RESPONSES OF PLASMA TRANSAMINASE ACTIVITY IN CYPRINUS CARPIO VAR. COMMUNIS TO MERCURY TOXICITY

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ABSTRACT

The present study reports the behavioural and enzymological responses in a freshwater teleost fish, Cyprinus carpio var. communis, exposed to acute and sublethal toxicities of mercuric chloride. During acute treatment, significant behavioural changes like erratic swimming, excess mucus secretion, increased opercular movements, etc. were noticed. During acute and sublethal treatments, both aspartate amino transferase and alanine amino transferase activity increased throughout the study period. Comparing the treatments, the changes in enzyme activities were found high in acute treatment and all the values were significant at 5% level. The above findings can be used as non-specific biomarkers of environmental pollutants.

Keywords: Mercuric chloride, AST, ALT, Cyprinus carpio

INTRODUCTION

Industrial effluents contaminating aquatic bodies contain a number of toxic chemicals, which in turn, exercise their effects on fish and pose threat to aquatic life. Sankar Narayan and Madhyastha (1985) reported that heavy metals are continually released into the aquatic environment from natural processes such as volcanic activity, weathering of rocks and industrial processes. The three heavy metals, which are toxic to most organisms at the least concentrations and probably never beneficial to living organisms are cadmium, mercury and lead. According to Dimou et al. (1989), Hg is a ubiquitous, highly toxic heavy metal, which is bioconcentrated through food chain. In India alone, about 180 t of Hg are introduced into the environment every year, which ultimately reach the aquatic systems. Hg accumulates in various organs of mammalian system and adversely affects their functions. In fishes, Hg is accumulated in the form of methyl mercury (WHO, 1986).

It is established that any kind of stress, not resulting in gross changes and mortality produces certain changes in the fish blood characteristics (Christensen et al., 1977). Methods of clinical diagnosis have been introduced in fish biology to assess the effects of pollutants (Lockhart and Metner, 1984). Mayer et al. (1992) reported that serum enzyme activities have been used extensively to provide simple accurate measures of organ dysfunction in mammals and have received greater attention from aquatic toxicologists.
Aspartate amino tranferase (AST) and alanine amino transferase (ALT) not only function as link enzymes between the protein and carbohydrate metabolism, but also serve as indicators of altered physiological or stress condition. AST and ALT enzyme activities have been used to demonstrate tissue damage in fish (Kristoffersson et al., 1974; Asztalos and Nemcsok, 1985). Hence, in the present study, the degree of enzyme responses (AST and ALT) of an economically important fish, Cyprinus carpio, to Hg toxicity was investigated.

MATERIAL AND METHODS

Specimens of C. carpio var. communis, a freshwater teleost fish, were collected from Tamil Nadu Fisheries Development Corporation Limited, Aliyar Fish Farm, Aliyar (India), and acclimatized to laboratory conditions for 20 days. During this period, the fish were fed ad libitum with rice bran and groundnut oil cake in the ratio of 3:1. Water was changed daily and aerated in order to reduce any accumulation of excretory products and to ensure sufficient oxygen supply. The tap water was analysed for physico-chemical features following APHA (1981) and the values were: pH 7.4±0.2; temperature 27±1.0°C; dissolved oxygen 6.4±0.02 mg/l; salinity 0.5±0.02%o and total hardness 6.0±1.0 mg/l.

Healthy fish with an average weight of 5 g and length of 8-9 cm were selected for the experimental study. The LC50 value of HgCl2 for 24 hours is 0.34 ppm (Finney, 1978). One-tenth of the acute value (0.034 ppm) was taken for sublethal studies according to Sprague (1971). Fish were exposed to acute concentration for 24 hours and sublethal concentration for 25 days. During sublethal exposure, fish were fed ad libitum and the toxicant was renewed daily. The physico-chemical parameters of the water were monitored regularly to maintain the same level. A common control was maintained. Fish from acute and from sublethal concentrations were removed at intervals of 24 hours and 5 days respectively with respective controls. Blood was drawn from the heart by puncturing it using an ice-cold microsyringe. The sample was centrifuged at 9000 rev/min for 5 minutes leaving a clear yellow fluid called plasma, which was used for the estimation of AST and ALT activities. AST (AST, EC 2.6.1.1) and ALT (ALT, EC 2.6.1.2) activities were estimated by 2, 4 DNPH method (Reitman and Frankel, 1957). The significance between the sample mean of control and experimental fish was tested using Students' 't' test.

RESULT

Behavioural changes constitute yet another index to measure toxicity. In the present study, during acute treatment of mercuric chloride, the fish exhibited the following behavioural changes: fast jerky movements, turning upside down, restlessness, erratic movement, mucus secretion from the body and increased opercular movements. However, fishes from the control did not show such behaviour changes.

Tables 1 and 2 show the changes in AST and ALT activity in the plasma of fish exposed to acute and sublethal
Table 1: Changes in aspartate aminotransferase activity and alanine aminotransferase activity in the plasma of *Cyprinus carpio* var. *communis* exposed to acute concentration (0.34 ppm) of HgCl₂

<table>
<thead>
<tr>
<th>Exposure period</th>
<th>Aspartate aminotransferase activity (U/ml)</th>
<th>Alanine aminotransferase activity (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
</tr>
<tr>
<td></td>
<td>66.067 ± 0.1566</td>
<td>226.186 ± 0.1146*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+242.35)</td>
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<tr>
<td>Acute treatment 24 h (0.34 ppm)</td>
<td></td>
<td>35.7040 ± 0.1295</td>
</tr>
<tr>
<td></td>
<td>66.067 ± 0.1566</td>
<td>126.492 ± 0.1194*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+254.00)</td>
</tr>
</tbody>
</table>

Table 2: Changes in aspartate aminotransferase activity and alanine aminotransferase activity in the plasma of *Cyprinus carpio* var. *communis* exposed to sublethal concentration (0.034 ppm) HgCl₂

<table>
<thead>
<tr>
<th>Exposure period (d)</th>
<th>Aspartate aminotransferase activity (U/ml)</th>
<th>Alanine aminotransferase activity (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
</tr>
<tr>
<td></td>
<td>61.074 ± 0.4608</td>
<td>76.586 ± 0.2729*</td>
</tr>
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<td></td>
<td></td>
<td>(+25.39)</td>
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<tr>
<td>5</td>
<td></td>
<td>54.170 ± 0.3095</td>
</tr>
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<td></td>
<td>42.215 ± 0.2439</td>
<td>56.477 ± 0.1453*</td>
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<td></td>
<td></td>
<td>(+33.78)</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>45.215 ± 0.2439</td>
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<tr>
<td></td>
<td>63.083 ± 0.0691</td>
<td>97.686 ± 0.1851*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(54.85)</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>57.477 ± 0.1452</td>
</tr>
<tr>
<td></td>
<td>84.473 ± 0.2857</td>
<td>142.398 ± 0.2793*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(54.85)</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>72.343 ± 0.2856</td>
</tr>
<tr>
<td></td>
<td>62.443 ± 0.3819</td>
<td>151.362 ± 0.1085*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+142.40)</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>90.574 ± 0.1958</td>
</tr>
</tbody>
</table>

Values are mean ± SE of five individual observations. Values in parentheses are percent changes over control.
Degree of freedom at 8t 0.05 = 2.306. *Values are significant at 5% level.
concentrations of HgCl₂. During the above treatment period, the enzyme activity increased both in acute and sublethal toxicities. In acute toxicity, maximum per cent increases of 242.35 of AST and 254.00 of ALT were noticed. During sublethal treatment also the AST and ALT activities increased and were directly proportional to the exposure period showing minimum per cent increases of 25.39 and 10.00 at the end of the fifth day and maximum per cent increases of 142.40 and 81.72 at the end of the 25th day, respectively. All the values were significant at 5% level.

DISCUSSION

Quantification of enzyme activity in plants and animals can serve as a valuable biomarker of pollutant exposure and effect. Toxicants can inhibit enzymes at very specific sites (e.g., esteratic site of acetylcholinesterase) or effects can be evoked by less specific interactions with various moieties like sulfhydryl groups (Mayer et al., 1992). Serum transaminases, specifically aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT), have been widely utilized in mammalian toxicology as a biomarker of specific organ dysfunction as a biomarker of specific organ dysfunction (Wroblewski and LaDue, 1956). ASAT is a non-specific cytosolic and mitochondrial enzyme found in a variety of tissues including liver, skeletal muscle, cardiac muscle and kidney (Verma et al., 1981). ALAT is also a cytosolic enzyme, but is more tissue-specific and is normally associated with liver. Recently, there has been increasing interest in using changes in enzyme activity in aquatic animals as an index reflecting metal toxicity. Both the alanine and aspartate transaminases are important in the diagnosis of liver damage caused due to the exposure to industrial chemicals.

The toxicity data in the present study show that HgCl₂ is toxic to C. carpio var. communis. Heavy metals are highly toxic, water soluble, non-degradable, vigorous oxidizing agents and are strongly bonded to many chemicals, especially polypeptides and proteins, and also to other materials which possess electron-rich functional groups such as sulfhydryl, amino and imidazole (Albert, 1965). Metals may bind to macromolecules inside the cells potentially with enzymes or change the concentration of cofactors or reactants by altering membrane permeability and indirectly affecting enzyme activity (Tucker and Matte, 1980).

Metals have been found to affect the activities of transaminases in fish. Fish exposed to acutely toxic concentrations of Cd, Hg, or Cu had increased transaminase activities (McKim et al., 1970). Alternatively, chronic exposure to Cu has been reported to decrease serum ASAT activity while chronic cadmium exposure does not affect activities of this transaminase in serum (McKim et al., 1970).

The degree of increase in the activity of cellular enzymes in serum depends primarily on the magnitude and severity of cell damage (Kristoffersson et al., 1974; Aszatalos and Nemcsok, 1985; Radhakrishnaiah, 1988). Tissue necrosis is one of the most used signs of damaged organisms (Chenery et al., 1981; Nemcsok, 1993). In the event of tissue necrosis, transaminases pass into the circulation.
from the damaged organ and their increased activities in the blood indicate the degree of tissue damage (Kristoffersson et al., 1974).

The enzymes AST and ALT are generally associated with cellular metabolic activity (Abston and Yarbrough, 1976). During cellular damage or lysis, transaminases are released. Elevated transaminases could be taken as a measure of compensatory mechanism as a consequence to impaired carbohydrate metabolism (Reddy and Venugopal, 1991). Radhakrishnaiah (1988) observed an increased accumulation of Cu in liver and gill of *Labeo rohita* and suggested that elevation or inhibition of enzyme activity may be due to the accumulation of heavy metals in these tissues leading to damage.

Kiran et al. (1990) reported that the increase in the levels of serum enzymes can be attributed to the efflux of enzymes from damaged liver and other organs into circulation. Hepatocellular disorder causes plasma AST and ALT levels to go up (Rao et al., 1992) and Moorthy et al. (1984) reported that the increase of AST activity could be a result of general tissue damage particularly liver, muscle and heart. Elevated AST and ALT levels were presumably due to damage of liver, but other organs may also have been damaged (kidney or/and gill). Reichenback-Klinke (1972), and Schreck and Lorz (1978) observed epithelial necrosis of the gills and glomerular atrophy of kidney when coho salmon, *Oncorhynchus kisutch*, was exposed to Cu.

According to Mayer et al. (1992), basically, there are four different processes that may suggest the responses of enzyme to specific or non-specific chemicals stress; they are (1) direct enzyme inhibition, (2) enzyme induction by specific classes of chemicals, (3) elevation of serum enzymes, viz., tissue damage and (4) alterations in enzyme activity as a result of changes in metabolic pathways or fluxes. The authors further added that enzyme activity is generally regulated such that specific substances or entire pathways may be homeostatically adjusted to compensate for endogenous or exogenous changes.

In the present study, the significant increase in AST and ALT levels during acute and sublethal studies may be due to accumulation of Hg in tissues leading to tissue (liver) damage resulting in release of these enzymes into blood or impaired carbohydrate metabolism. In the present study, long-term exposure enhanced the activities of enzymes suggesting that with increase in exposure period, the organism tries to mitigate the toxicant induced stress by increased rate of metabolism.

REFERENCES


