Impact Of Copper Cyanide On Metabolic Enzymes Of Fresh Water Fish Nile Tilapia (Oreochromis niloticus)

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ABSTRACT: In the River Nile and Manzalla lake some fishers use the cyanide for catching fish. So that the present study is a contribution to the assessment of the toxicity effects of copper cyanide on the Nile Tilapia (Oreochromis. niloticus) fish. Since biochemical assessment is a useful tool for measuring environmental quality. Short term toxicity experiments were conducted to study the effect of metal cyanide complex (copper cyanide) on the metabolic enzymes viz., lactate dehydrogenase (LDH), glucose-6phosphate dehydrogenase (G6PDH), aspartate amino transferase (AST) alanineamino transferase (ALT), acid phosphatase (AcP) and alkaline phosphatase (ALP) activity in (O. niloticus) fingerlings. A total of 45 fingerlings were (8±0.6 cm; 12±0.2 g) exposed to two sublethal concentrations (0.253 and 0.152 mg/L) for a period of15 days. Copper cyanide had significant (P> 0.05) effect on the metabolic enzymes, the highest activities were observed in the group exposed to 0.253 mg/L. Results suggest that metal cyanide complex significantly altered enzyme activities of fish in both the sublethal concentrations.

Key Words: *Oreochromis. Niloticus, Copper cyanide, acid phosphatase, lactate dehydrogenase.care; Sedation*

INTRODUCTION

Cyanide and its metals complexes are one of the most potentially harmful chemicals due to their adverse effects on non-target organisms, primarily due to the formation of complexes with metal ions that are present as enzyme cofactors. Most notably this occurs with Fe3+ ion in cytochrome, thereby inhibiting respiration and hence, oxidative phosphorylation, and when fish exposed to toxic concentrations of cyanide, their tissues get damaged, show abnormal behavior such as hyper-active and restless swimming, and movements such as burst swimming, jerking, partial jerking and increase in darting (1).The extent of cyanide toxicity in fish depends mainly on the rate of its detoxification in vivo (2). Fish occupy a prominent position in the field of toxicology; in studies concerning both human and ecological health. Much of the toxicological interest in cyanide has been focused on its rapid lethal action; however, the most widespread problems arising from cyanide are from chronic/ sub chronic exposures (3).Toxicity of cyanides to various aquatic organisms has been reviewed (4,5).Cyanide exposure is known to produce a variety of biochemical changes in animals (6) .Freshwater fish are the most cyanide-sensitive group of aquatic organisms tested, with high mortality documented at free cyanide concentrations >20 ug/L and adverse effects on swimming and reproduction at >5 ug /L (7). Studies carried out on freshwater fish species like Cyprinus carpio (8).Oreochromis mossambicus (9),Cirrhinus mrigala . In the River Nile and Manzalla lake some fishers use the cyanide in catching fish. So that the present study is a contribution to the assessment of the toxicity effects of copper cyanide on the O. niloticus juveniles. Since biochemical assessment is a useful tool for measuring environmental quality, the present work is aimed to study the effect of copper cyanide on key metabolic enzymes of fish.

Materials and methods

For the present study, freshwater O. niloticus (8±0.6 cm; 12±0.2 g) were collected from River Nile at Damietta during July 2018 .Fish were acclimated to the laboratory condition in glass aquarium (20 L) for subacute studies (10).Average water quality parameters during the present investigation were, temperature 27 ± 1°C, pH 7.6 ± 0.2, dissolved oxygen 7.1 ± 0.4 mg/l, hardness 21.2 ± 3.3 mg/l as CaCO3, phosphate 0.334 ± 0.001 µg/L, salinity 0.08 ppm, specific gravity 1.001 Fishes were fed with commercial fish feed pellets (3% of bodyweight) and water was renewed on every day to maintain water quality. The toxicant used in the present study was copper cyanide (97% purity). Total of 45 fingerlings of O. niloticus were divided into three groups (15 each). First two groups were exposed to two sub lethal concentrations (0.254 and 0.153 mg/L) of copper cyanide for 15 days and third group was maintained as control. These concentrations were selected on the basis of 1/3rd and 1/5th of 96 h LC50 (11). At the end of exposure period, fishes were sacrificed and tissues such as liver, gill and muscle were dissected and used for estimating the enzymatic activity. Results obtained were tested by one-way Analysis of Variance (ANOVA). ANOVA effects and treatments differences were considered significant when $p<0.05$. 5% percent of tissue homogenates were prepared in 0.25M ice-cold sucrose solution using a glass homogenizer and centrifuged. Supernatant is used for the estimation of enzymes viz, LDH, G6PDH, ALT and AST. LDH activity in different tissues was assayed following method of (12).The formazon extracted was measured spectrophotometrically at 495 nm in Gen way instrument, and the activity of enzyme was represented in µmol formazon/mg of tissue. Glucose 6-phosphatase was assayed according to the method of (13).Alanine aminotransferase and Aspartate aminotransferase was assayed by the method of (14). Acid Phosphatase was assayed by the method of (15).Where as for the estimation of AcP and ALP method of (16) modified by (17) was followed.

Results

Exposure of fish to both sub lethal concentrations of copper cyanide resulted significant changes in the enzymatic activity of the fish over a period of 15 days. The activity of LDH, G6PDH, ALT and AST exhibited increasing trend in all the tissues under cyanide treatment, where as the activity of AcP, ALP shown declining trend. Maximum increase in LDH activity was observed in gills (57.28%) at 1/3rd sublethal concentration and liver (38.62%) at 1/5th sub lethal concentration. Similarly G6PDH activity was found maximum in liver (49.58%) at 1/3rd sublethal concentration and in gills (30.84%) at 1/5th sublethal concentration (Table1). Fish exhibited higher ALT and AST activities in both sublethal concentrations. The maximum increase in ALT activity observed in gills (49.45% and 42.27%) at 1/3rd and 1/5th of sublethal concentrations. Similarly the activity AST also exhibited maximum increase in the gills (35.19% and 28.43%) at 1/3rd and 1/5th of sublethal concentrations (Table 2).

Table (1). Effect of sublethal concentrations of copper cyanide on LDH, (µmol formazon/mg

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LDH	Tissue	Control	Sublethal 1/3rd (0.253 mg/L))	Sublethal 1/5th (0.152 mg/L
	Liver	1.37 ± 0.03	1.95 ± 0.03	1.91 ± 0.03
	% Change		42.29	38.61
	Muscle	1.16 ± 0.22	1.61 ± 0.16	1.41 ± 0.13
	% Change		39.62	23.59
	Gills	1.61 ± 0.23	2.52 ± 0.15	2.12 ± 0.11
	% Change		57.28	31.67
G6PDH	Liver	6.18 ± 0.11	8.32 ± 1.06	7.62 ± 0.73
	% Change		36.68	25.25
	Muscle	1.89 ± 0.27	2.85 ± 0.31	2.17 ± 0.23
	% Change		49.58	15.63
	Gills	1.29 ± 0.24	1.71 ± 0.23	1.67 ± 0.31
	% Change		57.28	31.67

Data are means ± SD (n = 5) for an organ in a row followed by the same letter are significantly different ($p < 0.05$) from each other

Fig (1). Effect of sublethal concentrations of copper cyanide on LDH (µmol formazon/mg protein/h). in different tissues of O. niloticus

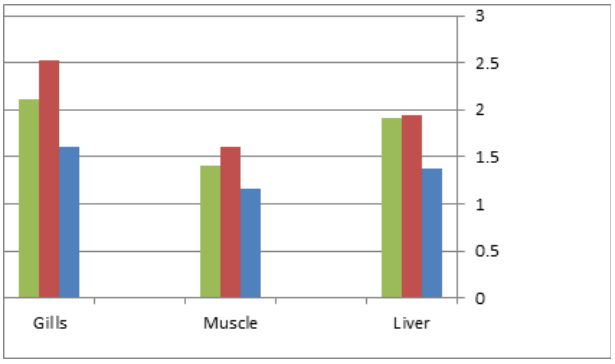


Fig (2) Effect of sublethal concentrations of copper cyanide on G6PDH activity (µmol of Pi formed/mg protein/h) in different tissues of O. niloticus

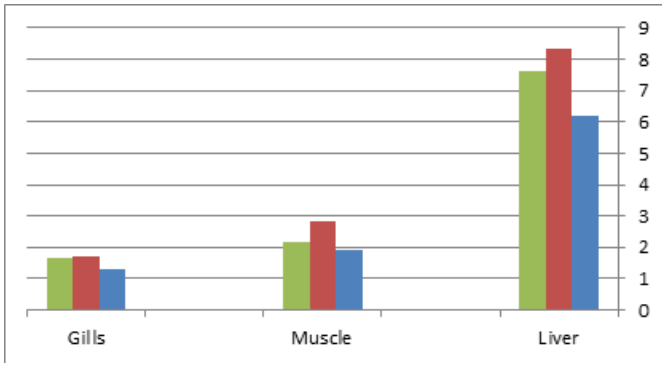


Table (2). Effect of sublethal concentrations of copper cyanide on A LT& AST (µmol of Pyruvateformed/mg protein/h) in different tissues of O. niloticus.

ALT	Tissue	Control	Sublethal 1/3rd (0.253 mg/L))	Sublethal 1/5th (0.152 mg/L
	Liver	6.41 ± 0.22	9.41 ± 0.12	8.94 ± 0.14
	% Change		46.77	39.49
	Muscle	3.38 ± 0.15g	4.81 ± 0.15	4.57 ± 0.20
	% Change		42.39	35.27
	Gills	4.85 ± 0.19	7.25 ± 0.21	6.90 ± 0.15
	% Change		49.45	42.27
AST	Liver	10.50 ± 0.11	13.60 ± 0.11	13.14 ± 0.07
	% Change		29.54	25.15
	Muscle	7.61 ± 0.12	9.95 ± 0.11	9.28 ± 0.09
	% Change		30.65	21.88
	Gills	12.65 ± 0.21	17.11 ± 0.20	16.25 ± 0.14
	% Change		35.19	28.43

Data are means ± SD (n = 5) for an organ in a row followed by the same letter are significantlydifferent ($p < 0.05$) from each other

Fig (3). Effect of sublethal concentrations of copper cyanide on A LT (µmol o f p yruvateformed/mg protein/h) in different tissues of O. niloticus

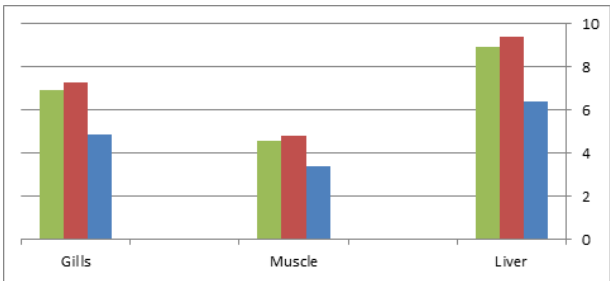


Fig (4). Effect of sublethal concentrations of copper cyanide on A ST (µmol o f p yruvateformed/mg protein/h) in different tissues of O. niloticus

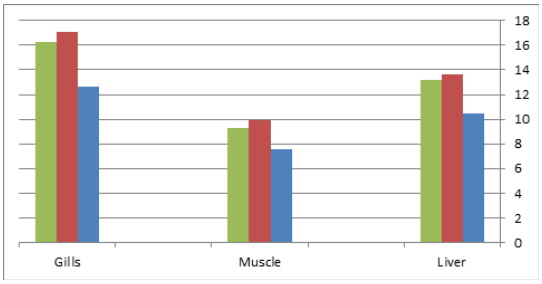


Table (3). Effect of sublethal concentrations of copper cyanide on AcP and ALP (µmol of p-nitrophenol formed/mg protein/h) in different tissues of O. niloticus

AcP	Tissue	Control	Sublethal 1/3rd (0.253 mg/L))	Sublethal 1/5th (0.152 mg/L
	Liver	4.10 ± 0.11	2.24 ± 0.21	2.76 ± 0.16
	% Change		-45.32	-32.75
	Muscle	2.63 ± 0.01	1.51 ± 0.06	1.58 ± 0.06
	% Change		-42.62	-39.86
	Gills	1.17 ± 0.03	0.71 ± 0.12	0.79 ± 0.12
	% Change		-38.83	-32.26
ALP	Liver	6.96 ± 0.21	4.05 ± 0.21	4.26 ± 0.16
	% Change		-41.76	-38.74
	Muscle	4.17 ± 0.08	3.04 ± 0.07	2.92 ± 0.02
	% Change		-27.06	-30.00
	Gills	2.69 ± 0.17	1.63 ± 0.06	1.81 ± 0.06
	% Change		-39.47	-32.74

Fig .(5) Effect of sublethal concentrations of copper cyanide on AcP (µmol of p-nitrophenol formed/mg protein/h) in different tissues of O. niloticus

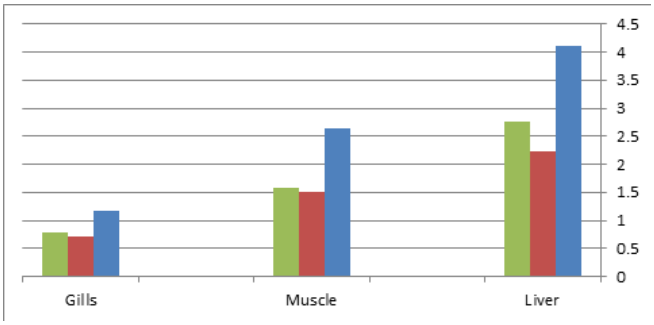
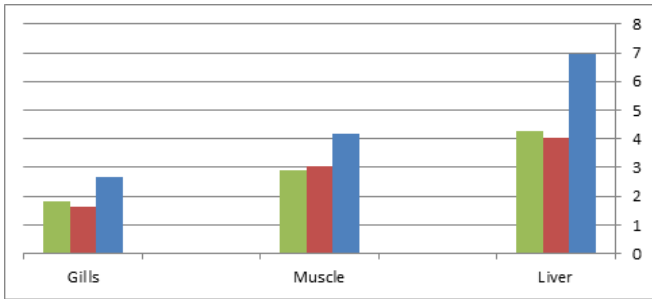


Fig .(6) Effect of sublethal concentrations of copper cyanide on ALP (µmol of p-nitrophenol formed/mg protein/h) in different tissues of O. niloticus.



Discussion

In the present study gill tissue exhibited maximum decrease in LDH activity compared to muscle and liver. This situation might favor anaerobic respiration due to the mild stress of hypoxia in C. catla .Similar observations were made by (8) in the fish C. carpio, exposed to sodium cyanide. While (18) reported reduced aggression in stressed fish. Fish exposed to copper cyanide slowly reached the water surface, probably due to gill damage that caused respiration malfunctions, or as a result of difficulties in gas exchange due to mucous accumulation. (19) Showed that the exposed fish had shown different modes of behaviors. During the first 14 days, the fish lost its equilibrium with excessive mucous secretion on its gill filaments and skin. On the other hand, tilapia showed a normal swimming behavior with no excessive mucus secretion at 21st and 28th days. (20) Reported that fish were seen swimming to the surface frequently with their opercula and mouths moving rapidly an indication that the Toxic effect of the heavy metals caused the depletion of the oxygen content of the medium. Swimming Activity and frequent surfacing reduced drastically, color change from black to pale with mucus covering the body were observed. The mucus covering the entire body of the test organisms might have resulted from response to the toxic effect of the heavy metals through excretion of some accumulated metals in their tissues. (21) Studied the influence of oxygen on the toxicity of KCN to rainbow trout and reported inverse relationship of cyanide to the oxygen consumption. Cyanide significantly stimulated G6PDH activity in the fish indicating mobilization of glucose through pathways other than glycolysis-Krebs cycle. High 6PDH activity indicative of high rates of HMP (Hexose monophosphate) shunt under stress condition as reported by (22) and substantiates the present work. The increase in the ALT and AST activities in our study supports earlier findings and serves as indicator of tissue damage (23). (24) Showed that serum glucose, liver function tests (AST, ALT and ALP) and kidney function tests (creatinine and uric acid) showed a significant increase, serum total proteins, albumin, globulin and total lipids showed a significant decrease. Both liver and gill tissues of the studied fish showed a reduction in GSH content and an elevation in MDA and GPx activities. Similar findings were also observed by (25) in C. punctatus. Who showed that both the activity was enhanced in all the tissues. Maximum increase in the ALT was observed in liver (42%), gill (40.65%) and muscle (34.81%). Similarly, AST also exhibited similar trend in the liver (50.62%), muscle (49.52%) and gills (40.93%). Significant increase phosphatase activity might be due to cellular damage. Similar observations were made by (25) in the fish C. baturachus, exposed to endosulfan and kelthan and attributed the observed changes as an indicator of hepatic tissue damage and dysfunction. As such, the fish can utilize stored proteins to overcome the toxic stress. Under toxic stress, the levels of key enzymes involved in proteins metabolism were changed. Increased activities of both aminotransferases indicated amplified transamination processes. The elevated levels of phosphatases may indicate the increase in the rate of phosphorylation and transport of molecules across the cell membrane. Since, cyanide has anti-phosphatase activity, so reduction in protein level may be due to the inhibition of alkaline phosphatase activity, as it plays an important role in protein synthesis (26).

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