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IMPACT OF COPPER CYANIDE ON THE KEY METABOLIC ENZYMES OF FRESHWATER FISH *CATLA CATLA* (HAMILTON)

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Abstract: Short term toxicity experiments were conducted to study the effect of metal cyanide complex (copper cyanide) on the key metabolic enzymes viz., lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), glucose-6 phosphate dehydrogenase (G6PDH), aspartate amino transferase (AST) alanine amino transferase (ALT), acid phosphatase (AcP) and alkaline phosphatase (ALP) activity in *Catla catla* juveniles. A total of 60 fingerlings were (2±0.5 cm; 1.5±0.2 g) exposed to two sublethal concentrations (0.253 and 0.152 mg/L) for a period of 15 days. Copper cyanide had significant (P> 0.05) effect on the key 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: Copper cyanide, enzymes, metabolism, subacute, *Catla catla*

Introduction

Pollution of the aquatic environment is a matter of concern. Around 1500 chemical substances are been listed as pollutants of freshwater ecosystem. Indiscriminate use of such chemicals lead to the contamination of our natural water resources such as lakes, reservoirs, rivers, ponds, paddy-fields, streams, and other low-lying areas all across the globe *(Dellinger et al., 2011).* These chemicals disturb the whole ecosystem, mainly the aquatic ones, leading to needless mortality of aquatic fauna, in particularly fish as revealed by several workers *(Hosetti et al., 2010; Osman et al., 2010; Suneetha, 2012).*

Among the different sources which cause environmental deterioration cyanide is the most important one *(Bhattacharya et al., 2009).* The use of cyanide in mining causes an unreasonable risk to the health of people, wildlife, and

fish *(Eisler and Wiemeyer, 2004)*. Apart from mining, cyanide is also used in photographic processes, production of synthetic rubber, chemical synthesis, manufacture of plastic, pesticides, dehairing of hides, laboratory processes, manufacture of dyes and pigments. These industries release an estimated amount of more than 14 million kg/yr of cyanide *(Dube and Hosetti, 2011)*. 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 Fe^{3+} ion in cytochrome, thereby inhibiting respiration and hence, oxidative phosphorylation *(Shwetha et al., 2012)*.

Algae and other macrophytes have the ability to tolerate higher environmental concentrations of cyanide, and show no adverse effects until 160mg/L or more when compared to fish and invertebrates *(Heath, 1991)*. Freshwater fish are the most cyanide-sensitive group of aquatic organisms tested, with high mortality documented at free cyanide concentrations >20 microg/L and adverse effects on swimming and reproduction at >5 microg/L *(Eisler and Wiemeyer, 2004)*. Studies carried out on freshwater fish species like *Cyprinus carpio (David et al., 2010)*, *Oreochromis mossambicus (Prashanth, 2012)*, *Cirrhinus mrigala (Shwetha et al., 2012)* found that when exposed to toxic concentrations of cyanide, their tissues get damaged, show abnormal behaviour such as hyper-active and restless swimming, and movements such as burst swimming, jerking, partial jerking and increase in darting.

The present paper is a contribution to the assessment of the toxicity effects of copper cyanide on the Indian major carp *C. catla*. 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 fish *C. catla* (2±0.5 cm; 1.5±0.2 g) were collected from State fisheries Department, Bhadra Reservoir Project, Shimoga and experiment was conducted in the laboratory at Department of Applied Zoology, Kuvempu University, Shimoga, Karnataka India. Fishes were acclimated to the laboratory condition in glass aquarium (20 L) for subacute studies *(APHA, 2005)*. Average water quality parameters during the present investigation were, temperature 27 ± 1°C, pH 7.2 ± 0.2, dissolved oxygen 6.3 ± 0.4 mg/l, hardness 23.2 ± 3.4 mg/l as $CaCO_3$, phosphate 0.39 ± 0.002 µg/L, salinity 0.01ppm, specific gravity 1.001 and conductivity less than 10 µS/cm. Fishes were fed with commercial fish feed pellets (Nova Aquatic Pvt Ltd, not less than 3% of body weight) and water was renewed on every day to maintain water quality.

The toxicant used in the present study was copper cyanide (97% purity), obtained from Loba chemicals Pvt. Ltd, Mumbai, Maharashtra, India. Total of 60 fingerlings of *C. catla* were divided into three groups (20 each). First two groups were exposed to two sublethal concentrations (0.253 and 0.152 mg/L) of copper cyanide for 15 days and third group was maintained as control. These concentrations were selected on the basis of $1/3^{rd}$ and $1/5^{th}$ of 96h LC_{50} *(Hosetti and Dube, 2010)*. 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 $p<0.05$.

Five 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, SDH, G6PDH, ALT and AST. LDH activity in different tissues was assayed following method of *Govindappa and Swami (1965)* and SDH by the method of *Nachlas et al. (1960)*. The formazon extracted was measured spectrophotometrically at 495 nm and the activity of enzyme was represented in µmol formazon/mg of tissue. For the estimation of G6PDH activity method described by *Bergmeyer and Bernt (1965)* was followed and the activity expressed as µM Pi liberated/mg protein/h, using phosphate standards *(Fiske and Subbarow, 1925)*. ALT and AST activity were estimated using method of *Reitman and Frankel (1957)*. Where as for the estimation of AcP and ALP method of *Kind and King, (1954)* modified by *Agorey (2010)* was followed.

Results

Exposure of fish to both sublethal concentrations of copper cyanide resulted significant changes in the key 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 SDH, AcP, ALP shown declining trend.

Table 1. Effect of sublethal concentrations of copper cyanide on LDH, SDH (μmol formazon/mg protein/h) and G6PDH activity (μmol of Pi formed/mg protein/h) in different tissues of *C. catla*

	Tissue	Control	Sublethal 1/3rd (0.253 mg/L)	Sublethal 1/5th (0.152 mg/L)
LDH	Liver	1.38 ± 0.03	1.97 ± 0.04	1.91 ± 0.04
	% Change		42.30	38.62
	Muscle	1.15 ± 0.20	1.60 ± 0.10	1.42 ± 0.16
	% Change		39.62	23.59
	Gills	1.59 ± 0.21	2.51 ± 0.19	2.10 ± 0.13
	% Change		57.28	31.67
SDH	Liver	0.74 ± 0.02	0.25 ± 0.02	0.33 ± 0.02
	% Change		-66.46	-55.79
	Muscle	0.75 ± 0.03	0.46 ± 0.01	0.50 ± 0.02
	% Change		-38.81	-33.40
	Gills	1.20 ± 0.02	0.80 ± 0.02	0.86 ± 0.02
	% Change		-33.14	-28.52
G6PDH	Liver	6.08 ± 1.11	8.32 ± 1.06	7.62 ± 0.73
	% Change		36.68	25.25
	Muscle	1.90 ± 0.37	2.84 ± 0.32	2.19 ± 0.21
	% Change		49.58	15.64
	Gills	1.28 ± 0.23	1.70 ± 0.22	1.68 ± 0.30
	% Change		32.43	30.84

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.

Maximum increase in LDH activity was observed in gills (57.28%) at 1/3rd sublethal concentration and liver (38.62%) at 1/5th sublethal 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. In contrast, SDH activity was declined in both the concentration, showing maximum inhibition in liver (66.46% and 55.79%), at both 1/3rd and 1/5th sublethal concentration (Table 1). 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 2. Effect of sublethal concentrations of copper cyanide on ALT (µmol of pyruvate formed/mg protein/h) in different tissues of *C. catla*

	Tissue	Control	Sublethal 1/3rd (0.253 mg/L)	Sublethal 1/5th (0.152 mg/L)
ALT	Liver	6.41 ± 0.22	9.41 ± 0.12	8.94 ± 0.14
	% Change		46.77	39.49
	Muscle	3.38 ± 0.15^{g}	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 significantly different ($p < 0.05$) from each other.

Table 3 shows the activity of acid and alkaline phosphatase activity in the fish exposed to both the sublethal concentration. Maximum inhibition in the AcP was observed in the liver (45.32%) at 1/3rd and muscle (39.86%) at 1/5th sublethal concentration, where as ALP activity exhibited maximum decline in liver (41.76 and 38.74%) at both the concentration.

Table 3. Effect of sublethal concentrations of copper cyanide on AcP and ALP (µmol of p-nitro phenol formed/mg protein/h) in different tissues of *C. catla*

	Tissue	Control	Sublethal 1/3rd (0.253 mg/L)	Sublethal 1/5th (0.152 mg/L)
AcP	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

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.

Discussion

Cyanide is one of the most toxic chemical to fish, as fish are one thousand times more sensitive to cyanide than human *(Hosetti and Dube, 2010)*. This active sensitivity of fish to cyanide therefore makes fish an excellent biomarker for the presence of cyanide in water *(Adeyemo, 2005)*. Pollution of the aquatic ecosystem stresses the animals and disturbs their metabolism by altering the enzyme activity, damage and dysfunction the tissues and hindering growth all that associated with biochemical changes (*Osman et al., 2010).* Analysis of biochemical parameters could help to identify the target organs of toxicity as well as the general health status of animals. It may also provide an early warning signal in stressed organism *(David et al., 2010; Prashanth, 2012).*

LDH is a tetrameric enzyme recognized as a potential marker for assessing the toxicity of a chemical *(Suneetha, 2012)*. In the present study gill tissue exhibited maximum decrease in LDH activity compared to muscle and liver. This may be due to the direct effect of the cyanide on disruption of the gill epithelium, resulting to the inhibition of cytochrome oxidase activity *(Bhattacharya et al., 2009)*. This situation might favor anaerobic respiration due to the mild stress of hypoxia in *C. catla*; thus, aerobic processes might be operating at a very slow rate. Similar observations were made by *David et al. (2010)* in the fish *C. carpio*, exposed to sodium cyanide. Further, conversion of pyruvate to lactate at the expense of NAD contributed to increase in LDH activity. Resultantly, to fulfill the energy demands, there may be increase in the operation of glycolysis under cyanide stress *(Rees et al., 2009).*

SDH is an important active regulatory enzyme of the Kreb's cycle of mitochondria. Depletion of SDH can be noticed from the present study in all tissues treated with sublethal doses of cyanide when compared to controls. Suppression of SDH activity in subacute conditions indicates derailment of metabolic cycle. This may also be due to the out come of mitochondrial disruption leading to a decrease in activities of oxidative enzymes *(Prashanth, 2012).* The induced decrease of SDH activity can be attributed to the ability of cyanide to inhibit mitochondrial enzymatic activities *(Shwetha et al., 2012).* Consequently, the decline in SDH activity shows a shifting of aerobic respiration to anaerobic respiration. The results of the present study are also in conformity with those of the earlier observations *(David et al., 2010*).

G6PDH enzyme is extra mitochondrial in location and highly specific for NADP as an electron acceptor *(Barroso et al., 2001)* and is the first enzyme in the pentose phosphate pathway. The effects of different chemical substances on the activity of G6PDH enzyme have been investigated in many *in vitro* and *in vivo* studies, performed with various organisms *(Murat et al., 2009).* In the present study cyanide significantly stimulated G6PDH activity in the fish indicating mobilization of glucose through pathways other than glycolysis-Krebs cycle. High

G6PDH activity indicative of high rates of PPP or HMP (Hexose monophosphate) shunt under stress condition as reported by *Surendranath et al. (1991)* and substantiates the present work.

Transaminases are important enzymes known to play a key role in mobilizing L-amino acids for gluconeogenesis and function as links between carbohydrate and protein metabolism under altered physiological, pathological conditions *(Prashnath, 2012; Shwetha et al., 2012).* Increase in the levels of these enzymes in liver, muscle and gills of fishes can be considered as a response to the stress induced by cyanide to generate ketoacid-like ketoglutarate and oxaloacetate for contributing to gluconeogenesis and/or energy production necessary to meet the excess energy demand under the toxic manifestation *(Okafor et al., 2008).* The increase in the ALT and AST activities in our study supports earlier findings and serves as indicator of tissue damage *(Okolie and Osagie, 2000).* Similar findings were also observed by *Naveed et al. (2010)* in *C. punctatus. Agrahari et al., (2007)* observed an increase in the ALT and AST of the catfish exposed to pesticide and opined that this increase in the activity of these enzymes is an indicator of cellular damage.

Acid phosphatase is a lysosomal enzyme that hydrolyses the phosphorous esters in acidic medium. This enzyme is hydrolytic in nature and acts as one of the acid hydrolyses in the autolysis process of the cell after its death. Alkaline phosphatase splits various phosphorous esters at alkaline pH, its activity is related to the cellular damage. Since, cyanide has anti-phosphatase activity; inhibition of phosphatase activity may be due to reduced protein synthesis, as such phosphatase plays an important role in protein synthesis *(Okolie and Osagie, 2000). Ogundele et al. (2010)* illustrated inhibition in ALP and AcP activity in the adult Wistar rats, administered with the cassava. *Inyang et al. (2011)* reported inhibition of AlP and AcP activity in the fish *C. gariepinus* resulting from the Diazinon exposure.

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Uticaj bakar-cijanida na ključne metaboličke enzime slatkovodnog Indijskog šarana (Hamilton)

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Rezime

Eksperimenti kratkoročne toksičnosti su sprovedeni sa ciljem da se prouči efekat metal cijanid kompleksa (bakar-cijanid) na ključne metaboličke enzime, aktivnost laktat dehidrogenaze (LDH), sukcinat dehidrogenaze (SDH), glukoza-6 fosfat dehidrogenaza (G6PDH), aspartat amino transferaze (AST) alanin amino transferaze (ALT), kisele fosfataze (ACP) i alkalne fosfataze (ALP) u mladim primercima Indijskog šarana. Ukupno 60 mladih šarana su (2 ± 0,5 cm, 1,5 ± 0,2 g) bili izloženi subletalnim koncentracijama (0,253 i 0,152 mg/l) u periodu od 15 dana. Bakar cijanid je imao signifikantan (p> 0,05) uticaj na ključne metaboličke enzime, najviše aktivnosti su zabeležene u grupi izloženoj koncentraciji od 0,253 mg/l. Rezultati ukazuju na to da metal cijanid kompleks značajno menja aktivnost enzima ribe u obe subletalne koncentracije.

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