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Multiple biomarker response in the fish, Labeo rohita due to hexavalent chromium

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Abstract*— Biochemical markers are a popular measure of toxic effects on organisms due to their assumed fast response and are usually assessed after acute exposure of the organism to the stressor xenobiotics. In the present research, hexavalent chromium toxicity indices of *Labeo rohita* using a battery of biomarkers such as Lactate dehydrogenase (LDH), Succinic dehydrogenase (SDH), Acetylcholinesterase (AchE), Alkaline phosphotase (ALP), Aspartate transaminase (AST) and Alanine transaminase (ALT) activities of the muscle, liver, brain and blood have been investigated. The results showed that LDH activity in the muscle decreased significantly ($p< 0.001$) in 24 hrs and ($p< 0.025$) in 48 hrs. But in contrast the SDH activity in the liver increased exponentially showing an overall percentage increase of 87.4 suggesting predominance of aerobic metabolism over anaerobic pathway in contrast to the pesticidal effect. ALT, AST and ALP increased significantly indicating liver and heart damage. Whereas, the activity of AchE in the brain decreased significantly ($p< 0.016$) in 24 hrs and thereafter the decrease was nonsignificant. This study highlights the significance and importance of using a set of integrated biomarker to assess the chromium toxicity in the fish, *Labeo rohita.

Keywords- Keywords- biomarker; hexavalent chromium; oxidative enzyme; transaminase; alkaline phosphatase; acetylcholinesterase; *Labeo rohita.*

I. INTRODUCTION

Fish are continued to be an extremely reliable component of an aquatic monitoring system because they integrate the effect of detrimental environmental changes as consumers which are relatively high in the aquatic food chain. The fish as a bioindicator species plays an increasingly important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment. The sudden death of a fish indicates heavy pollution; the effects of exposure to sub-lethal levels of pollutants can be measured in terms of biochemical, physiological or behavioural responses of the fish. Fish are very good biosensors of aquatic contaminants.

Biochemical markers are measurable responses to the exposure of an organism to xenobiotics. They usually respond to the mechanism of toxic activity. Biochemical markers detect the type of toxicity; in some of them, the magnitude of their response correlates with their level of pollution. The use of battery of biomarkers is more advantageous than the use of a single biomarker and offers an effective early warning system in biomonitoring of aquatic environment. The biochemical markers can detect early responses and prepathological alterations before other disturbances as disease, mortality or population changes occur.

In toxicological studies of acute exposure, changes the concentrations and enzyme activities which often directly reflect cell or organ damage in specific organs [1]. Hexavalent chromium is known to affect almost every functional site of fish, often due to their bioaccumulation and poor excretion. In general, any stress inducing substance will affect the respiratory metabolism of fish. Any alteration in the intermediary metabolism due to stress is bound to affect the activity of oxidative enzymes like LDH (EC 1.1.1.27) & SDH (EC 1.3.99.1). LDH is an important glycolytic enzyme which is present in all animal tissues [2]. The enzyme is involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress [3] and the alterations of normal LDH activity pattern are found to be the O_2 stress after exposure. LDH is a parameter widely used in toxicology and clinical chemistry to diagnose all the tissues and organ damage. SDH is a vital enzyme of the Krebs cycle which catalyzes succinate to fumarate. The other enzymes are alanine transaminase (ALT; EC: 2.6.1.2) and aspartate transaminase (AST; EC: 2.6.1.1) which participate in transamination reactions found predominantly in liver, cardiac cells and striated muscle tissues. Alkaline phosphatase (ALP; EC: 3.1.3.1) is present in almost all tissue of an organism especially in cell membrane. It catalyses the hydrolysis of monophosphate esters and also has wide substrate specificity. The functional activity of this enzyme increases during exposure to heavy metal toxicity. Increased activity of ALP has been found in such pathological process of liver impairment, kidney disfunction and bone diseases. Acetylcholinesterse (AchE; EC: 3.1.3.7) is necessary for biochemical function of the brain because it is a neurotransmitter and its reduction may impair the neurotransmission. Hexavalent chromium toxicity is associated with the formation reactive oxygen species (ROS) which may cause severe injury/damage to the hepatic and nervous tissues. Based on the above studies, we hypothesize that hexavalent chromium could accumulate in the liver and brain to such toxic levels which cause pathological alterations. Cellular damage releases the ALT and AST into blood stream and the levels of these enzymes have the potential to indicate hepato-toxicity. The biochemical

analyses of LDH, SDH, ALT, AST, ALP and AChE can be used for a rapid assessment of tissue and cellular damage within 96 hr. Very scarce data is available on the impact of hexavalent chromium on the above enzymes in *Labeo rohita*, a key ecological component inhabiting native fresh water ecosystems of India. The present paper is on the general use of biochemical measurements that can be used as biomarkers as diagnostic and prognostic tools for fresh water monitoring taking the fish *Labeo rohita* as a bioindicator species.

II. Material and Methods

Labeo rohita, common carp were obtained from the local hatchery and acclimatized according to the method of Kumari and Sinha [4]. Short term test of acute toxicity over period of 96 hrs was performed on the fishes following the renewal of bioassay. LC_{50} values were determined by EPA Probit Analysis Program [5].

Fishes were exposed intracoelomatically with $1/3^{rd}$ of 110 mg (LC_{50}) of potassium dichromate. After 24, 48, 72 and 96 hrs of exposure fishes were killed for different assays. The behavior and condition of the fishes were noted every 24 hr up to 96 hrs. Collection of blood was done according to the method of Kumari and Sinha [4].

Enzymatic Assays

The assay of LDH activity in muscle was done by the method of Elliot & Wilkinson [6]. Succinic dehydrogenase activity of the liver homogenate was measured following the method of Kun and Abood [7]. Alanine transaminase (ALT) and aspartate transaminase (AST) level in the blood was measured by kit from Span Diagnostic Ltd. by the method of Reitman and Frankel [8]. The assay of acetylcholinesterase (EC. 3.1.1.7) was done by the spectrophotometric method of Ellman *et al* [9] using DTNB (dithiobisnitrobenzoic acid) as chromogen and acetylthiocholine iodide as the substrate. The enzyme assay of alkaline phosphatase (ALP) was made according to the method of Bowers and McCommb [10].

III. Results and discussion

Acute toxicity (96 h LC_{50}) of hexavalent chromium for the fresh water fish, *Labeo rohita* was found to be 110 ppm of $K_2Cr_2O_7$ (Cr as 42.42 mg/l). When an organism is exposed to the xenobiotics, it undergoes inhibition or acceleration of the catalyzed reaction rate of the enzyme systems. The mechanism includes either changing the enzyme activity, the biochemical processes or directly affecting the enzyme molecule [11-14]. It is postulated that variations in the respiratory enzyme activities in particular serve as an early biomarker to assess the extent of pollution in the exposed animals [15, 16]. Respiratory distress is one of the important manifestations of acute chromium toxicity and is known to produce physiological imbalance in the fish, *Labeo rohita* exposed to $1/3^{rd}$ LC_{50} 96 hrs. In fish, mitochondrial respiratory enzymes such as lactate dehyrogenase (LDH) and succinic dehydrogenase (SDH) are involved in cellular respiration through glucose metabolism which yields ATP. Several authors [17-20] have reported that the disturbance in oxidative metabolism led to alteration in the whole animal oxygen consumption in different species of fish exposed to hexavalent chromium.

Table 1 shows that LDH activity in the muscle decreases significantly ($p < 0.001$) in 24 hrs and 48 hrs ($p < 0.028$) and thereafter the decrease in 72 and 96 hrs is not significant (Fig. 1). But in contrast, the SDH activity increases significantly in 24 hrs ($p < 0.006$), 48 hrs ($p< 0.046$), 72 hrs ($p < 0.004$) and returns to almost normalcy in 96 hrs (Fig. 1). The overall % increase in SDH activity was 87.4. The decrease in LDH activity with the concomitant exponential increase in SDH activity shows that aerobic pathway is predominant over the anaerobic metabolism. This is further substantiated by the decrease in blood glucose concentration (Fig. 3) suggesting that sustained ATP production is needed to meet the stress condition due to chromium toxicity (Tab.1). Earlier, it has been reported that during pesticidal stress anaerobic pathway predominates over aerobic pathway [12, 14]. It is concluded that in chromium toxicity aerobic cycle is predominant over the anaerobic cycle in contrast to the pesticidal effect.

Transaminases play an important role in carbohydrate and amino acid metabolism in the tissues of fish and other orgaisms. [12, 21, 22]. Alanine transaminase is a key metabolic enzyme released from the damaged hepatocytes. The enzyme shows a consistent increasing trend significantly ($p<0.001$) in 24 hr and in 48 hr ($p < 0.001$), in 72 hr ($p <0.002$) and 96 hr ($p < 0.042$). The overall increase is 222% which is quite high indicating a very high liver damage due to chromium toxicity. This is corroborated by the increase of ALP in 24 hr ($p< 0.03$), 48 hr ($p < 0.001$), 72 hr ($p< 0.001$) and 96 hr ($p < 0.001$) (Fig. 3). The overall % increase of ALP is 387.8% which indicate acute liver damage. Similar findings have been reported by Yang and Chen [23].

Similarly AST increased significantly ($p< 0.001$), 48 hr ($p < 0.002$), 72 hr ($p < 0.007$) and 96 hr ($p <0.001$) indicating heart and liver damage. The overall % increase of AST is 141% which is quite high suggesting acute heart and liver damage (Fig. 2).

Though the liver plays an important role in metabolic processes and detoxification of many xenobiotics, acute exposures of chromium may lead to accumulation in the liver and causing pathological alterations. Moreover, cell injury of certain organs like liver and heart to the release of tissue specific enzymes into the blood stream [24]. Significant increases in transaminases (ALT & AST) activity in the fish *Labeo rohita* could be possible due to leakage of enzymes across the damaged plasma membranes. As such, increased serum ALT & AST activities reflect a situation of liver and heart damage. This study indicates that ALT, AST, ALP, LDH, SDH can be used as biomarkers of tissue damage. Thus, the present study provides a new insight on hepato and cardiotoxicity following exposure to hexavalent chromium.

The behaviour and condition of the fishes in both the control and test solution was noted every 24 hr to 96 hr. wherein the fishes showed marked change in behaviour when exposed to chromium. Behavioural manifestations of acute toxicity like copious secretion of mucus, surfacing and ultimately lethargy indicating loss of equilibrium. It is well known that presence of glycoprotein in the mucous is indicative of its metal binding capacity of chromium

especially binds the SH groups [25]. Mucous coating prevents the further entry in the fish suggesting adaptive mechanism to survive chromium toxicity. It is interesting to note that the there was a significant decrease ($p<0.016$) in the brain AchE activity in 24 hr and thereafter the decrease was non-significant till 96 hrs. (Tab-1; Fig. 1). Loss of balance during swimming might be due to some neurological impairment in the central nervous system as evident by the inhibition of AchE by chromium. Similar findings have been reported by Devi and Fingerman [26]; Lata *et al* [27] and Patro [28]. These behavioural responses may be used as a tool in bio-monitoring program to monitor ecotoxicity risk of hexavalent chromium.

TABLE I. EFFECTS OF HEXAVALENT CHROMIUM IN THE FISH, *LABEO ROHITA*

Parameters	***Control***	***24 hr***	***48 hr***	***72 hr***	***96 hr***	***Over all % Change***
Muscle LDH (mg/g)	5.38 ±0.1	2.75 ±0.76 p<0.001	2.25 ±0.92 p<0.028	5.38 ±2.61 p<0.87	4.12 ±4.29 p<0.89	52 %
Liver SDH (µg of formagen /hr/g)	536 ± 37.09	827 ± 107.4 p< 0.006	700 ± 94.3 p< 0.046	1765 ± 423.35 p< 0.004	540 ± 79.30 p< 0.89	87.4 %
Plasma ALT (IU/L)	26.0 ± 1.7	95.89 ± 4.69 p< 0.001	90.68 ± 6.22 p< 0.001	43.38 ± 4.81 p< 0.002	66.28 ± 18.50 p< 0.024	222 %
Plasma AST (IU/L)	24.5 ± 2.13	60.473 ± 6.89 p< 0.001	57.018 ± 6.89 p< 0.002	69.658 ± 11.67 p< 0.007	60.847 ± 1.97 p< 0.001	141 %
Plasma ALP (IU/L)	99.0 ± 11.52	131.94 ± 14.77 p< 0.030	564.36 ± 64.70 p< 0.001	736.10 ± 30.93 p< 0.001	571.00 ± 53.64 p< 0.001	387.8 %
Plasma Glucose (IU/L)	41.99 ±1.82	38.39 ±0.66 p< 0.009	38.06 ±1.39 p< 0.013	35.81 ±0.82 p< 0.009	32.905 ±1.20 p< 0.004	13.7 %
Brain AchE ***µmol/min***	53.18 ± 11.64	36.99 ± 16.32 p< 0.016	45.96 ± 19.45 p< 0.613	44.6 ± 7.88 p< 0.322	42.96 ± 6.8 p< 0.144	27%

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