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ORIGINAL RESEARCH ARTICLE

Metabolic Changes In The Freshwater Fish *Catla catla*, Under Copper Cyanide Intoxication

B.B Hosetti *, Praveen N. Dube and Shwetha A

Toxicology Laboratory, Department of Applied Zoology, School of Biological Sciences, Jnana Sahyadri, Shankaraghatta - 577 451, Shimoga, Karnataka, India.

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ABSTRACT

Laboratory evaluations were made to assess the metabolic changes in the carp, *Catla catla* exposed to sublethal concentrations of copper cyanide. Fish were exposed to two sublethal (0.253 and 0.152 mg/L) concentrations for a period of 15 days. At the end of the exposure period, levels of total protein, free amino acids (FAA), protease, glutamine, urea, ammonia, glycogen, lactate and pyruvate were assessed in different functional tissues (liver, muscle and gill). Results exhibited significant ($p<0.05$) dose dependent decline in total protein, glycogen, ammonia and pyruvate, while the FAA, protease, urea, and lactate levels exhibited significant increase in its activity in both the concentrations. There was a significant recovery observed in all the above biochemical parameters, in all the tissues of fish after the 7^{th} d of the withdrawal of treatment. Significant alterations observed in the above parameters may be considered to be manifestation of stress induced by the toxicant and analysis of biochemical parameters in the fish tissue may serve as useful indices in environmental biomonitoring and surveillance.

Keywords: *Catla catla*, proteins, carbohydrates, metabolism, biochemistry

INTRODUCTION

Cyanide is one of the most toxic chemical substances on Earth. The extreme toxicity of cyanide arises from its readiness to react with other elements and hence interfere with normal biological processes (Okolie and Osagie, 1999). The molecular chemistry of cyanide and its chemical behavior in streams and sediments is complex and its toxicity is influenced by several factors, including acidity or alkalinity (Moran, 2004). There is much that is still unknown about the toxicity of cyanide and its degradation products. Free cyanide, as used in gold mining, is found to be the most toxic form. Most cyanide complexes are much less toxic than free cyanide, but weak acid dissociable complexes such as those of copper and zinc are relatively unstable and will release cyanide back to the environment (Shwetha and Hosetti, 2010). Metal-cyanide complexes and break down products (e.g. such as cyanates and thiocyanates), although less toxic than the free cyanide, can persist in the environment. These break-down products could cause long term effects in addition to the immediate effects from free cyanide (Moran, 2004).

Copper cyanide is an important compound for electroplating of copper and also used as a catalyst in polymerizations, and as insecticide, fungicide, and biocide in marine paints. The compound is useful reagent in organic synthesis (Richardson, 2005). It is ranked as one of the most hazardous compound to the ecosystem and human health (ATSDR, 1998). Although by themselves, it is much less toxic than free cyanide; its dissociation releases free cyanide as well as the metal cation which can also be toxic. Even in the neutral pH range of most surface water, copper cyanide complex can dissociate sufficiently to be environmentally harmful if in high enough concentrations (Hosetti et al., 2010). Stability of copper cyanide depends upon the pH of water and therefore, the potential environmental impacts and interactions (i.e. their acute or chronic effects, attenuation and re-release) can vary (Caruso, 1975).

Although there are different forms of cyanides, the biochemical action of cyanide is same upon

***Corresponding Author:** Dr. B.B. Hosetti, **Email:** basaling@yahoo.co.in, **Phone No:** +91-8282- 256308

entering the body. Once in the bloodstream, cyanide forms a stable complex with a form of cytochrome oxidase, an enzyme that promotes the transfer of electrons in the mitochondria of cells during the synthesis of ATP (Yen et al., 1995). Without proper cytochrome oxidase function, cells cannot utilize the oxygen present in the bloodstream, resulting in cytotoxic hypoxia or cellular asphyxiation. The lack of available oxygen causes a shift from aerobic to anaerobic metabolism, leading to the accumulation of lactate in the blood. The combined effect of the hypoxia and lactate acidosis is depression of the central nervous system that can result in respiratory arrest and death (Okolie and Osagie, 1999). At higher lethal concentrations, cyanide poisoning also affects other organs and systems in the body, including the heart.

The sensitivity of aquatic organisms to cyanide is highly species specific, and is also affected by water pH, temperature and oxygen content, as well as the life stage and condition of the organism. Fish and aquatic invertebrates are particularly sensitive to cyanide exposure (Eisler, 1991). Toxicity of cyanide has been unequivocally linked to the tropical ataxic neuropathy and epidemic spastic paraparesis (Okolie and Osagie, 1999). However not much is known about the possible toxic consequences of copper cyanide on the metabolic activity in fishes. Although it has been reported that cyanide induced some histopathological derangements in fish liver (Dixon and Leduc, 1981), the data obtained were not correlated with biochemical alterations. Therefore it becomes pertinent to study the deleterious effect of this toxicant on fish and their ecosystem. Exposure to cyanides elicits multiple biochemical and physiological adjustments in fish tissues (Doudoroff et al., 1966). Immediate responses include an activation of anaerobic glycolysis.

There are limited numbers of reports available on the effect of copper cyanide, an important industrial contaminant, on various biochemical constituents. Moreover, no work on Indian major carp, Catla (*Catla catla*) has been carried out specifically on these aspects. As freshwater aquaculture constitutes one-third of the total fish production in India and major carps being the dominant species (*C. catla*, *Labeo rohita*, and *Cirrhinus mrigala*). Therefore the present study was designed to examine the effect of copper cyanide on some biochemical parameters of an Indian carp *Catla* (*C. catla*) that forms an important candidate species in carp poly-culture systems.

MATERIALS AND METHODS

Healthy fingerlings of *C. catla* (length 2±0.5 cm; weight 1.5±0.2 g) were obtained from State Fisheries Department, B.R. Project, Shimoga, Karnataka, India and brought to the laboratory for acclimatization in large aquaria of 100 L capacity (100 x 35 x 50 cm) for a period of two weeks for further studies. Prior to stocking, fish were treated with 1% $KMnO_4$ to remove any dermal adherent. Water of the tank was changed daily to avoid any fungal and bacterial contamination. Dechlorinated tap water was used during the entire period of acclimatization and test condition, whose physico-chemical characteristics were analyzed following the methods mentioned in APHA (2005) and were found as follows, temperature 27±1 ºC, pH 7.4±0.2 at 27ºC, dissolved oxygen 6.8±0.5 mg/L, carbon dioxide 6.3±0.4 mg/L, total hardness 23.4±3.4 mg as $CaCO_3$/L, phosphate 0.39±0.002 µg/L, salinity 0.01ppm, specific gravity 1.001 and conductivity less than 10 µS/cm. Water was renewed every day and a 12-12 h photoperiod was maintained during acclimatization and test periods. In the laboratory, fish were fed regularly with commercial fish food pellets (Nova, Aquatic P. Feed, not less than 3% of their wet body weight).

Laboratory static renewal bioassay tests (APHA, 2005) were conducted to determine the 96h LC_{50} (0.76 mg/L) for juvenile carp. Accordingly two sublethal concentrations ($1/3^{rd}$ and $1/5^{th}$ of 96 h LC_{50}) were selected for the subchronic studies. The toxicant used in the present study was copper cyanide (97% purity), obtained from Loba chemicals Pvt. Ltd, Mumbai, Maharashtra, India. A total of 60 fingerlings were selected and divided into three equal groups with 20 fish each. Group one and two were exposed to two sublethal concentrations of copper cyanide (0.253 and 0.152 mg/L) and third group is maintained in cyanide free tap water served as control (20 L). After 15 d exposure, one third of the exposed fishes were transferred to cyanide free tap water for a period of 7 d (post-recovery period) in order to study the withdrawal effect. The experiment was repeated five times for each exposure and control groups. Analysis of variance was employed to calculate the significant difference among the exposed and the control groups. In all the cases the significance level was considered at $p<0.05$.

Fish were sacrificed at the end of the exposure (15 d) and recovery period (7 d) by severe blow jet on the head. Liver, muscle and gill tissues were removed and washed with distilled water. Tissue protein was estimated according to Lowry et al. (1951). Tissue samples were homogenized with 10% trichloroacetic acid and centrifuged at 1000 ×g for 10 min. Deproteinated supernatant was used for the determination of lactate (Huckabee 1961), free amino acids (FAA) by the addition of ninhydrin reagent (Moore and Stein, 1954) and protease activity by Davis and Smith (1955) method. Urea was estimated according to Natelson (1971) and Glutamine by using the method of acid hydrolysis described by Colowick and Kaplon (1967) and the values were expressed as µ moles / gm wet wt of tissue. Ammonia content was estimated with the Nesslers reagent as described by Bergmeyer (1965) the values were expressed as µmol/g wet wt of tissue. Glycogen content in the tissues was estimated by using the anthrone reagent as described by Caroll et al (1956). The homogenate (5%) was prepared in 5% trichloroacetic acid (TCA). Glycogen content was expressed as mg glycogen/g of tissue. Pyruvate level was measured according to Friedman and Haugen (1943). Homogenate (50 mg/ml) was prepared in 10% TCA. Sodium pyruvate was taken as standard and the values were expressed as mg pyruvate/g tissue.

RESULTS AND DISCUSSION:

Exposure to sublethal concentrations of copper cyanide (0.253 and 0.152 mg/L), caused significant dose dependent alteration in the nitrogenous, as well as carbohydrate metabolism of the fish *C. catla* in all the tissues (**Tables 1 and 2**).

Protein level significantly reduced, while FAA and protease activity increased significantly in all tissues of the exposed group when compared to control. Decrease in the total protein level in the liver tissue was 42.02% and 30.38%, the muscle to 40.60%, and 32.57%, while in the gills to 37.62% and 26.95% after treatment with 1/3rd and 1/5th sublethal concentrations. Whereas the FAA increased from 30.50% and 22.39% in the liver, 21.81% and 16.39% in the muscle and in the gills 32.31% and 23.64%. Similarly protease activity in the liver was 24.38% and 22.09%, muscle 27.85% and 21.28%, and in the gills it was 18.74% and 22.51%. Significant recovery was observed in protein, FAA and protease content after 7d post recovery. The decrease in total protein level with corresponding increase in the FAA and protease observed in the present study may be due to their degradation and also to their possible utilization for metabolic purposes (Prashanth and Neelgund, 2007). Bradbury et al. (1987) pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery. The quantity of protein is dependent on the rate of protein synthesis, or on the rate of its degradation. The quantity of protein may also be affected due to impaired incorporation of amino acids into polypeptide chains (Singh et al., 1996). Increases in the FAA levels were result of breakdown of proteins for energy and impaired incorporation of amino acids in the protein synthesis (Prashanth and Neelgund, 2007). Elevation in the FAA observed in the present study suggests high proteolytic activity due to the elevation of protease activity.

The nitrogenous end products, viz, urea and glutamine increased significantly, where as the ammonia content exhibited decrease in trend. Muscle tissue exhibited maximum decrease in the urea and glutamine content when compared to gill and liver (**Tables 1 and 2**). There was a significant recovery observed in the nitrogenous end products at the end of the recovery period. The reduction in ammonia content suggests that the ammonia might have been converted into non-toxic compounds, glutamine and urea, as evidenced in the present investigation. The elevated levels of glutamine and urea in liver, muscle and gill tissues reveal an enhancement of the biosynthesis of urea and glutamine might also be an adoptive response as a major pathway of detoxification of ammonia (Prahsnath and Neelgund, 2009). This indicates that this pathway may be beneficial to aquatic animals especially to fishes in detoxification and physiological compensation or adjustment to various exogenous and endogenous toxicants. The elevated glutamine may be utilized in the formation of amino acids in protein synthesis (David et al., 2004).

Levels of glycogen in the tissues decreased significantly in both the sublethal concentrations. Muscle tissue exhibited maximum decrease (-42.26 and -37.62%) followed by liver (-37.05 and -33.10%) and gill (-28.78 and -22.11%) when compared to control. Depletion of glycogen may be due to direct utilization for energy generation, a demand caused by active compound induced hypoxia (Shwetha and Hosetti, 2010). Similarly

pyruvate level exhibited decrease in trend due to higher energy demand during cyanide exposure. In consonance with the increase in lactate content, their was significant decrease in pyruvate and glycogen and this trend has been observed in all the tissue (**Tables 1 and 2**).

The decrease in pyruvate level suggests the possibility of a shift towards anaerobic dependence due to a remarkable drop in aerobic segment. The end product of glycolysis under anaerobic condition is lactic acid where as pyruvate level in the tissue may be taken as a measure of aerobic condition of tissue depending upon availability of molecular oxygen (Tiwari and Singh 2004). The decrease in pyruvate could be due to its conversion to lactate, or due to its mobilization to form amino acids, lipids, triglycerides and glycogen synthesis in addition to its role as a detoxification factor in cyanide toxicity (David et al, 2010). The increase in lactate corroborates with the corresponding decrease in pyruvate content of the tissues of fish exposed to copper cyanide. The increase in lactate also suggests a shift towards anaerobiosis as a consequence of hypoxia created under cyanide toxic impact leading to respiratory distress (Dube and Hosetti, 2010).

Table 1. Metabolic changes in different tissues of fish *C. catla,* after 15 days exposure to 1/3rd of 96 h LC_{50} (0.253 mg/L) of Copper cyanide

Parameters	Tissues	Control	1/3rd LC_{50} ( )	7 d Post Recovery
Total proteins (mg/gm wet wt)	Liver	42.98±0.05 (100)	24.92±0.08 (-42.02) ↓	38.24±0.05 (-11.04) ↓
	Muscle	31.56±0.05 (100)	18.75±0.15 (-40.60) ↓	27.40±0.07 (-13.18) ↓
	Gill	22.52±0.03 (100)	14.05±0.08 (-37.62) ↓	20.45±0.13 (-9.19) ↓
FAA (mg/gm wet wt)	Liver	3.31±0.03 (100)	4.32±0.31 (30.50) ↑	3.95±0.43 (19.34) ↑
	Muscle	5.25±0.12 (100)	6.39±0.19 (21.81) ↑	5.85±0.01 (11.51) ↑
	Gill	2.94+0.25 (100)	3.88±0.51 (32.31) ↑	3.41±0.01 (16.12) ↑
Protease (mg of amino acid /gm wet wt)	Liver	1.31±0.02 (100)	1.63±0.01(24.38) ↑	1.51±0.01 (15.05) ↑
	Muscle	0.84±0.09 (100)	1.07±0.05 (27.85) ↑	0.95±0.15 (13.47) ↑
	Gill	0.50±0.01 (100)	0.60±0.06 (18.74) ↑	0.56±0.12 (11.97) ↑
Glutamine (µmol/g wet wt)	Liver	24.91±0.01 (100)	37.10±0.06 (48.93) ↑	28.21±0.04 (13.25) ↑
	Muscle	8.58±0.08 (100)	13.57±0.01 (58.30) ↑	9.80 ±0.27 (14.34) ↑
	Gill	7.35±0.07 (100)	10.28±0.05 (39.82) ↑	8.18±0.37 (11.32) ↑
Urea (µmol/g wet wt)	Liver	10.32±0.4 (100)	12.17±0.12 (17.98) ↑	10.97±0.09 (6.35) ↑
	Muscle	8.51±0.04 (100)	10.80±0.08 (26.89) ↑	9.31±0.52 (9.41) ↑
	Gill	12.42±0.61 (100)	15.52±0.04 (24.95) ↑	13.30±0.98 (7.05) ↑
Ammonia (µmol/g wet wt)	Liver	7.19±0.22 (100)	5.61±1.09 (-21.99) ↓	6.36±0.11 (-11.64) ↓
	Muscle	3.37±0.15 (100)	2.45±0.51(-27.33) ↓	2.96±0.42 (-12.40) ↓
	Gill	4.82±0.19 (100)	3.15±0.30 (-34.61) ↓	4.16±1.14 (-13.67) ↓
Glycogen (mg / g wet wt)	Liver	28.32±0.38 (100)	17.83±0.18 (-37.05) ↓	24.50±0.27(-13.48) ↓
	Muscle	8.71±0.18 (100)	5.03±0.22 (-42.26) ↓	7.53±0.19 (-13.57) ↓
	Gill	4.11±0.25 (100)	2.93±0.12 (-28.78) ↓	3.70±0.23 (-9.95) ↓
Lactate (mg / g wet wt)	Liver	2.42±0.15 (100)	3.46±0.38 (43.03) ↑	2.81±0.83 (16.44) ↑
	Muscle	2.59±0.92 (100)	4.02±0.29 (55.15) ↑	3.04±0.71 (17.21) ↑
	Gill	2.33±0.13 (100)	3.81±0.32 (64.04) ↑	2.76±0.49 (18.54) ↑
Puruvate (mg / g wet wt)	Liver	8.94±1.71 (100)	5.20±0.19 (-41.89) ↓	7.31±0.16 (-18.23)
	Muscle	7.40±0.01(100)	3.64±0.03(-50.84) ↓	6.20±0.05(-16.20) ↓
	Gill	4.11±0.20 (100)	2.12±0.14 (-48.40) ↓	3.34±0.22(-18.63) ↓

Values are mean ± SE of Five replicates. Values in parentheses are % of control value ("↑" increase, "↓" decrease). All values are significantly different from the corresponding control at p<0.05 level of significance.

Table 2. Metabolic changes in different tissues of fish *C. catla,* after 15 days exposure to 1/5th of 96 h LC_{50} (0.152 mg/L) of Copper cyanide

Parameters	Tissues	Control	1/5th LC_{50}	7 d Post Recovery
Total proteins (mg/gm wet wt)	Liver	42.98±0.05 (100)	29.93+0.17 (-30.38) ↓	39.03+0.05 (-9.20) ↓
	Muscle	31.56±0.05 (100)	21.28+0.08 (-32.57) ↓	28.66+0.65 (-9.19) ↓
	Gill	22.52±0.03 (100)	16.45+0.05 (-26.95) ↓	20.72+0.18 (-8.02) ↓
FAA (mg/gm wet wt)	Liver	3.31±0.03 (100)	4.05±0.67 (22.39) ↑	3.94±0.01 (19.06) ↑
	Muscle	5.25±0.12 (100)	6.11±0.15 (16.39) ↑	6.07±0.02 (15.72) ↑
	Gill	2.94+0.25 (100)	3.63±0.10 (23.64) ↑	3.32±0.81 (12.97) ↑
Protease (mg of amino acid /gm wet wt)	Liver	1.31±0.02 (100)	1.60±0.02 (22.09) ↑	1.46±0.01 (10.81) ↑
	Muscle	0.84±0.09 (100)	1.02±0.04 (21.28) ↑	0.93±0.13 (10.40) ↑

Glutamine (µmol/g wet wt)	Gill	0.50±0.01 (100)	0.62±0.01 (22.51) ↑	0.55±0.72 (9.95) ↑
	Liver	24.91±0.01 (100)	34.37±0.03 (37.96) ↑	27.08 ±0.03 (8.70) ↑
	Muscle	8.58±0.08 (100)	12.92±0.02 (50.62) ↑	9.32±0.13 (8.71) ↑
Urea (µmol/g wet wt)	Gill	7.35±0.07 (100)	9.79±0.04 (33.14) ↑	7.65±0.03 (4.01) ↑
	Liver	10.32±0.4 (100)	11.95±0.03 (15.85) ↑	10.77±0.11 (4.36) ↑
	Muscle	8.51±0.04 (100)	10.41±0.94 (22.28) ↑	8.93±0.56 (4.92) ↑
Ammonia (µmol/g wet wt)	Gill	12.42±0.61 (100)	14.96±0.16 (20.43) ↑	12.97±0.01 (4.46) ↑
	Liver	7.19±0.22 (100)	5.91±1.13 (-17.92) ↓	6.51±0.61 (-9.52) ↓
	Muscle	3.37±0.15 (100)	2.37±1.03 (-29.61) ↓	3.13±1.00 (-7.42) ↓
Glycogen (mg / g wet wt)	Gill	4.82±0.19 (100)	3.72±0.20 (-22.88) ↓	4.42±1.61 (-8.31) ↓
	Liver	28.32±0.38 (100)	18.95±0.36 (-33.10) ↓	25.71±0.33 (-9.21) ↓
	Muscle	8.71±0.18 (100)	5.44±0.16 (-37.62) ↓	7.81±0.21 (-10.41) ↓
Lactate (mg / g wet wt)	Gill	4.11±0.25 (100)	3.20±0.15 (-22.11) ↓	3.81±0.17 (-7.30) ↓
	Liver	2.42±0.15 (100)	3.28±0.36 (35.87) ↑	2.74±0.75 (13.28) ↑
	Muscle	2.59±0.92 (100)	3.84±0.48 (48.15) ↑	2.94±0.28(13.58) ↑
Pyruvate (mg / g wet wt)	Gill	2.33±0.13 (100)	3.52±0.51 (51.28) ↑	2.58±0.29 (10.98) ↑
	Liver	8.94±1.71 (100)	8.27±0.11 (-7.55) ↓	5.71±0.23 (-36.09) ↓
	Muscle	7.40±0.01(100)	4.83±0.03(-34.71) ↓	6.72±0.05(-9.17) ↓
	Gill	4.11±0.20 (100)	3.18±0.19(-22.63) ↓	2.75±0.31(-32.98) ↓

Values are mean ± SE of Five replicates. Values in parentheses are % of control value ("↑" increase, "↓" decrease). All values are significantly different from the corresponding control at $p<0.05$ level of significance.

CONCLUSION:

The biological response of an aquatic organism to pollutants frequently induces changes at cellular and biochemical levels, leading to changes in the structure and function of the cells, tissues, physiology and behavior of the organism. Toxicity experiments showed that copper cyanide caused significant biochemical changes in the carp. It has been reported that alterations in the biochemical activities directly indicates major pathologic changes in cell membrane permeability or hepatic cell rupture, a signal of underlying pathological process (Hayes *et al.*, 2002). The above observations seems to confirm the results obtained in this study that the metal cyanide had impacted on the cell integrity as reflected in marked changes in some biochemical constituents.

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