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Full Paper

Effect of heavy metals on the level of vitamin E, total lipid and glycogen reserves in the liver of common carp (*Cyprinus carpio* L.)

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Abstract: The aim of this study is to examine some changes in the biochemical profile of the liver tissue of common carp (*Cyprinus carpio* L.) exposed to a sublethal concentration of heavy metal mixture (cadmium, chromium, nickel and lead). The biochemical profile, specifically glycogen, total lipid and vitamin E content in the liver tissue was examined and compared to that of the control group. The exposed group showed a marked decline in glycogen and vitamin E reserves. Conversely an increase in total lipid in comparison to control was observed. The result reflects the sensitivity of these biochemical parameters to the effects of sublethal levels of combined heavy metals for this the widely consumed freshwater fish.

Keywords: heavy metals, *Cyprinus carpio* L., toxicity, liver damage, glycogen, total lipids, vitamin E

Introduction

The toxicity of heavy metals to aquatic organisms has attracted considerable research attention, particularly as a result of the continuing anthropogenic mobilisation of the metals in the

environment. Like warm-blooded animals, changes due to heavy metals in fish occur because of injuries or infection which otherwise can be used to detect the dysfunction of the organs. These changes reflect the effect on fish survival, reproduction and growth [1]. Most of the heavy metal ions exhibit toxicity through the formation of coordination complexes and clusters in the animal cells [2]. Low concentration of heavy metals may induce a chronic stress which may not kill the individual fish but lower its size and body weight [3], thus reducing their ability to compete for food and habitat. Fish also have a tendency to bioaccumulate heavy metals and humans can therefore be at great risk through contamination of the food chain [4].

Cadmium causes poisoning in various tissues and animals [5-7]. It can react with polythiol groups of cellular macromolecules such as glycogen, lipids, amino acids. Cadmium bioaccumulated in tissues can replace the essential element zinc present in the enzymes carboxypeptidase [8] and metallothionein [9]. The metal causes oxidative damage by alteration of mitochondrial activity and genetic information [10-11]. In the environment, chromium exists primarily in the trivalent and hexavalent states, the latter being the predominant species in natural water [12]. Chromium in combination with nickel as trace metals function as potential health hazard that causes disturbances in gastrointestinal, hepatic and neurological activities. Hexavalent chromium generates reactive oxygen species (ROS) which increase risk for cellular and hepatic DNA fragmentation, enhance intracellular oxidised states, and decrease cell viability with necrosis and programmed cell death [13].

Nickel salts significantly increase the level of lipid peroxidation and simultaneously decrease glutathione level and glutathione peroxidase activity in the liver [14]. Lead exposure provokes adverse effect on the central nervous system as it is extremely toxic to most of the living things [15]. The assimilation of relatively small amounts of lead over a long period of time in the human body can lead to the malfunctioning of the organs [16].

Heavy metal contamination in aquatic environment exerts an extra stress on fish which tend to accumulate the heavy metals in metabolically active tissues and organs [17]. The liver is an important organ performing vital functions including biotransformation, migration of vitamins and lipids, glycogen storage, and release of glucose into the blood. Heavy metal chelation may disrupt the liver tissue by disintegrating the functional and structural properties of the cells.

Toxicity tests for xenobiotics serves as a sensitive index in predicting and preventing damage to aquatic life in receiving waters by regulating the toxic waste effluents [18]. Assessment of biochemical parameters in damaged fish organ can be used as a diagnostic tool to characterise such metal toxicity. There are considerable earlier information assessed by potential biomarkers indicating that heavy metals are responsible for many adverse effects in various types of fish and other animals [19-21]. In the majority of exotoxicological studies, effects of single metals on fish have been evaluated, while studies on the biochemical profile of fish subjected to the impact of combined heavy metals are limited. Therefore the aim of this study is to assess the biochemical changes in glycogen, lipid and vitamin E in common carp (*Cyprinus carpio* L.) in the case of liver injury by a model mixture of heavy metals, namely lead, cadmium, chromium and nickel.

Materials and Methods

The freshwater common carp (10-13 cm in length and weighing 35.70 ± 0.60 g) were collected from ponds of the southern districts of Tamilnadu, India and were acclimatised to laboratory conditions for a week. Twenty to twenty-five individuals were used for the experiments. All the fish were kept in batches under constant temperature ($25 \pm 1^\circ\text{C}$) with a controlled photoperiod of 12:12 hour light and dark cycle and constant filtration. Analytical grade cadmium chloride, lead nitrate, potassium chromate and nickel sulphate (supplied by BDH, India) were used as metal toxicant throughout the experiment. The fish used for this experiment were maintained in 200 L recirculating tanks, filled with dechlorinated tap water. The physicochemical characteristics of the water during heavy metal induction period and the water used in control pond are presented in the Table 1. The water in the control and experimental tanks was changed every 3 days.

Table 1. Water quality parameters measured in the control and experimental ponds during heavy metal induction period

| Water Quality Parameter | Mean \pm SD | | |
|---|-------------------|----------------|----------------|
| | Control | Day | Night |
| Temperature ($^\circ\text{C}$) | 27 ± 0.2 | 27 ± 0.2 | 27 ± 0.2 |
| pH | 7.20 ± 0.2 | 7.2 ± 0.1 | 7.1 ± 0.1 |
| Electrical conductivity ($\mu\text{S}/\text{cm}$) | 1255.00 ± 0.5 | 1650 ± 5 | 1650 ± 5 |
| Total dissolved solids(ppm) | 815.75 ± 0.3 | 1072 ± 5 | 1072 ± 5 |
| Alkalinity (ppm) | 140.50 ± 0.2 | 148 ± 1.2 | 147 ± 0.5 |
| Total hardness (ppm) | 280.45 ± 0.5 | 355 ± 5 | 355 ± 5 |
| Dissolved oxygen (%) | 7.2 ± 0.2 | 7.4 ± 0.3 | 7.3 ± 0.2 |
| Total ammonia (ppm) | 10.80 ± 0.2 | 12.2 ± 0.2 | 11.2 ± 0.3 |
| Salinity (%) | 14.6 ± 0.2 | 15 ± 0.5 | 14.8 ± 0.1 |

Note: The values are statistically significant at $p < 0.05$.

The fish were divided into two groups, with the first group serving as control and the other as experimental group. The experimental group was exposed to a sublethal 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. The heavy metal concentration was selected based on preliminary results, which showed a sublethal effect after a 32-day period of exposure.

The fish was fed with commercially available fish feed at a daily rate of 3-4 % body weight throughout the experiment. The control and the experimental groups were starved for 24 hours before experimentation. Five specimens of the control group and 5 specimens of the metal-exposed group were then sacrificed during each exposure period of 1, 8, 16 and 32 days. The liver tissues of both experimental and control fish were dissected out, blotted free of blood and weighed, and processed for biochemical analysis. Glycogen (in mg/mL of liver homogenate) was assayed by the method of Seifter et al. [22]. Total lipid (in g/L of liver homogenate) was

determined using a method described by Bragdon [23]. Vitamin E content (in $\mu\text{g/g}$ tissue) in liver homogenate was estimated by the method of Baker and Frank [24]. All the analyses were performed by adapting universally accepted standard protocols employing a Hitachi UV-Visible spectrophotometer. All experimental results are expressed as mean \pm SEM. Paired Student's t-test was employed, and $p < 0.001$ was considered significant.

Results and Discussion

The present study attempts to create awareness concerning the potential severe public health issues resulting from the toxic effects of heavy metals as pollutants from industrial, agricultural and urban wastes. The toxic effects of heavy metals on fish involve hepatotoxicity, neurotoxicity and nephrotoxicity [25]. Bioaccumulation of heavy metals and consequent alterations in gills, liver, kidney and flesh of common carp have been reported earlier [26].

The biochemical profile of the liver of the common carp exposed to a sublethal concentration of the combined heavy metal solution (5ppm) for the exposure period of 1, 8, 16 and 32 days is presented in Table 2. Glycogen reserves in liver tissue were depleted to a lower level compared to that of the control fish ($p < 0.001$). The declined glycogen level explains the formation of glucose, a major source of energy by glycogenolysis mechanism. The content of glycogen seemed to increase initially, then showed a marked decline at the end of the 16th day onwards as shown in Figure 1. Total lipid in liver tissue exhibited a significant ($p < 0.001$) increase after 32 days of exposure (9.17 ± 0.05 g/L) in comparison to the control value (5.66 ± 0.06 g/L) as reported in Table 2 and Figure 2. Vitamin E, a chain-breaking lipid-soluble antioxidant, showed significant progressively decreased activity ($p < 0.001$) in the liver of the treated groups for the periods studied (Table 2, Figure 3). The result indicated the loss of vitamin E in the liver tissue, which served as an effective marker for assessing the degree of damage in the liver tissue by the action of heavy metals.

The decreased glycogen concentration in the liver of common carp could be due to its enhanced utilisation as an immediate source to meet the energy demand under metallic stress. Depleted glycogen level under chromium stress reported in *Labeo rohita* [21] also supports our research findings. The carbohydrate source is stored as a reserve fuel in the liver and muscle tissues of fish for the endogenous derivation of energy during acute and chronic stress [27]. The decreased glycogen content as a result of hypoxic or anoxic condition activates the glycolytic enzymes via catecholamines that initially enhance glycogen concentration. It was also found that cadmium could decrease glycogen reserves in the liver and muscle tissues of *Cyprinus carpio* [28].

Table 2. The biochemical profile of liver of common carp (*Cyprinus carpio* L.) exposed to sublethal concentration of combined heavy metal solution (5ppm)

| Biochemical Profile | Duration of Exposure (Days) | Control Mean ± S.D | Experiment Mean ± S.D | % Change | Result |
|-------------------------|-----------------------------|--------------------|-----------------------|----------|-------------|
| Glycogen (mg/mL) | 1 | 0.63 ± 0.03 | 0.45 ± 0.02 | 28.57 | p < 0.001** |
| | 8 | 0.63 ± 0.03 | 0.58 ± 0.03 | 7.94 | p < 0.01* |
| | 16 | 0.63 ± 0.03 | 0.38 ± 0.02 | 39.68 | p < 0.001** |
| | 32 | 0.63 ± 0.03 | 0.32 ± 0.03 | 49.21 | p < 0.001** |
| Total lipid (g/L) | 1 | 5.66 ± 0.66 | 6.75 ± 0.06 | -19.25 | p < 0.001** |
| | 8 | 5.66 ± 0.66 | 7.14 ± 0.14 | -26.15 | p < 0.001** |
| | 16 | 5.66 ± 0.66 | 8.37 ± 0.14 | -47.88 | p < 0.001** |
| | 32 | 5.66 ± 0.66 | 9.17 ± 0.05 | -62.01 | p < 0.001** |
| Vitamin-E (µg/g.tissue) | 1 | 1800.85 ± 0.005 | 1640.67 ± 0.03 | 8.89 | p < 0.001* |
| | 8 | 1800.85 ± 0.005 | 1536.84 ± 0.04 | 14.66 | p < 0.001* |
| | 16 | 1800.85 ± 0.005 | 1290.25 ± 0.04 | 28.35 | p < 0.001* |
| | 32 | 1800.85 ± 0.005 | 1100.63 ± 0.03 | 38.88 | p < 0.001* |

Note: n = 5

** Highly Significant

* Significant

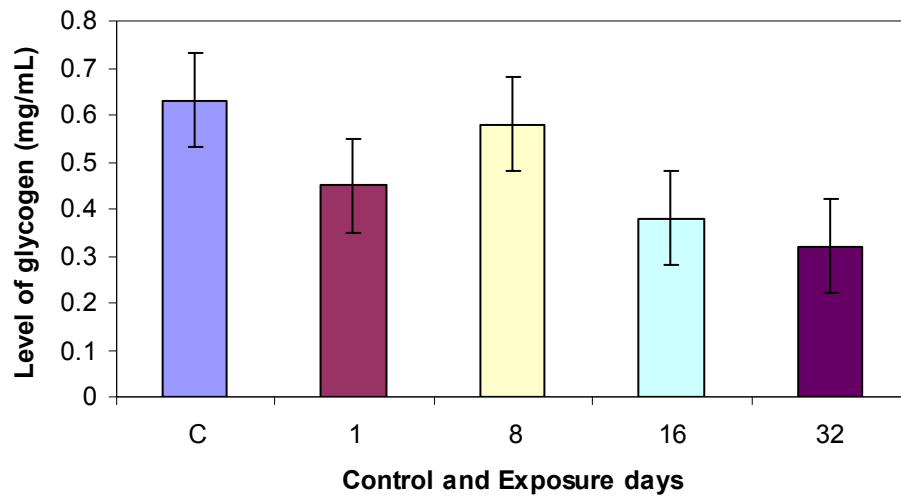


Figure 1. Level of glycogen in liver

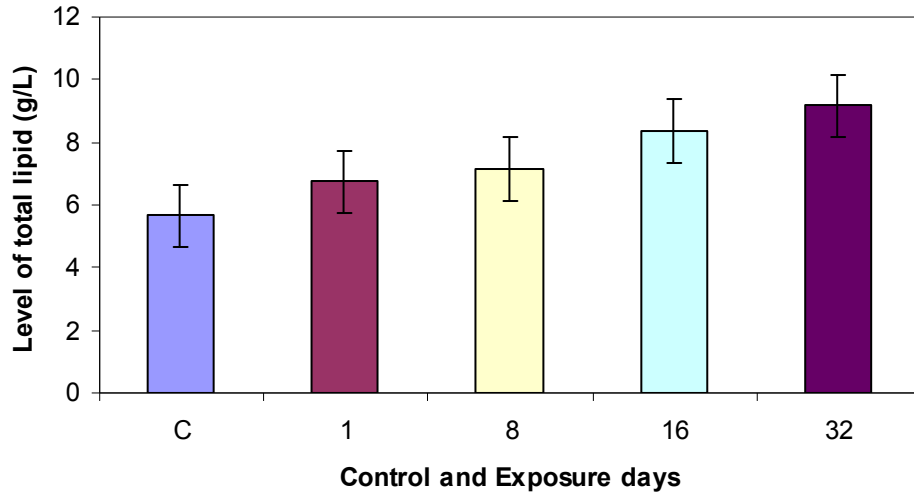


Figure 2. Level of total lipid in liver

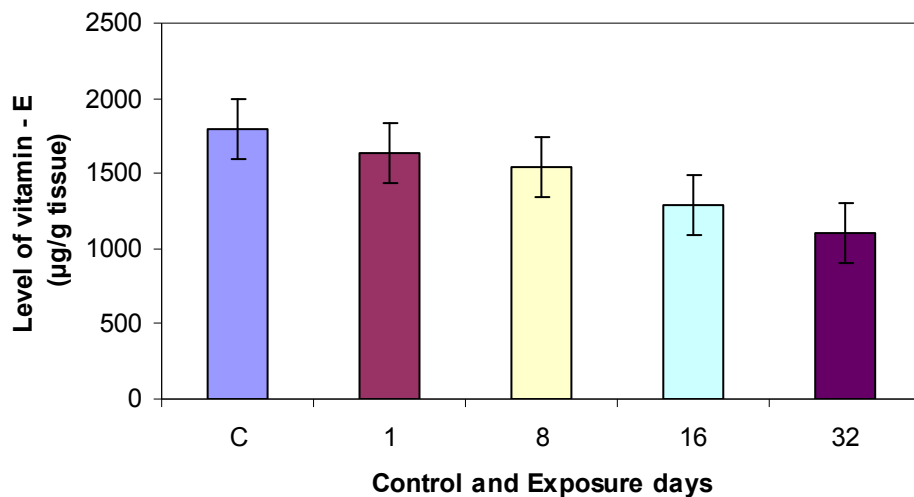


Figure 3. Level of vitamin E in liver

Indeed, prolonged stress after metal toxicity exerts weakness and hypoxic condition with the inability of hepatocytes to propagate the regular cellular metabolism [29]. The decreased glycogen content in fish, which was also observed during the present study, alters the enzymes of carbohydrate metabolism and might be utilised in the formation of glycoproteins and lipids [30].

In relation to the total lipid in this study, it was found that there was a significant increase of lipid in the heavy metal intoxicated groups as compared to control fish. Lipid blocks the metabolism of hepatic triglycerides due to the defective synthesis of very low density lipoproteins which are involved in the transport and mobilisation of triglycerides to extra hepatic tissues [31].

Results of this study show an abnormal increase in lipid, which may induce hyperlipidemia, premature atherosclerosis and excessive deposition of fat (obesity) loaded in carp tissues.

Vitamin E has a protective effect on the stabilisation of metabolic process in biological systems [32]. It performs a vital role in fish health by inactivating harmful oxy radicals stimulated by stress and environmental pollution. The reduced vitamin E could be attributed to the aggressive activation of heavy metals chelated in the liver. Numerous investigations documented vitamin E as exhibiting the greatest protection against heavy metal toxicity in humans and animals [e.g. 33-35]. In the present investigation the decreased vitamin E might be due to its utilisation as the first defense line in the protection of sub-cellular organelles and in the stabilisation of liver cell membranes against toxic reactive metabolites provoked by heavy metals.

Conclusions

The present study has demonstrated that the effects of sublethal concentration of combined heavy metals for the exposure period of 1, 8, 16 and 32 days proved to be toxic to common carp (*Cyprinus carpio L.*). Glycogen in the liver tissue was depleted to lower levels compared to that in the control fish, while the level of total lipid was significantly increased during the 32 days of heavy metal exposure, but that of vitamin E, like glycogen, significantly decreased. The result could seriously alter the ability of cells to counteract with heavy metals. This observation further implies that there is dire need to focus on the harmful influences of heavy metals on the biochemical activities of aquatic organisms and on the environment at large.

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