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Effects of heavy metal contamination on *Oreochromis niloticus* (Tilapia fish)

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Abstract

This study discusses the efecfts of heavy metal contamination in aquaculture ponds on *Oreochromis niloticus* (Tilapia fish). The effects of sub-lethal exposure to cadmium chloride and lead acetate on oxidative stress biomarkers (lipid peroxidation product) and antioxidant biomarkers (reduced glutathione) were investigated in *O. niloticus*. The determination of GSH was dependent on the precipitation of protein using a tungstate. Sulfuric acid solution and the formation of yellow color after reaction with 5, 5` dithiobis- 25-nitrobenzoic acid (DNTB) was measured at wave length 412 nm. There was a significant decrease in reduced glutathione (GSH) after exposure to different doses of cadmium chloride and lead acetate and a significant increase in lipid peroxidation product (LPO) after exposure to different doses of cadmium chloride and lead acetate. The investigation indicates that the cadmium chloride and lead acetate are elevating the level of lipid peroxidation which are toxic in Tilapia fish.

Keywords: cadmium chloride; lead acetate; 50% lethal concentration (LC_{50}); lipid peroxidation product; reduced glutathione.

Practical Application: Heavy metals are effecting on Tilapia fish by increasing the level of lipid peroxidation.

1 Introduction

Tilapia is a freshwater fish distributed throughout the tropical zones around the world. It lives in fresh water and is sold in markets and is an important cultural fish because it reproduces very easily and does not have feeding problems (Kalay & Canli, 2000). Tilapia can survive in poor environmental conditions because their resistance to disease is strong, and their respiratory demands are slight so that they can withstand low oxygen and high ammonia levels (Taweel et al., 2011). Fish are broadly used to biologically observer the degree of metal pollution in marine ecosystems and higher level of aquatic food chain. Toxic elemental contaminants are transmitted into human through ingesting of contaminated fish that leads to serious corrosion of human health standing (Mansour & Sidky, 2002). Metals are naturally occurring elements that become contaminants when human activities increase their concentrations above normal levels in the environment. Fish are the final organism in the aquatic food chain and a significant food source for many humans. Consequentially, heavy metals in aquatic environments are transferred throughout the food chain into humans (Taweel et al., 2011).

Tilapia is the most economically important farmed fish species (Ispir et al., 2011) and is an ideal species of organisms for an assessment study on effects of heavy metal contamination in aquaculture ponds (Taweel et al., 2011). So, the purpose of the present study was to determine the LC_{50} of cadmium and lead in *O. niloticus*.

2 Material and methods

2.1 Experimental animals

The study was carried out on 80 *Oreochromis niloticus*. The body weight and total body length of the fish ranged from 50-100 g and 10-15 cm, respectively. Fish were collected from El-Abbassa Fish Farm and transferred alive in a large plastic container to the laboratory where they were distributed in well-ventilated glass aquaria. The fish were acclimatized for 14 days before the onset of the experiment. Fish were fed commercial pellets and checked daily. The use of experimental animals in the study protocol was carried out in accordance with the ethical guidelines of the Medical Research Institute, Alexandria University.

2.2 Experimental design

The fish were classified into two groups as follows:

- Group 1. For the determination of 96-h LC_{50} (lethal concentration) of cadmium, 40 fish were used. Four concentrations of cadmium in the form of cadmium chloride ($Cd\ Cl_2$) were used (10, 20, 30, 40 mg/L), and each concentration was added into an aquarium containing 10 fish.

- Group 2. 40 fish were used for the determination of the 96-h LC_{50} value for lead in the form of lead acetate Pb

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$(CH_3COO)_2$. Four concentrations of lead acetate were used (50, 100, 150, 200 mg/L), and each concentration added into single aquarium containing 10 fish. Fish in each aquarium were continuously observed during the 96 h experiment period. Mortalities and time of death were recorded during the experiment period. These data were entered into SPSS 8.0, and probit analysis was used to determine the 96-h LC_{50} value for cadmium and lead (Singh et al., 2010).

- Group 3. This group studied the effect of ½ LC_{50} of each cadmium and lead on 50 fishes, and consisted of two subgroups:

Subgroup 1 determined the effect of ½ LC_{50} of cadmium chloride on some biological parameters after 1, 2, 4, and 6 days. The last aquarium served as control.

Subgroup 2 also determined the effect of ½ LC_{50} of lead acetate on the same parameters and for the same periods of exposure.

2.3 Tissue collection

Liver was weighed and homogenized immediately to give a 50% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose. The homogenate was centrifuged at 500 g for 10 min at 4 °C. The supernatant (10%) was used for biochemical determinations.

2.4 Statistical analysis

The results were expressed as the mean ± standard error of means (SEM). Data were statistically analyzed using Student's t-test with the program Statistical Package for Social Sciences (SPSS) version 0.8. The means of the data were considered significantly different at $p < 0.05$.

3 Results

These results study the effect of different concentrations of cadmium (Cd) and lead (Pb) individually administered to *Oreochromis niloticus*. Behavioral abnormalities were observed in fish exposed to different concentrations of Cd and Pb, including increases in breathing rate through increased movement of the operculum, increases in fish surfacing and panting for air, after which fish became lethargic, unconscious, and lost equilibrium. Finally, body activity and feeding decreased, resulting in bottom dwelling in the aquaria and death.

Table 1 shows the relationship between cadmium chloride concentrations (10, 20, 30, 40 mg/L) and mortality rates of *Oreochromis niloticus*. Mortality was virtually absent in the control, and found to be suitable for the LC_{50} upper and lower confidence limits.

According to analysis as shown in Table 2, the median lethal concentration (LC_{50}) of Cadmium in *Oreochromis niloticus* for 96 h of exposure was 28.01 ppm. The lower and upper lethal confidence limits for cadmium chloride indicate a wide range from 21.15 to 38.78 ppm, within which lies the concentration response for 96 h of exposure.

Table 3 shows the relationship between lead acetate concentrations (50, 100, 150, 200 mg/L) and mortality rates of *Oreochromis niloticus*. Mortality was virtually absent in the control and was found to be suitable for the LC_{50} upper and lower confidence limits.

According to the analysis as shown in Table 4, the median lethal concentration (LC_{50}) of lead in *Oreochromis niloticus* for

Table 1. Correlation between the Cadmium Chloride ($Cd\ Cl_2$) concentration and the mortality rate of *Oreochromis niloticus*.

Log Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Prob
1.00	10.0	0.0	.219	-0.21	0.02
1.30	10.0	3.0	2.549	0.45	0.25
1.48	10.0	6.0	5.534	0.46	0.55
1.60	10.0	7.0	7.571	-0.57	0.75

Table 2. LC_{50} value of cadmium chloride ($Cd\ Cl_2$) with lower and upper (95%) confidence limits.

	95% Confidence Limits		
Probit analysis	Concentration	Lower	Upper
0.01	8.52	1.12	13.84
0.05	12.08	2.86	17.39
0.10	14.54	4.68	19.76
0.15	16.49	6.48	21.65
0.50	28.01	21.15	38.78
0.80	43.06	33.04	104.06
0.90	53.92	38.89	186.95
0.95	64.93	44.11	305.98
0.96	68.54	45.72	353.50
0.99	91.99	55.33	778.25

96 h of exposure was 111.94 ppm. The lower and upper lethal confidence limits for cadmium chloride indicate a wide range of 66.13 to 183.28 ppm, within which likes the concentration response for 96 h of exposure.

Table 5 demonstrates the effects of individual administration of 1/2 LC_{50} of Cd and Pb on lipid peroxidation. Cd caused significant increases after the 4th day (+ 51.52%) and the 6th day (+ 93.18%), while the administration of 1/2 LC_{50} of Pb showed significant increase after 4th and 6th day (+ 68.94% and + 99.24%, respectively).

The individual administration of 1/2 LC_{50} of Cd and 1/2 LC_{50} of Pb showed significant decrease after the 4th day (-14.96% and -24.83%, respectively) and the 6th day (-22.45%, -31.97%, respectively) Table 6.

4 Discussion

Heavy metal pollution is the focus of biological monitoring in environmental and occupational health studies because of their widespread use (Hunaiti & Soud, 2000). They become toxic when they are not metabolized by the body and are accumulated in the soft tissues. Heavy metals may enter the human body through water, food, air, or absorption through the skin when humans come in contact with them in agriculture and in manufacturing, industrial, pharmaceutical or residential locations (Suleman et al., 2011).

Table 3. Correlation between lead acetate (Pb $(CH_3COO)_2$) concentration and mortality rate of *Oreochromis niloticus*.

Log Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Prob
1.70	10.0	2.0	1.72	0.27	0.17
2.00	10.0	4.0	4.47	-0.47	0.44
2.18	10.0	6.0	6.34	-0.34	0.63
2.30	10.0	8.0	7.51	0.48	0.75

Table 4. LC_{50} value of lead acetate (Pb $(CH_3COO)_2$) with lower and upper (95%) confidence limits.

	95% Confidence Limits		
Probit analysis	Concentration	Lower	Upper
0.01	15.38	0.087	37.08
0.05	27.51	0.681	52.91
0.10	37.51	2.02	64.40
0.15	46.23	4.19	73.96
0.50	111.94	66.13	183.28
0.80	229.54	152.17	1563.00
0.90	334.10	196.71	5730.59
0.95	455.51	239.61	17004.71
0.99	814.74	342.10	132658.82

Table 5. Effect of 1/2 LC_{50} of cadmium (Cd) or lead (Pb) on lipid peroxidation (LPO) in liver tissue of *Oreochromis niloticus*.

Concentration mg/L	Day of exposure			
	1 day	2 day	4 day	6 day
	nmol/g ± SE	nmol/g ± SE	nmol/g ± SE	nmol/g ± SE
Control	1.32 ± 0.11	1.32 ± 0.11	1.32 ± 0.11	1.32 ± 0.11
1/2 LC50 Cd % Change	1.55 ± 0.10 17.42	1.33 ± 0.15 0.75	2.00 ± 0.09 51.52.*	2.55 ± 0.09 93.18*
1/2 LC50 Pb% Change	1.39 ± 0.03 5.30	1.45 ± 0.08 9.85	2.23 ± 0.33 68.94*	2.63 ± 0.13 99.24*

Table 6. Effect of 1/2 LC_{50} of cadmium (Cd) or lead (Pb) on Glutathione (GSH) in liver tissue of *Oreochromis niloticus*.

Concentration mg/L	Day of exposure			
	1 day	2 day	4 day	6 day
	nmol/g ± SE	nmol/g ± SE	nmol/g ± SE	nmol/g ± SE
Control	5.88 ± 0.14	5.88 ± 0.14	5.88 ± 0.14	5.88 ± 0.14
1/2 LC50 Cd % Change	5.62 ± 0.21 -4.42	5.56 ± 0.22 -5.44	5.00 ± 0.19 -14.96*	4.56 ± 0.13 -22.45*
1/2 LC50 Pb % Change	5.52 ± 0.25 -6.12	5.02 ± 0.36 -14.63	4.42 ± 0.17 -24.83*	4.00 ± 0.14 -31.97*

Statistical analyses of results were performed between control (5) and treated (5). *Significant ($P < 0.05$). %: change from control.

The 96-h LC50 values of fish differ from metal to metal and from species to species (Gill & Pant, 1985; Shah & Altindağ, 2005; Alkahemal-Balawi et al., 2011).

Fish that are highly susceptible to the toxicity of one metal may be less or non-susceptible to the toxicity of another metal at the same concentration of that metal in the environment. Similarly, a metal which is highly toxic to one organism at low concentration may be less or non-toxic to other organisms at the same or even higher concentrations (Alkahemal-Balawi et al., 2011).

The heavy metals Cd, Pb, Al, and Zn are known to produce ROS and induce oxidative stress in certain plant species (Verma & Dubey, 2003). The production and accumulation of ROS inhibit the electron transfer chain in mitochondria. In general, the accumulated ROS consists of various amounts of hydrogen peroxide, hydroxyl ions, singlet oxygen, superoxide anions, lipid hydroperoxides, phospholipid hydroperoxides, etc. Excessive production of ROS disturbs the balance between the ROS and antioxidant agents (enzymes and antioxidant substances) in the cells (Latinwo et al., 2006).

Free radicals are created in normal and pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. Though, the uncontrolled production of oxygen-derived is involved in generating many diseases such as rheumatoid arthritis, cancer,and arteriosclerosis, as well as in degenerative manners associated with aging. Exogenous chemical and endogenous metabolic processes in the human body or in the digestive system might produce highly reactive free radicals, particularly oxygen-derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Almost all organisms are well protected against free radical damage by oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT), or by chemicals (non-enzymatic) such as ascorbic acid, a-tocopherol, carotenoids, polyphenols (Saleh et al., 2010).

Glutathione is a ubiquitous thiol-containing tripeptide that is involved in numerous processes that are essential for normal biological function, such as DNA and protein synthesis. It is predominantly present in cells in its reduced form (GSH), which is the active state. Among the several important functions of GSH, it contributes to the removal of reactive electrophiles (such as many metabolites formed by the cytochrome P-450 system) through conjugation by means of glutathione *S*-transferases (GSTs). GSH also scavenges ROS directly or in a reaction catalyzed by glutathione peroxidase (GPx) through the oxidation of two molecules of GSH to a molecule of glutathione disulphide (GSSG). The relationship between the reduced and oxidized state of glutathione, the GSH/GSSG ratio or glutathione redox status, is then considered as an index of the cellular redox status and a biomarker of oxidative damage because glutathione maintains the thiol-disulphide status of proteins, acting as a redox buffer (Peña-Llopis et al., 2003).

In agreement with a previous study, the level of GSH was significantly decreased in the liver tissue of the cadmium treated group compared to the control group. This decrease in GSH levels may be due to its consumption in the prevention of free radical-mediated lipid peroxidation (Koyuturk et al., 2006) Additionally, GSH may be consumed in the detoxification of heavy metals (Thévenod, 2003). Furthermore, it has been suggested that the decrease in GSH levels upon cadmium exposure might impair the degradation of lipid peroxides, thereby leading to its accumulation in the target organs (Sarkar et al., 1997). In contradiction to the current results, Kamiyama et al. (1995) reported an increase in GSH level in liver and kidney tissues after Cd injection, which could be explained as a protective mechanism.

The mechanisms of cadmium-induced damage include the production of free radicals that alter mitochondrial activity and genetic information (Burbure et al., 2006). In the current study, lipid peroxidation level (LPO) was significantly elevated in liver tissue of *O. niloticus* treated with cadmium compared to the control group, thus suggesting increased oxidative stress. These results were supported by Manca et al. (1991), who reported that LPO is an early and sensitive consequence of Cd exposure. Additionally, Hassoun & Stohs (1996), demonstrated that oxidative stress was induced following oral administration of cadmium chloride to rats. A similar results had been reported by Jurczuk et al. (2004). In addition, Jahangir et al. (2005) and Eybl et al. (2006) reported that cadmium is thought to induce lipid peroxidation, and this has often been considered to be the main cause of its deleterious influence on membrane-dependent function.

In this study, lead acetate induced decreases in glutathione and elevations in lipid peroxidation product in all fish under investigation and. This result is supported by Jahangir et al. (2005) and Eybl et al. (2006), who reported that lead is a ubiquitous environmental metal which can induce a broad range of physiological, biochemical and behavioral dysfunctions in fish. Oxidative stress has been proposed as a possible pathogenesis of lead toxicity. Previous studies reported that lead either decreased in antioxidant status, such as with GSH, or elevated LPO in lead-exposed animals. Lead showed the inhibition of several enzymes with functional SH groups. Reduced glutathione is tripeptide containing cysteine that has a reactive SH group. Normally, GSH plays a key role in cellular protection against oxidative stress by direct interaction of the SH group with reactive oxygen species (ROS), or involvement in the enzymatic detoxification reactions of ROS as a cofactor or a coenzyme.

The effects of lead might be attributed to its ability to generate reactive toxic action oxygen species. The Lead induce oxidative damage in several tissues by enhancing lipid peroxidation that occurs readily in the tissues, due to presence of membranes rich in polyunsaturated fatty acids (Mahmuda et al., 2020; Yacoub et al., 2021; Simukoko et al., 2022).

5 Conclusions

This investigation indicates that different doses of cadmium chloride and lead acetate elevate the level of lipid peroxidation, and decrease the level of glutathione content in fish.

Conflict of interest

The authors declared that they have no competing interests.

Availability of data and material

The data used to support the findings of this study are included within the article.

Author contributions

S.E.E., A.A.A., R.L.A. and M.F.E. contributed to study design. W.A.A. and H.M.Y. contributed to data acquisition. R.A.G. and M.A.A. organized the database, performed the statistical analysis. All authors revised, improved, read, and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

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