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Original Research Paper

Effects of Acute Waterborne Cadmium Exposure on Activities of Antioxidant Enzyme and Acetycholinesterase in the Fish Crimson Red Snapper (*Lutjanus Erythropterus*)

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ABSTRACT

Fish are widely used as biological monitors of variations in environmental levels of pollutants. To understand how the fish antioxidant, neural systems respond to the oxidative stress under acute Cd^{2+} exposures (0.005 mg/L, 0.025mg/L, 0.05mg/L, 0.25mg/L), the superoxide dismutase (SOD) activities and lip peroxidation (MDA) contents in the liver and gill, and acetycholinesterase (AChE) activities in the brain of *Lutijanus erythropterus* were measured respectively. The results showed that the hepatic SOD activities were induced significantly ($p<0.01$) on 24 and 48 hours of cadmium exposure, but inhibited on 168 hours ($p<0.01$), and impaired after 96 hours exposure from the content of MDA except the group of 0.005mg/L Cd^{2+}. While the SOD activities of branchial tissues were earlier than that of the hepatic in response to the cadmium exposure (about 6 and 12 hours), but the oxidative damage to the gills was less than the liver on 168 hours exposure. In addition, the activities of AChE in brain tissues were disturbed during 96 hours exposure, and inhibited on 168 hours exposure, which may have relations with the oxidative stress resulted from the cadmium exposure.

INTRODUCTION

The toxicity of heavy metals like cadmium, mercury, plumbum, etc. have been wide concerning threats to the coastal waters and human health due to the rapid development of Chinese industry (China 2010). Once they enter the water system will exert toxic effects on the receiving environment and also on the performance of biological physiological process (Aksu 2005), such as reducing considerable structural and functional change of protein and altering activity of enzymes by binding to their functional groups (sulphydryl, carboxyl, imidazol, etc.) (Risso-de 2000). Cadmium is a by-product of zinc production, and has been used for a long time in industry, the accumulation of Cd^{2+} in human body (principally in kidney and liver) can cause renal dysfunction and bone disease such as Itai-Itai (Cui 2005, Nordberg 1996). It is also carcinogenic in humans and laboratory organisms.

Monitoring the state of aquatic systems has traditionally been largely based on measurements of concentrations of contaminants in sediment and water column. However, the chemical monitoring alone is no longer considered meaningful or cost-effective (Pereira 2010). Additionally, it has become evident that measuring concentrations in tissues does not provide information needed to assess biological effects that contaminants may cause in organisms (Lehtonen 2006). Fish are widely used as biological monitors of pollution status in environmental levels of anthropogenic pollutants (Martinez 1999) due to their "early warning" role of biological effects (van der Oost 2003).

Heavy metals are capable of inducing oxidative stress in aquatic animals by distributing the antioxidants efficiency and enhancing the intracellular reactive oxygen species (ROS), which often prelude in DNA damage, lipid peroxidation (LPO) and enzyme inhibition (van der Oost 2003). The activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), etc. have been extensively used as biomarkers of oxidative stress (Ahmad 2008, Ahmad 2004, Ahmad 2006, Guilherme 2008, Pereira 2010). LPO estimation has also been found to have a high predictive value as a biomarker of effect (Guilherme 2008). Despite the numerous studies that have been conducted to date (Pedersen 1997), some key issues still remain to be addressed before biomarkers can be incorporated into routine environmental management protocols as reliable ecotoxicological tools. To our knowledge, few studies have been done on how the fish antioxidant system responds to the acute oxidative stresses caused by waterborne Cd^{2+} exposure.

Crimson red snapper (*Lutjanus erythropterus*) is an important marine food fish species in China with great potential for marine cage culturing industry. The present study was undertaken to elucidate the effects of acute Cd^{2+} exposures on activities of antioxidant enzyme and acetylcholinesterase (AChE), and to improve the potential application of them as biomarkers in marine environmental monitoring and risk assessment.

MATERIALS AND METHODS

Chemicals: All of chemicals used in this study were of analytical grade and purchased from Guangzhou Chemical Corp. Cadmium chloride ($CdCl_2$) was used to prepare the metal solutions.

Animals and treatments: Crimson red snapper were provided by Boyu Fish Breeding Farm, Sanya City (Hainan Province, China). The fish collected were of comparable size (4.95±0.79 cm) and weight (4.57±2.02 g). Glass of fish tanks of 50 L were used to accommodate the fish. The pH value of water was 7.7±0.1, and temperature was 26.60±0.82°C. The water in tanks was constantly aerated. During the acclimatising period, only the pure fish food was fed at 5.0 g per day per tank. To keep the water fresh, half of the water in each tank was replaced by newly prepared water every day after the fish had eaten up all the food. The total mortality of fish was 2% during the ten days acclimation period.

After acclimation to laboratory conditions, the fish were randomly divided into 5 groups. Each group consisted of 50 fish in one fish tank. Group 1 was the negative control, Groups 2, 3, 4 and 5 were diluted by the concentrations of Cd^{2+} with 0.005mg/L, 0.025mg/L, 0.05mg/L, 0.25mg/L respectively.

Tissues of gills, liver and brain were dissected out, rinsed by physiological salt water to remove blood remnants and stored at -70°C until analysis.

Biochemical determinations: The tissue was finely cut and about 0.20 g (total of 1.0g) of liver tissue from each fish was homogenized in 4 mL of 10 mM tris-sucrose buffer (pH 7.5, 0.01 M tris, 0.25 M sucrose and 0.01 M EDTA) in 1:4 wet wt./buffer volume ratio proportions using a glass douncer on ice. The homogenate obtained was then centrifuged at 7,000 rpm for 10 min at 4°C and the supernatants were immediately used as sources of protein content determination and activities of enzymes.

SOD activity was determined by an indirect method involving the inhibition of cytochrome reduction (McCord 1969). The reduction of cytochrome C by O_2^- was monitored by the absorbance increase at 550nm during 1 min (McCord 1969). The results of this enzymatic assay are given in units of SOD activity per milligram of protein (U mg^{-1}) where 1 U of SOD is defined as the amount of sample causing 50% inhibition of cytochrome C reduction (Barata 2005).

Lipid peroxidation was assessed by the thiobarbituric reactive species (TBARS) assay (Ohkawa 1979). Measurement of TBARS was carried out following the method of Correia et al. (2003) and Oakes & Van der Kraak (2003). TBARS concentrations were derived from an external standard curve of 1,1,3,3-tetramethoxypropane (also referred as malonadehyde acid; MDA) and the values expressed in nmols of MDA equivalents mg $proteins^{-1}$.

AChE activity was measured in brain tissue according the colorimetric method of Ellmann et al. (1961) and Barata (2005) using the acetylthiocholine as substrate. Enzymatic activity was measured in 5 min (linearity) at 37°C in a UV spectrophotometer fixed at 410 nm. Activity was calculated as micromoles of substrate hydrolysed $min^{-1}mg$ $proteins^{-1}$.

Protein concentration of tissues was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard.

All enzyme activity data were normalized by the total protein content in respective tissues.

Statistical analysis: The 96h LC_{50} were analysed using the probit-regressed methods, of which intervals were regulated with Feiller's test. And the correlation of equation was tested with Pearson correlation coefficient. Data were expressed as mean ± standard deviation (± SD).

Two-way analysis of variance was used to determine treatment and time effects on the various parameters studied. Mean separation was accomplished with Fisher PLSD test. The significance level in all instances was $p < 0.05$. SPSS 13.0 software packages were used for all statistical analyses.

RESULTS AND DISCUSSION

Doses and acute toxicity of cadmium: Behaviour provides a unique perspective linking physiology and ecology of an organism and its environment (Little 2001). To date, there are no standardized species or groups of species used for aquatic behavioural toxicology testing. Therefore, preliminary observations and assays are required to determine the feasibility of a particular species, and if aberrant behaviour patterns can be associated with specific exposure scenarios (Kane 2005). Also it is critical to carefully document observations of normal baseline behaviour under controlled conditions prior to behavioural testing with a chemical (Kane 2005). The behavioural alternations observed in *Lutjanus erythropterus* with exposure of cadmium were similar. Once exposed, the fish started flashing, after 12 hours or longer exposure, some lose the orientation and showed the abnormal swimming behaviours such as cockscrew swimming and

other erratic swimming. After 24 or 48 hours exposure, some swam much slower, followed by solitary time spent on or near the bottom. All the alternations were observed under concentrations exposure except the negative control group, and the quantities of abnormal fish increased with the elevation of concentration of cadmium.

The LC_{50} was 2.658mg/L in 96 hours of exposure. So, the experiential safety concentration i.e., 0.1 × 96h LC_{50} equalled 0.26mg/L.

Response of antioxidant system: Most organisms have baseline levels of antioxidant systems, involved in a variety of detoxification reactions, to assure the maintenance of balance between production and removal of endogenous ROS and other pro-oxidants (Barata 2005). Although one of the important features of antioxidant enzyme systems is their inducibility under conditions of oxidative stress, higher, equal or lower activities of various antioxidant enzymes have been observed in polluted compared to cleaner areas (Barata 2005), suggesting that antioxidant enzyme responses are transient and variable for different species, enzymes and chemicals (Livingstone 2001).

Enzymatic activities are regarded as fast prognostic indices of individual reaction to environmental stress, and should allow prediction of the consequences of pollution (Depledge 1994). The relationship between contaminant toxicity in animals, free radical processes and defensive responses of free radical scavenging system are thought to be crucial (Livingstone 1997).

Cadmium ions affected the hepatic antioxidant system. Fig. 1 indicated that during the first 12 hours exposure, the hepatic SOD activities were lower than the control group, but had no significant difference in 6 hours exposure in all. After 24 hours exposure, the hepatic SOD activities were induced significantly (p<0.01), which indicated the antioxidant system began to response to the acute cadmium stress. The results on 168 hours exposure suggested that the hepatic SOD activities were inhibited significantly (p<0.01), indicating that the hepatic antioxidant systems had been impaired to some extent under concentrations of cadmium exposure. The Fig.1 reflected the lipid peroxidation (LPO), suggesting that during the first 12 hours exposure, the hepatic tissues were not damaged in all. When exposed for 24 hours to 48 hours, the hepatic tissues were protected from ROS due to the activation of the antioxidant enzymes (such as SOD), although the concentrations of MDA varied. When the exposure was longer than 96 hours, the MDA were higher significantly; the hepatic tissues may have been damaged under conditions of cadmium due to the inefficient work of antioxidant enzymes.

Compared to the hepatic antioxidant system, the branchial system had its characteristics. The SOD activities were induced to some extent at the first 6 and 12 hours exposure, indicating that the branchial antioxidant system worked earlier than the hepatic to the cadmium exposure. The branchial SOD activities were inhibited on 12, 24, and 168 hours exposure, suggesting that the higher concentrations of cadmium inhibited the SOD activity. When exposed to 96 hours, the higher SOD activities indicated the protection of antioxidant enzyme to the gills have closed to the threshold of antioxidant system. When exposed to 196 hours, the branchial SOD activity was inhibited significantly (p<0.01). The subfigure MDA (gills) in Fig.1 showed that the branchial tissues were impaired after 48 hours of cadmium exposure, but the oxidative stress to the gills was less and earlier than to the liver, which accorded with the fact that the branchial tissues were just the main passage of cadmium ions but not the target organs (such as liver, kidney, etc.).

Inhibitory effects on the AChE: AChE activity was one of the most common biomarkers of neurotoxicity used in aquatic organisms (Durieux 2010). Studies have shown AChE inhibition caused by heavy metals (Bocquene 1995). The AChE activities in the brain tissues (Fig. 2) had no significant decrease on the first 6 and 12 hours exposure, except the highest cadmium concentrations (0.25mg/L) on 12 h (p<0.01). With the increase of concentrations of cadmium ions and exposure time, the activities of cerebric AChE were induced on 24h and 96h, but inhibited significantly on 48h and 168 hours exposure. This indicated that the cadmium caused the disturbance of AChE in brain tissues, which affected the *L. erythropterus* normal metabolism and physiochemical process.

CONCLUSIONS

In conclusion, under concentrations of acute Cd^{2+} exposure for 24 and 48 hours, the hepatic SOD activities were induced significantly (p<0.01), and began to be inhibited on 168 hours (p<0.01); the hepatic tissues were impaired after 96 hours exposure. While the SOD activities of branchial tissues elevated to some extent at the first 6 and 12 hours exposure, which worked earlier than the hepatic to the cadmium stresses, but the oxidative damage to the gills was less than to the liver on 168 hours exposure. The activities of AChE in brain tissues were disturbed on 96 hours exposure, and inhibited on 168 hours exposure. With the elevation of cadmium concentrations, the stress of oxidative to the antioxidant system, lip peroxidation and AChE enlarged in all. Therefore, considerations on the concentration of pollutants and exposure/response time should be taken to select the applicable biomarkers.

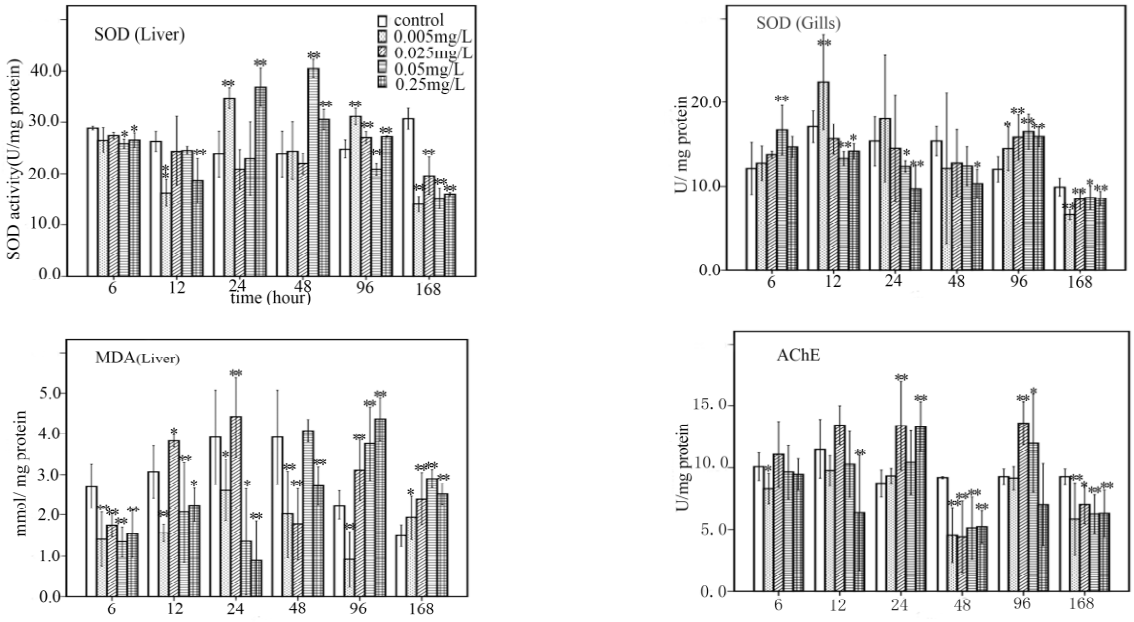

Fig.1: Antioxidant enzymatic activities and MDA contents in *L. erythropterus* exposed to Cd concentrations, ** indicates the value is significantly different from that of the control (p<0.01) and * means p<0.05.

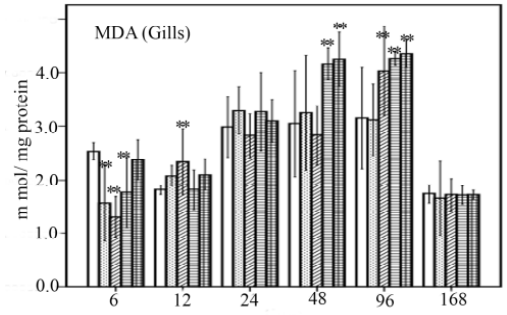

Fig. 2 Activities of AChE in the brain tissues of *L. erythropterus* exposed to Cd concentrations, ** and * mean p<0.01 and p<0.05.

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REFERENCES

Ahmad, I., Maria, V.L., Oliveria, M., Serafim, A., Bebianno, M.J., Pacheco, M. and Santos, M.A., 2008. DNA damage and lipid peroxidation vs. protection responses in the gill of *Dicetrachus labrax* L. from a contaminated coastal lagoon (Ria de Aveiro, Portugal). Science of The Total Environment, 406(1-2): 298-307.

Ahmad, I., Pacheco, M. and Santos, M.A., 2004. Enzymatic and non-enzymatic antioxidants as an adaptation to phagocyte-induced damage in *Anguilla anguilla* L. following *in situ* habor water exposure. Ecotoxicology and Environmental Safety, 57: 290-302.

Ahmad, I., Pacheco, M. and Santos, M.A., 2006. *Anguilla anguilla* L. oxidative stress biomarkers: An *in situ* study of freshwater wetland ecosystem (Pateria de Fermentelos,Portugal). Chemosphere, 65: 952-962.

Aksu, Z. 2005. Application of biosorption for the removal of organic pollutants: A review. Process Biochem., 40: 997-1026.

Barata, C., Lekumberri, I., Vila-Escal, M., Prat, N. and Porte, C. 2005. Trace metal concentration, antioxidant enzyme activities and susceptibility to oxidative stress in the tricoptera larvae *Hydropsyche exocellata* from the Llobregat river basin (NE Spain). Aquatic Toxicology, 74(1): 3-19.

Bocquene, G., Bellanger, C., Cadiou, Y. and Galgani, F. 1995. Joint action of combinations of pollutants on the acetylcholinesterase activity of several marine species. Ecotoxicol., 4: 266-279.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities protein utilising the principle of protein-dye-binding. Analitycal Biochemistry, 72: 248-254.

China, S.O.A.P.s.R.o. 2010. China's 2009 Communique on Marine Environment Quality. http://www.soa.gov.cn/hyjww/hygb/hyhjzlgb/A020707index_1.htm.

Correia, A.D., Costa, M.H., Luis, O.J. and livingstone, D.R. 2003. Age-related changes in antioxidant enyme activities, fatty acid composition and lipid peroxidation in whole body *Gammarus lacusta* (Crustacea: Amphipoda). J. Exp. Mar. Biol. Ecol., 289: 83-101.

Cui, Y., Zhu, Y.G., Zhai, R., Huang, Y., Qiu, Y. and Liang, J. 2005. Exposure to metal mixtures and human health impatcs in a contaminated area in Nanning, China. Environment International, 31: 784-790.

Depledge, M.H. and Fossi, M.C. 1994. The role of biomarkers in environmental assessment (2) Invertebrates. Ecotoxicology, 3: 161-172.

Durieux, E.D.H., Farver, T.B., Fitzgerald, P.S., Eder, K.J. and Ostrach, D.J. 2010. Natural factors to consider when using acetycholinesterase activity as neurotoxicity biomarker in Young-Of-Year striped bass (*Morone saxatilis*). Fish Physiology and Biochemistry, 37: 21-29.

Ellman, G.L., Courtey, K.D., Valentino Andres jr. and Featherstone, R.M.

1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemistry Pharmacology, 7(2): 88-90.

Guilherme, S., Valega, M., Pereira, M.E., Santos, M.A. and Pacheco, M. 2008. Antioxidant and biotransformation responses in Lia aurata under environmental mercury exposure - relationship with mercury accumulation and implications for public health. Marine Pollution Bulletin, 56: 845-859.

Kane, A.S., Salierno, J.D. and Brewer, S.K. 2005. Fish models in behavioral toxicology: Automated techniques, updates and perspectives. In: G.K. Ostrander (Ed.), Techiques in Aquatic Toxicology, Taylor & Francis, Baltimore, Maryland, pp. 559-582.

Lehtonen, K.K. and Schiedek, D. 2006. Monitoring biological effects of pollution in the Baltic Sea: Neglected-but still wanted? Marine Pollution Bulletin, 53: 377-363.

Little, E.E. and Brewer, S.K. 2001. Neurobehavioral toxicity in fish. In: D. Schlenk and W.H. Benson (Eds), Target Organ Toxicity in Marine and Freshwater Teleosts, New Perspectives: Toxicology and the Environment. Taylor & Francis, London, pp. 139-174.

Livingstone, D.R. 1997. Contaminated-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Pollut. Bull., 42: 656-666.

Livingstone, D.R. 2001. Contaminated-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Pollut. Bull., 42: 656-666.

Martinez, M., Del Ramo, J., Torreblanca, A. and Diaz-Mayans, J. 1999. Effect of cadmium exposure on zinc levels in the brine shrimp *Artemia parthenogenetica*. Aquaculture, 172(3-4): 315-325.

McCord, J.M. and Fridovich, I. 1969. Superoxide dismutase: An enzymatic function for erythrocuprein (hemocuperin). J. Biol. Chem., 244: 6049-6055.

Nordberg, G.F. 1996. Current issues in low-dose cadmium toxicology: Nephrotoxicity and carcinogenicity. Environemntal Science, 4: 133-147.

Oakes, F.D. and Van der Kraak, V.D. 2003. Utility of TBARS assay in detecting oxidative stress in white sucker (*Catotomus commersoni*) populations exposed to pulp mill effluent. Aquatic Toxicology, 63: 447-463.

Ohkawa, H., Ohishi, N. and Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Pedersen, S.N., Lundebye, A.K. and Depledge, M.H. 1997. Field application of metallothionein and stress protein biomarkers in the shore crab (*Carcinus maenas*) exposed to trace metals. Aquatic Toxicology, 37 (2-3): 183-200.

Pereira, P., Pablo, H.D., Vale, C. and Pacheco, M. 2010. Combined use of environmental data and biomarkers in fish (*Liza aurata*) inhabiting a eutrophic and metal-contaminated coastal system - Gills reflect environmental contamination. Marine Environmental Research, 69(2): 53-62.

Risso-de Faverney, C., Lafaurie, M., Girard, J.P. and Rahmani, R. 2000. Effects of heavy metals and 3-methylcholanthrene on expression and induction of CYP1A and metallothionein levels in trout (*Oncorhynchus mykiss*) hepatocyte cultures. Environ. Toxicol. Chem., 19: 2239-2248.

van der Oost, R., Beyer, J. and Vermeulen, N.P.E. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environmental Toxicology and Pharmacology, 13(2): 57-149.