Research Article
EFFECT OF LEAD (II) NITRATE ON BIO ENZYMEOLOGICAL STUDIES IN LIVER TISSUE OF FISH *Catla catla* (Hamilton)

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Abstract
Heavy metals are regarded as hazardous to aquatic ecosystems because of their environmental persistence and their tendency for bioaccumulation. Enzymes are biochemical macromolecules that control metabolic processes of organism, thus a slight variation in enzyme activities would affect the organism. In the present study, the effect of lead (II) nitrate (6 ppm for 7 days) intoxicated liver tissue of fish *Catla catla*. At median lethal concentration of lead (II) nitrate (6 ppm for 7 days) treated, the whole liver tissue showed on decreased level of phosphatase activity (ACP and ALP) and simultaneously increased level of transaminase activity (GOT and GPT). The results strongly suggested that, the chosen concentration of lead (II) nitrate might be affecting the fish metabolism.

Key words: Lead (II) nitrate, Liver, *Catla catla*, Phosphatase and Transaminase

1. Introduction

Nowadays, nature faces a serious problem of environmental pollution (Paramanandham *et al.*, 2011). Among various kinds of pollution, non-degradable heavy metals are regarded as hazardous to aquatic ecosystems because of their environmental persistence and their tendency for bioaccumulation. As the heavy metals are immutable, their biomagnifications has been reported in aquatic ecosystems. It may affect aquatic organisms if the organisms are sub-lethally exposed to them for the extensive time. Among the heavy metals, lead is known to alter the hematologic system of hosts by inhibiting the activities of several enzymes involved in heme biosynthesis. Enzymes are biochemical macromolecules that control metabolic processes of organism, thus a slight variation in enzyme activities would affect the organism.

The measurement of phosphatase activity is also useful as an indicator of liver function (Padmakumaran Nair *et al.*, 1998). Aminotransferases (GOT and GPT) are reliable marker enzymes of liver and they are the first enzymes to be used in diagnostic enzymology when liver damage has occurred (Kuchel and Ralston, 1988). These enzymes are involved in a variety of metabolic activities such as permeability, growth and cell differentiation, protein synthesis, absorption and transport of nutrients, gonadal maturation, and steroidogenesis. The liver disorder is a serious health problem (Kavitha and Jagadeesan, 2004). Thus, by estimating the enzyme activities in an organism, we can easily identify disturbances in its

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metabolism. The *Catla catla* fish was chosen for the present study for the following reasons; it is sensitive to toxicants. They are abundant and availability is high throughout year. They have economic and ecological importance. They can be reared easily in laboratory. In the present paper deals the disturbance of metabolism in *Catla catla* by exposing them to chosen concentration of lead nitrate.

2. Materials and Methods

*Catla catla*, the fish selected for the present study was collected from a fish farm near Sethiyathope, Cuddalore District, Tamil Nadu, India. Healthy fishes of comparable body weight (8 ± 1.04 g) and length (8 ± 1.55 cm) were selected for the study. The fishes were treated with 0.05% KMnO$_4$ solution for 2 min to clear any external infection. They were then transferred to 100 l capacity glass tanks filled with dechlorinated water, one week prior to the initiation of the experiment for acclimatization to laboratory conditions. A minimum of ten fishes were introduced in each tank. The tanks were provided with continuous aeration and were maintained under normal day-night light duration. Feeding was carried out with oilcake during acclimatization and stopped 24 hrs prior to experimentation. The water was exchanged after every 24 hrs. Every effort was made to provide healthy conditions for fish and no mortality occurred during this period. *Catla catla* fingerlings were divided in 2 groups, group I reared in metal free water and maintained as a control and group II was exposed to median lethal concentration of lead (II) nitrate (6 ppm) for 7 days. After 7 days, fish liver was collected for the bio enzymological studies in all two groups.

2.1. Estimation of acid phosphatase

The activity of acid phosphatase was assayed with the method of Tennis Wood *et al.* (1976). The liver tissue was homogenized in glass homogeniser, using 10 ml of distilled water and centrifuged at 3000 rpm for 10 minutes. 0.5 ml of supernatant was taken in a clean test tube and 0.5 ml of the substrate solution (p-nitrophenyl phosphate) and 0.5 ml of glycine buffer were added. The test tube with above solution was kept in water bath maintained at 37°C for 30 minutes. After completion of 30 minutes, the reaction was arrested in the extracts by adding 3.8 ml of 0.1N sodium hydroxide. The color formed at the end was read at 415 nm in UV-visible spectrophotometer (Spectronic-20 Bausch and Lamb). Values were expressed in μ moles of phenol liberated/min/100 mg protein.

2.2. Estimation of alkaline phosphatase

The activity of alkaline phosphatase was assayed with the method of Tennis Wood *et al.* (1976). The liver tissue was homogenized in glass homogeniser, using 10 ml of distilled water and centrifuged at 3000 rpm for 10 minutes. 0.5 ml of supernatant was taken in a clean test tube and 0.5 ml of the substrate solution (p-nitrophenyl phosphate) and 0.5 ml of glycine buffer were added. The test tube with above solution was kept in a water bath maintained at 37°C for 30 minutes. After completion of 30 minutes the reaction was arrested in the extract by adding 10 ml of 0.2 N sodium hydroxide. The color formed at the end was read at 415 nm in UV spectrophotometer (Spectronic-20, Bausch and Lamb). Values were expressed in μ moles of phenol liberated/min/100 mg protein.

2.3. Estimation of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT)

The activities of GOT and GPT were determined using the method of King (1965). The liver tissue was homogenized with 5 ml of phosphate buffer and centrifuged at 3000 rpm for 10 minutes. One ml of each substrate [for GOT activities - 1.33 g of L-aspartic acid and 15 mg of α-keto glutaric acid were dissolved in 20.5 ml of buffer and 1N sodium hydroxide to adjust the pH to 7.5 and made upto 50 ml with the phosphate buffer. GPT activities - 1.78 g of DL-alanine and 30 mg of α-keto glutaric acid were dissolved in 20 ml of buffer. The pH was adjusted to 7.5 with 1N sodium hydroxide and made upto 100 ml with buffer and then few drops of chloroform were added] was taken into clean test tubes and it was incubated for 5 minutes at 37°C. Then 0.2 ml of tissue homogenate was added in the test tubes and incubated for 1 hour in the case of GOT and 30
minutes for GPT. The reaction was arrested by adding 1.0 ml of DNPH reagent and tubes were kept at room temperature for 20 minutes. Then, 10 ml of 0.4 N sodium hydroxide solution was added and the color developed was read at 520 nm against the reagent blank in the UV spectrophotometer (Spectronic-20, Bausch and Lamb). A set of pyruvic acid was also treated in a similar manner for the standard. The activities of GOT and GPT values were expressed as IU/L.

3. Results and Discussion

In the normal untreated control, the levels of ACP and ALP activities in the liver tissue showed 3.58 ± 0.13 and 31.88 ± 1.02 respectively. At median-lethal dose of lead (II) nitrate treatment, the levels of ACP and ALP activities in the liver tissue is significantly decreased up to 1.90 ± 0.01 and 21.38 ± 1.34 μ moles of phenol liberated/minute/100 mg protein (% COUTC: - 46.92; -32.93) (Table 1). Phosphatase activities also serve as a diagnostic tool to assess toxicity stress of chemicals in the living organisms. In the present study, loss of ACP and ALP activities in the liver tissue of lead intoxicated fish was a consequence of changes in the permeability of plasma membrane in addition to change in the balance between synthesis and degradation of enzyme protein (Jagadeesan and Kavitha, 2006). Same type of decreasing trend was observed by Paramanandham et al. (2011) and Sastry and Gupta (2005) intoxicated mercuric chloride in Labeo rohita and Channa punctatus respectively. George et al. (2011) reported that, the murrel Channa striatus kidney and brain showed increasing trend in ACP and decreasing trend in ALP. It may be the different concentration, period of exposure and the species specific.

In the normal untreated control, the levels of GOT and GPT activities in the liver tissue showed 11.33±0.83 and 6.69±0.93. At median-lethal dose of lead (II) nitrate treatment, the levels of GOT and GPT activities in the liver tissue significantly increased up to 14.56±1.99 and 7.16±0.84 IU/L (% COUTC: + 28.51; + 7.03) (Table 1).

Table 1: Changes (Mean ± SD) in the level of ACP, ALP, GOT and GPT activities in liver tissue of fish, Catla catla treated with 7 days of median lethal concentration of lead (II) nitrate

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>Lead (II) nitrate Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACP (μ moles of phenol liberated/min/100 mg of protein)</td>
<td>3.58±0.13*</td>
<td>1.90±0.01*</td>
</tr>
<tr>
<td>%COUTCa</td>
<td></td>
<td>-46.92</td>
</tr>
<tr>
<td>ALP (μ moles of phenol liberated/min/100 mg of protein)</td>
<td>31.88±1.02*</td>
<td>21.38±1.34*</td>
</tr>
<tr>
<td>%COUTCa</td>
<td></td>
<td>-32.93</td>
</tr>
<tr>
<td>GOT (IU/L)</td>
<td>11.33±0.83*</td>
<td>14.56±1.99*</td>
</tr>
<tr>
<td>%COUTCa</td>
<td></td>
<td>+28.51</td>
</tr>
<tr>
<td>GPT (IU/L)</td>
<td>6.69±0.93*</td>
<td>7.16±0.84*</td>
</tr>
<tr>
<td>%COUTCa</td>
<td></td>
<td>+7.03</td>
</tr>
</tbody>
</table>

Mean ± SD (Mean of six individual observations); *Significance of 5% level of ANOVA; % change over untreated control
Transaminases play an important role at the junction between the carbohydrate and protein metabolism by interconverting the strategic compounds viz., ketoglutarate, pyruvate and oxaloacetate on one hand and alanine, aspartate and glutamate on the other hand. A close relationship exists between the mitochondrial intensity and transaminases level (Baintenico, 1974) and any modification in the organization of mitochondria might alter the enzyme associated with it. The increase in GOT and GPT was due to the hepatocellular necrosis, which caused an increase in the permeability of cell membrane resulting in the release of transaminase activity (Vandenberghe, 1995). Several investigators reported lead or metal induced increase in the level of GOT and GPT in culture cells as well as in experimental animals (Paramanandham et al., 2011; Abedi et al., 2013; Rana et al., 1996; Khandelwel et al., 2002).

4. Conclusion

The fish are therefore, directly exposed to either treated or untreated lead which may be toxic to them. Hence, the environmental awareness becomes more necessary, since fish forms delicious component of human food and further it is poor man’s dish, it may be concluded that the lead from distillery factories presently evaluated cause lethal effects found in the surrounding area whether terrestrial or aquatic. So, proper treatment of effluents is necessary to prerequisite system or environment.

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