EFFECT OF HEAVY METALS ON LIVER AND MUSCLE OF THE INDIAN MAJOR CARP CATLA CATLA FINGERLINGS

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ABSTRACT
The Indian Major carp fish *Catla catla* were collected from Kalyani Dam reservoir near Tirupati. The fishes were exposed to sublethal concentrations Lead chloride (PbCl2), Mercuric chloride (HgCl2) For 45 days. Effect of these heavy metals on various biochemical and metabolic enzymes of liver and muscle were observed. Histopathological changes in liver and muscle tissue of *catla catla* undergoes heavy metal stress. It is observed that increase in glutamate oxalo acetate transaminase and glutamate transaminase activities were noticed.

KEYWORDS: *Catla catla*, Lead chloride, Mercuric chloride, histopathology, glutamate oxalo acetate transaminase, glutamate transaminase.

INTRODUCTION
In last few decades global fisheries is facing constant decline in fish stocks both in coastal and inland water resources on account of constantly increasing water pollution. Contamination of fresh water with wide range of pollutions have become a matter of concern over last five decades (Vutukuru, 2005). Fresh water contamination with heavy metals causes devastating on ecological balance of all aquatic environments. Aquatic organisms becomes limited with the extent of pollution. Lead chloride and mercuric chloride are the heavy metals which are highly toxic to all animals including many aquatic organisms (Masud et al 2009, Atel, 2003).

Heavy metals enter in to human body through various rates including fish. Once absorbed to the body, inorganic metals are capable reacting with a variety of binding sites. Creed (1973)
reported that high concentration of heavy metals potentially interact with DNA and causes mutations in living systems. Although the effects of various heavy metals have been extensively studied in fishes. The main aim of this study is to investigate the effect of exposure to sublethal concentrations of Lead chloride and mercuric chloride on physiological modulations in commercially important and highly cultivable Indian major carp *catla catla*.

**MATERIALS AND METHODS**

*Catla Catla*

**CATLA CATLA**

Kingdom – Animalia  
Phylum – Chordata  
Sub-Phylum – Vertebrata  
Class – Actinopterygii  
Order – Cypriniformes  
Family – Cyprinidae  
Genus – Gibelion  
Species – Catla Catla

**Treatment procedure**

*Catla catla* fingerlings (8.0±0.52 cm in length & 10.0±1.0gm in weight) were obtained from the Andhra Pradesh government central fish farm, Kalyanidam, near Tirupati and immediately transferred to six cement aquaria (each 500 litres capacity), where they received unchlorinated continuous gentle flow of water from a deeply sunk bore well within the university campus. Fishes were acclimated to the laboratory conditions (25±1°C.12 hr light/dark cycle). Water quality parameters (TDS=600ppm, PH =6.75, D.O =5.8mg/l) were measured prior to throughout the experiment. The fishes were starved 24 hours before the
start of the experiments to avoid metabolic variations due to diet, if any lead chloride and mercuric chloride are used as test toxic heavy metals for lead and mercury respectively during the present research work.

After acclimation period, median lethal concentration for 72 hours of lead chloride and mercuric chloride were conducted by probit analysis method (Finney 1964). The LC\textsubscript{50} values for 72 hours as follows:

- Lead chloride: 5.34mg/l and
- Mercuric chloride: 5.70mg/l

Experimental groups received 1/10\textsuperscript{th} of LC\textsubscript{50} of Pbcl\textsubscript{2} and Hgcl\textsubscript{2} for 3,8,16,30 and 45 days. The heavy metal containing water medium was changed at every 24 hours inorder to maintaining a constant sublethal concentration of heavy metals.

![Fig. 1: Fingerlings of fish *Catla catla* Acclimatize to laboratory conditions.](image)

A group of acclimated fish ready for experimentation further divided into two main groups exposed to sublethal concentrations of Pbcl\textsubscript{2} and Hgcl\textsubscript{2} respectively. Each group was further divided in to six batches of ten individuals. In each group of these, the first five batches constitute the experimental groups. Each of which received daily 1/10\textsuperscript{th} of LC\textsubscript{50} of related heavy metal for 3,8,16 and 45 days. The sixth batch was served as control.

The individuals were sacrificed by cervical dislocation and liver and muscle tissues were quickly isolated and placed in chilled box immediately till for biochemical assays. Glycogen content of selected tissues was estimated by the method of carrol et al (1950). The activity levels of succinic dehydrogenase (SDH), lactate dehydrogenase (LDH) were estimated. Protein content was determined by using Folin phenol reagent (Lowry et al 1951).
RESULTS AND DISCUSSION

The sublethal levels of heavy metals PbCl$_2$ and HgCl$_2$ on glycogen contents in liver and muscle tissues of *catla catla* undergoes stress and decreased compared to that of the fish under control. The maximum decrease (52.51%) was observed on day 45. In liver tissue under mercuric chloride intoxication (Table-1). A steady decrease in the tissue glycogen content clearly indicates that effect of heavy metals are persistant under prolonged exposure periods. The change suggesting that glycogen utilization by anaerobic glycolysis perhaps to meet the energy warranted by intoxicated environment. In the present study it is evident that the heavy metals are hepatotoxins from the maximum drop in liver tissue activity. Sastry and Shukla (1990) reported that the muscle glycogen content was decreased due to PbCl$_2$ toxicity. In murrel fish Similar findings were reported by Kaviraj and Das (1994) in fish and other aquatic organisms and Tilak et al (2009) in fresh water edible fish.

Table 1: Differences in glycogen content in muscle and liver of *catla catla* exposed to 1/10$^{th}$ sublethal concentration of heavy metals. Values are expressed in mg/g weight of tissue.

<table>
<thead>
<tr>
<th>HEAVY METAL</th>
<th>TISSUE</th>
<th>EXPERIMENTAL DAYS (PERIOD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
<td>3</td>
</tr>
<tr>
<td>PbCl$_2$</td>
<td>Liver</td>
<td>9.63±0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-11.838)</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>10.54±0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-9.602)</td>
</tr>
<tr>
<td>HgCl$_2$</td>
<td>Liver</td>
<td>9.75±0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-14.68)</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>10.98±1.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-8.296)</td>
</tr>
</tbody>
</table>

It is observed that there is a decrease in the activity levels of glycogen, SDH, and MDH shown a steady decrease (Table -2). LDH activity has showed gradual increase (Table-3) up to 45 days exposure over the control. In living organisms energy is produced by the synthesis of ATP from ADP which results in the oxidation of certain prone compounds such as Succinate, Lactate, malate etc (Mayes 1977).
Table 2: Differences in succinate dehydrogenase levels (SDH) in Liver and muscle tissues of *catla catla* exposed to 1/10th sublethal concentrations of Heavy metals.

<table>
<thead>
<tr>
<th>HEAVY METAL</th>
<th>TISSUE</th>
<th>EXPERIMENTAL DAYS (PERIOD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
<td>3</td>
</tr>
<tr>
<td>PbCl₂</td>
<td>Liver</td>
<td>0.675±0.038</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>0.498±0.029</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>Liver</td>
<td>0.869±0.049</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>0.326±0.039</td>
</tr>
</tbody>
</table>

It shown that the variations in biochemical parameters are helpful for monitoring the pathological status of fish induced by toxic stress. Transaminase activity levels are being elevated in serum during pathological conditions. The variations of Transaminases are tissue and species specific. Hence they can be used as indicators of toxic pollution. In the present study both AIAT & AAT activities are increased and maximum percentage elevations are recorded on day 45 and the highest elevation was observed in the liver tissue (94.6% and 86.4%) under stress.

Table 3: Differences in Malate dehydrogenase levels (MDH) in Liver and muscle tissues of *catla catla* exposed to to 1/10th sublethal concentrations of Heavy metals.

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Tissue</th>
<th>Control</th>
<th>3</th>
<th>8</th>
<th>16</th>
<th>30</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>0.64±0.004</td>
<td>0.058±0.004</td>
<td>0.051±0.012</td>
<td>0.048±0.0012</td>
<td>0.043±0.020</td>
<td>0.039±0.05</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>0.226±0.018</td>
<td>0.214±0.014</td>
<td>0.202±0.006</td>
<td>0.186±0.034</td>
<td>0.164±0.012</td>
<td>0.145±0.037</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>Liver</td>
<td>0.258±0.019</td>
<td>0.231±0.015</td>
<td>0.219±0.012</td>
<td>0.185±0.019</td>
<td>0.149±0.026</td>
<td>0.126±0.039</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>0.231±0.006</td>
<td>0.216±0.008</td>
<td>0.207±0.013</td>
<td>0.183±0.022</td>
<td>0.162±0.024</td>
<td>0.133±0.029</td>
</tr>
</tbody>
</table>

The total protein content in liver and muscle tissue of *catla catla* exposed to two heavy metals PbCl₂ and HgCl₂ for a period of 45 days. A significant decrease in protein content was observed in all tissues of heavy metals intoxicated fish. The variation in distribution of protein suggests difference in metabolic calibre of various tissues. The similar decreasing trend in total proteins suggests difference in metabolic tissues. The similar decreasing trend in total proteins was also reported in the liver and tissue of fish under sublethal and lethal concentrations of fenevalerate by Anitha susan etal 1999. A significance decrease was
reported in all tissues of Ctenopharyngodon under fenvalerate intoxication (Tilak & yacobu 2002). A gradual and significant decrease in protein was reported at channapuncatatus. When treated with technical grade alachlor and 50% e-c1asso(Tilak etal 2009).

SUMMARY AND CONCLUSION
The present work indicates that the two heavy metals caused considerable and significant changes in the intermediate metabolism of catla catla and maximum changes are recorded in the liver tissues under intoxication over 45 days period. These variations have assumed that the fish yields high energy demand to overcome the stress indicated by the existence of hypoproteinamea in all tissues of fish. However, no mortality was recorded over 45 day intoxicated period from the point of fish production it is recognised that the protein in the tissues is utilized as energy source resulting in decrease of productivity in culture ponds.

It shows that the variations in biochemical parameters are helpful for monitoring the pathological status of fish induced by toxic stress.

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