. Egypt. J. Histol., 9: 77-85.

El-Banhawy, M.A., S.M. Sanad, S.A. Sakr, I.A. El-Elaimy and H.A. Mahran, 1993. Histopathological studies on the effect of anticoagulant rodenticide Brodifacoum on the liver of rat. J. Egypt. Ger. Soc. Zool., 17: 369-393.

El-Demerdash, F.M., M.I. Yousef, F.S. Kedwany and H.H. Baghdadi, 2004a. Cadmium induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: Protective role of vitamin E and b-carotene. Food Chem. Toxicol., 42: 1563-1571.

El-Demerdash, F.M., M.I. Yousef, F.S. Kedwany and H.H. Baghdadi, 2004b. Role of a-tocopherol and ß-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato-biochemical parameters and semen quality of male rats. J. Environ. Sci. Health. B, 39: 443-459.

El-Sokkary, G.H., G.H. Abdel-Rahman and E.S. Kamel, 2005. Melatonin protects against lead-induced hepatic and renal toxicity in male rats. Toxicology, 213: 25-33.

Elezabi, M.M., S. Elserafy, R. Heckmann, K.S. Eldeen and M.N. Seddek, 2001. Effects of some toxicants on the freshwater fish *Oreochromis niloticus*. J. Egypt. Ger. Soc. Zool., 36: 407-429.

Erkmen, B., M. Caliskan and S.V. Yerli, 2000. Histopathological effects of cyphenothrin on the gills of *Lebistes reticulatus*. Vet. Hum. Toxicol., 42: 5-7.

Fanta, E., F.S. Rios, S. Romao, A.C.C. Vianna and S. Freiberger, 2003. Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. Ecotoxicol. Environ. Saf., 54: 119-130.

Fathalla, M.M., M.A. Ashry and H.S. Gaber, 2001. The effect of water pollution on histological pattern of gill: Liver and kidney of Nile tilapia *Oreochromis niloticus*. J. Egypt. Ger. Soc. Zool., 36: 229-241.

Figueiredo-Fernandes, A., A. Fontaínhas-Fernandes, F. Peixoto, E. Rocha and M.A. Reis-Henriques, 2006a. Effect of paraquat on oxidative stress enzymes in Tilapia, *Oreochromis niloticus*, at two levels of temperature. Pest. Biochem. Physiol., 85: 97-103.

Figueiredo-Fernandes, A., A. Fontaínhas-Fernandes, R.A.F. Monteiro, M.A. Reis-Henriques and E. Rocha, 2006b. Effects of the fungicide mancozeb in the liver structure of Nile tilapia, *Oreochromis niloticus* assessment and quantification of induced cytological changes using qualitative histopathology and the stereological point-sampled intercept method. Bull. Environ. Contam. Toxicol., 76: 249-255.

Figueiredo-Fernandes, A., J.V. Ferreira-Cardoso, S. Garcia-Santos, S.M. Monteiro, J. Carrola, P. Matos and A. Fontainhas-Fernandes, 2007. Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. Pesq. Vet. Bras., 27: 103-109.

Folmar, L.C., 1993. Effects of chemical contaminants on blood chemistry of teleost fish: A bibliography and synopsis of selected effects. Environ. Toxicol. Chem., 12: 337-375.

Gabr, S.A., 1986. Physiological, biochemical and morphological studies on the effect of insecticides on the fishes of high Dam Lake. Ph.D. Thesis, Faculty of Science, Assiut University, Egypt.

Gadagbui, B.K.M., M. Addy and A. Goksoyr, 1996. Species characteristics of hepatic biotransformation enzymes in two tropical freshwater teleosts, tilapia (*Oreochromis niloticus*) and mudfish (*Clarias anguillaris*). Comparat. Biochem. Physiol. Part C: Pharmacol. Toxicol. Endocrinol., 114: 201-211.

Gann, P.H. and F. Khachik, 2003. Tomatoes or lycopene versus prostate cancer: Is evolution anti-reductionist?. J. Natl. Cancer Inst., 95: 1563-1565.

Garcia-Santos, S., A. Fontainhas-Fernandes and J.M. Wilson, 2006. Cadmium tolerance in the Nile tilapia (*Oreochromis niloticus*) following acute exposure: Assessment of some ionoregulatory parameters. Environ. Toxicol., 21: 33-46.

Gill, T.S., J.C. Pant and J. Pant, 1988. Gill, liver and kidney lesions associated with experimental exposures to carbaryl and dimethoate in the fish (*Puntius conchonius* ham.). Bull. Environ. Contam. Toxicol., 41: 71-78.

Gill, T.S., J. Pande and H. Tewari, 1990. Hepatopathotoxicity of three pesticides in a freshwater fish (*Puntius conchonius* Ham). J. Environ. Sci. Health., 25: 653-663.

Gluth, G. and W. Hanke, 1985. A comparison of physiological changes in carp, *Cyprinus carpio*, induced by several pollutants at sublethal concentrations. I. The dependency on exposure time. Ecotoxicol. Environ. Safety, 9: 179-188.

Gualdi, R., G. Casalgrandi, G. Montosi, E. Ventura and A. Pietrangelo, 1994. Excess iron into hepatocytes is required for activation of collagen type I gene during experimental siderosis. Gastroenterology, 107: 1118-1124.

Hacking, M.A., J. Budd and K. Hodson, 1978. The ultrastructure of the liver of the rainbow trout: Normal structure and modifications after chronic administration of a polychlorinated biphenyl Aroclor 1254. Can. J. Zool., 56: 477-491.

Hadley, C.W., E.C. Miller, S.J. Schwartz and S.K. Clinton, 2002. Tomatoes, lycopene and prostate cancer: Progress and promise. Exp. Biol. Med., 227: 869-880.

Hanke, W., G. Gluth, H. Bubel and R. Muller, 1983. Physiological changes in carps induced by pollution. Ecotoxicol. Environ. Safety, 7: 229-241.

Hara, K., M. Yoshizuka and S. Fujimoto, 1994. Toxic effects of bis (tributyltin) oxide on the synthesis and secretion of zymogen granules in the rat exocrine pancreas. Arch. Histol. Cytol., 57: 201-212.

Heath, A.G., 1995. Water Pollution and Fish Physiology. CRC Press, Boca Raton, Fla.

Heber, D. and Q.Y. Lu, 2002. Overview of mechanisms of action of lycopene. Exp. Biol. Med., 227: 920-923.

Hernandez-Munoz, R., M. Diaz-Munoz, V. Lopez, F. Lopez-Barrera and L. Yanez *et al*., 1997. Balance between oxidative damage and proliferative potential in an experimental rat model of CCl_4-induced cirrhosis: Protective role of adenosine administration. Hepatology, 26: 1100-1110.

Hinton, D.E. and D.J. Lauren, 1990. Integrative histopathological effects of environmental stressors on fishes. Am. Fish. Soc. Symp., 8: 51-66.

Hogan, J.W. and C.O. Knowles, 1968. Degradation of organophosphates by fish liver phosphatases. J. Fish. Res. Board Can., 25: 1271-1579.

Houston, A., S. Blahut, A. Mourad and P. Amirtharaj, 1993. Changes in erythron organization during prolonged cadmium exposure: An indicator of heavy metal stress?. Can. J. Fish. Aquat. Sci., 50: 217-222.

Humason, G.L., 1979. Animal Tissue Techniques. W.H. Freeman and Co., San Francisco.

Hussein, S.Y. and I.A.A. Mekkawy, 2001. The effects of lead-exposure and lead-clay interaction on the growth performance, biochemical and physiological characteristics and histopathology of *Tilapia zillii*. Bull. Fac. Sci., Assiut Univ., 30: 65-97.

Jacobson-Kram, D. and K.A. Keller, 2001. Toxicology Testing Handbook: Principles, Applications and Data Interpretation. Marcel Dekker, New York, ISBN: 9780824700737, Pages: 428.

Kadry, S.M., 1989. Studies on the effect of cypermethrin on the tissues of the freshwater catfish Clarias lazera. Egypt. J. Histol., 12: 271-276.

Karlsson-Norrgren, L., P. Runn, C. Haux and L. Forlin, 1985. Cadmium-induced changes in gill morphology of zebrafish, *Brachydanio rerio* (Hamilton-Buchanan) and rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol., 27: 81-95.

Khidr, B.M., E.T. Wassif, S.Y. Hussein and I.A.A. Mekkawy, 2001. Studies on the effect of the herbicide atrazine on the liver and kidney of the fresh water catfish *Chrysichys auratus*. J. Egypt. Ger. Soc. Zool., 34: 283-300.

Kock, G., M. Triendl and R. Hofer, 1996. Seasonal patterns of metal accumulation in Arctic char (*Salvelinus alpinus*) from an oligotrophic Alpine lake related to temperature. Can. J. Fish Aquat. Sci., 53: 780-786.

Krinsky, N.I., 2001. Carotenoids as antioxidants. Nutrition, 17: 815-817.

Larsson, A., C. Haux and M. Sjobeck, 1985. Fish physiology and metal pollution results and experiences from laboratory and field studies. Ecotoxicol. Environ. Saf., 9: 250-281.

Lemaire-Gony, S. and P. Lemaire, 1992. Interactive effects of cadmium and benzo-a-pyrene on cellular structure and biotransformation enzymes of the liver of the European eel *Anguilla anguilla*. Aquat. Toxicol., 22: 145-159.

Lloyd, R., 1992. Pollution and Freshwater Fish. Fishing News Books, London.

Mahmoud, A.A., 1999. Cytogenetic studies on the effect of some chemical pollution on a fresh water fish. M.Sc. Thesis, Faculty of science, Zagazig University (Benha branch), Egypt.

Manca, D., A.C. Richard, B. Trottier and G. Chevalier, 1991. Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicology, 67: 303-323.

Martin, M.H. and P.J. Coughtrey, 1982. Biological Monitoring of Heavy Metal Pollution. Applied Science Publishers, London.

Mason, C.F., 1991. Biology of Freshwater Pollution. Longman Science and Technology, Harlow England.

Matos, H.R., V.L. Capelozzi, O.F. Gomes, P.D. Mascio and M.H.G. Medeiros, 2001. Lycopene inhibits DNA damage and liver necrosis in rats treated with ferric nitrilotriacetate. Arch. Biochem. Biophys., 396: 171-177.

Matthiessen, P. and R.J. Roberts, 1982. Histopathological changes in the liver and brain of fish exposed to endosulfan insecticide during tsetse fly control operations in Botswana. J. Fish Dis., 5: 153-159.

McManus, J.F.A., 1946. Histological demonstration of mucin after periodic acid. Nature, 158: 202-202.

Mekkawy, I.A.A., S.Y. Hussein, M. Abd El-Nasser and S.M. Ahmed, 1996. Comparative studies on the effects of herbicide atrazine on some blood constituents and protein electrophoretic patterns of *Oreochromis niloticus* and *Chrysichthyes auratus* at Assiut, Egypt. J. Egypt Ger. Soc. Zool., 19: 283-319.

Mekkawy, I.A.A. and F.E. Lashein, 2003. The effect of lead and cadmium on LDH and G-6-PDH isozyme patterns exhibited during the early embryonic development of the teleost fish, *Ctenopharyngodon idellus* with emphasis on the corresponding morphological variations. Proceeding of the 26th Annual Larval Fish Conference, July 22-26, 2002, Bergen, Norway.

Mekkawy, I.A.A., U.M. Mahmoud, E.T. Wassif and M. Naguib, 2010. Effects of cadmium on some haematological and biochemical characteristics of *Oreochromis niloticus* (Linnaeus, 1758) dietary supplemented with tomato paste and vitamin E. Fish Physiol. Biochem., 37: 71-84.

Meyers, M.S., O.P. Olson, L.L. Johson, C.S. Stehr, T. Hom and U. Varunasi, 1992. Hepatic lesions other than neoplasms in subadult flatfish from puget sound, washington: Relationships with indices of contaminant exposure. Mar. Environ. Res., 34: 45-51.

Mohamed, F.A.S., 2009. Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. World J. Fish Mar. Sci., 1: 29-39.

Mohamed, S.H., 2006. Studies on the protective effect of Melatonin, vitamin C and vitamin E on Lead-Induced oxidative stress on *Clarias gariepinus*. M.Sc. Thesis, Assiut University, Egypt.

Mohamed, S.M., H.Z. Khalid, I.H. Magdy and F.M. Karima, 2005. The clinical signs, histopathological and physiological status associated with acute and chronic exposure to Benzo (A) pyrene in the cultured fish *Oreochromis niloticus*. J. Egypt. Ger. Soc. Zool., 47: 283-312.

Mowry, R.W., 1956. Alcian blue techniques for histochemical study and acidic carbohydrates. J. Histoch. Cytochem., 4: 407-407.

Muramoto, S., 1981. Vertebral column damage and decrease of calcium concentration of fish exposed experimentally to cadmium. Environ. Pollut., 24: 125-133.

Muriel, P., O.R. Suarez, P.M. Gonzalez and L. Zuniga, 1994. Protective effect of S-adenosyl-l-methionine on liver damage induced by biliary obstruction in rats: A histological, ultrastructural and biochemical approach. J. Hepatol., 21: 95-102.

Naziro, M., A. Karao and A.O. Aksoy, 2004. Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats. Toxicology, 195: 221-230.

Niemela, O., S. Parkkila, S. Yla-Herttuala, J. Villanueva, B. Ruebner and C.H. Halsted, 1995. Sequential acetaldehyde production, lipid peroxidation and fibrogenesis in micropig model of alcohol-induced liver disease. Hepatology, 22: 1208-1214.

Okajima, E., M. Tsutsumi, S. Ozono, H. Akai and A. Denda *et al*., 1998. Inhibitory effect of tomato juice on rat urinary bladder carcinogenesis after N-butyl-N-(4-hydroxybutyl) nitrosamine initiation. Jap. J. Cancer Res., 89: 22-26.

Ortuno, J., A. Cuesta, M. Esteban and J. Meseguer, 2001. Effect of oral administration of high vitamin C and E dosages on the gilthead seabream (*Sparus aurata* L.) innate immune system. Vet. Immunol. Immunopathol., 79: 167-180.

Oulmi, Y., R.D. Negele and T. Braunbeck, 1995a. Cytopathology of liver and kidney in rainbow trout *Oncorhynchus mykiss* after long-term exposure to sublethal concentrations of linuron. Dis. Aquat. Org., 21: 35-52.

Oulmi, Y., R.D. Negele and T. Braunbeck, 1995b. Segment specificity of the cytological response in rainbow trout (*Oncorhynchus mykiss*) renal tubules following prolonged exposure to sublethal concentrations of atrazine. Ecotoxicol. Environ. Saf., 32: 39-50.

Pacheco, M. and M.A. Santos, 2002. Biotransformation, genotoxic and histopathological effects of environmental contaminants in European ell (*Anguilla anguilla* L.). Ecotoxicol. Environ. Saf., 53: 331-347.

Popper, H. and F. Schaffner, 1957. Liver Structure and Function. McGraw-Hill Book Company Inc., New York, London.

Popper, H. and G. Kent, 1975. Fibrosis in chronic liver diseases. Clin. Gastroenterol., 4: 315-332.

Pregiosi, P., P. Galan, B. Hebeth, P. Valeix and A.M. Roussel *et al*., 1998. Effects of supplementation with a combination of antioxidant vitamins and trace elements, at nutritional doses, on biochemical indicators and markers of the antioxidant system in adult subjects. J. Am. Coll. Nutr., 17: 244-249.

Rainbow, P.S. and S.L. White, 1989. Comparative strategies of heavy metal accumulation by crustaceans: Zinc, copper and cadmium in a decapod, an amphipod and a barnacle. Hydrobiologia, 174: 245-262.

Rainbow, P.S., 1985. The biology of heavy metals in the sea. Int. J. Environ. Stud., 25: 195-211.

Ranganna, S., 1976. Manual of Analysis of Fruits and Vegetable Products. Tata McGraw Hill, New Delhi, India, Pages: 634.

Rao, A.V. and S. Agarwal, 1999. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases a review. Nutr. Res., 19: 305-323.

Richmonds, C. and H.M. Dutta, 1989. Histopathological changes induced by malathion in the gills of bluegill *Lepomis macrochirus*. Bull. Environ. Contam. Toxicol., 43: 123-130.

Rodriguez, A., A. Cuesta, M.A. Esteban and J. Mesegure, 2004. The effect of dietary administration of the fungus *Mucor circinelloides* on non-specific immune responses of gilthead seabream. Fish Shellfish Immunol., 16: 241-249.

Sanders, M.J., 1997. A field evaluation of the freshwater river crab, *Potamonautes warreni*, as a bio-accumulative indicator of metal pollution. M.Sc. Thesis, Rand Afrikaans University, South Africa.

Sarkar, S., P. Yadav and D. Bhatnagar, 1997. Cadmium-induced lipid peroxidation and the antioxidant system in at erythrocytes: The role of antioxidants. Trace Elem. Med. Biol., 11: 8-13.

Schwaiger, J., K. Fent, H. Stecher, H. Ferling and R.D. Negele, 1996. Effects of sublethal concentrations of triphenyltriacetate on rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol., 30: 327-334.

Shaikh, Z.A., T.T. Vu and K. Zaman, 1999. Oxidative stress as a mechanism of chronic cadmium induced hepatotoxicity and renal toxicity and protection by antioxidants. Toxicol. Applied Pharmacol., 154: 256-263.

Shen, Y. and S. Sangiah, 1995. Na^+, K^+-ATPase, glutathione and hydroxyl free radicals in cadmium chloride-induced testicular toxicity in mice. Arch. Environ. Contam. Toxicol., 29: 174-179.

Smith, C.E. and R.G. Piper, 1975. Lesions Associated with Chronic Exposure to Ammonia. In: The Pathology of Fish, Rubelin, W.E. and G. Migaki (Eds.). University of Wis. Press, Madison, pp: 497-514.

Soengas, J.L., M.J. Agra-Lago, B. Carballo, M.D. Andres and J.A.R. Veira, 1996. Effect of an acute exposure to sublethal concentration of cadmium on liver carbohydrate metabolism of Atlantic salmon (*Salmo salar*). Bull. Environ. Contam. Toxicol., 57: 625-631.

Svegtiati Baroni, G., L. D'Ambrosio, G. Ferretti, A. Casini and A. Di Srio *et al.*, 1998. Fibrogenic effect of oxidative stress on rat hepatic stellate cells. Hepatology, 27: 720-726.

Tapiero, H., D.M. Townsend and K.D. Tew, 2004. The role of carotenoids in the prevention of human pathologies. Biomed Pharm., 58: 100-110.

Teh, S.J., S.M. Adams and D.E. Hinton, 1997. Histopathological biomarkers in fetal freshwater fish populations exposed to different types of contaminants stress. Aquat. Toxicol., 37: 51-70.

Van Dyk, J.C., G.M. Pieterse and J.H.J. van Vuren, 2007. Histological changes in the liver of *Oreochromis mossambicus* (Cichlidae) after exposure to cadmium and Zinc. Ecotoxicol. Environ. Saf., 66: 432-440.

Velmurugan, B., M. Selvanayagam, E.I. Cengiz and E. Unlu, 2007. Histopathology of lambda-cyhalothrin on tissues (gill, kidney, liver and intestine) of *Cirrhinus mrigala*. Environ. Toxicol. Pharmacol., 24: 286-291.

Vendemiale, G., I. Grattagliano, M.L. Caruso, G. Serviddio, A.M. Valentini, M. Pirrelli and E. Altomare, 2001. Increased oxidative stress in dimethylnitrosamine-induced liver fibrosis in the rat: Effect of N-acetylcysteine and interferon-&alpha: Toxicol. Applied Pharmacol., 175: 130-139.

Verbost, P.M., G. Flik, R.A. Lock and S.E. Wendelaar-Bonga, 1987. Cadmium inhibition of Ca^{2+} uptake in rainbow trout gills. Am. J. Physiol., 253: R216-R221.

Verity, M.A. and A. Reigth, 1967. Effect of mercurial compounds on structure-linked latency of lysosomal hydrolases. Biochem. J., 105: 685-690.

Visioli, F., P. Riso, S. Grande, C. Galli and M. Porrini, 2003. Protective activity of tomato products on *In vivo* markers of lipid oxidation. Eur. J. Nutr., 42: 201-206.

Walter, J.B. and M.S. Israel, 1974. General Pathology. Churchill Livingstone, New York, London.

Wassif, E.T., B.M. Kider, S.Y. Hussen, I.A. Mekkawy and H.I. Hassan, 2000. Effects of the herbicide Atrazine on the structure of some organs of the Nile fish *Oreochromis niloticus*. Egypt. J. Aquat. Biol. Fish., 4: 197-234.

Wertz, K., U. Siler and R. Goralczyk, 2004. Lycopene: Modes of action to promote prostate health. Arch. Biochem. Biophys., 430: 127-134.

Woodall, A.A., S.W.M. Lee, R.J. Weesiea, M.J. Jacksonb and G. Brittona, 1997. Oxidation of carotenoids by free radicals: Relationship between structure and reactivity. Biochim. Biophys. Acta, 1336: 33-42.

Xianquan, S., J. Shi, Y. Kakuda and J. Yueming, 2005. Stability of lycopene during food processing and storage. J. Med. Food, 8: 413-422.

Yamamoto, M. and N. Egami, 1974. Sextual differences and age changes in the fine structure of hepatocytes in the medaka, *Oryzias latipes*. J. Fac. Sci. Univ. Tokio, 413: 199-210.

Young, G., C.L. Brown, R.S. Nishioka, L.C. Folmar, M.C. Andrews, J.R. Ashman and H.A. Bern, 1994. Histopathology, blood chemistry, physiological status of normal and moribund striped bass (*Morone saxatilis*) involved in summer mortality (die-off) in the Sacramento-San Joaquin Delta of California. J. Fish Biol., 44: 491-512.

Yousef, M.I., G.A. Abdallah and K.I. Kamel, 2003. Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. Anim. Reprod. Sci., 76: 99-111.

Yuness, H.A.M., 2005. Role of Melatonin in reducing hypoxia-induced oxidative stress. M.Sc. Thesis, Assiut University, Egypt.

Zhang, Y., Y. Wang, R. Yu, S. Zhang and Z. Wu, 2008. Effects of heavy metals Cd^{2+}, Pb^{2+} and Zn^{2+} on DNA damage of loach *Misgurnus anguillicaudatus*. Front. Biol. China, 3: 50-54.