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Biochemical changes of antioxidant enzymes in common carp (*Cyprinus carpio* L.) after heavy metal exposure

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Abstract: Accidental industrial spills may lead to a high concentration of toxic metals in the aquatic environment, as well as affecting freshwater ecosystems with acute and chronic toxicity. Of all aquatic species, fish are particularly sensitive to waterborne contamination and are recognized as bioindicators for water quality monitoring. The objective of the present study was to evaluate the biochemical changes of the enzymatic defense systems in fresh water fish, the common carp (*Cyprinus carpio* L.), when they are exposed to a heavy metal contaminated aquatic system. The fish were systematically exposed to heavy metal solutions such as cadmium, lead, nickel, and chromium at a sub-lethal level for a period of 32 days. The analytical result indicates that heavy metal toxicity in fish organs gradually increases during the exposure period and slightly decreases at the 32nd day. The activity of the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), in the fish were increased. This observation clearly indicates the defensive nature and the adaptive mechanism of cells against free radical induced toxicity. All the results were statistically significant at $P < 0.001$.

Key words: Common carp (*Cyprinus carpio* L.), antioxidant enzymes, lipid peroxidation, oxidative stress, heavy metals

Introduction

The contamination of fresh water systems with a wide range of pollutants has become a matter of concern over the last few decades (1). The natural aquatic bodies were extensively contaminated with heavy metals released from domestic, industrial, and other man-made activities. This may have serious effects on the ecological balance of the recipient environment. The organisms present in the aquatic environment may accumulate the toxic metals, which ultimately affect not only the productivity and reproductive capabilities of the organisms, but also the health of the human beings that depend on the organisms as a major source of protein.

In aquatic animal species, fish are the inhabitants that cannot escape from the detrimental effects of the pollutants (2). Studies carried out on various fish species have revealed that heavy metals may alter the biochemical parameters both in tissues and in the blood (3,4).

Aerobic organisms generate reactive oxygen species (ROS), such as superoxide anion radical (O_2), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$), because of oxidative metabolism. The hydroxyl radicals can initiate lipid peroxidation (LPO) in tissues. To attenuate the negative effects of ROS, fish possess an antioxidant defense system like other vertebrates that utilizes enzymatic and non-enzymatic

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mechanisms. The most important antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST). The non-enzymatic defense includes Vitamin–E, C, and A, glutathione, carotenes and ubiquinol$_{10}$ (5).

The antioxidants protect the organism against oxyradical damage, such as DNA strand breaks, protein oxidation and the induction of lipid peroxidation (6). The increase in the activated form of molecular oxygen species due to overproduction and/or to the inability to destroy them may lead to damage in the DNA structure and thus may cause mutations, chromosomal aberrations, and carcinogenesis. A shift to a more oxidative state or any imbalance between production and degradation of reactive oxygen species (ROS) in animal tissues may cause lipid peroxidation, plasma membrane alterations, and inactivation of enzymes (7). The use of the biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed fish. Oxidative stress biomarkers have rapidly increased in the field of ecotoxicology. Therefore, it has been suggested that they could be used in environmental monitoring systems (8). Several metals have been reported to elicit oxidative stress in aquatic organisms (9).

Common carp (*Cyprinus carpio* L.) is an important commercial species around the world that caters to large populations. It has an adaptive response in a polluted aquatic environment. The direct exposure of gills in the water medium has been dominantly accepted as they are the main site to water contamination and toxicity (10,11). Heavy metals in contaminated water bodies are normally present in ionic forms. These ions, when exposed to fish species, cause deleterious effects on organs, such as the liver, gills, gonads and components of the blood (12).

In toxicological studies of acute exposure, changes in concentrations and enzyme activities often directly reflect cell damage in specific organs. The liver is an important organ involved in metabolic processes and in the detoxification of xenobiotics. In some situations, heavy metals may accumulate in the liver to toxic levels and cause pathological alterations (13).

In these research studies, the biochemical changes of the enzymatic defense systems in the common carp (*Cyprinus carpio* L.) under a heavy metal contaminated aquatic environment were evaluated.

Materials and methods

The fresh water common carp (*Cyprinus carpio* L.) (11–13 cm in length and with an average weight of 35.70 ± 0.60 g) used for the chronic toxicity studies were collected from the ponds of the southern districts of Tamil Nadu, India. They were acclimated to laboratory conditions for 10 days prior to the experiments. The fish were kept in batches of 20–25 individuals with a photoperiod of a 12:12 h light and dark cycle. During the experiment, the fish were fed with commercially available fish feed at a daily rate of 3%-4% body weight throughout the experiment. The fish were starved for 24 h before experimentation.

Analytical graded cadmium chloride, lead nitrate, potassium chromate, and nickel sulphate supplied by BDH (India) were used as the metal toxicant for the experiments. The fish were divided into 4 groups with the first group serving as control and the others as experimental groups. The experimental groups were exposed to a combined heavy metal solution with a sub-lethal concentration of 5 ppm (1/10th of LC_{50} / 48 h) daily for 1, 8, 16, and 32 days.

The fish were humanly sacrificed and the perfused liver and kidney tissues were taken for enzyme assays. The post-mitochondrial fraction from the pooled liver and kidney samples were washed in ice-cold 1.15% KCl solution blotted and weighed. The tissues were homogenized in 4 volumes of homogenizing buffer (50mM Tris-HCl mixed with 1.15% KCl and pH adjusted to 7.4) using Teflon homogenizer. The resulting homogenate was centrifuged at 16,000 g for 15 min in a Beckman L5-50B centrifuge at 0-4 °C. The supernatant was decanted and stored at –20 °C until analysis.

The antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), were assayed using a Hitachi UV-Visible spectrophotometer. The detailed analytical procedure was outlined in the literature (14).

Data obtained from the experiments were analyzed, and the results were expressed as mean ± S.D. The results were evaluated using Student's t test.

Values of $P < 0.001$ were considered statistically significant.

Results

The level of malondialdehyde (MDA) in the liver tissue of the experimental fish was found to be in the range of 1.340 ± 0.020 to 2.540 ± 0.025 during the initial and at the 32nd day of heavy metal exposure. The antioxidant enzyme activities, such as superoxide dismutase (SOD), were in the range of 6.239 ± 0.004 to 7.334 ± 0.004 in the liver and they were 2.443 ± 0.004 to 3.267 ± 0.007 in the kidney. The activity of catalase (CAT) in the liver was 0.949 ± 0.004 to 1.293 ± 0.002 and in the kidney, it was in the range of 0.938 ± 0.003 to 1.062 ± 0.002.

The glutathione peroxidase (GPx) activity in liver was in the range of 4.156 ± 0.003 to 4.165 ± 0.003 and in kidney, it was 1.639 ± 0.002 to 1.839 ± 0.003. The activity of glutathione-S-transferase (GST) in liver was in the range of 2.015 ± 0.003 to 3.285 ± 0.003 and it was in the level of 0.986 ± 0.001 to 1.445 ± 0.003 in the kidney. These values were statistically significant at $P < 0.001$.

Discussion

The liver tissues in fish are more often recommended as an environmental indicator of water pollution than any other organs. The toxicants cause a disturbance in the physiological state of the fish, which affects the enzyme activity. It then causes distortions in the cell organelles, which may lead to the elevation in the activity of various enzymes.

The heavy metal toxicity stimulates the oxidative stress and the antioxidant enzymes are induced as a defense mechanism. Oxidative lesions in various organs of the common carp (*Cyprinus carpio* L.) have recently been related to liver tumor formation in fish from polluted environments (15). The major mechanism for metal toxicity appears to be direct and indirect damage to the mitochondria (via) depletion of glutathione, an endogenous thiol (SH–) group containing antioxidant, which results in excessive free radical generation and mitochondrial damage (16).

The chronic administration of heavy metals resulted in a gradual increase in hepatic and renal antioxidant defense (17), which was also confirmed in the present study by the increased bioaccumulation of heavy metals loaded in the carp tissues. The penetration of cadmium in the gills, liver, kidney, and flesh antagonizes the antioxidant (MnSOD) in hepatocyte mitochondria, depletes glutathione through the generation of free radicals, causes lipid peroxidation, and directly causes uncoupling of mitochondrial respiratory chain activity (18,19).

Cadmium exposure is also associated with increased risk for osteoporosis and fracture (20). Occupational exposure to lead results in plasma levels of 40 mg lead/100 mL blood, which reduces sperm concentration in men (21). The levels of chromium and nickel were found to be significantly decreased when compared to cadmium and lead toxicity in the gills, liver, kidney, and flesh. This indicates a synergistic role in the perturbation of cadmium and lead accumulated in the organs and it can cause damage to the cells.

The balance between the production of oxidants and the scavenging of those oxidants by antioxidants determined the extent of lipid peroxidation in various organs of the fish. The malondialdehyde (MDA) level generally increased in the organs during 32 days of exposure (Figure 1). Many studies have demonstrated that lipid peroxidation and oxidative stress increase in tissues of different species of aquatic organisms, because of being exposed to environmental stress (22). However, it is by no means a general rule that exposure to a pollutant increases the malondialdehyde level.

In the literature, some authors have reported that lowered malondialdehyde levels in fish sampled in a site contaminated by metal and organic compounds (23) and others have shown that fish show no response when exposed to azinphosmethyl and 2,4–dichlorophenoxy acetic acid (24). The increased level of malondialdehyde observed in 4 organs of a fresh water fish, the common carp (*Cyprinus carpio* L.), agreed with the previous investigation carried out with tilapia that had been exposed to microcystins (25).

The defensive free radical scavenger, superoxide dismutase (SOD), triggers an induction response in heavy metal intoxicated groups. This indicates that

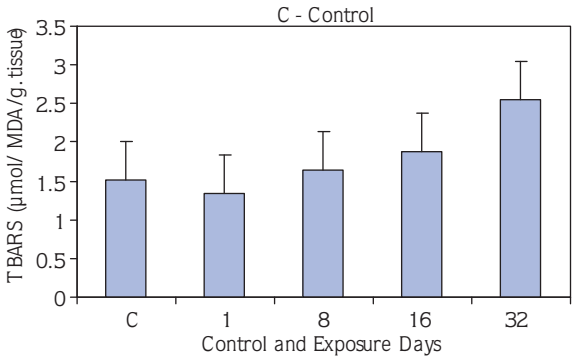

Figure 1. Level of TBARS in the liver.

more protein is required to protect cells against superoxide radicals. The superoxide dismutase level was found to be increased up to 16 days of exposure and then decreased at the 32nd day in the liver and kidney tissues (Figure 2). However, the hepatic superoxide dismutase showed increased activity when compared to kidney superoxide dismutase. This indicates the chronic stress developed by the heavy metals. The increased superoxide dismutase activity in the liver and kidney of the common carp (*Cyprinus carpio* L.) may be explained as a compensation mechanism against heavy metal intoxication, which was similar to the results observed with increased superoxide dismutase activity after exposure to pollutants (26).

The peroxy radical H_2O_2 was trapped by catalase that primarily occurs in peroxisomes. The target function of catalase is to protect the cells from the accumulation of H_2O_2 by dismuting it to form H_2O and O_2 or by using it as an oxidant where it works as a peroxidase. No discernible effects were observed in

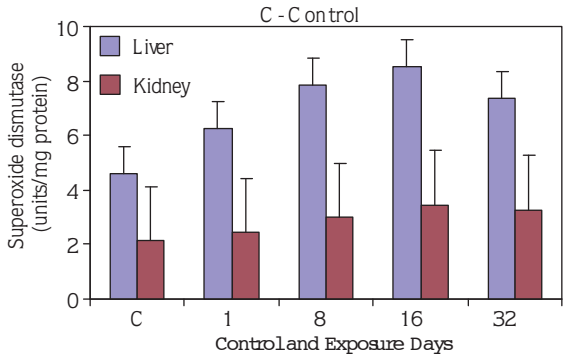

Figure 2. Level of superoxide dismutase in the liver and kidney.

the catalase activity of the liver initially, but after 8 days of exposure, the activity increased in the liver. Thereafter, the catalase activity gradually decreased (Figure 3). Similarly, the level of catalase activity gradually increased up to 16 days of exposure and then decreased at the 32nd day in the kidney tissues of the common carp (*Cyprinus carpio* L.). This indicates a reduced activity to protect the cells against H_2O_2. It was reported that the enhanced superoxide dismutase and catalase activities in the hepatocytes of the common carp (*Cyprinus carpio* L.) could be induced by microcystin (27). The induction of catalase in the liver was an adaptive response of the cells to mitigate the toxicity for the prolonged exposure of 32 days. Evidence suggested that high concentration of copper inhibited catalase activity in the liver, gills, and muscle (12).

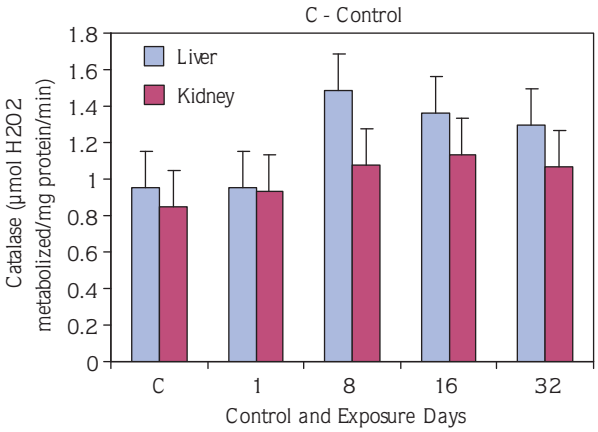

Figure 3. Level of catalase in the liver and kidney.

Glutathione peroxidase (GPx) is the most important peroxidase that has been postulated to protect the erythrocytes from damage by H_2O_2. It catalyzes the glutathione dependent reduction of hydroperoxides and of hydrogen peroxide. Therefore, it is hypothesized that this enzyme may protect tissues against oxidative damage due to lipid peroxidation. Environmental pollutants may induce glutathione peroxidase activity. The level of glutathione peroxidase in the liver was found to be increased gradually during the exposure period with heavy metals, which fell down at the end of the 32nd day. It was found to be slightly increased in the kidney on the successive exposure days, which caused a decrease

on the 32nd day of exposure (Figure 4). Significant increase in glutathione peroxidase activity was observed predominantly in the liver and kidney tissues of the carp. This indicates the protective role of the enzyme against lipid peroxidation, which also reflects similar findings with an increased level of glutathione peroxidase in microcystin induced toxicity (27). The liver is a major target organ for ingested oxidants that increase in glutathione peroxidase activity. This probably reflects an adaptation to the oxidative conditions to which the fish have been exposed (28).

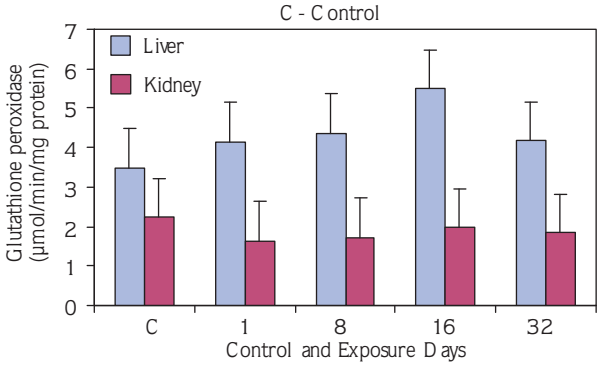

Figure 4. Level of glutathione peroxidase in the liver and kidney.

Glutathione-S-transferase (GST) is an abundant cytosolic antioxidant involved in conjugation of toxic reactive metabolites. The higher tripeptide content is involved in the activation of γ-glutamylcysteine synthetase, one of the enzymes involved in glutathione synthesis (29). The enzyme glutathione-S-transferase was found to be increased in the liver during the exposure days. The kidney enzyme activity slowly increased on the successive exposure days and later showed decreased activity on the 32nd day like other antioxidants (Figure 5). The higher glutathione-S-transferase activity observed in the liver of the carp after heavy metal toxicity indicates an augmented detoxification activity in the liver tissue. The kidney

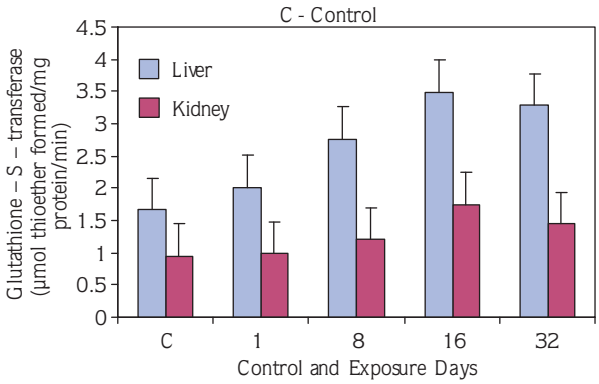

Figure 5. Level of glutathione-S-transferase in the liver and kidney.

also shows prominent response in glutathione-S-transferase activity, but less when compared to the liver. The glutathione-S-transferase detoxifies a number of environmental carcinogens, reactive nucleophile, and epoxides intermediates. The increased glutathione-S-transferase assay was suggested as a useful tool for biomonitoring oxidative stress (30). The findings reveal that heavy metals create harmful effects by generating reactive oxygen species that damage the cells by disturbing the fluidity balance. They also suggest that the heavy metals could make molecular complexes with cell protein thiols and develop toxic effects on the cells towards dysfunction. However, it was counter balanced by the production of antioxidants to suppress the free radicals and protect the cells against oxidative damage.

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