Effects of Cadmium on Some Histopathological and Histochemical Characteristics of the Kidney and Gills Tissues of Oreochromis niloticus (Linnaeus, 1758) Dietary Supplemented with Tomato Paste and Vitamin E

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ABSTRACT
Tomato and their variable products attracted the attention of many authors as antioxidants in mammals and fishes due to the high content of different micronutrients including lycopene. The latter micronutrient was proofed in many studies to be valid in cancer treatments especially for prostate and breast cancer. So, the present study was carried out to investigate the potential protective effects of tomato paste (9 mg lycopene/kg b.wt.) in comparison with vitamin E (50 mg kg\(^{-1}\) b.wt.) against the impacts of cadmium (Cd) toxicity (4.64 mg L\(^{-1}\); \(1/4\) of 96 h-LC\(_{50}\)) on fishes exposed for 15 and 30 days. Cd impacts were evaluated in terms of histopathological and histochemicals characteristics. Kidneys from fishes exposed to concentration of cadmium for 15 days showed swelling and hypertrophy of tubules with nuclear deterioration and pyknosis. Glomerular alternation was also observed. But in case of longer exposure (30 days), a severe damage in the structure of the kidney was noticed (anged from degeneration to necrosis). The gill exhibited severs hyperplasia and proliferation of chloride cells as well as significant shortened length and reduced secondary gill lamellae. Glycogen content decreased in the kidney reflecting decline in carbohydrates materials and decrease in the brush border of renal tubules in comparison with the control group. Gills also showed increase in the number of mucus and chloride cells. These Cd-induced parameters were significantly improved with supplementation of vitamin E and/or tomato paste. These findings demonstrated the beneficial supplementation of vitamin E and/or tomato paste in reducing the harmful effects of Cd.

Key words: Fishes, cadmium, tomato paste, vitamin E, kidney, gills

INTRODUCTION
Today, one of the serious problems in the world is the environmental pollution which affects the health of aquatic ecosystems and physiological changes of aquatic animals. The biochemical and physiological parameters of these animals especially fishes are tools and biomarkers for evaluating pollution impacts (Kock et al., 1996; Mekkawy et al., 2011, 2012). Oreochromis niloticus attracted the attention of toxicologists and biological scientists (Figueiredo-Fernandes et al., 2006; Garcia-Santos et al., 2006).

Heavy metals occur naturally in the environment and are found in varying levels in all ground and surface waters (Martin and Coughtrey, 1982). The aquatic animals experience unnaturally
high levels of these metals which are have initial effects at cellular or tissue levels before significant (Van Dyk et al., 2007). Cadmium fish toxicity and factors controlling such toxicity were discussed and reviewed by many authors including Heath (1995).

The pollutant-induced histological characteristics of different fish organs lead to understanding the adverse impact on a given aquatic ecosystem (Meyers et al., 1992; Young et al., 1994). The teleostean kidney and gills are first organs to be affected by chemicals in the aquatic environments (Thophon et al., 2003; Mekkawy et al., 2008). Tubule degeneration, changes in the corpuscle and reduction of Bowman’s space are the most common alterations found in pollutant-induced kidney of fishes (Takahima and Hibiya, 1965). The gill functions including respiration, ion regulation and acid-base balance (McDonald, 1983) are sensitive to low concentrations of numerous pollutants (Dalela et al., 1979).

In concern with animals and mammals, the Cd-free radical generations and the antioxidant defense were evaluated especially with supplementation of the natural antioxidants like tomato carotenoids and vitamin E (α-tocopherol) (Clinton, 1998; Bramley, 2000; Krinsky, 2001; Visioli et al., 2003; Tapiero et al., 2004). Inactivation of the harmful free radicals produced through normal cellular activity and from various stressors was achieved with these naturally occurring antioxidants (El-Demerdash et al., 2004; Mekkawy et al., 2011, 2012). So, investigators’ attention has been directed to the assessment of the relative antioxidant potency of vitamin E and carotenoids (Heber and Lu, 2002; El-Demerdash et al., 2004; Wertz et al., 2004). Among these antioxidant, lycopene has a higher antioxidant potential than α-tocopherol and β-carotene (Woodall et al., 1997; Rao and Agarwal, 1999).

Based on the aforementioned finding, the present study was suggested as an extension of those of Mekkawy et al. (2011, 2012) and aimed to study the protective role of tomato paste and/or vitamin E on the cadmium-induced histopathological characteristics of the Nile fish, Oreochromis niloticus (Linnaeus, 1758). Lycopene was considered as a guide to manage tomato paste manipulation in this study. To what extent one can benefit of tomato paste as fish diet supplementation and as a source of lycopene that work in an additive or synergistic manner with other phytonutrients is a question to be answered. Do the current results have applicable role in human studies under heavy metal toxicity is another 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 wet weight twice daily. Feces and residual food were aspirated regularly. The water temperature, pH and Dissolved Oxygen (DO) concentrations were measured daily (24.2±.0.8°C, 6.8±0.11 pH and 6.5±0.89 mg L⁻¹ DO, respectively) with photoperiod of 12 light/12 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
Table 1: The fish groups exposed to cadmium (4.64 mg L\(^{-1}\)) and tomato paste (9 mg lycopene kg\(^{-1}\) b.wt.) and vitamin E (50 mg kg\(^{-1}\) b.wt.) and their combinations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C</th>
<th>VE</th>
<th>Tp</th>
<th>VE+Tp</th>
<th>Cd</th>
<th>Cd+VE</th>
<th>Cd+Tp</th>
<th>Cd+VE+Tp</th>
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<tr>
<td>Cadmium (mg L(^{-1}))</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>4.64</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>9</td>
<td>9</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Vitamin E (mg kg(^{-1}))</td>
<td>0</td>
<td>50</td>
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</tr>
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C: Control, VE: Vitamin E, Tp: Tomato paste, Cd: Cadmium

spectrophotometrically according to the methods of Ranganna (1977) and Choudhari and Ananthanarayan (2007). The lycopene concentration in the tomato paste was 30.28 mg kg\(^{-1}\). In addition to lycopene, tomato paste composition include water, proteins, carbohydrates, fibers, calcium, potassium, zinc, copper, manganese, iron, vitamin C, vitamin E, β-carotinoids and other phytonutrients (Okajima et al., 1998). The authors refer to tomato paste through the text in terms of lycopene.

**Experimental design:** Fishes were weighed, measured and classified randomly into 8 groups (14 fish/tank) according to doses of Cadmium, lycopene, vitamin E and their combinations (Table 1).

**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 kidney and gills 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 μm in thickness and then stained by following stains: Harris’s hematoxylin and eosin stain (HE) (Bancroft and Stevens, 1982).

**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). The positively stained materials have been proved to be glycogen as verified by PAS-technique with and without pretreatment with diastase.

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**

**Histopathological and histochemical studies**

**Kidney**

**Control kidney and treatment with lycopene (9 mg kg\(^{-1}\)) or vitamin E (50 mg kg\(^{-1}\)) and their combinations:** The control kidney of Oreoichromis niloticus consists of tremendous number of nephrons, the functional units of the kidney. Each nephron is composed of renal corpuscle (Malpighian corpuscle), renal and collecting tubules. The renal corpuscle is roughly spherical.
Fig. 1(a-d): Transverse sections of control and treated fish kidney for 30 days showing Renal Corpuscles (RC) and Renal Tubules (RT). (H and E x400), (a) Control, (b) Lycopene treatment, (c) Vitamin E treatment and (d) Vitamin E and lycopene simultaneous treatment

consisting a double membrane capsule (Bowman’s capsule) enclosing a tuft of blood capillaries (glomerulus). There is a space in between the glomerulus and the capsule which called the Bowman’s space (Fig. 1a).

The renal tubules are numerous and their cross sections exhibit a round or oval outline with a narrow lumen. Their lining coat is comprised of columnar epithelial cells with markedly eosinophilic cytoplasm and centrally located nuclei. (Fig. 1a). The collecting tubules are lined with cubical or low columnar epithelial cells with basally located nuclei (Fig. 1a). Lycopene or vitamin E and their combinations administrated to fishes showed the same structure of the kidney of the control group after 15 and 30 days (Fig. 1b-d).

Histochemically, PAS-technique displayed a positive reaction mainly at the brush border and the basement membrane of the renal tubules. Renal (Malpighian) corpuscles appeared moderately reacted (Fig. 2a). The application of PAS reaction has presented great carbohydrates materials localization in the renal tissue when lycopene and/or vitamin E were administrated to fish after 15 and 30 days (Fig. 2b-d).

**Treatment with 4.64 mg L⁻¹ of cadmium:** Examination of kidney sections after 15 days of cadmium exposure showed hypertrophies of glomeruli, extravasated erythrocytes (haemorrhage) between renal tubules, rupture of the renal corpuscles with disorganization in the glomerular tuft, indistinct lumen and focal tubular necrosis and necrotic area were observed (Fig. 3a, b).
Fig. 2(a-d): Transverse sections of control and treated fish kidney for 30 days showing marked PAS reactivity in brush border (BB) and the basement membrane (BM) of the renal tubules and renal corpuscle (RC), (PAS-reaction, x400), (a) Control, (b) Lycopene treatment, (c) Vitamin E treatment and (d) Vitamin E and lycopene simultaneous treatment.

In addition to the previous changes after 30 days of exposure, the majority of tubules showed indistinct lumen. Focal tubular necrosis was also noticed. Rupture of Bowman’s capsule and the glomerular tuft, hypertrophies of glomeruli, haemorrhage (Hr) between renal tubules, degeneration of the epithelial cells lining the renal tubules and degeneration of the parietal cell of renal corpuscles. Proliferation of renal tubules and some renal tubules were dilated, accumulation of lipid droplets and necrotic (Fig. 3c, d).

Examination of PAS-treated sections of Cd-administered fish showed great decline in carbohydrate materials as reflected by the feebly stained renal corpuscle after 15 and 30 days. Also, the basement membranes and brush borders of the renal tubules were suffering from a great deficiency of carbohydrate materials. (Fig. 3e, f).

**Treatment with 4.64 mg L\(^{-1}\) cadmium plus 9 mg kg\(^{-1}\) lycopene:** Cd-treated fishes dietary supplemented with lycopene for 15 days showed proliferations of renal tubules with degeneration of the epithelial cells lining the renal tubules (Fig. 4a). The kidney tissue of the examined sections retained its normal structure after 30 days of exposure and dietary supplemented with lycopene (Fig. 4b).
Fig. 3(a-f): Transverse sections of treated fish kidney exposed to (4.46 mg L⁻¹) cadmium (a, b and e) for 15 days and (c, d and f) for 30 days, Showing haemorrhage (Hr) between renal tubules, hypertrophies of glomeruli (HYT) and necrosis (N), (H and E x400). Showing rupture of the renal corpuscles (RRC) and necrosis (N), (H and E x400). Showing haemorrhage (Hr) between renal tubules, hypertrophies of glomeruli (HYT) and accumulation of lipid droplets (L), (H and E x400). Showing necrotic (N) area, (H and E x400), (e) Showing a remarkable depletion of carbohydrate materials in brush border (BB) and the basement membrane (BM) of the renal tubules and renal corpuscle (RC), (PAS-reaction, x400). (f) Showing increase depletion of carbohydrate materials in the different components of the kidney tissue, (PAS-reaction, x400)
Fig. 4(a-d): Transverse sections of treated fish kidney exposed to lycopene and cadmium (9 mg kg⁻¹ and 4.46 mg L⁻¹, simultaneous), (a and c) for 15 days and (b and d) for 30 days, (a) Showing proliferation of renal tubules (RT), (H and E x400), (b) Showing the general structure of renal corpuscle (RC) and renal tubule (RT), (H and E x400), (c) Showing a moderate, PAS-reaction in brush border (BB) and the basement membrane (BM) of the renal tubules and renal corpuscle (RC), (PAS-reaction, x400), (d) Showing increase in kidney carbohydrate materials, (PAS-reaction, x400)

The application of PAS reaction has presented moderate carbohydrates materials localization in the renal tissue when lycopene was administered to Cd-treated fish for 15 and 30 days (Fig. 4c and d).

**Treatment with 4.64 mg L⁻¹ cadmium plus 50 mg kg⁻¹ vitamin E:** Vitamin E administration to Cd-treated fish showed a hyaline degeneration of renal tubules with irregular scattered pyknotic nuclei after 15 day of exposure (Fig. 5a). After 30 days, an improvement in the structure of the kidney tissue was revealed. Generally repairing of the majority of morphological changes induced by vitamin E administration were noticed (Fig. 5b).

Histochemically, PAS-technique revealed the protective role of vitamin E in preservation of carbohydrate materials after its administrated to the Cd-treated fish after 15 and 30 days (Fig. 5c, d).
Fig. 5(a-d): Transverse sections of treated fish kidney exposed to vitamin E and cadmium (50 mg kg⁻¹ and 4.46 mg L⁻¹, simultaneous), (a and c) for 15 days and (b and d) for 30 days, (a) Showing a hyaline degeneration (HD) of renal tubules with irregular scattered pyknotic nuclei (PK), (H and E x400), (b) Showing more or less normal structure of renal corpuscle (RC) and renal tubules (RT), (H and E x400), (c) Showing a moderate, PAS-reaction in brush border (BB) and the basement membrane (BM) of the renal tubules and renal corpuscle (RC), (PAS-reaction, x400) and (d) Showing good PAS-reactivity in the most of kidney tissue, (PAS-reaction, x400)

**Treatment with 4.64 mg L⁻¹ cadmium, 9 mg kg⁻¹ lycopene and 50 mg kg⁻¹ vitamin E:** Administrations of lycopene and vitamin E to the Cd-treated fish exerted beneficial protective effect against cadmium-induced morphological alterations in the kidney. Some cellular degeneration in the lining epithelium of the renal tubules and disconnection of renal tubules were detected (Fig. 6a). In addition, slightly dilatation of Bowman’s space and hyaline degeneration in some renal tubules was also observed after 15 days of exposure (Fig. 6a). After 30 days of exposure, the kidney of the examined sections retained clearly its normal structure (Fig. 6b).

The application of PAS reaction has presented moderate carbohydrates materials localized in the renal tissue when lycopene and vitamin E were administered to Cd-treated fish after 15 and 30 days (Fig. 6c, d).
Fig. 6(a-d): Transverse sections of treated fish kidney exposed to vitamin E, lycopene and cadmium (50.9 mg kg⁻¹ and 4.46 mg L⁻¹, simultaneous), (a and c) for 15 days and (b and d) 30 days, (a) Showing slightly dilation of Bowman’s space (BS), disconnection of renal tubules (DRT) and cellular degeneration in the lining epithelium of the renal tubules (RT), (H and E, x400), (b) Showing more or less normal structure of kidney tissue, (H and E, x400), (c) Showing slight accumulation of carbohydrate materials in brush border (BB) and the basement membrane (BM) of the renal tubules and renal corpuscle (RC), (PAS-reaction, x400), (d) Showing good PAS-reactivity in the most of kidney tissue, (PAS-reaction, x400)

Gills

Control gills and treatment with lycopene (9 mg kg⁻¹) or vitamin E (50 mg kg⁻¹) and their combinations: There are four gill arches on each side of the buccal cavity. Each arch is composed of numerous gill filaments which have two rows of Secondary Lamellae (SL) that run perpendicular to each filament. Each lamella is made up of two sheets of epithelium delimited by many Pillar Cells (PC) which are contractile and separate the capillary channels. One to two erythrocytes are usually recognized within each capillary lumen. Chloride Cells (CC) are identified as large epithelial cells with light cytoplasm, usually present at the base of lamellae. Mucous Cells (MC) and pavement cells (PV) are also present in the epithelium of the filament and at the base of lamellae, but they lack the light cytoplasm and are smaller than chloride cells. The gill filaments are covered with squamous pavement cells (PV). The Mucous Cells (MC) are mostly observed in the
Fig. 7(a-d): Longitudinal Sections of control and treated fish gills for 30 days showing blood capillary (BC), chloride cells (CC), mucous cells (MC), pillar cells (PC), primary lamellar epithelium (PLE), pavement cells (PV) and secondary lamellae (SL), (H and E x400), (a) Control, (b) Fish gills exposed to 9 mg kg\(^{-1}\) of lycopene, (c) Fish gills exposed to 50 mg kg\(^{-1}\) of vitamin E, (d) Fish gills exposed to (50 and 9 mg kg\(^{-1}\)) of vitamin E and lycopene simultaneous

inter-lamellar epithelium between two Secondary Lamellae (SL) and at the distal tip of the Primary Lamellar Epithelium (PLE) (Fig. 7a). Lycopene or vitamin E and their combinations administrated to fishes showed the same structure of the control gills after 15 and 30 days (Fig. 7b-d).

The application of PAS reaction has presented a large amount of carbohydrate materials are observed in the epithelial cells of the primary lamellae. Few amount of mucous cells secretion are present and basement membrane is distinct. (Fig. 8a-d).

The application of alcian blue plus periodic acid sheif (AB-pH 2.5 and PAS) reaction has presented a few numbers of mucous secreting cells at the base of the secondary lamellae (purple color of neutral mucin) and the basement membrane is distinct. (Fig. 9a-d).

**Treatment with 4.64 mg L\(^{-1}\) of cadmium:** Examination of gill sections after 15 days of cadmium exposure showed fusion of 2nd lamellae, vascular congestion or lamellar aneurysm that led to clubbing of the tips of 2nd lamellae in the gills (Fig. 10a and b). Disorganization of the pillar cell system and subepithelial edema were seen in the secondary lamellae (Fig. 10b). After 30 days of
Fig. 8(a-d): Longitudinal Sections of control and treated fish gills for 30 days showing mucopolysacharides spread over the 2nd lamellae. Well distinct basement membrane (BM), mucous cell (MC) and mucous secretions (MS), (PAS-reaction, x400), (a) Control, (b) Fish gills exposed to 9 mg kg^{-1} of lycopene, (c) Fish gills exposed to 50 mg kg^{-1} of vitamin E, (d) Fish gills exposed to (50 and 9 mg kg^{-1}) of vitamin E and lycopene simultaneous.

Fig. 9(a-d): Longitudinal Sections of control and treated fish gills for 30 days showing the basement membrane (BM) is distinct and mucous cells (MC) at the base of the secondary lamellae (purple color of neutral mucopolysacharides), (AB-PAS reaction, x400), (a) Control, (b) Fish gills exposed to 9 mg kg^{-1} of lycopene, (c) Fish gills exposed to 50 mg kg^{-1} of vitamin E, (d) Fish gills exposed to (50 and 9 mg kg^{-1}) of vitamin E and lycopene, respectively.
Fig. 10(a-d): Longitudinal sections of fish gills exposed to 4.46 mg L⁻¹ of cadmium (a and b) for 15 days and (c and d) for 30 days. (H and E x400), (a) Showing fusion (F) of 2nd lamellae and clubbing of the tips of 2nd lamellae in the gills, (b) Showing aneurysm (A), subepithelial edema (E) and fusion (F) of 2nd lamellae, (c) Showing extensive curling and hypertrophy (HYT) of 2nd gill lamellae, (d) Showing severe congestion (Cog) of 2nd gill lamellae. Shortening in the length (ShL) of gill lamellae and wear and tear (W and T).

exposure to the same dose of cadmium, extensive hypertrophy of 2nd gill lamellae was observed in the gills (Fig. 10c). Shortening in the length of gill lamellae, wear and tear in the 2nd gill lamellae, damage in pillar cell system (Fig. 10d).

Examination of PAS-treated sections of Cd-treated fish showed great decline in carbohydrate materials as reflected by the feebly stained basement membranes. The number of the mucous cells increased after 15 and 30 days. Also a slimy layer of mucous substance was noticed spread over the pavement cells of the 2nd lamellae (Fig. 11a, b).

Examination of AB-PAS treated sections of Cd-treated fish showed numerous mucous secreting cells in the inter-lamellar epithelium between two secondary lamellae and rupture in basement membrane after 15 days and the numbers of mucous cells increased after 30 days (Fig. 11c, d).

**Treatment with 4.64 mg L⁻¹ cadmium plus 9 mg kg⁻¹ lycopene:** Lycopene administration plus cadmium to fish showed desquamation in respiratory wall (pavement cells of the 2nd lamellae) with rupture of this wall. Subepithelial edema in the secondary lamellae after
Fig. 11(a-d): Longitudinal sections of fish gills exposed to 4.46 mg L\(^{-1}\) of cadmium (a and c) for 15 days and (b and d) for 30 days, (a) Showing a remarkable depletion in carbohydrate materials of gill tissue and basement membrane (BM), (PAS-reaction, x400), (b) Showing a remarkable more depletion of carbohydrate materials in gill tissue and basement membrane (BM) and large amount of mucous secretion (MS), (PAS-reaction, x400), (c) Showing mucous cells (MC) and rupture basement membrane (RBM), (AB-PAS reaction, x400), (d) Showing ruptured basement membrane (BM) and numerous mucous cells (MC), (AB-PAS reaction, x400).

Fig. 12(a-b): Longitudinal sections of fish gills exposed to (4.46 mg L\(^{-1}\) cadmium and 9 mg kg\(^{-1}\) lycopene) (a) for 15 days and (b) for 30 days, (H and E x400), (a) Showing subepithelial edema (E) and desquamation of respiritory wall (RW), (b) Showing the general structure of gill tissue as in control one.
Fig. 13(a-d): Longitudinal Sections of fish gills exposed to (4.46 mg L\(^{-1}\) cadmium and 9 mg kg\(^{-1}\) lycopene simultaneous), (a and c) for 15 days and (b and d) for 30 days. (a and b) Showing, moderate amount of carbohydrate materials and mucous secretion (MS) in gill tissue and basement membrane (BM), (PAS reaction, x400), (c and d) Showing distinct the basement membrane (BM) and small number of mucous cells (MC) (AB-PAS reaction, x400).

15 days was noticed (Fig. 12a). After 30 days of exposure to the same dose of cadmium, the gill retained its normal structure (Fig. 12b). Reflecting the increased protective effects of lycopene with time.

The application of PAS reaction has presented moderate carbohydrates materials localization in the gill tissue and few amount of mucous cells secretion was observed in the epithelial cells of the PL, when lycopene was administered with cadmium to the fish after 15 and 30 days (Fig. 13a, b).

The application of AB-PAS reaction has presented a few number of mucous cells which were observed in the epithelial cells of the PL and slight basement membrane after 15 and 30 days (Fig. 13c, d).

**Treatment with 4.64 mg L\(^{-1}\) cadmium plus 50 mg kg\(^{-1}\) vitamin E:** Vitamin E administration with cadmium to the fish showed hyperplasia of interlamellar epithelium, severe blood congestion of secondary lamellae and hypertrophy in pavement cells or respiratory wall. Subepithelial edema
Fig. 14(a-b): Longitudinal sections of fish gills exposed to (4.46 mg L\(^{-1}\) and 50 mg kg\(^{-1}\)) cadmium and vitamin E simultaneous, (a) for 15 days and (b) for 30 days. (H and E, x400), (a) Showing severe blood congestion (Cog) of secondary lamellae epithelial, subepithelial edema (E), epithelial hyperplasia (HYP) and hypertrophy in pavement cell (PV), (b) Showing more or less normal structure of gill tissue as in control one

was seen in the gills after 15 days of exposure (Fig. 14a). After 30 days of exposure to the same dose of Vitamin E plus cadmium, the protective effects of vitamin E upon the gill tissue structures were recorded. (Fig. 14b).

The application of PAS and AB-PAS reaction has presented great decline in carbohydrate materials as reflected by the feebly stained of the gill tissues and basement membrane. A few numbers of mucous cells were observed in the epithelial cells of the PL when vitamin E was administered with cadmium to the fish after 15 and 30 days (Fig. 15a-d).

**Treatment with 4.64 mg L\(^{-1}\) cadmium, 9 mg kg\(^{-1}\) lycopene and 50 mg kg\(^{-1}\) vitamin E:** Administrations of lycopene and vitamin E with cadmium to the fish reflected the beneficial protective effect against cadmium-induced morphological alterations in the gills. However, there were blood congestion, fusion of secondary lamellae, hyperplasia of interlamellar epithelium and rupture in respiratory wall after 15 days of exposure (Fig. 16a). After 30 days of exposure to the same dose of cadmium, the gills retained its normal structure with small edema (Fig. 16b). Accordingly, the protective effect of lycopene and vitamin E increased to some extent with time of treatment.

The application of PAS reaction has presented moderate carbohydrates materials localization in the gill tissue and few numbers of mucus cells were observed in the epithelial cells of the Primary Lamellae (PL) when lycopene and vitamin E were administered to Cd-treated fish after 15 and 30 days (Fig. 17a, b).
Fig. 15(a-c): Longitudinal sections of fish gills exposed to (4.46 mg L\(^{-1}\) and 50 mg kg\(^{-1}\)) cadmium and vitamin E simultaneous, (a and c) for 15 days and (b and d) for 30 days, (a and b) Showing, small amount of carbohydrate materials in gill tissues and basement membrane more or less distinct (BM). (PAS reaction, x400), (c and d) Showing few numbers of mucus cells (MC) and basement membrane (BM) more or less distinct, (AB-PAS reaction, x400)

Fig. 16(a-b): Longitudinal Sections of fish gills exposed to (4.46 mg L\(^{-1}\), 50 and 9 mg kg\(^{-1}\)) cadmium, vitamin E and lycopene simultaneous, (a) for 15 days and (b) for 30 days. (H and E, x400), (a) Showing blood congestion (Cog), fusion (F) of secondary lamellae, hyperplasia (HYP) of interlamellar epithelium and rupture in respiratory wall (RW). (b) Showing more or less normal structure of gill tissue with small edema (E)
Fig. 17(a-d): Longitudinal sections of fish gills exposed to (4.46 mg L$^{-1}$, 50 and 9 mg kg$^{-1}$) cadmium, vitamin E and lycopene simultaneous, (a and c) for 15 days and (b and d) for 30 days, (a and b) Showing moderate amount of carbohydrate in gill tissue and well distinct basement membrane (BM), (PAS reaction, x400), (c and d) Showing good AB-PAS reactivity in the most of gill tissue and few numbers of mucous cells (MC), (AB-PAS reaction, x400)

The application of AB-PAS reaction has presented few number of mucous cells compared with cadmium treated and small ruptured in basement membrane after 15 and 30 days (Fig. 17c and d).

**DISCUSSION**

Heavy metal-induced cell injury on the molecular and subcellular levels of biological organization lead to degenerative and neoplastic diseases in target organs of fishes (Polmar, 1993; Pacheo and Santos, 2002; Cick and Engin, 2005; Jacobson-Kram and Keller, 2001). Therefore, many authors referred to the histopathological and biochemical changes to be useful indicators of toxicity in fish organs (Schwaiger et al., 1996; Teh et al., 1997; Mekkawy et al., 2011, 2012). These indicators were reported by many authors for vital organs such as gills, liver and kidney (Singhaseni and Tesprateep, 1987; Gill et al., 1988; Richmonds and Dutta, 1989; Barlas, 1999; Erkmen et al., 2000; Cengiz et al., 2001; Cengiz and Unlu, 2002). To what extent this situation was revealed in Cd-stressed *O. niloticus* fed supplemented diet with tomato paste and or
vitamin E is considered. Mekkawy et al. (2011, 2012) reported that tomato paste and vitamin E expressed high protective potentials against cadmium-induced biochemical changes especially liver transaminases and liver histopathological alterations.

The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only involved in removal wastes from blood but it is also responsible for selective reabsorption which helps in maintaining volume and pH of blood and body fluids and erythropoiesis (Iqbal et al., 2004). So, the kidney in addition to gills are firstly impacted by contaminants in the water (Thophon et al., 2003).

Under pesticides and heavy metals induced stress, different histopathological alterations were recorded in fish kidney (Wester et al., 1985; Forlin et al., 1986; Barlas, 1999; Wassif et al., 2000; Fathalla et al., 2001; Khidr et al., 2001; Thophon et al., 2003; Bernet et al., 2004; Hussein, 2005; Peebuuaa et al., 2006; Mohamed, 2009). These alterations include hydropic and vacuolar degeneration in tubular cells, dilatation of tubular lumen, dilated Bowman's capsule and necrosis were also observed. Similarly, histopathological alterations were recorded in the present study.

The histopathological alterations observed in O. niloticus exposed to 4.64 mg L\(^{-1}\) cadmium chloride after 15 and 30 days were hypertrophies of glomeruli, hemorrhage between renal tubules, rupture of Bowman's capsule and the glomerular tuft, degeneration of the epithelial cells lining the renal tubules, hyaline degeneration of renal tubules with irregular scattered pyknotic nuclei and dilatation of Bowman's space. In more severe cases, the degenerative process leads to tissue necrosis. The present results are in agreement with those observed in Puntius conchonius exposed to CdCl\(_2\) (Gill et al., 1989), Heteropneustes fossilis exposed to mercury intoxication (Bano and Hassan, 1990), Cyprinus carpio exposed to malathion and sevin (Dhanapakiam and Premalatha, 1994) and Labeo rohita exposed to hexachlorocyclohexane (Das and Mukherjee, 2000). Velmurugan et al. (2007) recorded sever changes in the kidney tissues of Cirrhinus mrigala exposed to (0.3 and 0.6 ppb) of lambda-cyhalothrin for 10 days. These changes includes narrowing of the tubular lumen, cloudy swelling of epithelial cells of renal tubules, necrosis in tubular epithelium, contraction of the glomerulus and expansion of space inside the Bowman's capsule. While, Benli et al. (2008) reported displayed glomerulonephritis and hyperemia in the kidney of Oreochromis niloticus exposed to different concentrations of ammonia.

In the present study, vacuolar, nuclear degenerations and necrosis were observed. These results were in agreement with Hawkins et al. (1980) who found vacuolar degeneration and tubular necrosis after the acute exposure of Leiostomus xanthurus to 10, 15 and 25 mg L\(^{-1}\) CdCl\(_2\). Robbins and Angell (1976) mentioned that this damage could be attributed to autolysis action of lysosomal enzymes released into these cells or due to sufficient accumulation of the toxin in the epithelial cells or due to reabsorption of excreted protein molecules which are greatly represented in the glomerular filtrate due to cellular damage. Tu (1991) mentioned that this damage might indicate disruption of active transport process which includes reabsorbance and excretory functions of the tubular cells.

Hyaline degeneration in tubular cells was recorded in the present study might be the result of a glomerular alteration or an increased permeability of the glomerular filter (Bucher and Hofer, 1995). Generally, The kidney considered as one of the main targets for cadmium accumulation (Brown et al., 1994; Allen, 1995; Cinier et al., 1997; Mekkawy et al., 2008; Mekkawy et al., 2007) even if its concentration in the water is very low (Kock et al., 1995; Mekkawy et al., 2008, 2007).
In the present investigation, the PAS positive materials decreased in the ruptured basement membrane and the brush border of the renal tubules of the kidney of fish exposed to cadmium chloride. This finding is consistent with other studies which showed a reduction in carbohydrate materials following exposure to heavy metals (Mohamed, 2006) and exposure to herbicides (Wassif et al., 2000; Khidr et al., 2001). Increased glucose blood concentration (hyperglycemia) which observed in the present study may be explaining the decrease of carbohydrates content in the kidney.

Fish gills are multifunctional organs involved in ion transport, gas exchange, acid-base regulation and waste excretion (Laurent, 1984; Heath, 1987; Moyle and Cech, 1982; Perry, 1997; Wendelaar Bonga, 1997; Wong and Wong, 2000; Thophon et al., 2003; Zayed and Mohamed, 2004). Dietary cadmium can enter the branchial epithelium from the blood compartment and it has been suggested that it is excreted via the gills (Handy, 1996; Harrison and Klaverkamp, 1989). Whatever the original source, progressive accumulation of heavy metals in the gills can induce structural damage to the gills (Pratap et al., 1989; Wendelaar Bonga, 1997; Thophon et al., 2003).

Mohamed and Gad (2005) and Mohamed (2008) and as a consequence plasma ion homeostasis may be disturbed (Pratap et al., 1989).

In the present study, the effects of cadmium on gill structure of O. niloticus were particularly severe since they serve as a major organ for osmotic regulation and respiration. The most frequent histopathological changes were observed in the gills (Balah et al., 1983; Sorensen, 1991).

Cadmium was taken up across the epithelial layer of fish gills (Verborg et al., 1987, 1989) via calcium channels (Farag et al., 1994; Wicklund-Glynn et al., 1994). Calcium is known to exert considerable control over the permeability of the gills and displacement of calcium could stimulate ion loss and water uptake (Reid and McDonald, 1991). Verborg et al. (1987) postulated that cadmium affects calcium balance in rainbow trout. It also induces damage in gill structure of zebra fish, rainbow trout and tilapia (Pratap and Bonga, 1993; Karlsson-Norrgren et al., 1985; Part et al., 1985).

Among these changes were observed in the present study, fusion and curling of 2nd lamellae, shortening in the length, vacuolar congestion (lamellar aneurysm) led to clubbing of the tips of 2nd lamellae, subepithelial edema, hypertrophy of 2nd lamellae and necrosis of gill epithelium and severe hyperplasia of interlamellar epithelium. These results in agreement with (Erkmen et al., 2000; Elezabi et al., 2001; Fathalla et al., 2001; Congiz and Unlu, 2002, 2003, 2006; De Silva and Samayawardhena, 2002; Caliskan et al., 2003; Mohamed et al., 2005; Velmurugan et al., 2007; Benli et al., 2008; Bhattacharya et al., 2008; Mohamed, 2008, 2009; Pandey et al., 2008).

According to Skidmore (1964) and Burton et al. (1972) epithelial lifting, swelling and hyperplasia of lamellar epithelium (edema) recorded could serve as a defense by increasing the distance across which water borne irritants must diffuse to reach the blood stream. Tietge et al. (1988) who stated that the separation of secondary lamellar epithelium leads to the formation of a lymphoid space. Enlargement of lymphoid space is associated with presence of lymphatic fluid, extrusion of the fluid from the central capillaries causes vascular stasis.

Fusion of 2nd lamellae was observed in the present study. Hughes et al. (1979) and Sergers et al. (1984) mentioned that lamellar fusion could be protective in that, it diminishes the amount of vulnerable gill surface area to slow the entry of toxicant.

The secondary lamellae showed capillary congestion or aneurism, similar to those reported in Gnathionemus petersii exposed to 10 mg L\(^{-1}\) of cadmium for 6 h (Alazemi et al., 1996). This lamellar aneurism resulted from the collapse of the pillar cell system and the breakdown of vascular
integrity with a release of large quantities of blood that push the lamellar epithelium outward (Alazemi et al., 1996). García-Santos et al. (2006) reported that this lesion can induce changes in pillar cell of normal structure, with consequent loss of their support function and probably, were responsible for the emergence of lamellar aneurisms in fish exposed to cadmium. Similar results were observed by Thophon et al. (2003) in Lates calcarifer exposed to cadmium. However, Mallatt (1985) suggests that these lesions are rarely associated to metals exposure.

The histochemical results in the present investigation illustrated a hyper mucous secretion (as shown by PAS positive reactions) in the gills may be due to the physiological reaction of the fish towards the sever pollutants as one of granules and the excessive mucous secretion may be formed as a result of metal binding proteins (Onosaka and Cherian, 1981; Bucher and Hofer, 1993; Lappivaara et al., 1995). The large quantity of mucous secretion which was observed in O. niloticus acts as a defense mechanism against cadmium and other several toxic substances (Sellers et al., 1975; McDonald, 1983; Handy and Eddy, 1991; Mazon et al., 1999). The regular sloughing of mucous from the surface of gills into the media helps to remove the bound pathogens, toxicants and foreign matters including metal-compounds which adhere to the gills (Lock and van Overbeeke, 1981; Powell et al., 1992).

The mucous coat covering the gill epithelia is composed mainly of glycoproteins that have electro-negative charges (as shown by AB and PAS positive reactions at pH 2.5). It is perhaps also due to the well established ability of these glycoproteins to trap heavy metal ions (Lock and van Overbeeke, 1981). According to Daoust et al. (1984), exposure to heavy metals very often alters the chemical composition or thickness of mucous layer that may disturb the normal ability to recognize different cell types. They concluded that this is due to contact stress and may also be due to transformation of electrically charged properties of the epithelial cells which favors adhesion between the cells of two neighboring Secondary Lamellae (SL) which has been a very common manifestation of the toxic impact of a large number of xenobiotics, including cadmium salt. This leads to extensive fusion of Secondary Lamellae (SL), causing a drastic reduction in the respiratory surface area. Several other xenobiotics are also known to induce fusion of the Secondary Lamellae (SL) of gills (Leino et al., 1987; Dutta et al., 1996; Wendelaar Bonga, 1997; Cengiz and Unlu, 2002; Cengiz and Unlu, 2003).

In the present study, administration of antioxidants Tomato paste (lycopene) as dietary supplementation remarkable protective effects and restored the general structure and the carbohydrate contents of the kidney. Karahan et al. (2005) reported ameliorative in histopathological and morphological changes in the kidney treated with lycopene against Gentamicin-induced Oxidative Stress (GEN) toxicity in Rats. Atessahin et al. (2005) also recorded decrease in histopathological and morphological changes in the kidney treated with cisplatin-induced nephrotoxicity in Rats fed lycopene. So, one can conclude that lycopene or tomato paste micronutrients scavenge or quench free radicals liberated due to cadmium toxicity in the kidney and thus preventing their damage. Such role of lycopene is confirmed by Heber and Lu (2002), Stahl and Sies (2003) and Visioli et al. (2003).

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 kidney. The present results are in agreement with those observed in kidney of Clarias gariepinus exposed to vitamin E against Lead-indu.induced oxidative stress (Mohamed, 2006), in kidney of rats exposed to vitamin E against cisplatin-induced oxidative stress (Naziroglu et al., 2004) and exposed to vitamin E and probucol against gentamicin-induced nephrotoxicity.
The effect of dietary Vitamin E on renal tissue damage and lipid peroxidation was investigated by Hamazaki et al. (1988) and Iqbal et al. (1988) following treatment with ferri-nitrotoltriacetate (Fe-NTA) in rats. Hanafy and Soltan (2004) also referred to the valid effects of vitamin E pretreatment on subacute toxicity of mixture of Co, Pb and Hg nitrate-induced nephrotoxicity in rats.

CONCLUSION

In conclusion, the present study emphasizes on the protective role of tomato paste (9 mg lycopene/kg BW) in comparison with vitamin E (60 mg kg⁻¹ BW) against the impacts of cadmium toxicity (4.64 mg L⁻¹; ⅓ of 96h-LC50) on the kidney and gills of O. niloticus exposed for 15 and 30 days. This protective role was emphasized by the current authors studying liver histology and biochemical parameters of the same species for the same doses.

REFERENCES


