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Effect of Arsenic and Chromium on the Serum Amino-Transferases Activity in Indian Major Carp, *Labeo rohita*

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Abstract: Arsenic and hexavalent chromium toxicity results from their ability to interact with sulfahydryl groups of proteins and enzymes, and to substitute phosphorus in a variety of biochemical reactions. Alanine aminotransferase (ALT; E.C: 2.6.1.2) and Aspartate amino transferase (AST; EC 2.6.1.1) play a crucial role in transamination reactions and can be used as potential biomarkers to indicate hepatotoxicity and cellular damage. While histopathological studies in liver tissue require more time and expertise, simple and reliable biochemical analysis of ALT and AST can be used for a rapid assessment of tissue and cellular damage within 96 h. The main objective of this study was to determine the acute effects of arsenic and hexavalent chromium on the activity of ALT and AST in the Indian major carp, *Labeo rohita* for 24 h and 96 h. Significant increase in the activity of ALT ($P < 0.01$) from controls in arsenic exposed fish indicates serious hepatic damage and distress condition to the fish. However, no such significant changes were observed in chromium-exposed fish suggesting that arsenic is more toxic to the fish. These findings indicate that ALT and AST are candidate biomarkers for arsenic-induced hepatotoxicity in *Labeo rohita*.

Keywords: Arsenic, chromium, acute toxicity, *Labeo rohita*, serum aminotransferase, biomarkers

Introduction

Arsenic exerts its toxic effects through an impairment of cellular respiration by inhibition of various mitochondrial enzymes, and the uncoupling of oxidative phosphorylation. Arsenic toxicity results from its ability to interact with sulfahydryl groups of proteins and enzymes, and to substitute phosphorus in a variety of biochemical reactions [1]. Chromium continues to be in widespread use in industry, paints, metal plating as corrosion inhibitor and its particulates enter the aquatic medium through effluents discharged from tanneries, textiles, and electroplating, mining, dyeing, and printing industries, photographic and pharmaceutical industries. In the environment, chromium exists primarily in the trivalent and hexavalent forms but the hexavalent form predominate the trivalent form in natural waters. Cr (VI) compounds have been found to be mutagenic and carcinogenic in a variety of test systems [2]. A large number of industrial estates have been established in the pre-catchments of many aquatic bodies in Hyderabad City, the area under study in this investigation, and the occurrence of arsenic and chromium in many compartments of the ecosystem is well documented. *Labeo rohita* is a widely consumed teleost fish that commonly inhabits native aquatic bodies. This fish can be exposed to arsenic and hexavalent chromium through a variety of anthropogenic activities, including, but not limited to, its release through industrial activities.

In toxicological studies of acute exposure, changes in concentrations and enzyme activities often directly reflect cell and organ damage in specific organs [3]. Arsenic and hexavalent chromium are known to affect almost every functional site of fish, often due to their bioaccumulation

and poor excretion. Alanine aminotransferase (ALT; E.C: 2.6.1.2) and aspartate amino transferases (AST; EC 2.6.1.1) participate in transamination reactions and found predominantly in liver, cardiac cells and striated muscle tissue. Recent studies have pointed out that arsenic toxicity is associated with the formation of reactive oxygen species, which may cause severe injury/damage to the nervous system [1]. Based on the above studies, we hypothesize that these metals could also accumulate in the liver to toxic levels and cause pathological alterations. Cellular damage releases the ALT and AST into the blood stream and the levels of these enzymes have the potential to indicate hepatotoxicity [4]. While histopathological studies in liver tissue require more time and expertise, simple and reliable biochemical analysis of ALT and AST can be used for a rapid assessment of tissue and cellular damage within 96 h. Serum ALT and AST are usually elevated in serious hepatic damage and the extent of organ damage is dependent on the type of toxicant, its mechanism of action and duration of exposure [5]. Little data are available on the impact of arsenic and hexavalent chromium on the serum ALT and AST in *Labeo rohita*, a key ecological component inhabiting native freshwater ecosystems in India. The objectives of the present study were to determine the acute toxicity of arsenic and hexavalent chromium and to measure the serum levels of the aminotransferase enzymes alanine aminotransferase and aspartate amino transferase in *Labeo rohita* in response to arsenic and chromium toxicity as potential biomarkers of hepatotoxicity.

Materials and Methods

Fish Collection and Maintenance

Juvenile specimens of *Labeo rohita* (5–6 cm long; weight: 2.5 ± 0.25 g) were used for the toxicity tests. These were collected from the Undasagar fish farm located near Bandalaguda, Hyderabad, India, transported from the farm in oxygenated polyethylene bags to the laboratory and immediately transferred into glass aquaria of 100 L capacity containing well-aerated unchlorinated ground water. The fish were allowed to acclimate for 15 days before the experiments. They were fed with rice bran ad libitum during the acclimation period. The fish were subsequently transferred into 50 L glass aquaria for easy handling during the experiments. Only fish which were healthy and showed active movements were used for the experiments. The physico-chemical characteristics of the water used for holding and experiments such as pH, temperature, DO, hardness, alkalinity, sulphates and chlorides are 7.0 ± 0.5, 26.0^{O}C – 27.0^{O}C, 6.0 –8.0 mg/L, 220 mg/L, 432 mg/L, 1.47 mg/L and 95.74 mg/L, respectively.

Determination of Median Lethal Concentration (96-h LC_{50})

The acute toxicity of arsenic and chromium to *L. rohita* was determined using a standard 24-h static renewal technique [6]. Desired concentrations of arsenic and chromium were prepared by adding aliquots of 1% AS_2O3 and $K_2Cr_2O_7$ stock solutions to known quantities of water. The initial experiments were conducted on a minimum of two random concentrations to determine the mortality of the fish within the range of 5–95%. The tests were repeated three times to check for the reproducibility of the results. Thirty fish were tested at each concentration in 50 L glass aquaria. The loading of fish into the test chambers was according to the recommendations given by US EPA [6]. No distinction was made between sexes since immature fish were used. At the end of each 24 h period, water containing the toxicant from each aquarium was siphoned into a plastic bucket numbered to match the aquarium. Fish were carefully transferred into these buckets by nylon net. Each aquarium was filled with fresh water so as to maintain the desired concentrations of arsenic trioxide and potassium dichromate, and fish were reintroduced into their respective aquaria. Tests were subsequently conducted using four concentrations of arsenic trioxide – i.e. 20.0 mg/L, 25.0 mg/L, 30.0 mg/L, 40.0 mg/L; six concentrations of potassium dichromate-i.e. 20.0 mg/L, 40.0 mg/L, 50.0 mg/L, 60.0 mg/L, 75.0 mg/L, 80.0 mg/L, respectively, which resulted in the mortality of the fish within the range of 5–95%. Thirty fish (three batches of ten each) were exposed to each concentration separately. Controls without toxicant were also run simultaneously. Behavioral manifestations and the condition of the fish were noted every 24 h up to 96 h. between the experiments, the aquaria were carefully washed to eliminate residual metal adsorption to the walls. The fish that failed to respond even to strong tactile stimuli were considered to be dead and removed immediately. The number of dead fish was recorded for each concentration of the toxicant, and the data were used to determine the median lethal concentration (LC_{50}) by means of probit analysis [7]. The corresponding results were generated with a computer program. The regression equations were calculated by the method of least squares, and 96 h LC_{50} values were derived from the equation presented in Table 1.

Table 1: Median Lethal Concentration and 95% fiducial limits of arsenic trioxide and potassium dichromate (96 h LC_{50})

S.No	*Toxicant*	*Regression Equation (Log +2)* $(Y = (\bar{y} - b\,\bar{x}) + b\,x)$	*96h LC_{50} (mg/L)*	*95% Fiducial Limits (mg/L)*
1.	As_2O_3	-20.12 +7.27x	28.30 ± 0.23	26.1- 30.40
	Arsenic as	-	21.10	19.6 - 22.6
2.	$K_2Cr_2O_7$	-5.9091+1.77x	61 ± 0.18	60.99 - 61.00
	Chromium as	-	21.56	21.56-21.56

Blood is drawn by cutting the tail aseptically and Sahli's pipette rinsed with EDTA was used to draw blood dripping out from the cut and then drained into a sterile eppendorff tube. The pooled up blood of three fishes was taken as a single sample for 96 h LC_{50} concentrations of arsenic and chromium for 24 h and 96 h. At least three replicates were taken in clean sterilized test tubes. The blood is apportioned and treated for enzymatic investigations. The blood in the eppendorff tube was maintained at room temperature for coagulation. After coagulation, the uncoagulated part of blood was sucked in to centrifuging tubes. This was centrifuged for 10 min at 3,000 rpm and the supernatant contains serum, which was used for enzyme assays. Serum ALT and AST activity was assayed following the modified International Federation for Clinical Chemistry (IFCC) method laid down in Monozyme enzyme kits (Hyderabad, India) using UV-VIS Spectrophotometer (Systronics). Unpaired Student's 't' test was applied to examine the significance of the difference between the control and experimental data.

Results and Discussion

The toxic effects of As^{+3} and Cr^{+6} on the survival and biochemical profiles i.e. serum ALT activity of *Labeo rohita* exposed to lethal concentrations were critically examined and the corresponding results are compared and discussed with those of other fish exposed to arsenic, chromium and other heavy metals. The 96 h LC_{50} value for As_2O3 was found to be 28.30 mg/L (As as 21.1mg/L) and the 95 % fiducial limits are 26.1 mg/L (As as 19.6 mg/L) to 30.4 mg/L (As as 22.6 mg/L) while that of chromium was found to be 61mg/L and the 95 % fiducial limits are 60.99 mg/L (Cr as 21.56 mg/L) to 61.0 mg/L (Cr as 21.56 mg/L). The mortality ranged from 10% to 93.3% and increased with a corresponding increase in the toxicant concentration and also duration of the exposure demonstrating both time and concentration dependent responses. Copious mucous secretion, loss of scales, grouping, erratic swimming, surfacing and darting movements and loss of equilibrium associated with convulsions were observed. These observations were more pronounced in higher concentrations of both the metals used as a function of time.

Serum ALT and AST levels

In the present investigation, serum ALT levels of *L. rohita* exposed to arsenic trioxide showed an activity of 641.18 IU/L at 24 h and 798.11 IU/L at 96 h exposure (Figure 1), which was significant when compared with the control that showed 487.25 U/L at 24 h and 399.7 IU/L at 96 h, respectively. Though there was an increase in serum levels of ALT in fishes exposed to chromium for both 24 h and 96 h, it was not significant (P>0.05) when compared with control fish (Figure 2). Similar trends were also observed in serum AST of fish exposed to arsenic that showed an activity of 1532. 77 IU/L at 24 h and 1622.39 IU/L at 96 h exposure from the control that showed 1085.72 U/L at 24 h and 861.58 IU/L at 96 h, respectively, while there is no significant increase of AST in fishes exposed to chromium (Figures 3 and 4).

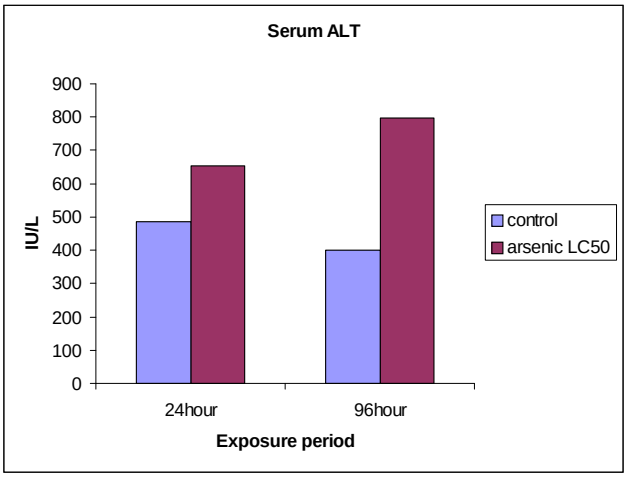

Figure 1: The toxic effect of arsenic trioxide (28.30 mg/L) on the ALT activity of *Labeo rohita* at the end of 24 and 96 h exposure periods (P<0.01)

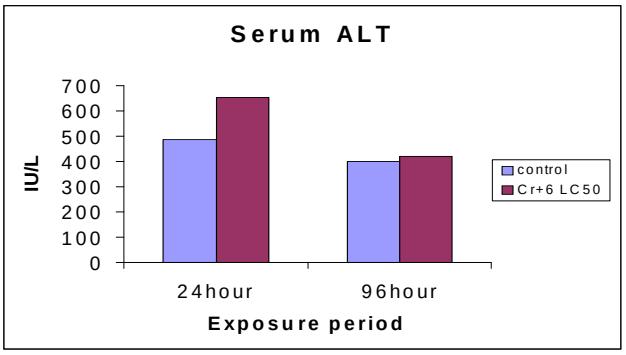

Figure 2: The toxic effect of potassium dichromate(61 mg /L) on the ALT activity of *Labeo rohita* at the end of 24 and 96 h exposure periods (P>0.05)

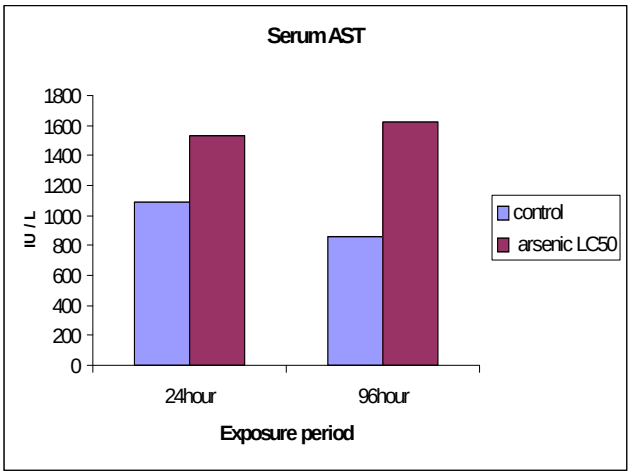

Figure 3: The toxic effect of arsenic trioxide (28.30 mg/L) on the AST activity of *Labeo rohita* at the end of 24 and 96 h exposure periods (P<0.01)

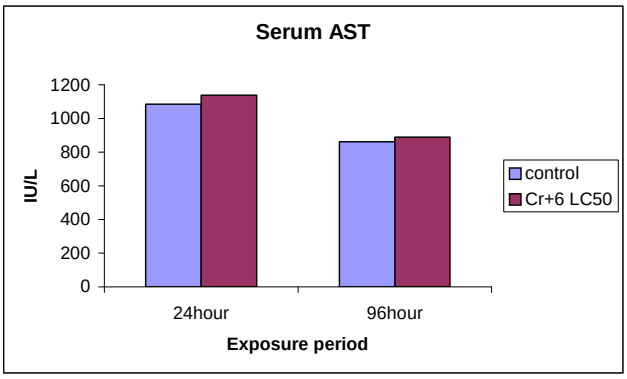

Figure 4: The toxic effect of potassium dichromate (61 mg /L) on the AST activity of *Labeo rohita* at the end of 24 and 96 h exposure periods (P>0.05)

Though the liver plays an important role in metabolic processes and detoxification of many xenobiotics, acute exposures to metals like arsenic and chromium may lead these metals to accumulate in the liver and cause pathological alterations [8]. Moreover, cell injury of certain organs like liver leads to the release of tissue-specific enzymes into the bloodstream [9]. Significant increase in transaminases (AST and ALT) activity in fish exposed to arsenic could be due to possible leakage of enzymes across damaged plasma membranes and/or the increased synthesis of enzymes by the liver. Although it's precise biochemical functions in the *Labeo rohita* are not fully understood, arsenic administration increased serum AST and ALT activities of fishes reflecting a situation of tissue damage. Though not directly related to arsenic toxicity, earlier studies also demonstrated an increased activity of ALT and AST and hepatocyte ultra structure of common carp, *Cyprinus carpio* after gallium exposure [10].

Research indicates that ALT and AST can be used as biomarkers of cellular damage in blood plasma, protein degradation and liver damage [11]. The major findings of this study are that arsenic is relatively more toxic substance than chromium in *Labeo rohita*, exposed to median lethal concentrations. The fish showed significant increase in serum ALT and AST levels suggesting serious hepatic damage. Arsenic and hexavalent chromium are extensively used in many industrial processes, and it is essential to be aware of their toxicity in aquatic environments. Despite its deleterious hepatotoxicity effects, very few studies were carried out on the effects of arsenic on the liver [12]. The present study provided new insights on the hepatotoxicity of arsenic, and indicated that ALT and AST activity can be used as a biomarker of hepatotoxicity. In contrast, though an increase in the ALT activity was observed in the fish exposed to chromium at the end of 24 h, the difference is not significant (P>0.05) and it is similar to that of the control fish at the end of 96 h. The maximum duration required for observing arsenic and chromium alterations in serum is found to be 96 h. Significant increase (P<0.01) in the activity of serum AST and ALT in fish exposed to arsenic indicates putative hepatic damage and distress to the fish. Chromium also induced the serum AST activity but this induction is not statistically significant (P>0.05) even at the end of 96 h. Further studies on the liver ultra structure could possibly explain the pathological alterations in liver under sub-lethal and chronic exposures.

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