

EFFECT OF ARSENIC ON THE ENZYMES OF THE ROHU CARP, *LABEO ROHITA* (HAMILTON, 1822)

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ABSTRACT. – Juvenile Rohu carp, *Labeo rohita*, with an average weight of 3.70 ± 0.03 grams were exposed to 96 and 144 $\mu\text{g/L}$ of arsenic concentrations for 30 days under laboratory conditions. The effect of arsenic on the activity levels of acid phosphatase, alkaline phosphatase, glutamate-pyruvate transaminase and glutamate-oxaloacetate transaminase in muscle and liver tissues of the fish were assessed. Enzyme activities were reduced and a significant variation in the activities of these enzymes was noted after exposure to arsenic.

KEY WORDS. – Arsenic, freshwater fish, *Labeo rohita*, enzymes.

INTRODUCTION

Various organic and inorganic wastes in industrial and domestic effluents are responsible for pollution. Non-degradable heavy metals are regarded as hazardous to aquatic ecosystems because of their environmental persistence and their tendency for bioaccumulation (Das et al., 2001). As heavy metals are immutable, their biomagnification has been reported in aquatic ecosystems. Heavy metals may affect aquatic organisms if the organisms are sub-lethally exposed to them for a long time. It has been reported that heavy metals affect various biochemical parameters of the fish liver (Jana & Bandyopadhyaya, 1987; Lomte & Sontakke, 1992). Arsenic contamination is mainly caused by the use of the arsenic pesticides, industrial activities and mining operations (Chakraborty et al., 1998). Due to increasing levels of arsenic in the ground water in Bangladesh and West Bengal, considerable attention has been given to the study of the effect of arsenic on human beings and selected mammals (Biswas et al., 1998). However, there are only a few reports on the physiological and biochemical responses of fish to arsenic (Leah et al., 1992). Enzymes are biochemical macromolecules that control metabolic processes of organisms, thus a slight variation in enzyme activities would affect the organism (Roy, 2002). Thus, by estimating the enzyme activities in an organism, we can easily identify disturbances in its metabolism. In this study, we monitored the disturbance of metabolism in *Labeo rohita* by exposing them to two concentrations of arsenic.

MATERIALS AND METHODS

Thirty juveniles of the freshwater fish, *Labeo rohita*, were collected from the nursery pond of the Aarey colony, Mumbai. They were approximately 15 - 20 days old and weighed 3.70 ± 0.03 grams. These fishes were brought to the laboratory and acclimatized for 15 days in glass aquaria ($36 \times 24 \times 24$ cm) containing aged tap water. Aged tap water (water stored for 24 hours) was used throughout the study to minimize mortality of the fishes. During acclimatization, the aquarium water was maintained under the following conditions: an oxygen level of 6.00 - 6.50 mg/L, pH 7.2 - 7.5 and temperature 27 - 29°C. These conditions were sustained during the entire length of the experiments and the fishes were fed with commercial fish pellets. After the acclimatization period, the juvenile *L. rohita* were divided into 3 groups of 10 and each group was transferred to an aquarium measuring $12 \times 8 \times 8$ cm. The first two groups were exposed to sub-lethal arsenic concentrations of 96 and 144 $\mu\text{g/L}$, respectively. The third group functioned as a control and was not exposed to any chemicals.

Sodium arsenicicum ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) was used for preparation of the sub-lethal dose of arsenic. One gram of sodium arsenicicum was dissolved in 100 ml distilled water to get an aliquot. Dilutions were then made from this aliquot to get the required concentration of arsenic. The test media was changed daily with fresh addition of the toxicant. After one month, all the fish were sacrificed and liver and muscle tissues were removed. The tissues were then blotted and weighed before homogenization. The tissues were homogenized using a glass homogenizer with chilled distilled water and were centrifuged at 10,000 rpm for 15 minutes.

Table 1. Enzyme activities in the liver of *Labeo rohita* after exposure to arsenic. Activity is expressed as μM pyruvate formed per mg protein (mg^{-1} protein) in 30 minutes.

<i>Labeo rohita</i> Groups	Arsenic Concentration ($\mu\text{g/L}$)	GOT μM pyruvate formed in 60 minutes (mg^{-1} protein)	GPT μM pyruvate formed in 30 minutes (mg^{-1} protein)	ACP μM pyruvate formed in 30 minutes (mg^{-1} protein)	ALP μM pyruvate formed in 30 minutes (mg^{-1} protein)
(Values are mean of five determinations \pm standard deviation)					
Control	0	19.44 \pm 3.34	9.50 \pm 3.29	0.27 \pm 0.14	0.76 \pm 0.31
First group	96	18.46 \pm 4.15	6.91 \pm 1.10	0.07 \pm 0.01	0.74 \pm 0.07
Second group	144	13.09 \pm 6.46	1.88 \pm 1.29	0.04 \pm 0.06	0.64 \pm 0.05

GOT = glutamate-oxaloacetate transaminase; GPT = glutamate-pyruvate transaminase; ACP = acid phosphatase; ALP = alkaline phosphatase.

Table 2. Enzyme activities in the muscles of *Labeo rohita* after exposure to arsenic. Activity is expressed as μM pyruvate formed per mg protein (mg^{-1} protein) in 30 minutes.

<i>Labeo rohita</i> Groups	Arsenic Concentration ($\mu\text{g/L}$)	GOT μM pyruvate formed in 60 minutes (mg^{-1} protein)	GPT μM pyruvate formed in 30 minutes (mg^{-1} protein)	ACP μM pyruvate formed in 30 minutes (mg^{-1} protein)	ALP μM pyruvate formed in 30 minutes (mg^{-1} protein)
(Values are mean of five determinations \pm standard deviation)					
Control	0	15.23 \pm 3.37	1.95 \pm 0.70	0.20 \pm 0.01	0.43 \pm 0.02
First group	96	11.19 \pm 4.69	1.81 \pm 1.27	0.05 \pm 0.05	0.40 \pm 0.30
Second group	144	8.16 \pm 4.39	1.12 \pm 8.47	0.02 \pm 0.01	0.36 \pm 0.02

GOT = glutamate-oxaloacetate transaminase; GPT = glutamate-pyruvate transaminase; ACP = acid phosphatase; ALP = alkaline phosphatase.

The supernatant was then used for quantifying the enzymes. Glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) from the supernatant was measured using calorimetric determination with 2,4-dinitrophenylhydrazine (Bergmeyer, 1956). The acid and alkaline phosphatases (ACP, ALP, respectively) were assessed using *p*-nitrophenylphosphate (Bergmeyer, 1956). Protein content of the tissues was estimated by the Lowry method (Lowry et al., 1951).

RESULTS AND DISCUSSION

No mortality was observed during the 30 day exposure period in the fish exposed to both concentrations of arsenic. In both the liver and muscle tissues, a pronounced effect of arsenic was noted on acid and alkaline phosphatase (ACP and ALP, respectively). Activities of glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) also decreased in the liver and muscles of the treated fishes. The mean and standard errors of arsenic concentrations in the tissues for each exposure concentration are given in Tables 1 and 2.

A significant decrease in all enzymatic activity was noted in liver of the juvenile *Labeo rohita* exposed to the higher concentration of arsenic (144 $\mu\text{g/L}$), whereas the decrease was not significant at the lower concentration (96 $\mu\text{g/L}$), except in case of ACP. The ACP activity showed a significant decrease in both experimental groups compared to the control group. However, the decrease was not distinguishable between the two experimental groups (Tables 1 & 2).

It has been shown that the liver is the prime location for removing xenobiotics and biocides in fishes (Roy, 2002). The metabolic pathways in liver of fish are affected by various pollutants, including organic and inorganic chemicals due to the alteration of cellular enzymatic activities. The decreased activities of GOT, GPT, ACP and ALP, indicate disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane transport system. Such damage to cell organelles has been reported in various studies (e.g., Karatas & Kalay, 2002; Roy, 2002).

The variation in enzyme activities in the freshwater fish exposed to various pollutants and heavy metals, in particular, have been reported (e.g., Nemcsok, 1981; Sojbeck et al., 1984; Begum & Vijayaraghavan, 1995; DeSmet et al., 2001). However, there is very little data on the effect of arsenic toxicity to enzymes in fishes. It has been reported that the variation in enzyme activities in heavy metal treated fish is due to increased permeability of the cell as well as the direct effect of the heavy metal on the tissues (Roy, 2002). Therefore, significant depletion of enzymes in the fishes exposed to arsenic observed in the present study can be attributed to increased arsenic levels in the tissues. Furthermore, accumulation of arsenic in liver and muscles could be the possible reason for variation of enzyme activities. Such correlation between accumulation of heavy metals in fish tissues and abnormal enzyme activities has been reported in the exposure of the freshwater fish *Channa punctatus* to cadmium (Dubale & Shah, 1981).

Arsenic, in the form of arsenate, can also resemble phosphate which is used by cells for energy and signaling. By displacing

phosphate in enzymes or signaling proteins, arsenic can block energy production and normal cell signaling (Dartmouth toxic Metal Research, 2005). Decreases in phosphatase activity levels shown in the present study might be due to the increased arsenic level in the water and its accumulation in the tissues of the fishes.

As arsenic toxicity induces oxidative stress, the antioxidant enzymes (especially the glutathione-dependent enzymes), react to defend against arsenic toxicity. Allen & Rana (2004) showed that activities of glutathione-S-transferases, glutathione peroxidase, glutathione reductase and catalase decreased in the liver and kidney as a result of arsenic in the liver and kidney. This can be correlated with the decrease in GOT and GPT activities in the fishes exposed to arsenic in this study.

In summary, we have shown that arsenic concentrations in the range of 96 - 144 µg/L in an aquatic ecosystem are harmful to juvenile *Labeo rohita* and this significantly reduces the activity of their enzymes (GOT, GPT, ACP and ALP).

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