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Copper-Influenced Changes in Lactate Dehydrogenase and G-6-PDH Activities of the Freshwater Teleost, *Labeo rohita*

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Copper contamination in freshwater ecosystem is considerably increasing in recent years with the release of effluents from firms manufacturing pesticides, fungicides, fertilizers, electrical equipments, explosives, antifouling paints and iron and steel, in addition to copper mining. It is a group IB metal having high toxic to aquatic fauna, particularly to fresh water fishes, which serve staple food for human beings (Spehar *et al.,* 1982). It has high affinity to nitrogen, oxygen and/or sulphur ligands of proteins and plays a predominant role in enzymes, such as oxygen binding haemocynine and tyrosinase (Frieden, 1979). The literature on the toxic effects of copper in freshwater fishes is very scanty (Noel-Lambat *et al.,* 1992; Dixion and Sprague, 1981; Moore and Ramamurthy, 1984). Hence, an attempt was made to study the impact of lethal and sublethal concentrations of copper on the LDH and G-6-PDH activities of gill, liver, brain and muscle of the freshwater teleost, Labeo rohita (Hamilton). As the period of exposure to any heavy metal has considerable influence on the metabolic changes (Venkataramana and Radhakrishnaiah, 1987) the study is made at 1, 2 and 3 days on exposure to lethal and 1, 15 and 30 days to sublethal concentrations of copper.

MATERIALS AND METHODS

Teleost L. rohita weighing around 10±1g were procured from local Fisheries Department and were maintained in the laboratory in large glass aquaria containing dechlorinated tap water (Temperature 28±1°C, PH 7.6±0.2, dissolved oxygen 5.76±0.4 mg/l, total hardness 100±5 mg/l $CACO_3$ and light period of 12 h/1 day). The water was changed every day to renew the oxygen content. The fish were fed with commercial fish pellets everyday and were adopted tolaboratory conditions for 15 days prior to experimentation. The fish were exposed to different concentrations of copper prepared from cupric chloride salt. LC 50 values for 96 hrs were determined from per cent and

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probity mortality verses log concentration curves (Finney, 1971) and were subsequently verified by dregstedt and Behren's method (Carpenter, 1975). The 95 hr LC 50 value of copper was found to be 1.2 mg/l. One fifth of LC 50 value (0.24 mg/l) was taken as sublethal concentration and employed in the present study. After the stipulated time of exposure to lethal (1, 2 and 3 days) and sublethal (1, 15 and 30 days) concentrations of copper, the gill, liver, brain and muscle were isolated from the fish survived and transferred to cold fish ringer solution for the estimation of Lactate dehydrogenase and G-6-Phosphate dehydrogenase activities. The 5% tissue homogenation prepared in 0.25 M ice-cold sucrose solution was centrifuged and supernatant was used as enzyme source. The LDH activity was assayed by the method of Srikantan and Krishnamoorthy (1955) as modified by Govindappa and Swami (1965).

RESULTS AND DISCUSSION

The present study revealed that the lactate dehydrogenase activity significantly ($P<0.05$) increased with a corresponding decrease in the glucose -6 - phosphate dehydrogenase activity in the gill, liver, brain and muscle of the fish, *L. rohita* from 1 to 3 days of exposure to lethal concentration of copper (Table 1).

Some behavioural changes were also observed in the fish exposed to lethal and sublethal concentrations of copper. In lethal concentration, the fish developed irritability, irregular erratic swimming and jumping restlessness, coagulated mucus film covering over gill epithelium, shrinkage in epithelium, the colour of gill changed from red to brown and increase in the opercular movement at 1 day of exposure and drastic decrease in its movement at 2 and 3 days of exposures. Similar behavioural changes were also reported by Ansari (1984) in Channa punctatus and Mystus Vittatus exposed to lethal concentration of copper stress. The drastic decrease in opercular movement at 2 and 3 days of exposures may be due to the reduced supply of oxidative metabolism (Venkataramana and Radhakrishnaiah 1987) and the intestine filled with water was observed may be due to the failure of osmoregulatory mechanism. Probably, the coagulated muucus film and necrosis on gill epithelial cells may cause the suffocation leading to death of the animal in lethal concentration. Against to lethal concentration, the fish exhibited sluggishness and uncoordinated movements and less in opercular movement, particularly, in early exposure period, but slowly the behaviour of the animal recovered near to normal state at later exposure periods. The less decrease in opercular movement at 30 days of exposure may be due to the activation of microsomal drug metabolizing enzymes which bring about the biodegradation of pollutants to reduce toxicity. The general disorders in the behaviour and over all decline in physical stability of the fish exposed to lethal concentration may be due to drastic disruption in physiological and biochemical functions and water balance, where as in sublethal concentration, the recovery of the fish near to

normal state may be due to the process of adaptation achieved by the activation of detoxification mechanisms and metal elimination pathways.

The drastic increase in the LDH activity, over the time of exposure, indicates the induction of anaerobic glycolysis to meet the required energy demands. Burton *et al.* (1972) reported the decrease in pyruvate level and an increase in lactate level with an increase in the lactate dehydrogenase activity in rainbow trout Salmo gairdneri under Zinc stress. The decrease in the glucose - 6 - phosphate dehydrogenase activity over the time of exposure, indicates the suppression of HMP shunt leading to slowing down of the flow of metabolites to much needed biosynthetic pathways of necleic acids, lipids, steriods, TCA cycle and electron transport.

The drastic elevation in the LDH activity in the four organs exposed to lethal concentration indicates a high accumulation of metal in it leading to the disruption of structural organisation and increase in permeability of cells, whereas the drastic decrease in the G-6-PDH activity may be due to derangement in architectural integreity and permeability of mitochondria causing blockage of HMP shunt thus, the death of the fish in lethal concentration of copper could be with the added reasons like high glucose level in blood and more lactate accumulation in tissues.

The LDH activity significantly ($P<0.05$) increased at 1 day of exposure less at 30 days and moderate at 15 days of exposure to lethal concentration of copper. In contrast, the G - 6- PDH significantly ($P<0.05$) increased at 30 days of exposure (Table 1). The increase in the LDH activity at 1 day of exposure period indicates the sudden suppression in oxygen consumption leading to the stimulation of anaerobic glycolysis to meet required energy demands, whereas the recovery of its activity near to normal state at 30 days of exposure may indicate a metabolic shift from anaerobic to aerobic through Acetyl CoA barrier, as reported by Swami *et al.* (1983). Correspondingly, the elevation in the G - 6 - PDH activity in the organs indicates the actively operation of HMP shunt through G - 6- PDH. The strategic step of the animal may be to sequest the metal and to maintain the dynamic functioning of the organ systems in sublethal toxic stress. This may not be possible in lethal concentration, because the bound copper has some finite limit with regard to intracellular storage. Suppression of the enzyme activities in the organs of the fish exposed to lethal and sublethal concentrations of copper may be due to dual effect of copper.

Differences observed in the degree of increase in the LDH activity and decrease in the G- 6- PDH activities in the four organs of the fish exposed to lethal and sublethal concentrations can be attributed to the concentration of the metal accumulated in them.

The glycolysis and enzymes are much more predominant in the muscle of the fish. Several physiological and pathological conditions were known to elevate and inhibit the cellular enzymes (Cahill, 1971). In this study high concentration of copper might have caused greater damage to a greater enhancement in the LDH activity. However, the recovery in the LDH activity near to normal state in it on exposure to sublethal concentration indicate the structural stability leading to shift to aerobic to anaerobic phase. Naturally, the G- 6- PDH activity is low in the skelethal muscle than to the other organs of the animal (Harper, 1977) and hence its role in the synsthesis of pentose may be less. It is also found true in this study. The drastic decrease in the G- 6- PDH activity in the muscle of the fish exposed to lethal concentration indicates the greater disruption in mitochondrial permeability caused inhibition in HMP shunt, whereas in sublethal concentration the increase in this enzyme activity may indicate the repair of its structural configuration by the synthesis of aminoacids and lipids. Next to the muscle, a high increase in the LDH activity and decrease in the G- 6- PDH activity were observed in the gill of the fish exposed to lethal concentration indicate the disruption of gill lamella and isolation of cellular fragments. In contrast, in the sublethal concentration less increase in the LDH activity and more increase in the G- 6- PDH activity coudl aid fortification of its structural rigidity of perform both osmo and inoregulatory and respiratory functions, as the activities of Na^+ - K^+ and Mg^{2+} ATP ases are the lipid bound enzymes responsible for active sodium, potassium and magnesium transport (Renfro *et al.,* 1974). Liver being the centre for detoxification and the site for glycogen and lipid storage plays a key role in the synthesis of glycogen and lipids and its oxidation pathways. The increase in the LDH activity and decrease in the G- 6- PDH activity in this organ exposed to lethal concentration indicate the accumulation of copper in it leading to the failure of the homeostatic mechanism where as less elevation in the LDH activity and more increase in the G- 6- PDH activity are observed in the liver of the fish exposed to sublethal concentration indicate more oxidation of lactate and lipogenesis in the consruction of membranes for cellular and sub-cellular membrane bound organelles and to protect the tissue from toxic stress. The changes in the enzymes activities in the brain of the fish exposed to lethal concentration of copper are relatively less. This situation might have arised due to less accumulation of copper in its cellular fragments resulted the decrease in the oxidative metabolism and pentose pathways. However, in the sublethal concentration the recovery in the LDH activity at lethal exposure and the gradual elevation in the G- 6- PDH activity observed in it could indicate the production of total lipids through pentose pathways for the maintenance of proper lipid water balance in the neuronal membrane for effective functioning of membrane permeability and nerve transmission.

Thus, it is inferred that exposure to lethal toxic stress of copper leads suppression of energy yielding enzymes causing to the death of the fish,

Table 1. Changes in LDH and G-6-PDH activities (μM formozan/mg protein /h).

Organ	Exposure Period (in Days)						
	Control	Lethal			Sublethal		
		1	2	3	1	15	30
LDH ACTIVITY							
Gill	0.145	0.218	0.258	0.278	0.197	0.188	0.178
S.D.±	0.052	0.040	0.050	0.043	0.028	0.222	0.028
%	--	(50.4)	(77.9)	(91.7)	(35.9)	(28.9)	(20.3)
Liver	0.650	0.962	1.102	1.235	0.871	0.827	0.799
S.D.±	0.042	0.040	0.068	0.053	0.027	0.038	0.100
%	--	(48.0)	(69.53)	(90.0)	(34.0)	(27.0)	(19.5)
Brain	0.686	0.926	1.302	1.396	0.892	0.859	0.771
S.D. ±	0.050	0.044	0.050	0.040	(0.040)	(0.038)	0.068
%	--	(34.9)	(89.79)	(99.8)	(30.0)	(25.0)	(11.9)
Muscle	0.698	1.117	1.308	1.396	0.698	0.908	0.867
S.D.±	0.045	0.045	0.042	0.040	0.020	0.030	0.036
%	--	(60.0)	(60.0)	(99.8)	(40.1)	(29.9)	(22.1)
G-6-PDH Activity							
Gill	0.300	0.155	0.110	0.045	0.345	0.395	0.484
S.D.±	0.030	0.024	0.030	0.030	0.028	0.030	0.030
%	--	(-48.3)	(-63.3)	(-85.0)	(15.0)	(29.2)	(49.5)
Liver	0.348	0.208	0.109	0.069	0.370	0.456	0.525
S.D.±	0.025	0.029	0.030	0.023	0.030	0.029	0.030
%	--	(-40.2)	(-68.87)	(-80.2)	(14.2)	(29.0)	(40.0)
Brain	0.200	0.133	0.102	0.075	0.220	0.243	0.275
S.D. ±	0.030	0.029	0.030	0.029	0.030	0.036	0.029
%	--	(-33.5)	(-49.0)	(-62.5)	(10.2)	(20.0)	(35.0)
Muscle	0.180	0.126	0.109	0.071	0.197	0.231	0.262
S.D.±	0.032	0.019	0.011	0.015	0.030	0.018	0.030
%	--	(-30.0)	(-39.45)	(-60.5)	(09.2)	(20.3)	(32.0)

Each value is mean of ten estimations

S.D.: Standard Deviation

Values in parentheses indicate increase / decrease (-) over control.

whereas in sublethal concentration stress, though there was sudden disturbances observed at initial exposure period, this enzymes were recovered near to normal state at later exposure period might be due to the activation of metal detoxification and elimination pathways. A little increment in the accumulation of the metal in the organs beyond finite limit may lead to behavioural changes and create wide spread disaster in the normal physiology ultimately resulting the death of the fish may be used as simple valuable devices for determining the environmental pollution by copper.

REFERENCES

Ansari IA (1984) Studies on the toxicity of copper sulphate on Channa punctatus (Bloch) and Mystus vittatus (Bloch): Determination of LC50 values. Acttaciencis Indica 10: 154-160.

Burton TD, Jones AH, Caixs TJ (1972) Acute zinc toxicity to rainbow trout, Salmo gairdeneri confirmation of the hypothesis that death is related to tissue hypoxia, Fish Res Bd Canada 29: 1463-1466.

Cahill GF (1971) Physiology of insulin in man. Diabetes 20: 785-799.

Carpenter PL (1975) In : Immunology and Serology, 3rd Edition, WB, Saunder Company, Philadelphia, London, Toronto. p. 254.

Dixon DH, Sprague JB (1981) Copper bioaccumulation and hapatoprotein synthesis during accumulation of copper by juvenil rainbow trout. Aquat Toxicol 1: 69-81.

Finney DJ (1971) Probit analysis, 3rd edition, London and New York, Cambridge Univesity Press: p.333.

Frienden E (1972) Ceruloplasmin: The serum copper transport protein with oxidase activity, In: Copper in the Environment. Part - 2, Health effects, (Ed) J.U. Niraga, Wiley, New York, pp.242-284.

Govindappa S, Swami KS (1979) Electrophoretic characteristics of subcellular components and their relation to enzyme activities in the amphibian muscle fibre. Indian J Exp Biol 3: 209-212.

Harper HA (1977) Metabolism of lipids, regulation of carbohydrate and lipid metabolism. In : Review of Physiological Chemistry, 16th Edition, Lange Medical Marurer Company Ltd., pp.280-336.

Moore JW, Ramamurthy S (1984) In: Heavy metals in natural waters : Applied monitoring and impact assessment: Ed. Desanto RS Springer - Verlag, New York, Berlin, pp.77-99.

Noel - Lambat F, Gerday C, Disteche A (1978) Distribution of Cd, Zn and Cu in liver and gills of Eel, Anquilla anquilla, with special reference to Biochem Physiol 64C: 177-178.

Renfro JLB, Schmidt-Nielson D, Miller DB, Allens J (1974) Methyl mercury and inorganic mercury uptake, distribution and effect on osmoregulotory mechanisms in fishes. In: Pollution and physiology of marine organisms, (Eds. F.J. Vernberg and W.B. Vernberg). Academic press, New York, San Fransisco, pp.101-122.

Spehar RC, Christensen GM, Curtis C, Lemke AE, Norberg TJ, Pickering OH (1982) Effects of Pollution on freshwaer fish. J Wat Pollut 54: 877-922.

Srikantan TN, Krishnamoorthy CR (1955) Tetrazolium Test for dehydrogenase. J Sci Ind Res 14: 206-207.

Swami KS, Jayantha Rao KS, Sathyavelu Reddy K, Krishnamoorthy K, Lingamurthy G, Chetty CS, Indira K (1983) The possible metabolic diversions adapted by the freshwater mussel to counter the toxic metabolic effects of selected pesticides. Indian J Comp Anim Physiol 1: 95-106.

Venkataramana P, Radhakrishnaiah K (1987) Lethal and sublethal effects of copper on the protein metabolism of the freshwater teleast, Labeo rohita (Hamilton). Trends Life Sci 2: 1-7.