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Toxicological Effects of Lead On Certain Enzymological and Biochemical Parameters in *Cirrhina Mrigala*

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Abstract

Lead is found to be the most toxic of heavy metals so the present research is designed to study toxicological effects of lead on certain enzymological and biochemical parameters in tissue and blood. Fish were exposed to sublethal dose of lead chloride ($PbCl_2$) for 40 days. The tissue extracts were taken and tested for the activity of key enzymes of glycolysis and Kreb's cycle along with their biochemical components. In blood, the level of glucose increased by 115.38%. Pyruvic acid and lactic acid decreased by 25.97% and 37.5% respectively. In liver, the glycogen content increased by 104.2%. The activity of glucose-6- phosphatase increased in liver and gills. Inhibition in the activity of LDH in liver and muscle indicates decreased rate of glycolysis. Elevation in the activity of PDH and SDH in liver, gill and muscle shows that in fish the rate of oxidative metabolism is increased to withstand the toxic stress.

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Introduction

Heavy metals are common persistent pollutants of aquatic ecosystem entering them through numerous diverse anthropogenic and natural sources (Moore, 1991). Aquatic systems are very sensitive to heavy metal pollutants and the gradual increase in the level of such metals in aquatic environment, mainly due to anthropogenic sources, became a problem of primary concern (Thirumvalan. R, 2010). This is due to their persistence, as they are not usually eliminated either by biodegradation or by chemical means, in contrast to most organic pollutants.

Lead (Pb) is one of the most toxic of heavy metals and its compounds are included in the grey list of international conventions (Taylor *et al.*, 1985). Lead enters the aquatic environment through erosion and leaching from soil, lead-dust fall out, combustion of gasoline, municipal and industrial waste discharges, run-off water deposits from streets and other surfaces as well as precipitation (Department of Water and Forestry (D.W.A.F.), 1996). Lead that is emitted into the atmosphere can be inhaled, or it can be

ingested after it settles out of the air. It is rapidly absorbed into the bloodstream and is believed to have adverse effects on the central nervous system, cardiovascular system, kidneys, and the immune system (Bergeson, Lynn L, 2008).

Chemical changes disturb the equilibrium (homeostasis) of ecosystems and thus prevent their normal functioning. In polluted water bodies, concentrations of compounds containing both essential metals and those playing no part in an organism's functioning (lead) may increase to toxic levels (Jezierska and Witeska 2001). The above affect individual development of plants and animals. Among animal species, fish are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa *et al.*, 2004; Clarkson, 1998; Dickman and Leung, 1998). Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems (Farkas *et al.*, 2002; Yousuf and El-Shahawi, 1999). So an attempt has been made in the present research to study the effect of heavy metal lead (Pb) on certain physiological activities and biochemical parameters in tissue and blood.

Materials and Method

The fish, *Cirrhina mrigala* were purchased from the local fish market having an average length 12±3 cm and wt 200±2 gms The fish were then kept in different aquaria for conduction of various experiments. The fish were acclimatized to laboratory conditions in aquaria for a few days. In one aquarium the fish were kept as control specimens given the same food and environment as that of the experimental fish except that they were not given the dose of heavy metal compound. Inorganic salt of heavy metal lead namely lead chloride anhydrous ($PbCl_2$) was the experimental toxicant. To observe the chronic effects of lead, sublethal dose (1/10 concentration of 96 hr LC_{50}) of the heavy metal compound was given for 40 days. During exposure period the fish were conditioned to feeding on packed fish food at the rate of 2% of body weight. The fish were fed once daily at 11am.

For the estimation of activities of enzymes of glycolysis and kreb's cycle, 10% of W/V homogenates were prepared in 0.25 M sucrose solution for tissues namely liver, gills and muscles. The homogenates were refrigerated in cold at 1000x g for 20 min. and clear supernatant fluids were used as the source of enzymes. The activities of Lactate Dehydrogenase (LDH), Pyruvate Dehydrogenase (PDH), Succinate Dehydrogenase (SDH) enzymes were estimated by the triphenyltetrazolium chloride method of Srikantan and Krishnamoorthi (1995). The activity of Glucose-6-Phosphatase was determined by adopting the method of Swanson (1955). Glycogen in liver was estimated by the method of Hassid and Abrahim (1957). The method of Lowery *et al.* (1951) was adopted for the estimation of total proteins.

Blood from the caudal vessel of both control and experimental fish was drawn with the help of heparinized needles for the estimation of the levels of glucose, lactic acid and pyruvic acid. Glucose was determined by the method of Folin and Wu (1929). Pyruvic acid was determined by the method of Friedmann and Haugen (1944). Lactic acid in blood was

estimated according to the method of Barker (1963). The significance of the difference between control and experimental means was calculated by Students't' test (Wardlaw, 1985)

Observation and Results

Enzymological Studies

LIVER

The activity of Glucose-6-Phosphatase increased by 38.91% after 40 days of exposure. The activity of lactate dehydrogenase (LDH) decreased after 40 days of exposure (39.45%). Increase was observed in the activity of pyruvate dehydrogenase (PDH) and succinate dehydrogenase (SDH) after 40 days of exposure by 103.5% and 40.47% respectively.

GILLS

Increase in the activity of Glucose-6-phosphatase was observed after 40 days (9.27%). The activity of lactate dehydrogenase decreased after 40 days of exposure. However, increase of 27.78% was recorded in the activity of pyruvate dehydrogenase (PDH) after 40 days of exposure. The activity of succinate dehydrogenase (SDH) increased after 40 days (41.49%).

Muscle

Decrease was observed in the activity of lactate dehydrogenase (LDH) after 40 days of exposure. However, no significant change was observed in the activity of PDH during exposure period. Increase by 40.13% was observed in the activity of Succinate dehydrogenase (SDH) after 40 days of exposure.

Biochemical Studies

Blood

The blood glucose level increased after 40 days of exposure by 115.38%. The fish were hypolectemic after chronic exposure to lead. Pyruvic acid level decreased in the blood after 40 days of exposure by 25.97%.

Liver

On chronic exposure to lead, glycogen content of liver increased by 104.2%.

ENZYMES	CONTROL	40 DAYS
GLUCOSE-6- PHOSPHATASE [a]	233.39±0.02	324.22±0.04***
LACTATE DEHYDROGENASE [b]	4.79±0.02	2.90±0.03***
PYRUVATE DEHYDROGENASE [b]	3.14±0.04	6.39±0.02***
SUCCINATE DEHYDROGENASE [b]	5.09±0.01	7.15±0.04***

Values are mean±SD; n=6, NS= not significant
*significant, *p<0.05, **p<0.01, ***p<0.001
[a]µg inorganic phosphate/mg Protein/hr
[b]µg formazon/mg Protein/hr

Table-1: Alterations In Liver Enzyme Activities In *Cirrhina Mrigala* Exposed To Lead (Pb) For 40 Days

ENZYMES	CONTROL	40 DAYS
GLUCOSE-6- PHOSPHATASE [a]	27.72±0.05	30.29±0.01***
LACTATE DEHYDROGENASE [b]	4.87±0.02	4.73±0.08*
PYRUVATE DEHYDROGENASE [b]	3.73±0.04	5.60±0.04***
SUCCINATE DEHYDROGENASE [b]	6.49±0.01	8.66±0.05***

Values are mean±SD; n=6, NS= not significant
*significant, *p<0.05, **p<0.01, ***p<0.001
[a]µg inorganic phosphate/mg Protein/hr
[b]µg formazon/mg Protein/hr

Table-2: Alterations in Gill Enzyme Activities In *Cirrhina Mrigala* Exposed To Lead (Pb) For 40 Days

ENZYMES	CONTROL	40 DAYS
LACTATE DEHYDROGENASE [b]	8.03±0.02	6.69±0.01***
PYRUVATE DEHYDROGENASE [b]	11.05±0.04	10.97 ± 0.07NS
SUCCINATE DEHYDROGENASE [b]	4.51±0.03	6.32±0.03***

Values are mean±SD; n=6, NS= not significant
*significant, *p<0.05, **p<0.01, ***p<0.001
[b]µg formazon/mg Protein/hr

Table-3: Alterations In Muscle Enzyme Activities In *Cirrhina Mrigala* Exposed To Lead (Pb) For 40 Days

PARAMETERS	CONTROL	40 DAYS
BLOOD Glucose [a]	0.13±0.01	0.28±0.01***
Lactic Acid [a]	0.08±0.01	0.05±0.05**
Pyruvic Acid [a]	0.77±0.03	0.57±0.03***
LIVER Glycogen [b]	0.70±0.05	1.43±0.07***

Values are mean±SD; n=6, NS= not significant
*significant, *p<0.05, **p<0.01, ***p<0.001
[a] mg/ml of blood
[b] mg/gm wet weight of tissue

Table-4: Alterations In Biochemical Parameters In *Cirrhina Mrigala* Exposed To Lead (Pb) For 40 Days

Discussion

The toxic effects of heavy metals arise from their action on biological systems. 'Enzymes' are the common targets of toxicants and undergo marked alterations in their activities with the period of exposure and tissue.

After 40 days of exposure, blood sugar level increased showing increased rate of glycogenolysis as the activity of G-6-Pase also increases after 40 days but simultaneous increase in the liver glycogen may be due to the increased rate of glycogensis. Hinston *et al.* (1983) reported an increase in the blood glucose level in the fish *Channa punctatus* exposed to pollutants. R. Vinodhini *et al.* (2008) also observed increase in blood glucose level in *Cyprinus carpio* after exposure to mixture of heavy metals, lead (Pb), cadmium (Cd), chromium (Cr) and nickel (Ni) for 32 days. Patil and Dhande (2000) also reported increased blood glucose level in *Channa punctatus* exposed to mercuric chloride. Almeida *et al.* (2001) also found increase in blood glucose level in *Oreochromis mossambicus* exposed to cadmium.

Decrease in lactic acid content and increase in the liver glycogen shows increased rate of glycogenesis i.e. formation of liver glycogen from lactic acid. Sastry *et al.* (1987), Sastry and Sunita (1983) and Lowe Jinde and Niimi (1984) reported decrease in lactic acid in the liver of *Heteropneustes fossilis* and *Channa punctatus* exposed to lead (Pb) and chromium (Cr) respectively.

Decrease in the activity of lactate dehydrogenase (LDH) in liver, gills and muscle on chronic exposure to lead (Pb) indicates decreased rate of glycolysis. Similar decrease in LDH activity was noted in the early developmental stages of African Catfish, *Clarias graiepines* on exposure of lead nitrate ($Pb(NO_3)_2$) (Alla G. M. Osman *et al.* 2006). Singhal (1994) also observed decrease in activity of lactate dehydrogenase (LDH) in liver, gill, muscle and kidney of catfish *Hetropneustes fossilis* on exposure to sublethal concentrations of lead nitrate ($Pb(NO_3)_2$).

Inhibition in the activity of LDH is evidenced by decrease in the pyruvic acid level of blood showing decreased rate of glycolysis. Similar

decrease in pyruvic acid was recorded by Rajeshwari *et al.* (1990) in *Clarias batrachus* on exposure to cadmium.

Oxidative metabolism prevailed in liver and gills and muscles after 40 days of lead exposure. The evidence in favour of this comes from the increase noted in the activity of PDH and SDH in liver, gill and muscle (40 days). Impairment of glycolysis and favouring of oxidative metabolism shows that pyruvic acid utilized in the oxidative metabolism was derived from an alternative source. Similar increase in the PDH and SDH activities was observed in different tissues of *Channa punctatus* exposed to chromium (Sastry and Sunita, 1983). Increase in SDH activity was noted on exposure of cadmium for 30 days in *Channa punctatus* (Sastry *et al.*, 1997).

Conclusion

Increase in the activity of SDH and decrease in LDH in different tissues clearly shows that in fish, the rate of oxidative metabolism is increased to withstand the toxic stress. Elevation in the activity of enzymes of Kreb's cycle in different tissues suggest that metabolic rate of fish exposed to lead(Pb) was higher than control fish,

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