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Full Paper

Effect of heavy metals induced toxicity on metabolic biomarkers in common carp (*Cyprinus Carpio* L.)

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Abstract: This research paper presents the pathological effects of a sub-lethal concentration of heavy metals (cadmium, lead, nickel, and chromium) on common carp (*Cyprinus carpio L.*). Total protein and levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in the liver tissue were measured. Compared with the control group a significant decrease of total protein ($p < 0.001$) was ascertained in the experimental group. The ALP on the other hand was significantly higher ($p < 0.001$). The values of ALT, AST, and LDH significantly decreased in the first day and then progressively increased afterwards ($p < 0.001$). The above results on the biochemical profile indicate marked hepatotoxic effects of heavy metals in common carp.

Key words: marker enzymes, transaminases, metal toxicity, common carp, *Cyprinus carpio* L.

Introduction

Human activities have led to accumulation of toxic metals in the aquatic environment [1-2]. The adverse input of diverse industrial wastes has aggravated the problem of contamination, and sewage disposal has greatly enhanced the addition of heavy metals into the aquatic ecosystem. Trace element pollution of the sediment in rivers, lakes, estuaries and bays caused by industrialisation has been reported by many researchers around the world [3-5].

This problem has become complex because of the non-degradability of the inorganic pollutants. They are continuously released into the aquatic environment both from natural processes like volcanic activity and from rapid industrialisation. Heavy metals have received particular attention among the non-degradable toxic chemicals due to their adverse effects on aquatic life forms [6].

Heavy metals are the most noxious pollutants owing to their diverse effects. Some metals are soluble in water and readily absorbed into the living organisms. Metal ions of high toxicity are known to cause deleterious impact on organs and blood level in fish [7-8]. They form metal complexes with the structural proteins, enzymes and nucleic acids and interrupt their functions. For example, cadmium is a non essential, non-biodegradable element reported to be a major contaminant that causes adverse effects on the aquatic system [9-11]. Lead pollutant induces lipid peroxidation in tissues and causes an irreversible damage to the respiratory organs of fish [12-13]. Nickel induces a morphological transformation and chromosomal aberration in cells [14]. Nickel in combination with cobalt induces convulsions, DNA strand breakage and organ damage [15]. Hexavalent chromium is relatively mobile in the environment and is acutely toxic, mutagenic, teratogenic and carcinogenic to aquatic organisms [16].

Enzymes are necessary for normal cellular metabolism including that of the liver, and the degenerative changes due to the combined metal toxicity exhibited in the liver alter the level of a number of its enzymes. For example, lactate dehydrogenase (LDH) is released from the liver after its cellular damage and failure due to organophosphate insecticide intoxication [17]. LDH, spartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) are released in acute and chronic liver disorders. These enzymes are biomarkers of acute hepatic damage, thus their bioassay can serve as a diagnostic tool for assessing necrosis of the liver cells [18-19].

The aim of the present study is to determine the toxicity of combined heavy metals on the activity of the marker enzymes (AST, ALT, ALP and LDH) in order to measure the degree of organ damage in the liver of the common carp (*Cyprinus carpio* L.), an economically important species around the world.

Materials and methods

Freshwater common carps (10 – 13 cm long and weighing 35.70 ± 0.60 g) were collected from ponds of southern districts of Tamilnadu, India and were acclimatised to laboratory conditions for a week. Twenty to twenty five individuals were used for the experiment. They were kept in batches in 200L recirculating tanks filled with dechlorinated tap water under constant temperature (25 ± 1^{o}C) with a controlled photoperiod of 12:12-hour light-and-dark cycle and constant filtration. The physico-chemical characteristics of the tap water used in this study were as follows: pH 7.20, electrical conductivity 1255 µS/cm, TDS 815.75 mg/L, alkalinity 140.5 mg/L, total hardness 280.45 mg/L, Ca 120.25 mg/L, Mg 75.2 mg/L, Na 15 mg/L, K 8 mg/L, Cl^- 148.55 mg/L, SO_4^{2-} 68.65 mg/L and total NH_3 10.8 mg/L. The water in the control and experimental tank was changed every 3 days.

The fish were divided into two groups, with one group serving as control and the other as experimental group. The latter group was exposed to a concentration of 5 ppm of a combined (Cd + Pb + Cr + Ni) metal solution containing 1.25 ppm of each metal ion (1/10th of LC50/48h) for a period of 32 days. Analytical grade cadmium chloride, lead nitrate, potassium chromate, and nickel sulphate

supplied by BDH were used as metal toxicants. The selected heavy metal concentration was based on preliminary results, which showed it to be sub-lethal after a 32-day period of exposure. No fish mortality was observed during the experiment.

The fish were fed with a commercially available fish feed at a daily rate of 3-4 % body weight throughout the experiment. The fish were starved for 24 hours before experimentation. Five specimens of the control and 5 specimens of the metal-exposed group were sacrificed during each exposure period of 1, 8, 16 and 32 days. The post-mitochondrial fraction from the pooled liver samples was washed in ice-cold 1.15% KCl solution, blotted and weighed. The tissues were homogenised in 4 volumes of homogenising buffer (50 mM Tris-HCL mixed with 1.15% KCl and the pH adjusted to 7.4) using a Teflon homogeniser. The resulting homogenate was centrifuged at 16,000g for 15 min at 0-4^{o}C a Beckman L5-50B centrifuge. The supernatant was decanted and stored at -20^{o}C until analysis.

The level of total protein in the liver tissue was assayed by the method of Lowry et al. [20]. The value of protein was expressed as g/L. Alkaline phosphatase activity was estimated by the method of Balasubramanium et al. [21]. The results were expressed as IU/L. Transaminases activity was estimated by the method outlined by Bergmeyer and Bernt [22] and Splittstoesser et al. [23]. The results were expressed as U/L. The enzyme lactate dehydrogenase activity was estimated by the method of King [24]. The enzyme activity was expressed as IU/L.

The data obtained from the experiments were analysed, and the results were expressed as mean ± S.D. The results were evaluated using Student's t-test. The values of $p < 0.001$ were considered statistically significant.

Results and Discussion

The levels of metabolic marker enzymes in the liver of the common carp (*Cyprinus carpio* L.) exposed to a combined heavy metal solution for a period of 1,8,16 and 32 days were determined. The level of total protein showed a significant decrease ($p < 0.001$) after several stages of exposure (Figure 1). The enzyme alkaline phosphatase in the liver was increased significantly after 1, 8, 16, and 32 days (Figure 2). Alanine aminotransferase and aspartate aminotransferase exhibited a significant decrease in the first day compared to control and then progressively increased in the successive exposure periods (Figures 3, 4). Lactate dehydrogenase activity showed a similar trend (Figure 5).

The mean values of total proteins were significantly decreased in the exposed fish compared to control (Figure 1). This implicates that the bioaccumulation of heavy metals triggers the oxidative stress in the liver cells by the generation of reactive oxygen species. The defensive surface proteins antagonise the toxic radicals resulting in elimination of protein from the liver cells. The lowered level of total protein in plasma, muscle and liver reflects the capacity of protein synthesis and denotes the osmolarity of the blood and liver impairments. Hence, it is a valuable indicator in the diagnosis of toxicity in fish.

In the present study the decrease in total protein might be due to several pathological processes induced by heavy metals including plasma dissolution, renal damage and protein elimination in the urine, a decrease in liver protein synthesis, and alteration in hepatic blood flow and/or hemorrhage into the peritoneal cavity and intestine. The present findings are in good agreement with previous reports of decreased level of soluble protein and RNA content in the liver [25].

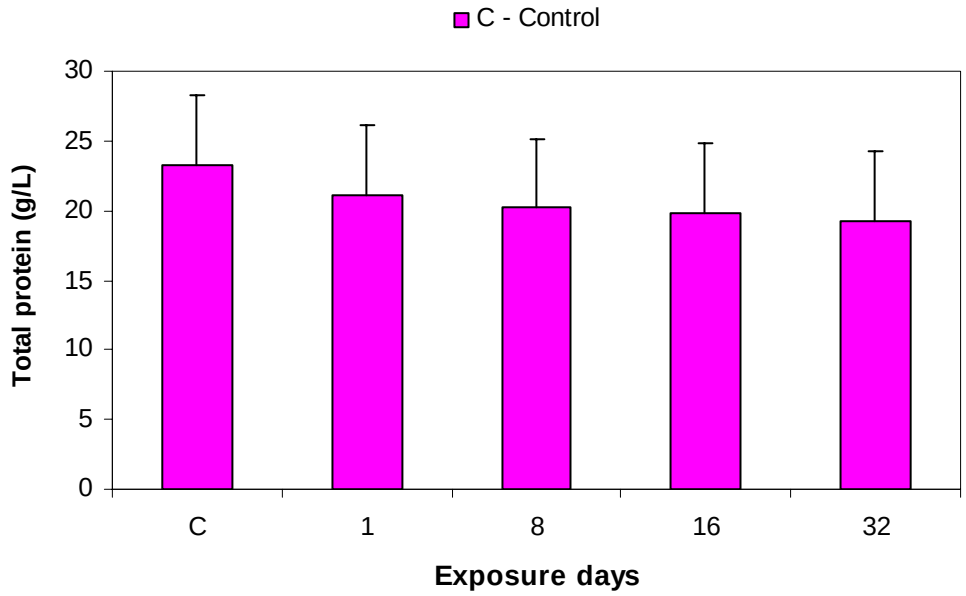

Figure 1. Level of total protein in liver

The alkaline phosphatase is composed of several isoenzymes that are present in practically all tissues of the body, especially in cell membranes. It catalyses the hydrolysis of monophosphate esters and has a wide substrate specificity. The functional activity of this enzyme was found to increase during the exposure with heavy metals as an adaptive response in mitigating the metal toxicity. Increased stimulation of alkaline phosphatase has previously been found in such pathological processes as liver impairment, kidney dysfunction and bone disease [26-27]. This supports our present study with increased activity of alkaline phosphatase in experimental fish when compared to the control (Figure 2).

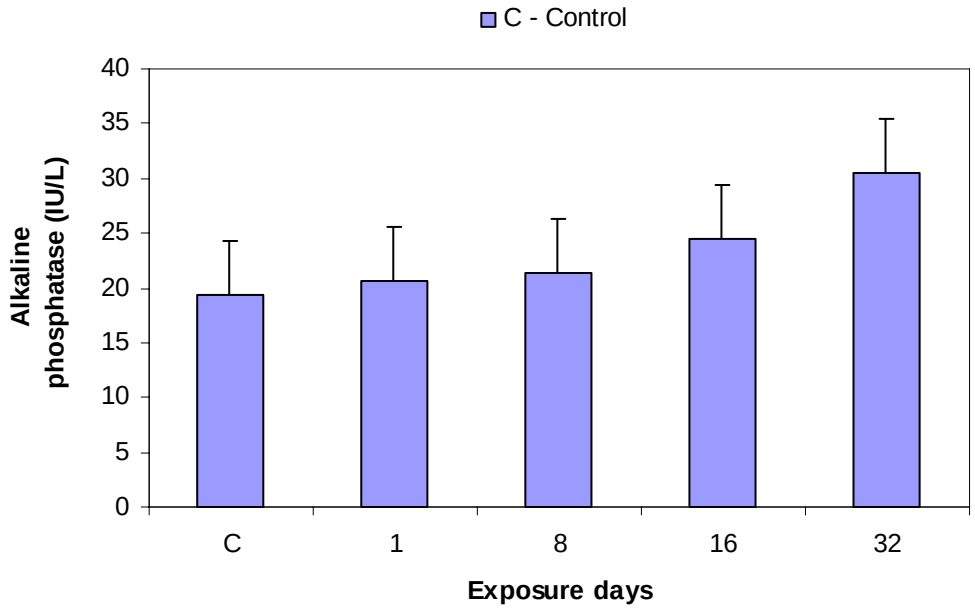

Figure 2. Level of alkaline phosphatase in liver

Transaminases play an important role in carbohydrate and amino acid metabolism in the tissues of fish and other organisms [28]. Alanine aminotransferase is a key metabolic enzyme released on the damage of hepatocytes. The enzyme shows a decreasing level in the first day and from then onwards its level increased steadily in the injured liver (Figure 3), indicating its adaptive response to its leakage into the blood stream due to the metal toxicity. Also, the alanine aminotransferase has a part in transforming protein to glycogen, which is the major reserve fuel of the body during the stress-induced toxicity in the liver .This result is in accordance with the results of previous investigators on fresh water fish [29]. The results indicate that under the influence of different heavy metals or in a state of stress, the damage of tissues and organs may occur with concomitant elevation and liberation of transaminases into the circulation.

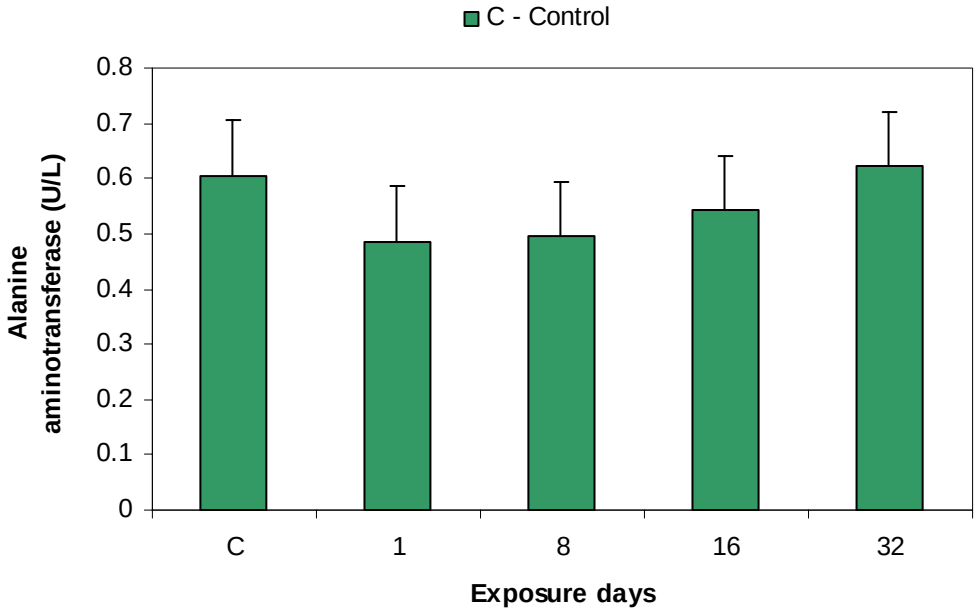

Figure 3. Level of alanine aminotransferase in liver

The aspartate aminotransferase belongs to the plasma non-functional enzymes which are normally localised within the cells of liver, heart, gills, kidneys, muscle and other organs. Monitoring of liver enzyme leakage into the blood has proved to be a very useful tool for toxicity studies in the liver. The present observation on the freshwater common carp revealed a slight decrease of the aspartate transaminase level in the liver during the first exposure day followed by a significant increase onwards (Figure 4). This is supported by a reported alteration in the enzyme activity due to the inhibitory effect of metals [30].

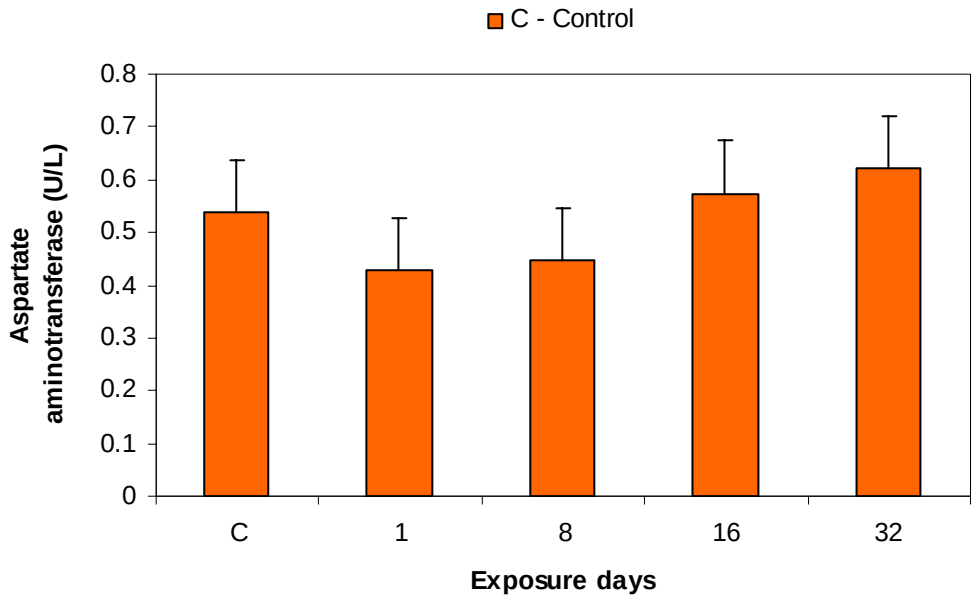

Figure 4. Level of aspartate transaminase in liver

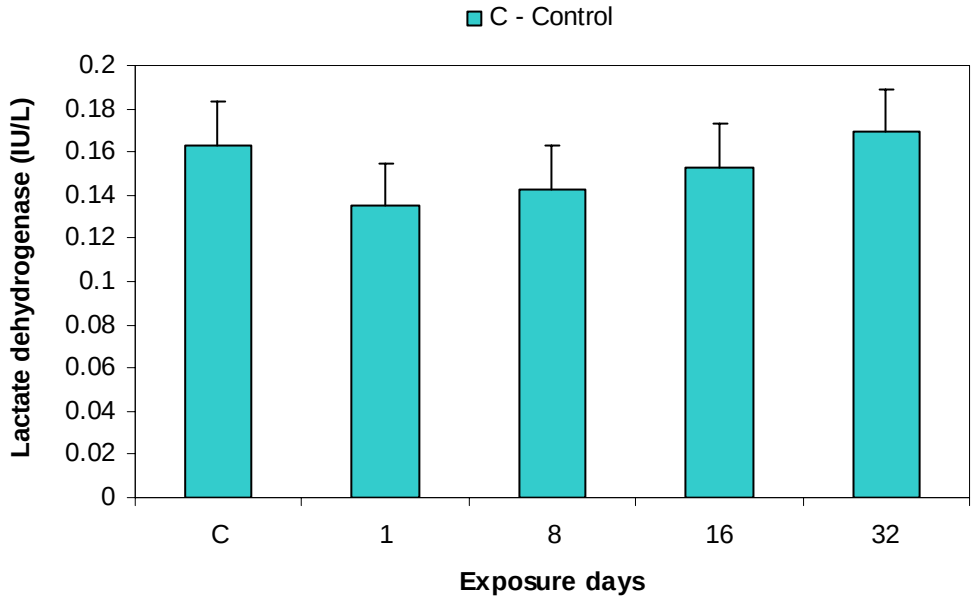

Figure 5. Level of lactate dehydrogenase in liver

The lactate dehydrogenase is an anaerobic enzyme involved in the conversion of pyruvate to lactate in the Embden Meyerhoff pathway. This enzyme shows an increasing activity during a strenuous muscle exercise. In this investigation the level of the lactate dehydrogenase in the liver homogenate was found to decrease in the one-day exposed fish with subsequent significant increase afterwards (Figure 5). The increased level of the lactate dehydrogenase may be due to an alternative anaerobic glycolytic pathway in conversion of lactate to pyruvate for the production of glucose, which is a major

source of energy during stress induced by heavy metals. The variation of lactate dehydrogenase activity can thus be used as another sensitive index for assessing heavy metal toxicity.

Conclusions

It can be concluded that fishes show various stress responses comparable to other vertebrates. It is of interest to note that a significant rise in alkaline phosphatase in the liver and a drastic fall of total protein were observed in the present findings. Alanine aminotransferase, aspartate aminotransferases and lactate dehydrogenase exhibit a significant initial decrease in the first day before increasing progressively in the following 8th, 16th and 32nd day. This indicates that an exposure of common carp to a sub-lethal concentration of combined heavy metals causes liver damage. Continuous accumulation of toxic heavy metals by common carp may affect hepatic function and cause cellular degeneration. Furthermore, the results provide evidence that enzyme biomarkers can be used as sensitive indicators of aquatic pollution.

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