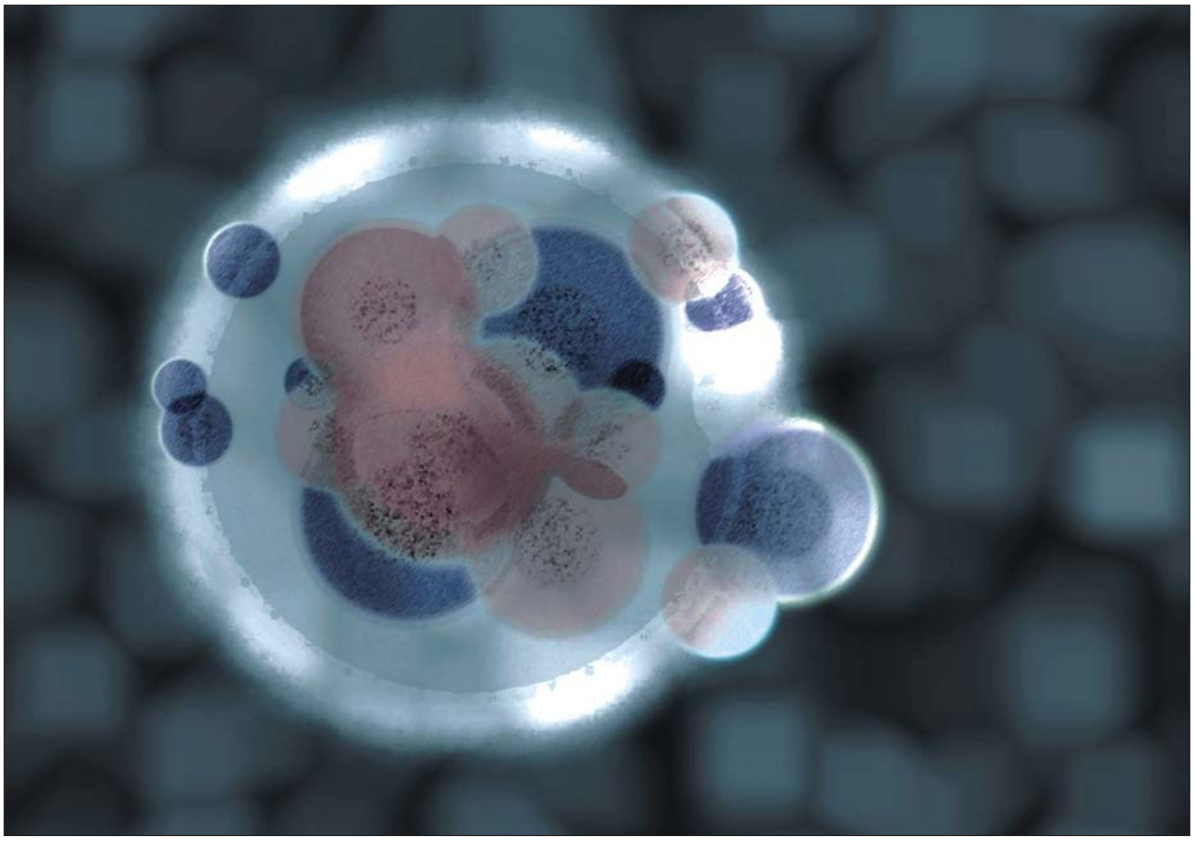

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences 5 (3): 260-265, 2005
ISSN 1727-3048

Biochemical Effects of Short-term Cadmium Exposure on the Freshwater Fish, *Oreochromis niloticus*

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Abstract: The influences of cadmium, a well known environmental pollutant, on different biochemical parameters in fresh water fish, *Oreochromis niloticus* exposed to sublethal concentration of 9.3 ppm, 0.5 of 96 h LC_{50}, for 1, 4 and 7 days were investigated. Significant increases in the levels of blood glucose and the activities of glutamic pyruvic acid transaminase, glutamic oxaloacetic acid transaminase and alkaline phosphatase were observed in the fish treated with cadmium during all experimental periods. The levels of blood triglycerides and total protein were statistically elevated at second and last periods. Insignificant alterations in the levels of blood cholesterol were noted. The observed hyperglycaemia induced by cadmium might be explained in part by increasing rate of glycogenolysis or gluconeogenesis. The observed hypertriglyceridaemia and the elevations of glutamic pyruvic acid transaminase, glutamic oxaloacetic acid transaminase and alkaline phosphatase may be due to liver dysfunction. The observed hyperproteinaemia in the fish, *Oreochromis niloticus* following cadmium administration is possibly attribute to disorder of protein metabolism. However, the significance of the toxic effects of sublethal concentration of cadmium on the various biochemical parameters studied is discussed.

Key words: Biochemical parameters, blood, cadmium, *Oreochromis niloticus*, toxicity

INTRODUCTION

Heavy metals are recognized as one of the most hazardous environmental pollutants and they toxic to many living organisms. Environmental exposure to heavy metals has been reported to cause disease in human and other mammals[1]. In recent years there has been a growing interest about the effects of heavy metals on the health of fish. This aspect is of great importance with development of fisheries, which are often located in rivers, ponds or estuaries subjected to industrialization or mining practices[2]. Contamination of freshwater with heavy metals causes devastating effects on ecological balance of the aquatic environments. The diversity of aquatic organisms becomes limited with the extent of pollution. Cadmium is among the heavy metals most toxic to many aquatic organisms[3-5]. Also, cadmium is a carcinogenic metal[6-8]. Cadmium effects have been already recorded as affecting different metabolic and physiological variables[9-14].

The aim of this study was to investigate the effect of exposure to a sublethal concentration of cadmium on the blood of adult Tilapia, *Oreochromis niloticus* during different time periods from 1 to 7 days. The Tilapia was chosen as an experimental animal on the basis of some important criteria. The organism is representative of an ecologically important group. Moreover, it is widely edible and available, is amenable to laboratory testing, easily maintained and genetically stable so uniform populations can be tested. In addition, other purposes including type of test, sensitivity to the pollutants and consistency in response to the pollutants were taken into consideration.

MATERIALS AND METHODS

Experimental animals: Adult healthy specimens of *Oreochromis niloticus* (200±10.2 g weight) were obtained from a local hatchery. They were adapted to laboratory conditions for at least 2 weeks in several 200 L glass aquaria containing well aerated tap water (pH = 7.1±0.1, hardness as $CaCO_3$ = 94±2 ppm, oxygen concentration= 8.9±0.1 ppm and temperature = 22±1°C). Thereafter, groups of 10 fish each were also adapted for 1 week in 100 L experimental glass aquaria. During the adaptation period they were fed 3 times daily on commercial fish pellets (35% protein) and they were fasted for 24 h prior to any experiment.

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Toxicity bioassay: After adaptation period, preliminary experiments were conducted to determine the median lethal concentration at 96 h (96 h LC_{50}) for cadmium. Various concentrations of cadmium as Cd $(NO_3)_2.4H_2O$ (10, 12, 14, 16, 18, 20, 22 and 24 ppm Cd) were prepared in eight 100 L glass aquaria. Groups of 10 fish were exposed to each test concentration of cadmium for 96 h. The ninth group served as control. The exposure and control tests were replicated twice. The median lethal concentration at 96 h (96 h LC_{50}) was calculated according to the method of the American Public Health Association[15] and was found to be 18.6 ppm.

Cadmium exposure: To trace the effects of cadmium on blood, three groups of 10 fish each were exposed to 9.3 ppm of cadmium, 0.5 of the calculated 96 h LC_{50}, in 100 L glass aquaria. Fish were sampled for biochemical investigations at 1, 4 and 7 days following exposure to cadmium. Three groups of control fish in tap water were sampled for each specified period. During the experimental periods the test medium was daily renewed to sustain nominal concentration of cadmium.

Biochemical measurements: For blood analysis, the caudal peduncle was cut, blood was collected in non-heparinized tubes. The blood immediately centrifuged at 1500 rpm for 10 min. Serum was then removed and stored at 4°C prior to immediate determination of biochemical parameters, glucose, cholesterol, triglycerides, total protein, glutamic pyruvic acid transaminase (GPT), glutamic oxaloacetic acid transaminase (GOT) and alkaline phosphatase (ALP). Blood glucose was estimated using the method of Trinder[16]. Blood cholesterol was measured according to the procedure of Pearson *et al.*[17]. Blood triglycerides was determined using the method of Rice[18]. The method of Lowry *et al.*[19] was carried out to determine the value of total protein. The activities of blood GPT and GOT were estimated according to the methods of Reitman and Frankel[20]. To determine the activity of blood ALP, Bessey *et al.*[21] method was used.

Statistical analysis: The significance of differences between control and experimental data was statistically analyzed using the Student's t-test. $p \leq 0.05$ was taken as the level of significance.

RESULTS AND DISCUSSION

The levels of blood glucose and the activities of GPT, GOT and ALP were significantly increased from the corresponding control values throughout the periods of exposure (Table 1). The blood triglycerides and total protein values were markedly elevated at the second and last periods. Insignificant changes in the levels of blood cholesterol were noted during the periods of cadmium treatment (Table 1).

The present study demonstrated that the fish *Oreochromis niloticus* exposed to sublethal concentration of cadmium displayed a significant elevation in the level of blood glucose after one day till the end of the experimental period. Similar observations have been reported by Benson *et al.*[22], Early *et al.*[23], Fu *et al.*[24], Partap and Bonga[25], Hontela *et al.*[26] in fish and rat treated with cadmium. Additionally, significant hyperglycaemia were noted by Nath and Kumar[27] in the catfish, *Heteropneustes fossilis* exposed to nickel, Radhakrishaniah *et al.*[28] and Van Vuren *et al.*[29] in the fish, *Labeo rohita* and *Claries gariepinus* subjected to copper and James *et al.*[30] in the teleost, *Oreochromis mossambicus* exposed to lead.

Table 1: Levels of biochemical parameters (X±SD)in the serum of control (n = 5) and in fish (n = 6) exposed to 9.3 ppm cadmium

		Exposure period (day)		
Parameters	Treatment	1	4	7
Glucose (mg 100 mL^{-1})	C	82.80±1.68	85.26±1.44	100.26±1.35
	T	347.76±2.46*	158.94±1.30*	155.17±2.23*
Cholesterol (mg 100 mL^{-1})	C	162.24±1.18	169.65±1.85	184.32±1.53
	T	161.85±1.13	170.82±1.84	186.03±1.75
Triglycerides (mg 100 mL^{-1})	C	197.67±2.07	204.16±1.52	190.96±1.60
	T	196.85±1.11	336.16±1.69*	217.04±1.52*
Total protein (g 100 mL^{-1})	C	2.73±0.20	2.81±0.06	2.53±0.07
	T	2.84±0.06	3.11±0.13*	3.05±0.07*
GPT (U L^{-1})	C	51.40±2.30	57.60±3.54	48.60±2.79
	T	63.33±2.42*	85.67±2.25*	152.13±3.31*
GOT (U L^{-1})	C	189.40±3.89	175.80±4.64	160.60±6.11
	T	320.83±4.31*	342.50±3.08*	355.33±3.62*
ALP (U L^{-1})	C	11.60±1.82	12.80±0.84	16.80±3.58
	T	14.33±1.07*	16.17±1.60*	26.17±1.47*

C indicates Controls, T indicates Treated, * Statistically significant at $p \leq 0.05$, Student's t-test

Wedemeyer and McLeay[31] stated that high levels of blood glucose are caused by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stresses. A variety of stressors stimulate the adrenal tissue, resulting in increased level of circulating glucocorticoids[25,26,32-34] and catecholamines [35-37]Both of these groups of hormones produce hyperglycaemic. Several authors[30,38-42] observed significant decreases in the levels of muscle and/or liver glycogen with significant hyperglycaemia in fish exposed to cadmium, lead and chromium. Förlin *et al.*[43] demonstrated that the apparent glycogen depletion in trout hepatocytes following exposure to cadmium may reflect an accelerate rate of glycolysis, which would deplete the liver glycogen stores. The decrease in liver glycogen is due to glycogenolysis and normally the decrease of tissue glycogen content runs parallel to a elevation of glucose in serum[26,30,32]. Additionally, Kozaric *et al.*[44] reported that the liver glycogen content of carp (*Cyprinus carpio*) proved significantly decreased, but the activities of hexokinase and glucose phosphate isomerase were elevated. They suggested an intensive glycogenolysis in cadmium-intoxicated carp. Also, Sastry and Shukla[45] stated that the activities of hexokinase, glucose-6-phosphataes, lactate dehydrogenase, succinate and malate dehydrogenases decreased in the liver of teleost fish, *Channa punctatus* exposed to cadmium. They suggested that glycolysis and gluconeogenesis were impaired in liver. It can not excluded that the possibility that cadmium exert a reducer effect on the content of liver and muscle glycogen in *Oreochromis niloticus*. However, the elevation of blood glucose is a response to the increased rate of glycogenolysis or gluconeogenesis.

Exposure of cadmium induced a significant rise in blood triglycerides level in the experimental animals, *Oreochromis niloticus*. Several studies demonstrated that the levels of blood triglycerides were significantly increased in the animals treated with different metals[46,47]. There are a number of reports indicating that pollutants (e.g., cadmium, lead and mercury)influence thyroid function[26,34,48-50]. Since decreased thyroid secretion (hypothyroidism) greatly increases triglycerides level in the blood, the observed hypertriglyceridaemia may be due to hypothyroidism induced by cadmium and/or liver dysfunction because the liver is the principle center of lipid metabolism[51].

The observed hyperproteinaemia in the fish, *Oreochromis niloticus* following cadmium administration is possibly due either to (1) water loss in the serum, (2) the relative changes in the mobilization of blood protein, or (3) elevated *de novo* synthesis. These findings and explanation are comparable to those considered by Hilmy *et al.*[52], Ruparelia *et al.*[53] and Gopal *et al.*[54] on toad and fish exposed to cadmium, lead, mercury, copper and nickel.

The data of the present study showed that the exposure of cadmium caused significant elevations in the activities of blood GPT, GOT and ALP. Several investigations showed that these blood enzymes were highly increased in the fish treated with cadmium, zinc and copper[22,46,55,56]. Shakoori *et al.* [57] reported that the increase of blood enzymatic activity is either due to (1) leakage of these enzymes from hepatic cells and thus raising levels in blood, (2) increased synthesis and (3) enzyme induction of these enzymes. Also, Tietz[51] and Campbell *et al.*[58] reported that these enzymes liberate to the blood stream when the hepatic parenchyma cells are damaged. Förlin *et al.*[43] and Thophon *et al.*[59] found structural and ultrastructural damage in the liver of rainbow trout and white seabass following cadmium exposure. Moreover, Shimizu and Morita[60], Habeebu-Sultan *et al.*[61] demonstrated that cadmium induced hepatic necrosis in rats and mice. Finally, De Smet and Bulst[62] reported that the concentrations of free amino acids and the activities of proteases were increased in gill,liver and kidney of carp, *Cyprinus carpio* exposed to cadmium. Also, they observed an increase in the activities of GOT and GPT and they suggested that the observed proteolysis is intended to increase the role of protein in the energy production during cadmium stress.

The results indicated that cadmium produces severe toxic effects in fish blood. Also, *O. niloticus* is incapable of adapting to changes in the water quality caused by the addition of sublethal concentration of cadmium. Further studies are required to investigate the influences of chronic exposure to cadmium.

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