Alexandria Journal of Veterinary Sciences 2014, 40:29-43
ISSN 110-2047, www.alexjvs.com
doi: 10.5455/ajvs.48333

Physiological and Oxidative Stress Biomarkers in the Freshwater Nile Tilapia, *Oreochromis Niloticus* L., Exposed to Sublethal Doses of Cadmium

Ahmed M. EL-Gazzar[1], Khalid E. Ashry[1], Yasser S. El-Sayed[2]

1 Department of Veterinary Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Alex University, Edfina-Behara, Egypt

2 Department of Veterinary Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt

Key words: Nile tilapia (*Oreochromis niloticus*); heavy metals; Toxicity; Biomarkers; Lipid peroxidation

ABSTRACT:

This study was designed to investigate the possibility of cadmium (Cd) to induce oxidative stress and biochemical perturbations in Nile tilapia liver and gills and the role of Vitamin C (Vit. C) in alleviating its toxic effects. Nile tilapia fish were randomly divided into four groups of thirteen each, group one served as control without any treatment, group two exposed to Cd (5mg/liter water), group three supplemented with vitamin C (Vit.C) (500mg/kg diet), and group four exposed to Cd plus Vit. C. The exposure to Cd caused increase in Liver aminotransferases (AST and ALT), elevation in lipid peroxidation (LPO), activity of catalase (CAT) enzyme, and the activity of glutathione *S*-transferase (GST). The urea and creatinine levels were not affected. However, reduction in the activity of glutathione peroxidase (GPx) was observed. An increase in reduced glutathione (GSH) content was also observed and in gills there were no significant changes in LPO, antioxidant enzymes activity and GSH level. Vit.C supplementation in Cd-induced oxidative stress of Nile tilapia maintained Liver AST and ALT near normal level and modulated LPO, CAT, GST, GPx and GSH level in liver. It is concluded that Vit.C scavenges reactive oxygen species and render a protective effect against Cd toxicity.

***Corresponding Author:** Ahmed M. EL-Gazzar; elgazar86@gmail.com

1. INTRODUCTION

Contamination of the aquatic ecosystem by industrial and agricultural pollutants affects the health of fish, either directly by uptake from the water, or indirectly through their diet of vegetation, invertebrates or smaller fish. Since fish are part of the natural diet of both aquatic mammals and birds, as well as providing an increasingly important protein source for humans, their population and health is of major concern (Kime, 1995). The important environmental pollutants are those that tend to accumulate in organisms, those that are persistent because of their chemical stability or poor biodegradability, and those that are readily soluble and therefore environmentally mobile (Hellawell, 1986). Heavy metals cannot be destroyed through biological degradation. When exposed to higher concentrations, organs of aquatic animals may accumulate

heavy metals (Pelgrom et al., 1995, Grosell et al., 1996, Kalay et al., 1999 and Mazon et al., 2002). Heavy metals accumulated in the tissues of fish catalyze redox reactions that generate reactive oxygen species (ROS) which may lead to environmental oxidative stress and, therefore, cause biochemical, molecular and morphological alterations in fish (Varanka et al., 2001 and Monteiro et al., 2005).

Among the heavy metals, cadmium (Cd) is considered as a major aquatic pollutant in many parts of the world. It is a nonessential heavy metal; however, it is considered as one of the most toxic water contaminants and could cause toxicity at each level in organisms (Rashed, 2001). Cd is a highly toxic, since it causes deleterious effects in organisms at low levels of exposure (Cope et al., 1994). Various toxic effects of cadmium have been reported, as well as its accumulation mainly in fish kidneys, liver, and gills (Hollis et al., 1999; McGeeret al., 2000). Cd toxicity in fish has been studied at different levels e.g., behavioral parameters (Eissa et al. 2010). Furthermore, it can damage gills (Wong and Wong, 2000), causes skeletal deformities (Wicklund-Glynn et al., 1994), as well as adverse effects on growth, reproduction, respiratory functions, and osmoregulation (Pratap and Wendelaar Bonga, 1990). Cd dissolved in the water medium can be absorbed through the gills and then distributed in unequal concentrations in different fish tissues. It is not a redox-active metal, but it could indirectly generate oxidative stress and free radicals (Cuypers et al. 2010), which could cause lipid peroxidation and thus DNA damage (Bagchi et al. 1996). Therefore, at biochemical levels, antioxidant systems have great potential to indicate the cellular responses to the toxic effects of Cd (Cirillo et al. 2012).

To protect themselves against heavy metals and other toxic materials generating oxidative stress, aerobic organisms have evolved complex antioxidant defense systems. Antioxidant systems are beginning to receive attention as a biological means to reduce damage to aquatic organisms (McFarland et al., 1999; Wedderburn et al., 2000; Lionetto et al., 2003; Pandey et al., 2003; George et al., 2004). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione –s– transferase (GST) and antioxidant materials such as glutathione (GSH), ascorbic acid, metallothionein (MT), and α-tocopherol have been found in the livers of Aquatic organisms (Basha and Rani, 2003; Hansen et al., 2006). Antioxidant enzymes are considered as sensitive biomarkers in environmental stress before hazardous effects occur in fish, and are important parameters for testing water for the presence of toxicants (Heath, 1987; Geoffroy et al., 2004).

Current approaches to minimize the severity of Cd toxicity include enhancement of its sequestration and elimination by using different treatment methods. Considering the relationship of Cd exposure with oxidative stress and elemental homeostasis, we can speculate that the administration of antioxidants and natural biomolecules may be protective in Cd toxicity. Ascorbic acid (Vitamin C) is an essential nutrient in aqua – feed and is an indispensable nutrient required to maintain the physiological processes of different animals, including fish (Tolbert, 1979), however most of the fish species, including tilapia are not capable of Vitamin C biosynthesis (chatterjee, 1973). It is an important dietary antioxidant and in the general absence of metal ion-catalyzed reactions, it is qualitatively the single most important plasma antioxidant (Kang et al. 1998). It has been known to protect all classes of lipids from oxidation under a number of relevant types of oxidant stress (Coa et al. 1998). It is also significantly decreases the adverse effect of ROS that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in several diseases (You et al. 2000). The antioxidant potential of ascorbic acid is not only attributed to its ability to quench ROS, but also to its ability to generate other small molecule antioxidants, such as α-tocopherol, glutathione and β-carotene (Evans P et Halliwell B, 2001).

Therefore, the present study aimed to (1) characterize the chronic sublethal effects of Cd exposure i.e., the levels of liver and gill oxidative stress markers, and serum biochemical parameters, (2) evaluate the possible protective effect of dietary supplementation of Vitamin C against Cd toxicity in adult Nile tilapia.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

- Cadmium chloride (CdCl2) was purchased from Lab Service Co. Egypt and dissolved in water by 5mg/l and vitamin C was purchased from Algomhoria CO. Egypt and added to the ration by 500mg/kg diet. The assay kits used for biochemical measurements of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine levels, lipid peroxide (malondialdehyde, MDA), catalase (CAT), Reduced glutathione, glutathione-s-transferase (GST) and glutathione peroxidase (GPx) were purchased from Biodiagnostic and/or Biotechnology Co., ARE. All other chemicals were of reagent grade and were commercially available from local scientific distributors in Egypt.

2.2. Animals and experimental design

A total of 120 healthy adult Nile tilapia *O. niloticus* individuals (60 ± 5 g bwt) were obtained from Lafranyh Farm, El-Beheira, Egypt. The fish were acclimatized for 2 weeks prior to the experiment in glass aquaria (dimensions, 90 × 30 × 50 cm) filled with 120 L de-chlorinated tap water; the aquaria were aerated and the tilapia were maintained in a laboratory environment with a photoperiod (12-h light and 12-h dark cycle) and a temperature of 29 ± 2°C. Dissolved oxygen 6.5 mg/L, pH 6.9 ± 0.4, and electrical conductivity 219 ± 2 µmho/cm of aquaria water were determined using the Hack Method (Sigma Laboratory); conditions were closely monitored and kept stable during the experiments. The fish were fed a commercially prepared ration (Zoo-Control Co., Egypt) twice daily of 3% of total body weight. The experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH), and the study protocol was approved by the local authorities of Alexandria University. Every effort was made to minimize the number of fish used and their suffering.

2.2.1. Serum biochemical parameters

Biochemical examinations of the Nile tilapia were performed on surviving fish. The body surfaces were cleaned and blotted dry with adsorbent paper. Blood samples, collected from the caudal vessel using disposable 3-cc syringes and 21-gauge needles, were transferred into Eppendorf tubes without anticoagulants for serum separation, as described for the assessments of serum urea and creatinine levels, and AST, ALT, activities, which were spectrophotometrically (LABOMED Co., Lab. American Inc., USA) estimated according to the manufacturer's instructions.

2.2.2. Antioxidants and lipid peroxidation biomarkers

Tissue homogenates were separately prepared from frozen liver and gill samples in 10 volumes of 0.1 M Tris-EDTA buffer (pH 7.4), centrifuged at 1,000 × *g* at 4°C for 30 min. Aliquots of the supernatant were utilized for the following spectrophotometric assessments. The GSH level was assayed using a method based on the reductive cleavage of 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) by a sulfhydryl (-SH) group to yield a yellow color . The reduced chromogen (absorbance measured at 412 nm) is directly proportional to the GSH concentration. The GST activity in tissue homogenates were assayed spectrophotometrically according to the method of Habig and Pabst Jakoby, (1974). By measuring the conjugation of 1-chloro- 2,4- dinitrobenzene (CDNB) with reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample. The activity of the GPx enzyme in tissue was determined colorimetrically using kits from Bio-diagnostic Company. The assay is an

indirect measure of the activity of GPx. The GSSG that is produced upon the reduction of organic peroxide by GPx is recycled to its reduced state by GR. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm (A_{340}), providing a spectrophotometric means for monitoring GPx enzyme activity. To assay GPx, an aliquot of tissue homogenate was added to a solution containing GSH, GR, and NADPH. The enzyme reaction was initiated by adding the substrate, tert-butyl hydroperoxide, and the A_{340} was recorded. The rate of the decrease in A_{340} is directly proportional to the GPx activity in the tested sample. The activity of CAT was measured according to the method described by Aebi (1984). The CAT reacts with a known quantity of H_2O_2, and the reaction is stopped after 1 min with a CAT inhibitor. In the presence of peroxidase, the remaining H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore, with a color intensity inversely proportional to the amount of CAT in the sample. The absorbance was measured at 510 nm. Lipid peroxidation was assayed by measuring MDA formation, as described by Ohkawa et al. (1979). Thiobarbituric acid reacted with MDA in acidic medium at a temperature of 95°C for 30 min to form a TBA reactive product. The absorbance of the resulting pink product was measured at 534 nm.

2.3. Statistical analysis

SPSS statistical package version 17.0 for Windows was used for all data analysis. One-way ANOVA was carried out to illustrate the significant difference between the different geoups within different periods of exposure. All data are expressed as means ± standard deviation (SD) of the means, and the levels of significance were represented for each other. A *p*-value less than 0.05 are considered statistically significant.

3. RESULTS

Our results concerned with determining the subchronic impact of cadmium exposure (5 mg/L) and the possible protective effect of vitamin C supplementation (500 mg/kg diet) on some biochemical parameters including; liver and kidney functions, and antioxidant status and lipid peroxidation levels, and molecular parameters including; MT, and antioxidants enzymes in adult Nile tilapia fish.

3.1. Biochemical findings

3.1.1. Liver and kidney functions

In relation to control group, Cd significantly ($p<0.05$) increased the serum levels of AST at 21 and 42 days, and ALT at 42 days of exposure period which indicate hepatotoxicity, however it has no any effect on serum levels of urea and creatinine (Table 1). Vit C alone has no effect on the measured hepatorenal biochemical parameters, fortunately it returned the increased levels of AST and ALT to their normal values in Cd + Vit C-treated fish.

3.1.2. Antioxidants and lipid peroxidation finding

3.1.2.2. Reduced glutathione.

Table 2 showed that there are no significant ($p>0.05$) changes in hepatic GSH levels at 7 days of exposure between groups; however, they are markedly ($p<0.001$) increased upon Cd exposure at 21 and 42 days in a time-dependent manner. Vitamin C supplementation significantly ($p<0.05$) increased GSH levels at only 42 days of exposure. In gills, GSH levels non-significantly ($p>0.05$) changed in Cd exposed groups in relation to controls, and it was significantly ($p<0.05$) increased upon vitamin C supplement alone.

3.1.2.3. Glutathione-s- transferase

There were no significant ($p>0.05$) changes in the activity of hepatic GST enzyme in Vit.C and Cd + Vit.C treated groups compared with the control (Table 3), however it was significantly ($p< 0.05$) increased in Cd exposed fish at 7, 21and 42 days in a time-dependent manner. In gills, there are also no significant ($p>0.05$) changes in the activity of GST enzyme between groups; except its increase significantly ($p<$

0.05) in Vit. C supplemented group at 21 days of exposure period.

Table 1 Effect of Cd and /or vitamin c on the hepatic and renal function indices (AST, ALT, urea and creatinine contents) at 7, 21 and 42 days of exposure period in adult Nile tilapia fish.

Parameter	Groups	Period of exposure		
		7 days	**21 days**	**42 days**
AST (U/L)	CTR	6.00±1.0	5.33±0.58^{b}	6.33±0.58^{b}
	Cd	6.67±1.53	8.0±1.0^{a}	10.0±1.0^{a}
	VC	4.00±0.0	4.67±0.58^{b}	4.67±1.16^{b}
	CdVC	5.33±1.53	5.33±1.53^{b}	5.0±1.0^{b}
ALT (U/L)	CTR	13.00±5.20	20.67±4.04	14.67±1.16^{b}
	Cd	15.33±6.81	13.33±9.82	23.0±4.36^{a}
	VC	16.33±6.51	12.00±6.25	13.0±3.00^{b}
	CdVC	14.33±7.51	12.67±3.06	14.0±1.73^{b}
UREA (mg/dl)	CTR	7.00±1.0	6.00±1.0	6.00±1.0
	Cd	7.67±3.06	6.67±1.16	7.33±1.53
	VC	7.67±1.16	6.67±1.53	6.33±1.16
	CdVC	9.00±1.0	6.67±0.58	7.33±2.52
Creatinine (mg/dl)	CTR	0.30±0.1	0.300±0.0	0.267±0.06
	Cd	0.30±0.1	0.333±0.15	0.333±0.06
	VC	0.30±0.0	0.233±0.06	0.333±0.06
	CdVC	0.33±0.12	0.267±0.12	0.233±0.06

Mean values within the same column of the same organ carrying different letters are significantly different from each other (p< 0.05). CTR= control group Cd = cadmium group, Vc=Vitamin C group, Cdvc = Cadmium + Vitamin C group ALT= alanine transaminase enzyme AST = aspartate transaminase enzyme

Table 2 Effect of Cd and/or vitamin C on GSH level (mmol/g wet tissue) in the liver and gills of adult Nile tilapia at 7, 21 and 42 days exposure period.

Organ	Group	Period of exposure		
		7 days	**21 days**	**42 days**
Liver	CTR	0.76±0.12	0.87±0.08^{b}	0.69±0.08^{b}
	Cd	0.48±0.06	2.18±0.30^{a}	3.71±0.42^{a}
	VC	0.64±0.15	0.85±0.11^{b}	0.96±0.08^{b}
	CdVC	1.14±0.49	1.33±0.41^{a}	3.80±0.20^{a}
Gills	CTR	0.32±0.09	0.24±0.13^{b}	0.20±0.05^{b}
	Cd	0.40±0.14	0.36±0.11^{ab}	0.33±0.07^{ab}
	VC	0.56±0.09	0.49±0.06^{a}	0.51±0.18^{a}
	CdVC	0.48±0.03	0.40±0.04^{ab}	0.21±0.08^{ab}

Mean values within the same column of the same organ carrying different letters are significantly different from each other ($p< 0.05$). CTR= control group Cd = cadmium group, Vc=Vitamin C group, Cdvc = Cadmium + Vitamin C group

Table 3 Effect of Cd and/or vitamin C on GST activity (U/g wet tissue) in the liver and gills of adult Nile tilapia after 7, 21 and 42 days of exposure period.

Organ	Group	Period of exposure		
		7 days	21 days	42 days
Liver	CTR	0.13±0.02[b]	0.26±0.03[b]	0.20±0.06[b]
	Cd	0.45±0.10[a]	0.46±0.05[a]	0.47±0.07[a]
	VC	0.49±0.05[a]	0.50±0.08[a]	0.43±0.07[a]
	CdVC	0.40±0.07[a]	0.55±0.00[a]	0.53±0.11[a]
Gills				
	CTR	0.29±0.03	0.26±0.03[b]	0.31±0.03
	Cd	0.19±0.08	0.23±0.02[b]	0.41±0.08
	VC	0.39±0.17	0.48±0.09[a]	0.53±0.18
	CdVC	0.23±0.04	0.29±0.04[b]	0.34±0.09

Mean values within the same column of the same organ carrying different letters are significantly different from each other ($p< 0.05$). CTR= control group Cd = cadmium group Vc=Vitamin C group Cdvc = Cadmium + Vitamin C group

Table 4 Glutathione peroxidase (GPx, U/g tissue) activities in the liver (A) and gills (B) of adult Nile tilapia exposed to Cd and/or Vit.C for 42 days. All values are expressed as means ± SD.

Organ	Group	Period of exposure		
		7 days	21 days	42 days
Liver				
	CTR	55.30±9.49	52.27±9.82	53.38±4.29[a]
	Cd	47.93±3.91	45.70±7.96	35.41±2.45[b]
	VC	45.74±4.04	48.12±6.04	47.21±6.80[ab]
	CdVC	48.03±2.42	41.03±1.26	48.03±4.54[a]
Gills				
	CTR	44.63±7.75	45.97±8.08	52.30±5.02
	Cd	52.00±13.00	65.61±14.39	61.97±14.79
	VC	52.00±11.53	57.07±22.65	65.40±11.53
	CdVC	60.30±16.96	54.67±5.85	79.23±16.07

Mean values within the same column of the same organ carrying different letters are significantly different from each other ($p< 0.05$). CTR= control group Cd = cadmium group Vc=Vitamin C group Cdvc = Cadmium + Vitamin C group

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3.1.2.4. Glutathione peroxidase

The hepatic GPx activity was significantly ($p<0.05$) decreased in Cd-exposed group at 42 days of exposure compared with the control (Table 4), and without any significant ($p>0.05$) changes in Vit.C-supplemented groups. In gills, there were no any significant ($p>0.05$) changes in GPx activity between groups at different times of exposure.

3.1.2.5. Catalase

In liver, there were no significant ($p>0.05$) changes in CAT activity at 7 and 21 days of exposure period compared with the control (Table 5). However, CAT activity significantly

(p<0.05) increased in Cd exposed fish at 42 days of exposure period. And in gills, there were no significant (p>0.05) changes in CAT activity at all experimental period.

Table 5 Catalase (CAT, U/g tissue) activity in the liver and gills of adult Nile tilapia fish (*Oreochromus niloticus*). All values are expressed as means ± SD.

Organ	Group	Period of exposure		
		7 days	21 days	42 days
Liver				
	CTR	2.32±0.22[b]	2.38±0.35[b]	2.44±0.13[b]
	Cd	3.18±0.14[ab]	3.49±0.31[a]	3.51±0.19[a]
	VC	2.70±0.06[ab]	2.58±0.25[b]	2.68±0.05[b]
	CdVC	3.10±0.61[ab]	2.55±0.37[b]	2.78±0.33[b]
Gills				
	CTR	3.37±0.06	3.03±0.31[ab]	2.83±0.45
	Cd	2.33±0.65	2.73±0.23[b]	2.73±0.40
	VC	3.13±0.46	3.23±0.38[a]	3.17±0.21
	CdVC	2.80±0.36	2.87±0.06[b]	2.97±0.25

Mean values within the same column of the same organ carrying different letters are significantly different from each other (p< 0.05). CTR= control group Cd = cadmium group Vc=Vitamin C group Cdvc = Cadmium + Vitamin C group

Table 6 Malondialdehyde (MDA) levels in the liver and gill of Nile tilapia exposed to cd and/or vit. C for 42 days exposure period and overall treatment means. All values are expressed as means ± SD

Organ	Group	Period of exposure		
		7 days	21 days	42 days
Liver				
	CTR	17.82±4.05[b]	18.66±5.07[b]	17.03±1.91[b]
	Cd	145.03±18.83[a]	92.93±9.9[a]	116.00±9.04[a]
	VC	12.83±0.96[b]	12.73±2.78[b]	9.53±0.83[b]
	CdVC	117.08±9.25[a]	76.43±10.04[a]	46.57±34.33[b]
Gills				
	CTR	141.01±18.79	113.45±16.29	167.03±19.87[ab]
	Cd	162.89±13.31	203.05±38.01	233.20±63.62[a]
	VC	127.98±24.65	112.77±56.89	123.59±23.45[b]
	CdVC	166.82±78.33	183.50±65.78	235.59±13.21a

Mean values within the same column of the same organ carrying different letters are significantly different from each other (p< 0.05). CTR= control group Cd = cadmium group Vc=Vitamin C group Cdvc = Cadmium + Vitamin C group

3.1.2.5. Malondialdehyde (MDA) levels.

In relation to control group, **Table 6** showed that in liver MDA level significantly (p<0.05) increased in Cd-exposed group and returned to normal level in Cd + Vit.C-supplemented group. However, it was unchanged in vit.C group. In gills, MDA level

insignificantly (p>0.05) increased in Cd and Cd + Vit.C group at 42 days of exposure.

4. DISCUSSION

The contamination of water by metal compounds is a worldwide environmental problem. Heavy metals, such as cadmium, are released into the environment by industries and reach streams and rivers via run-off from unregulated waste disposal (Nriagu et al., 1998). Cadmium is a highly toxic heavy metal, since it causes deleterious effects in organisms at low levels of exposure (Cope et al., 1994). The present study demonstrates the effect of sub-lethal Cd exposure for 42 days on several pollution indicative biomarker enzymes and the possible protective effects of Vitamin C in O. niloticus.

Serum biomarkers such as ALT, AST, urea and creatinine have been used to detect cellular damage in liver and kidneys, and measure the responses to metals (Yang and Chen 2003; M. O'Neill et al. 1998; Congleton and La Voie, 2001). It was emphasized that their measurement can be useful as a diagnostic tool in fish toxicology to identify their general health status and target organs affected by toxicants (Zikic et al. 2001; McDonald and Grosell 2006). In the present study, exposure of Nile tilapia to chronic sublethal concentration of Cd significantly increased the serum activity of AST at 21 and 42, and ALT at 42 days of exposure. these results indicate liver injury. Previously, sublethal concentration of Cd caused significant increases in AST and ALT of common carp after 7 and 15 days (Shalaby, 1997) and O. niloticus after 15 and 30 days (Mekkawy et al. 2010). The increased concentration of transaminases activity in blood in Cd-exposure may be attributed to the hepatocellular damage or cellular degradation induced by the cadmium, perhaps in liver, heart or muscles (Öner et al. 2008). Interestingly, Vit. C supplementation in cd-exposed fish returned the increased values of AST and ALT to the normal values as that of controls. The results obtained herein, revealed that dietary Vit. C may decrease the tissue injury in intoxicated fish. Abdel-Tawwab et al. (2001) reported that dietary fed of ascorbic acid (Vit.C) returned the decreased level of AST and ALT in O. niloticus exposed to mercury and they concluded that ascorbic acid (vitamin C) (>500 mg/kg diet) be used to be efficient in toxicity reduction and enhancing fish tolerance to environmental stress. The different levels of ascorbic acid prevented the inhibition of AST activities in Tilapia zillii intoxicated with copper (Ghazaly, 1994). Therefore, it could be concluded that dietary ascorbic acid is efficient in the reduction of Cd toxicity.

The antioxidant enzymes have been shown to work in a cooperative or synergistic manner to protect against oxidative stress and tissue-specific damage. These enzyme systems have been proposed as a biomarkers of reactive oxygen species (ROS) mediating contaminant exposure and as a potential tool in environmental risk assessment (Kohen and Nyska 2002; Livingstone 2001). The ROS are detoxified by a set of antioxidant enzymes that protect macromolecules such as proteins, lipids and nucleic acids against damage (Lushchak et al. 2001; Ozmen et al. 2004). Several studies revealed that exposure to contaminants, including Cd, in aquatic ecosystems can enhance the intracellular formation of ROS which could cause oxidative damage to biological systems (Ercal et al. 2001; Ferreira et al. 2006). Earliest biological indicators of toxicant-induced oxidative stress are observed as shifts in enzymatic activity or changes in other biochemical systems. Antioxidant systems include superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase, glutathione peroxidase (GPx) and glutathione (GSH) are the protective agents identified as early indications of cellular susceptibility to oxidant injury caused by free radicals (Vijayavel et al., 2004).The antioxidant defense systems are divided into two categories: enzymatic (e.g., SOD, CAT, GPx and GST) and non-enzymatic (e.g., GSH). In fish, metal exposure alters the GSH status (Maracine and Segner, 1998). In the present study, GSH level in the liver of O. niloticus increased

under Cd exposures especially at 21 and 42 days of exposure period and the level of GSH not changed in gills. A similar observation has been reported by Atli and Canli (2008) and Firat O. et al. (2008) that GSH levels increased in liver of O. niloticus exposed to Cd. They concluded that the induction of GSH is probably due to the primary defense system involving GSH in protecting the fish from oxidative stress and they suggested that Cd induces increased synthesis of hepatic GSH in O. niloticus; the formed GSH results in an important decline in bio-available metallic cations. Moreover, most of radicals generated by metal-mediated reactions can be neutralized by GSH and GSH is considered as a first line of cellular defense against metals by chelating and detoxifying them, scavenging oxyradicals and participating in detoxification reactions catalyzed by glutathione peroxidases (Sies, 1999). Also El-Demerdash et al., (2004) reported that Cd has high affinity to GSH, where a sulfhydryl, an amino, and two carboxylic acid groups, as well as two peptide linkages, represent reactive sites for metals. In addition, the elevated GSH in tilapia may be related to the tolerance of tilapia to Cd stress. Studies have shown that tilapia can tolerate high levels of waterborne Cd (U.S. Environmental Protection Agency, 2001; Garcia-Santos S., 2006). The high tolerance to Cd may require high basal and induced GSH to ameliorate Cd toxicity, since elevated levels of GSH correlate with environmental stress tolerance (May 1998).

Glutathione-S-transferases form a family of multifunctional phase II biotransformation enzymes present in the cytosol of most cells, which catalyze the conjugation of glutathione to a variety of compounds and also involved in the transport and elimination of reactive compounds that carry other indirect antioxidant functions (Livingstone 2003). In the present study, there is no any significant change in gill GST but hepatic GST activity significantly increased due to Cd exposure. Previosly Cd exposure increased GST activity in the liver of O. niloticus exposed to 5 mg/L for 30 days (Basha and Rani, 2003), 3 mg/L for 5, 10, and 20 days (Xu Z. and Bai S., 2007), and 2 mg/L for 8 days due to (Lin Y. et al. 2011) and they indicated that the increased level of GST in liver showed a possible shift toward a detoxification mechanism under long term exposure to the heavy metal Cd2+.

The GPx detoxifies H2O2 or organic hydroperoxides that produced in LPO (Halliwell and Gutteridge 1999). In the present study, Cd exposure significantly decreased the activity of hepatic GPx at 42 days of exposure and not affects GPx activity in gills. Our results are in accordance with a previous study (Lin et al., 2011) that highlighted the different responses of GPx to Cd in the gill and liver tissues. They found that GPx activity was stimulated in the gill of fish exposed to 0.016, 0.08 and 0.4 mg/L of Cd but not in fish exposed to 2 mg/L Cd. On the other hand, Cd significantly inhibited GPx activities in the fish livers of all the Cd treatment groups and they returned these apparently different responses to Cd in the gills and in the livers to their suggestion that GPx showed a tissue-specific response to Cd exposure and they depend on Tissue specific alternations of CAT and SOD which documented in O. niloticus exposed to diazinon (Durmaz. et al., 2006).

Also, they reported that after scrutinization of Cd burdens in these two tissues, it seemed more likely that Cd caused a hormetic response of GPx in O. niloticus (characterized by a low dose of stimulation and a high dose of inhibition) and the inhibition of GPx was probably the direct toxic effect of Cd on GPx as Cd has high affinity to bind –SH groups and therefore deactivates the enzyme activity, as reported in other studies (Karmakar. et al., 1999; Giguère. et al., 2005). Also, a similar hormetic effect of Cd on GPx was observed in a marine fish (Salaria basilisca) which had an increased activity of GPx after 14 days exposure but a decreased activity after 28 days of exposure to Cd (Messaoudia I. et al., 2009). Furthermore Berntssen M. et al., (2000) demonstrated that inhibited GSH-GPx activity may give rise to increased formation of free radicals, which can cause subsequent oxidation of fatty acids.

Catalase a primary antioxidant defense component protects fish from oxidative stress by converting the hydrogen peroxide to oxygen and water (Atli & Canli, 2007). In the present study there were no any significant changes in CAT activity in Gills. However, CAT activity increased in Cd exposed fish at 21 and 42 days of exposure. Previously Atli and Canli, (2007) and (2010) reported that CAT activity increased in liver of O. niloticus due to an effective antioxidant defense system acting against oxidative stress caused by metal exposures and/or compensating for the decrease in other antioxidant enzymes, such as GPX. On the other hand, no significant change in the CAT activity may be attributed to the increase in non- enzymatic mechanisms such as GSH. Also, Basha and Rani (2003) demonstrated that there were simultaneous increases in the levels of CAT activity in the liver following Cd exposure of O. niloticus. They indicated that there was a possible shift toward a detoxification mechanism under long term Cd exposure.

Lipid peroxidation has been considered as the first step of cellular membrane damage by heavy metals (Viarengo, 1989; Ahmad, 1995; Livingstone, 2001). In the present study, MDA level significantly increased in liver and its level not changed in gills of Cd-exposed fish. Our result is in agreement with (Lin Y. et al. 2011) in which they demonstrated that Cd increased MDA level in liver of O. niloticus exposed to 2 mg/l and not increased in gills. They indicated that the increase in lipid peroxidation implied that excess ROS had been produced in the liver upon exposure to Cd. The increase in lipid peroxidation was also observed in O. niloticus exposed to 3 mg/L (Almeida et al. 2009) and to 4.64 mg/L Cd (Mekkawy et al. 2010). And other fish species challenged with Cd (Berntssen et al. 2000; Asagba et al. 2008).

Vitamin C is an important dietary antioxidant and significantly decreases the adverse effect of reactive species such as reactive oxygen that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are associated in several diseases (You. et al. 2000).In the present study vitamin c supplementation in Cd-exposed fish significantly increased GSH level, GST activity, returned the GPx and CAT enzymes activity to their normal level and decreased LPO level in liver. Previously, Vijayavel. et al. (2004) reported that Administration of ascorbic acid to the copper stressed fish decreased LPO level and increase the activities of CAT, GPx and GSH level due to the ability of ascorbic acid to directly react with various reactive oxygen species as well as to interfere with oxidation processes in lipid and cellular compartment (Ramanathan et al., 2002).

Singh and Rana, (2007) found that GSH status improved on ascorbic acid co-treatment also, ascorbate co-treatment restored GST enzyme activity in male rats and this was considered as an adaptive response facilitated by ascorbic acid against arsenic induced stress

Li et al., (2001) and Nandi et al., (2005) demonstrated that co-treatments of ascorbic acid and arsenic inhibited lipid peroxidation in liver and kidney due to its antioxidative property as Ascorbic acid is well known to inhibit oxidative damage to membranes.

Gupta and Kar, (1987) reported that vitamin C can prevent increased lipid peroxidation levels resulting from cadmium toxicity and they concluded that vitamin C could serve as an effective antioxidant against restraint stress induced pro-oxidant status and increase the antioxidant enzyme activity in rat brain (Zaidi and Banu, 2004) and liver (El-Sokkary, 2008).

Also, our results probably ascribed to the properties of vitamin C closely related to the immunological system performance, antioxidant action, maintenance of the integrity and fluidity of biological membranes (Brake, 1997) and control oxidizing reaction of fatty acids. The latter function is particularly important in keeping cellular respiration and avoiding cell death (Verthac and Gabaudan, 1994).

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