Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

Int. J. Modn. Res. Revs.

Volume 2, Issue 12, pp 569-572, December, 2014

ISSN: 2347-8314

ORIGINAL ARTICLE

EFFECT OF CADMIUM CHLORIDE ON ANTIOXIDANT STATUS IN LIVERS TISSUE OF FRESH WATER FISH, *OREOCHROMIS MOSSAMBICUS*

***T.Vivekananthann**

Department of Zoology, Annamalai University, Annamalai Nagar-608002, Tamilnadu

Article History: *Received 11th Nov,2014, Accepted 30th Dec,2014, Published 31st December,2014*

ABSTRACT

In the present study the level of lipid peroxidation, reduced glutathione (GSH), glutithione peroxidase (GPx), catalase (CAT) and superoxide dimutase (SOD) were observed in both control and cadmium exposed fish, Oreochromis mossambicus. The present study showed the level of lipid peroxidation was increased and reduced glutathione (GSH), glutithione peroxidase (GPx), catalase (CAT) and superoxide dimutase (SOD) were decreased in control and cadmium exposed fish. The present study concludes that cadmium chloride could damage the liver tissue in the fresh water fish, *Oreochromis mossambicus*

.**Keywords:** Cadmium chloride, *Oreochromis mossambicus*, Liver, lipid peroxidation, antioxidants

.

1.INTRODUCTION

The pollution of ecosystems by heavy metal is a world wide problem (Bryan, 1976.) Industrialization, Population growth and the resulting waste material lead to pollution of the environment in air, water, soil and living organisms. The chief sources of the waste matter are automobile emissions, industrial effluent, household chemicals released into sewage system etc. These chemicals entering the ecosystem affect man, animal life, plant life and materials and exert serious health and ecological problems (Ober *et al*.,1987).

Heavy metals are widely found in natural environment mostly representing severe health hazards in organism [Bamennan and Schiesty, 1996]. The toxicological effects of pollution are due to their high persistence and accumulation in the organisms [Goyer, 1996]. Although suitable concentration of heavy metals play a vital role in metabolic pathways when their concentration exceed the threshold level, they act as physiological biochemical and behavioral inhibition in the organisms.

Cadmium is major contaminants of aquatic environments that are toxic towards aquatic organisms (Munger *et al*., 1999) even at concentrations found in natural waters (Pelgrom *et al.,* 1994). Metals are present in very low concentrations in natural aquatic ecosystems (Nussey, 1998). The most important heavy metals in water pollution are zinc, copper, lead, cadmium, mercury, nickel and chromium (Abel, 1989; Seymore, 1994; Viljoen, 1999).Cadmium is found in the effluent of fertilizer, electroplating, pigment and paint industries. It is found in the effluent of metal plating, tanning, rubber and photographic industries and also found in the effluent of plating and electrical industries(Pelgrom *et al.*, 1994).

Corresponding author ***Dr.T. Vivekananthan****, Asst. Professor, Dept. Of Zoology, Annamalai University-608002*

Fishes are sensitive to contaminants of the water and pollutants may damage certain physiological and biochemical processes when they enter the organs of the fish(Tulasi *et al*.,1992). The heavy metal in the tissue of fishes may cause various physiological defects and mortality(Torres *et al*.,1987). The fishes which are largely being used foe the assessment of the quality of the aquatic environment and can cause bioindicator of environmental pollution (Dautrempuits etal.,2004;Lopes*et al*.,2001).

Liver is one of the most multifaceted and active organs in higher animals. In a vertebrate body, the liver is the most important target organ as it is the chief metabolic and detoxification center. It is the site for numerous and varied metabolic activities, including synthesis of bile which contains bile salts, bile pigments, cholesterol and lecithin. All toxins pass through liver at some point or the other; the liver may manifest the highest toxin concentrations as also the most clearly discernible structural and functional impacts. Within the hepatic cells, virtually all the reactions or intermediary metabolism take place.

2.MATERIALS AND METHODS

Procurement of experimental animal

The fresh water fish,*Oreochromis mossambicus* were collected from the fish farm located in Puthur,Nagai District, 15 Km away from the Uinversity campus. This fishes were brought to the laboratory and transferred to the rectangular fibre glass tanks (100X175cm) of 500liters capacity containing chlorine free aerated well water.

Acclimatization of animals

The fresh water fish, *Oreochromis mossambicus* were acclimatized for a minimum period of 15 days in the laboratory

conditions at room temperature(28±1ºC) before subjecting them for screening test. These fingerlings were fed with artificial food pellets on alternative days and the water renewed every 24 hours. The tanks were rinsed with potassium permanganate or acroflavine (2mg/l) to prevent fungal attack. The fresh water fish, *Oreochromis mossambicus* were critically screened for the signs of disease, stress,physical damage and mortality. The injured, severely diseased, abnormal and dead fishes were discarded. The feeding was discontinued 24 hours before the beginning of the experiment to reduce the excretory products in the test trough as suggested by Arrora *et al*., (1972). During the acclimatization, the fishes were reared in tank until there was less than 10 percent mortality in 4 days perior to the beginning of the test as suggested by Anderson(1977). The water in the experimental trough was changed daily and also aeration was stopped to avoid the possible oxidation of the toxicants.

Selection of cadmium chloride

The toxicant,cadmium chloride was used for the present experimental studies. It is rarely found in pure state, it is present in various types of rocks and soils and in water as well as in coal and petroleum. The sulfate, nitrate and halides are soluble in water. In the present investigation cadmium chloride has been selected for the present experimental study.

Experimental design

The toxicant exposure was done by 24 hour or renewal bioassay system. For analysis sublethal toxicity, 3 groups of 10 fish each were exposed separately and cadmium chloride (6ppm : 10 % 96 hours LC_{50}). Solution prepared in well water. The experimental medium was prepared by dissolving cadmium chloride at 6 ppm having dissolved oxygen 5.8 ppm, PH7.4, water hardness 30.3mg/l (APHA *et al*., 1992) and water temperature 28± 2 C. Each group was exposed to 50 l of the experimental medium. Parallel groups of 10 fish each were kept in separate aquaric containing 50 l of well water as control. Feeding was allowed in the experimental as well as control groups every day for a period of 3 hours. Before the reneval of the medium through out the tenature of the experimental.

Estimation of LC_{50} value

Perior to the commencement of the experiment, 96 hr medium lethal concentration as (96 hr LC_{50}) of cadmium choloride for oreo choromis mossambicus was estimated (Hamilton *et al* 1977). And 24 hrs renewal bioassay system and was found to be 60 ppm after 5% trimming.

BIOCHEMICAL STUDIES

After experiment, the fish each from the respective experimental as well as control groups were sacrificed. The gills, liver and kidney were isolated from the fish and used for various study. The lipid peroxidation was estimated by the method of Nichans and Samuelson (1968). The level of reduced glutathione was determined by the method of Beutler and Kellay, (1963).The level of glutathione peroxide activity was determined by the method of Rotruck *et al*. (1973).The activity of catalase was determined by the method of Sinha (1972).SOD activity was assayed by the method of Kakkar *et al*. (1984).

Statistical analysis

Statistical significance was evaluated by using ANOVA followed by Duncan Multible Range Test (DMRT) Duncan (1957).

3.RESULTS

The level of lipid peroxidation exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of cadmium. The lipid peroxidation in liver tissue was found to be increased for 7 days and 14 days respectively. The percent change over control were 42.18 and 5/8.63 respectively (Table1).

The level of glutathione exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of cadmium. The glutathione in liver tissue was found to be decreased for 7 days and 14 days respectively. The percent change over control were -19.07 and-31.49 respectively (Table 1).

The level of glutathione peroxidase exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of cadmium. The glutathione peroxidase in liver tissue was found to be decreased for 7 days and 14 days respectively. The percent change over control in the muscle were -24.20 and -35.66 respectively (Table1).

The level of lipid peroxidation exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of cadmium. The catalase in liver tissue was found to be decreased for 7 days and 14 days respectively. The percent change over control were -24.20 and -41.67 respectively (Table1).

The level of superroxide dismutase exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of cadmium. The superroxide dismutase in liver tissue was found to be decreased for 7 days and 14 days respectively. The percent change over control were -17.9442.18 and -33.42 respectively (Table1).

Table 1 Level of lipid peroxidation(LPO) and antioxidants in the liver tissue of fresh water fish *Oreochromis mossambicus* exposed with sub-lethal concentration of cadmium chloride

Tissues	Control	7 days	14days
Lipid peroxidation (nmoles/g wet wt.of tissue)	0.851±0.65	1.21±0.75* 42.18	1.35±0.25* 58.63
Reduced glutathione µmoles/g wet wt.of tissue)	23.75±1.82	19.22±0.12* -19.07	16.27±0.56* -31.49
Glutathione Peroxidase(µmoles/mg protein /min)	0.157±0.33*	0.119±0.87* -24.20	0.101±0.38* -35.66
Catalase(µmoles/mg protein /min)	8.59±0.11*	6.51±0.99 -24.21	5.01±0.87* -41.67
Superoxide dismutase(units /mg protein)	7.69±1.01	6.31±29* -17.94	5.12±0.15* -33.42

Mean ± S.D. of six individual observation * Significance ($p<0.05$) Group I compared with group II and III.

4.DISCUSSION

Lipid peroxidation (LPO) is a chemical mechanism capable of disrupting the structure and the function of the biological membranes that occurs as a result of free radical attack on lipids. The LPO may also play an indirect role in the conversion of procarcinogen to ultimate carcinogens (Bagchi et al., 1996, 1996). The LPO is measured as TBARS is the end product of lipid peroxidation. The level of TBARS of the tissue was increased with increasing dose of heavy metal, causing tissue damage (Hwang and Wang, 2001).

Antioxidants such as GSH, SOD, CAT and GP_X are the main defense against O_2^- and H_2O_2 mediated injury. Antioxidants both in enzymatic and non-enzymatic, together with the substance that are capable of either reducing ROMs or preventing their formation, form a powerful reducing buffer which affects the ability of the cell to counteract the action of oxygen metabolites

forming the protective mechanism which maintains the lowest possible level of the ROMs inside the cell (Sies, 1993). In view of this, the present work has been designed to evaluate the lipid peroxidation and antioxidant status in the selected tissues of *Oreochromis mossambicus* exposed to sublethal concentration of cadmium chloride.

Heavy metals such have the ability to produce reactive oxygen species, resulting in lipid peroxidation, and, depletion of sulfhydryl groups (Stohs and Bagchi, 1995).The increased lipid peroxidation caused by mercury also leads to the formation of hydroperoxides that are removed by GSH with the help of GPx. Both the reactions lead to depletion of GSH. The toxicant may inhibit the enzymes directly impairing the functional groups or indirectly rendering the supply of glutathione.

Heavy metals accumulated in the tisses of fish may catalyze reactions that generate reaqctive oxygen species (ROS) which may lead to environmental oxidative stress. Defensive mechanisms to counteract the impact of ROS are found in many mammalian species including aquatic animals such as fish. These systems include various antioxidant defense enzymes such as superoxide dismutases which catalyze the dismutation of superoxide radical to hydrogen peroxide, catalyzed action on hydrogen peroxide, glutathinone S-transferase family possessing detoxifying activities towards lipid hydroperoxides generated by heavy metals (Tjalkens *et al*.,1998). Heavy metal promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxides. The ROS enhances the peroxides and reactive hydroxyl radicals (Miller *et al*, 1991; Hussan *et al*, 1999). These lipid peroxides and hydroxyl radicals may cause cell membrane damage and thus destroy the cell. Heavy metal also inhibits the activities of free radicals quenching enzymes such as catalase, superoxide dismutase and glutathione peroxidase (Benov *et al*., 1990). It is well known that mercury increases the level of oxygen reactive intermediates in tissues and cells by the depletion of their cellular antioxidant systems (Stacey and Kappus, 1982; Christie and Casta, 1984; Woods, 1988).

In the present study, the level of glutathione and GPx, CAT, SOD significantly decreased but the level of LPO content increased in the gill, liver and kidney tissues of *Oreochromis mossambicus* when treated with sub-lethal concentration of cadmium chloride for 7days and 14days. This results could be rerlated to the alteration in the antioxidants enzyme activities and lipidperoxidation which may cause biochemical dysfunction in the tissues.The present study showed that the increased level of LPO content suggested that the excess production of ROS by mercury toxicity might be explained by its ability to produce alteration in mitochondria by blocking the permeability transition pore (Nilcoli *et al.*, 1995) and alteration in mitochondrial electron transport chain. These events cause the oxidative phospharilation uncoupling and subsequent increase in ROS production (Salducci *et al.*, 1999).

Farombi *et al*.,(2007) reported that the levels of heavy metals on biomarkers of oxidative stress as surrogate bioindicators of aquatic pollution in *Clarias gariepinus.* Sies,(1993) reported that the depletion in GSH and GPx result in the involvement deleterious oxidative changes due to accumulation of heavy metal.The heavy metals which led to induction of lipid peroxidation and alteration in the antioxidant enzymes in the organs of the fish. The concentration of heavy metal was increased in the giil,liver and kidney tissues. This may be due to the fact that gills serve as the respiratory organ in fishes through which metal ions are absorbed (Bebianno *et al*.,2004).

Farombi *et al*.,(2007) indicated significant elevation of lipid peroxidation and decreased antioxidant level in all the organs. The appa2007rent increase in lipid peroxidation may be attributed to the accumulation of the heavy metals in the organs of heavy metals in the various organs. Metal catalyzed formation of ROS capable of damaging tissues such as DNA, proteins and lipids is well documented (Pandey *et al*.,2003; McCord,1996).

Farombi *et al*.,(2007) the activities of SOD, GST and the redox sensitive thiol compound GSH were elevated in all the organs. The increasing level of lipid peroxidation in these organs may be a response to oxidative stress caused by the presence of heavy metals. The accumulation of heavy metals might have led to the production of superoxide anions which led to H_2O_2. SOD catalytically scavenges superoxide radical which appears to be an important agent of toxicity of oxygen and this provides a defense against this aspect of oxygen toxicity. (Kadar *et al*.,2005). GSH is known to be a substrate for the activity of GST. The apparent increase in GSH levels with concomitant elevation in the activity of GST in the organs suggests an adaptive and protective role of this biomolecule against oxidative stress induced by the heavy metals. The decreased CAT activity may be due to the flux of superoxide readicals which have been shown to inhibit CAT activity (Stanic *et al*.,2005).

The present study showed enhanced LPO and decreased activities of antioxidant enzymes in various tissues of mercury intoxicated rats. The decreased activities of antioxidant represent increased utilization due to oxidative stress (Santhakumari *et al.*, 2004). SOD is an important defense enzyme, which converts superoxide radicals to hydrogen peroxide (Rana *et al*.,1996). CAT is a heme protein, which decomposes hydrogen peroxidase and protects the tissue from highly reactive hydroxyl radicals (Chance *et al*, 1982). The reduction of these enzymes may be due to oxidative stress of metal intoxication. The decreased level of protein content in all tissues also confirm the reason for the depletion of this enzyme.

Iasmach *et al*., (2000) have reportedthat the study of changes in the activities of some antioxidants enzymes and the level of lipid peroxidation as an index of pollution using fish from Warri and Ethioper Rivers. Studies on antioxidant status during a free radical challenge can be used as an index of protection against the development of lipid peroxidation in experimental animals (Banerjee *et al*., 1999; Banerjee, 1999).

The antioxidant enzymes SOD and GSH-Px are active scavengers free radicals, and hence are involved protecting against potential cell injury and neuropathological conditions (Hussain *et al*., 1999). Dorval *et al*., (2005) have studied the altered antioxidant (CAT, GPX and GSH), lipid peroxidation and plasma cortisol, and thyroid hormone levels in fish sampled in areas impacted by agrichemicals.

5.REFERENCES

Bagchi,D., Bagchi,M., Hassoun, E.A., Stohs,S .J., 1996. cadmium-induced excretion of winary lipid metabolites, DNA damage, glutathione depletion, and hepatic lipid peroxidation in Sprague-Dawley rats. Biol. Trace Elem. Res. 52,. 143-154.

Banerjee B.D, 1999. The influence of various factors on immune toxicity assessment of pesticide toxicity assessment of pesticide chemical, Toxicol letters 107.21.

Banerjee B.D., Seth V, Bhattacharya A and Chakraborthy A. 1998. Oxidative stress in human poisoining cases following malathion and propoxur ingestion, Toxicol letters 95 (suppl.1) 58.

Bebianno, M. J.; Geret, F.; Hoarau, P. seraphim, M. A. Coelho, M.R. Gnassia-Barelli, M.; Romeo, M.2004. Biomarkers in Ruditapes decussates: a potential bioindicator species. *Biomarkers.* 2004, 9, 305-30.

Bebianno, M. J.; Geret, F.; Hoarau, P. seraphim, M. A. Coelho, M.R. Gnassia-Barelli, M.; Romeo, M.2004. Biomarkers in Ruditapes decussates: a potential bioindicator species. *Biomarkers.* 2004, 9, 305-30.

Benov, L.C., Bendev, I.C.and Monovich, O.H. 1990. Thiol antidotes effect on lipid peroxidation in mercury-posioned rats. *Chem. Boil. Interact*. 76:321.

Bryan, G.W. 1976 some effects of heavy metal tolerance in aquatic organisms. In: Lockwood A.P.M. (ed.) Effects of pollutants on aquatic organisms. Cambridge University press. Cambridge, England.. 7-34.

Chance, B., Green Stein, D.S., Roughton, R.J.W. 1982. The mechanism of catalase action steady state analysis Arch. *Biochem. Biophys*. 37: 301-339.

Chandravathy, V.M. and Reddy, S.L.N. 1999. Effect of lead on antioxidant enzymes activities and Lipid peroxidation in old male mice. *Mus musculus. J. Environ. Biol.* 20: 103-106.

Dorval J, Leblond, V.S.and Hontela, A., 2005. Oxidative stress and loss of cortisol secretion in adrenocortical cells of raibow trout (Oncorhynchus mykiss) exposed in vitro to endosulfan, an organochlorine pesticide. *Aquat Toxicol.* 63: 229-241.

Farombi, E.O., Adelowo, O.A, and Aimuko, Y.R. 2007. Biomarkers of Oxidative stress and heavy metal levels as indicators of Environmental pollution in African Cat fish (Clarias gariepinus) from Nigeria Ogun River. *Int. J. Envriron. Res. Public Health*, 4. 158-165.

Fukuzawa, K., Takase. S., and Tsukatani, H. 1985. The effect of concentration on the antioxidant effectiveness of 2-tocopherol in lipid peroxidation induced by superoxide free radicals. Arch. Biochem.Biophys. 240, 117-120.

Gill, K.D., Pal, R. and Nath, R. 1989. Effect of cadmium on lipid peroixdation and antioxidant enzymes in undernourished wealing rat brain. *Pharmacol. Toxicol*. 65: 73-77.

Goyer, R.A. 1996. Toxic effects of metals. In : Klassen, C.D (Ed) Casarett and Doull's toxicology: The basic science of poisons. 5th ed. McGraw-Hill, New York.

Gul, s.: Belge-Kurutas, E.; Yildiz, E.; saha, a.; Doran, F.2004. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyprinidae) living in Seyha Dam Lake, Turkey. *Environ Int*. 30, 605-9.

Hussain, S., Atkinson, A., Thompson, S.J. and Khan, A.T. 1999. Accumulation of mercury and its effect on antioxidant enzymes in brain, liver and kidney of mice. *J. Environ. Sci. Health*. 34 : 645-660.

Hwang, D.F. and Wang, L.C. 2001. Effect of taurine on toxicity of cadmium in rats. *Toxicology*. 167 : 173-180.

Isamah, G.K., S.O. Asagba and H.A.B. Coker, 2000. Comparative evaluation of the cells of some antioxidant enzymes and lipid peroxidation in different fish species on two rivers in the western Niger Delta. Bull. Envrion. Contam. Toxicol. 65: 351-356.

Kadar, E.; costa, V.; santos, R.S.2005. Distribution of micro-essential (Fe, Cu, Zn) and toxic (Hg) metals in tissues of two nutritionally distinct hydrothermal shrimps. *Sci Total Environ*. 65-70.

Kono, Y.; fridovich, I.1982. superoxide radical inhibits catalase. *J. Biol. Chem*. 257, 5751-4.

Meister, A. 1995. Glutathione metabolism. Methods. Enzymol. 251: 3-7.

Miller, O.M., Lund, B.O. and Woods, J.S. 1991. Reactivity of Hg (II) with superoxide : evidence for the catalytic dismutation of superoxide by Hg (II). *J. Biochem. Toxicol*. 6 : 293-298.

Munger, C.; Hare, L.; Craig, A. and Charest, P-M. 1999. Influence of exposure time on the distribution of cadmium within the cladoceran *Ceriodaphnia dubia*. *Aquatic Toxicology*. 44: 195-200.

Nilcoli, A., Basso, E., Petronilli, V., Wenger, R.M. Bernard, P. 1995. Interaction of cyclophilin with the mitochondiral inner membrane and regulation of the permeability transition pore and cyclosporine A sensitive channel. *J. Bio. Chem.* 271: 2185-2192.

Ober, G.A., Gonzalez, M. and Maria, I.S. 1987. Heavy metals in molluscan, crustacean and other commercially important Chilean marine coastal waters species. *Bull. Environn. Contam. Toxicol*. 38: 534-539.

Pelgrom, S.M.G. J.; Lamers, L.P.M.; Lock, R.A.C.; and Wendelaar Bonga, S.E. 1995a. Interactions between copper and cadmium modify metal organ distribution in mature tilapia, *Oreochromis mossambicus. Environ. Poll.*. 90 (3): 415-423.

Rana, S.V.S., Singh, R. and Verma, S. 1996. Protective effects of few antioxidants on liver functions in rats treated with cadmium and mercury. *Ind. J. exp. Biol*. 34: 177-179.

Romeo, M., Bennan, N., Gnassia – Barell: M., lafaurime, M., and Grnad, J.P 2000. cadmium and copper display different responses towards oxidative stress into kidney of the sea bass. *Aquat. Toxicol*. 48. 185-194.

Salducci, M.D., Chauvet-Monges, A.M., Berland, Y., Dussd, B., Elsen, R. Crevat, A. 1992. The restoration of ATP synthesis may explain the protective effect of calcium antogonist against cyclosporine A nephrotoxicity. *Life. Sci.* 50: 2053-2058.

Santhakumari, D., Srinivasalu, S. and Subramanian, S. 2004. Effect of fluoride intoxication on lipid peroxidation and antioxidant status in experimental rats. *Toxicology* 204 : 219-228.

Sarkar, S., Yadav, P. and Bhatnagar, D. 1998. Lipid peroxidative damage on cadmium exposure and alterations in antioxidant system in rat erythrocytes : a study with relation to time. *Biometals* 11: 153-157.

Sies, H., 1993. Damage to plasmid DNA by singlet oxygen and its protection. *Mut. Res.* 299: 183-191.

Stacey, M.H. and Kappus, H. 1982. Cellular toxicity and lipid peroxidation in respond to mercury. *Toxicol. Appl. Pharmacol.* 63: 29-35.

Stanic, B.; andric, N.; zoric, S.; Grubor-Lajsic, G.; Kovacevic, R.2005. Assessing pollution in the Danube river near Novi Sad (Serbia) using several biomarkers in sterlet (*Acipenser ruthenus* L.). *Ecotoxicol Environ Safety*, 2005, 2.423-427.

Stohs, S.J. and Bagchi, D. 1995. Oxidative mechanism in the toxicity of metal ions. *Free. Radic. Boil. Med.* 18: 321-336.

Tjalkens, R.B.; valerio, L./g. Jr.; Awasthi, Y. C.; Petersen, D.R.1998. Association of glutathione S-transferase isozyme-specific induction and lipid peroxidation in two inbred strains of mice subjected to chronic dietary iron overload. *Toxicol Appl. Pharmacol.* 151, 174-81.

Tulasi, S.J.; Reddy, P.U.M. and Ramano Rao, J.V. 1989. Effects of lead on the spawning potential of the fresh water fish, *Anabas testudineus. Bull. Environ. Contam. Toxicol.* 43: 858-863.

*****