A New Record on Cadmium Chloride Induced Oxidative Stress Response In Fresh Water *Anabas Testudineus* Bloch

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ABSTRACT

The present work aimed to estimate the effect of different concentrations of CdCl$_2$ on antioxidant activities of freshwater fish, *Anabas testudineus*. 96-h LC$_{50}$ of CdCl$_2$ was 7ppm. Levels of liver protein content was increased after 14 days of exposure to different concentrations of CdCl$_2$ (2ppm, 3ppm and 4ppm). The hepatic SOD and CAT activity was significantly increased with in 7 days of exposure to all the three concentrations of CdCl$_2$ while the GST activity was decreased.

**Key words:** *Anabas*, CdCl$_2$, Super oxide dismutase, Catalase, Glutathione-S-Transferase.

1. Introduction

Pollution of aquatic environment arises from almost all current human activities, ranging from mining and processing of heavy metals, combustion of fossil fuels for transport, heating and electricity generation, pesticide using, food processing, production and disposal of domestic wastes, plastics and a wide variety of miscellaneous industrial processes. When there were chemicals accumulated in the water, it becomes unsuitable for drinking and other household purposes, irrigation and fish cultivation and also animal communities living in them may suffer seriously. Heavy metals exert a broad spectrum of effects on aquatic organisms, especially on the fish species. Heavy metals are soft but highly toxic as they compete for binding with essential metals and consequently they interfere with sulphydryl groups that play an important role for normal function of enzymes and structural proteins.

Heavy metals including cadmium (Cd$^{2+}$) have been shown to act as endocrine disrupting compounds in fish [1]. Endocrine disruptors are environmental chemicals that when absorbed in to the body either mimics or block hormones and disrupts the body’s normal physiological functions [2].
Out of the several heavy metals in the industrial waste streams, cadmium is reported to be associated with the effluents of battery, electroplating and metal finishing, mining and metallurgy and paints and dye industries [3]. Common compounds of cadmium include cadmium chloride [CdCl₂], cadmium oxide, cadmium sulphide and cadmium acetate. The target organs for Cd²⁺ toxicity have been identified as liver, placenta, kidneys, lungs, brain and bones in higher vertebrates [4]. Bio-enhancement of Cd²⁺ transfer along a food chain was studied by [5] and fish are reported to be used as biological indicators to assess water pollution [6].

The present work was aimed to determine the antioxidant effects of sublethal concentrations of CdCl₂ in freshwater Teleost, Anabas testudineus. Evidences are now accumulating that even low levels of pollutants can disrupt the functioning of the endocrine system of fish [7].

2. MATERIALS AND METHODS

2.1 Fish and Aquaria

Prior to experiment the animal model Anabas testudineus Bloch collected from local suppliers were acclimatized for two weeks in a glass aquaria filled with dechlorinated tapwater under laboratory conditions [natural photoperiod and temperature 26±2°C]. The fish were fed with protein rich feed on alternate days. Cadmium chloride [CdCl₂.H₂O RM – 469 – 100g purchased from HiMedia, Mumbai] was used as the test chemical and sublethal doses [80, 120 and 160ppm] of cadmium chloride were finalized after determining the LC₅₀ of Cd²⁺. After stipulated periods of exposure, fish were sacrificed. Then liver was excised straight away and frozen immediately at −80°C (NBS, USA) for enzymes assay. The blood was collected from caudal artery and centrifuged at 10,000 r.p.m for 10 minutes in a high speed refrigerated centrifuge [Eppendorf, Germany]. The supernatant was collected and kept in an ultra low freezer at -80°C until biochemical analysis.

2.2 Homogenate preparation and Protein measurement

The chilled liver tissue was blotted, weighed and a 10% homogenate of liver was prepared in cold sucrose (0.25M) using potter Elvehjem type homogeniser with teflon pestle (Arthur Thomas, USA) and centrifuged at 10,000 rpm for 10 minutes. All the operations were carried out at 4°C. The supernatant was collected and used for enzyme’s assay. The liver protein concentration for all the enzyme studies was determined using Bradford method.

2.3 Assay of Enzymes

Spectrophotometric determination of specific activities of hepatic antioxidant enzymes such as super oxide dismutase (SOD, EC.1.15.1.1), catalase (CAT EC.1.11.1.6) and glutathione S–transferase (GST EC.1.11.1.8) were assayed as per standard protocols.
2.4 Statistics
Data were collected from six animals in each group. Statistical analysis was done by SPSS statistical package. Data were analyzed by one – way analysis of variance, and groups that were not significantly different in Duncan’s multiple range tests were considered homogenous. Difference between groups was considered significant when $P<0.05$.

3. RESULTS
Exposure of fish to different concentrations of CdCl$_2$ revealed significant changes in the antioxidant enzyme activities studied at different periods of time in *Anabas testudineus* Bloch. The toxic impact of CdCl$_2$ on hepatic antioxidant enzymes were clearly analysed on the basis of the bio assay test, and in comparison with control fish. Cadmium chloride caused effect in the form of hyper hepatic proteinemia on exposure to all the three sublethal doses of CdCl$_2$ (2, 3 and 4mg/L) [Fig 1]. An increase in SOD and CAT activity was observed for all the three periods (7, 14 and 28 days) on exposure to higher doses of CdCl$_2$ (3 and 4mg/L) [Fig 2] and [Fig 3]. Activity of GST showed an increase at first and then decreased and restored to control value at the final period of exposure [Fig 4].

![Total Hepatic Protein Content](image1)

![Hepatic CAT activity](image2)
Fig. 1-4 represents total hepatic protein content, SOD, Catalase and GST activity respectively. The significant difference between the groups was analysed by one-way analysis of variance, mean values of groups with different superscripts letters are significantly different (P<0.05) as determined by Duncan’s multiple range test.

4. DISCUSSION

The present study clearly reveals that exposure to sublethal doses of CdCl₂ has specific influence on the activities of hepatic antioxidant enzymes in *Anabas testudineus*. Exposure to CdCl₂ significantly increased the liver protein content. Fish are responding to various stressors by a series of biochemical and physiological stress reactions, so called secondary stress responses comparable to those of higher vertebrates. Energy gain or loss in fish is controlled not only by carbohydrates but also by other
macronutrients like proteins. An exposure to the heavy metal toxicant CdCl₂ showed an increase in liver protein in *Catla catla* [8].

In the present study an increase in the liver protein content was observed. Protein is the major source of energy, material for growth and is responsible for the meaty flavour of the fish flesh [9]. Changes in the protein metabolism caused by the activation of the synthesis or by the protein degradation and the activation or inhibition of certain enzymes such as aspartate amino transferase [AST] and alanine amino transferase [ALT] were reported among the adverse effects caused in the liver by toxic substances [10].

An antioxidant is defined as any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate. Super oxide dismutase [SOD] is one of the key enzymes that clears reactive oxygen species [ROS] and avoids cellular oxidation damage *in vivo*. It has been demonstrated that SOD is concerned with the resistant pollution stress of organism. SOD catalyzes O₂⁻ dismutation to H₂O₂ and O₂ [11]. Catalase [CAT] is a major secondary antioxidant defense component that primarily works to catalyze the decomposition of H₂O₂ to H₂O. Glutathione S-transferase [GST] catalyses the conjugation of glutathione with a large number of compounds bearing an electrophilic center, the carcinogens and a variety of non substrate ligands [12].

Increase in the activities of antioxidants has been reported to be a general response of fish when exposed to aquatic pollutants and significant increase in antioxidant enzymes (SOD, CAT and GST) may be regarded as a protective mechanism that fish adopt on exposure to the heavy metal pollutant CdCl₂. In the present study a decrease in GST activity on exposure to higher dose of CdCl₂ was in agree with the previous findings in which the activities of GST in plasma and liver was significantly decreased due to CdCl₂ administration in male rats resulted from stress [13].

5. CONCLUSION

On the whole, the results of this study highlight the stress to which freshwater fish are exposed through the uncontrolled discharge of heavy metals in the aquatic environment. It could be concluded that cadmium chloride induced deleterious effect on the activities of antioxidant enzymes in fish. Cadmium can cause an induction or inhibition of a variety of cellular enzymes in fish

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7. REFERENCES


