Studies on the biochemical changes in the fresh water fish *Labeo rohita* (HAM) exposed to the cadmium combined with cadmium selenium.

B. Aruljothi and P. Sangeetha
Assistant professor ,Government Arts college C.Mutlur
Chidambaram,Cuddalore Dist,Tamilnadu-608001

Abstract

The present study evalutes toxicity of cadmium and its impact on biochemical constituents like structural enzymes (AST and ALT) ,metabolic enzymes (LDH and SDH),antioxidant(GSH and GPx),total protein and lipid levels in some selected tissues in the freshwater fish *labeo rohita* and how selenium counters its toxic manifestations and metal antitode by some biochemical studies.the evaluation of AST and ALT activities are highly sensitive and useful criteria in predicting the early cell injury .Increased activities of AST and ALT in cadmium treated fish clearly signifies the increased gluconeogenesis. A significant depression of cellular metabolism enhancement of SDH and LDH activities were observed in the various tissues of cadmium treated fishes. Cadmium induced the early oxidative stress by producing free radicals and thus caused the lipid peroxidation and defects in the antioxidant status (GSH, GPx and TSH)of the fish .Significant decline in the tissue protein level in cadmium exposed labeo rohita clearly indicate the protein catabolism as a source of energy under metal stress. The metal chelator ,selenium had been proved to a better antitode against cadmium toxicity by neutralizing the toxic biochemical effects of cadmium. It can be concluded that the use of selenium have the capacity to alleviate many of the harmful effects of cadmium.

Key words: toxicity of cadmium, freshwater fish, gluconeogenesis, chelator and selenium

INTRODUTION

The increasing industrialization, urbanization and developmental activities to cope up with the population explosion have brought inevitable water crisis. The usage of metals by man has increased in the last few decades and many of the industries eliminated them through their effluents. Cadmium is becoming an ever more widely used metal as protective coatings for iron, copper and steel cadmium electroplated rods are used in radio and television. Cadmium salt are used as antihelminthics, ascaricides and antiseptic is also extensively used in storage batteries, photography, ceramic industry, lithography, petroleum refineries and phosphate fertilizer industries (ATSDR,2008).

Cadmium is a well-known heavy metal toxicant with a specific gravity 8.65 times greater than water (Lide, 1992). Heavy metals become toxic when they are not metabolized by the body and accumulated in the soft tissues. The target organs of cadmium toxicity have been identified as liver, placenta, kidney, lungs, brain

and bones (Nordberg *et al.* 2007).Consuming fish (or) other animals that have accumulated cadmium may pose a threat to human health. Cadmium concentration in cat fish muscle tissue increased with increasing concentrations in their food and significantly reduced fish growth (Ruangsomboon S and Wongrat L., 2006). Chronic exposure of cadmium causes multifocal damage to lungs and also increases the total lipid content, LDH, MDH, GDH activities in catfish (Abdul kareems and Owolabi, 2014).

Selenium is an essential micro nutrient to fishes and plays a crucial role in enzyme GP_X, phospholipid, Hydroperoxide GP_X and 5-Deiodinase in the form of Seleno cysteine (Bock et al., 1991). The neutralizing effect of selenium against various compounds of mercury and cadmium in fish, mice and rat using different parameters (Shaffi et al., 2001, vineeta shukla et al., 2002). In the present investigation an attempts have been made to ascertain the toxic impact of cadmium on structural enzymes (AST and ALT), metabolic enzymes (LDH & SDH), antioxidant (GSH, GP_X) total lipid and total protein levels in some selected tissues of freshwater fish *Labeo rohita* and its recovery through the concomitant treatment with selenium.

Materials and methods:

Labeo rohita fingerlings were collected from in and around Chidambaram. The fingerlings were 20±2g in average weight and 15±2cm in average length. They were stocked and maintained in the laboratory condition for 30 days in cement tanks of 100 litre capacity containing aerated well water with 50 numbers of fingerlings in each tank to avoid overcrowding. They were feed with boiled egg and earthworm pieces. LC_{50} was determined by following renewal bioassay and was calculated by Finney (1971) probit analysis method.The biochemical constituents *viz,* structural enzymes, metabolic enzymes, antioxidant, total lipid and protein were estimated by standard procedures is gill, liver, kidney and muscle tissue of the healthy fish (control) and those from the fish exposed to sub-lethal and lethal concentration of cadmium and cadmium combined with selenium.

The activity of AST and ALT was determined by the method of Reitman and Frankle(1957), Succinate dehydrogenase (SDH) was estimated by the method of Nachlas *et al.*, 1960. Lactate dehydrogenase (LDH) was estimated by the method of Govindappan and Swami 1965. The lipid peroxidation in tissue was determined by quantitation the TBARS by the method of Hogberg *et al.*, 1974. GSH in tissue were determined according to the method of Beutler and Kelley (1963). GP_X was estimated by the method of Rotruck *et al.*, 1973.

Total sulfhydryl group were estimated in tissue by the method of sedlack and Lindsay 1968 using DTNB as the coloring reagent. The total protein content in tissue was estimated by the method of Lowry et al., 1951. All the grouped data were statistically evaluated and the significance of various treatment were calculated using students “t” test (Benett and Franklin, 1976).

Results and Discussion:

The activities of AST and ALT was observed in animals treated with sub-lethal concentration of cadmium in all the tissues when compared with control group(Table1 and Table 2). The elevated levels of these enzyme activities were recorded significantly (P< 0.001, P<0.01) near to their normal levels in animal treated with cadmium and selenium. Sub-lethal concentration of cadmium showed a progressive increase (P<0.001) of LDH and significant decreases (0.01 and 0.05) SDH activity in all the tissues in comparison with control group(Table3). The cadmium treated group showed the constructive effects of selenium against cadmium toxicity. Cadmium with selenium showed a significant (P<0.05) decreases in the level of lipid peroxidation with the maximum of decreases (P<0.001) in liver when compared with cadmium treated group that implies the protective nature of selenium against cadmium induced toxicity(Table 4). The depleted levels GSH(Table 5) were reverted significantly (P<0.05) near to their normal level with the maximum in (P<0.001) gill tissue of cadmium along with selenium treated group in comparison with cadmium treated group showed the neutralizing effects of selenium against. A perusal decreases (P<0.05) in the activity of Glutathione peroxidase in all the tissue of Cadmium treated animals in comparison with control group signified the toxic nature of cadmium on cellular antioxidants(Table 6). A significant reversal (P<0.05) of GSHPx, activity in cadmium along with selenium treated group in comparison with cadmium treated group indicated the protective role of selenium in cadmium induced toxicity(Table 7). A significant (P<0.01 and 0.05) reversal of TSH levels with the maximum in gill (P<0.001) near to their normal levels in cadmium along with selenium treated groups showed the protective nature of selenium against cadmium toxicity. In cadmium along with selenium treated group the declined protein contents(Table 8) were reverted significantly (P<0.01) near to normal level with the maximum in liver tissue (P<0.001). when compared with cadmium treated group showed a protective efficacy of selenium against cadmium toxicity.

Sobha *et al*., 2007 state that the toxicity of cadmium and its impact on biochemical constituents like glucose, glycogen, total protein, lipid and free aminoacids in the freshwater fish edible carp catla catla as cadmium bioaccumulation can affect humans through biomagnification.Manikandan *et al.*, 2016 reported biochemical changes in freshwater fish *Channa punctatus* exposed to lead nitrate.

Many investigators have reported the protective nature of selenium against heavy metals mercury(Yiin et al, 1999, Shaffi *et al*, 2001, Jamba *et al*, 1997). The results also suggest that biochemical parameters are key indicators to assess the toxic influence of cadmium on the energy metabolism and antioxidants status of an organisms and also study the toxic impacts of cadmium on structural enzymes (AST and ALT), metabolic enzymes (SDH and LDH), antioxidants (GSH, GP and TSH), total protein and lipid peroxidation of different visual organs (gill, liver, kidney, muscle) in *Labe orohita* find out the influence on the presence of protective agent against cadmium toxicity, the protective agent used selenium had been proved to a better

antitode against cadmium toxicity by neutralizing the toxic biochemical effects of cadmium in gill, liver, kidney, and muscle tissues.

Table 1 : The levels of AST content in the tissues of*Labeo* combination with Selenium

Tissue	Enzymes	Control (Group – I)
Gill	AST	0.19±0.25
Liver	AST	0.07±0.02
Kidney	AST	0.07±0.03
Muscle	AST	0.06±0.04

Table 2; levels of ALT content in the tissues of *Labeorohita* treated with Cadmium and Cadmium in combination with Selenium for 30 days

Tissue	Enzymes	Control (Group-I)	Cadmium (Group-II)	Cadmium+Selenium (Group-III)
Gill	ALT	0.07±0.01	0.51±0.06	0.19± 0.01
Liver	ALT	0.04±0.01	0.74±0.04	0.13 ±0.47
Kidney	ALT	0.06±0.01	0.70±0.03	0.14± 0.01
Muscle	ALT	0.03±0.01	0.36±0.04	0.07± 0.01

Values were expressed as µ mole ALT/g wet wt. of the tissue.

Values are mean ±SD of 5 individual observations.

Comparisons were made between Group I & Group II & Group III.

P values <0.05*; <0.01**; <0.001***.

Table 3: The levels of LDH and SDH content in the tissues of *Labeorohita* treated with Cadmium and Cadmium in combination with Selenium for 30 days

Tissue	Enzymes	Control (Group-I)	Cadmium (Group-II)	Cadmium+Selenium (Group-III)
Gill	LDH	0.25± 0.02	1.51± 0.03	0.41 ±0.02
	SDH	0.28± 0.03	0.14± 0.01	0.32± 0.03
Liver	LDH	2.27± 0.02	2.83± 0.02	0.71± 0.02

	SDH	0.59± 0.03	0.28± 0.01	0.49± 0.02
Kidney	LDH	0.11± 0.02	2.52± 0.07	0.88±0.02
	SDH	0.34± 0.01	0.11± 0.07	0.28± 0.01
Muscle	LDH	0.56 ±0.01	1.26 ±0.03	0.61± 0.01
	SDH	0.65± 0.01	0.12± 0.08	0.46± 0.01

Table 4: The levels of Lipid Peroxidation (LPO) Content in the Tissues of *Labeorohita* treated with Cadmium and Cadmium in combination with Selenium for 30 days

Tissues	**Control (Group-I)**	**Treatments**	
		Cadmium (Group-II)	**Cadmium+Selenium (Group-II)**
Gill	0.31 ± 0.007	0.51 ± 0.007	0.39 ± 0.007
Liver	0.53 ± 0.01	0.99 ± 0.01	0.72 ± 0.009
Kidney	0.46 ± 0.007	0.76 ± 0.008	0.61 ± 0.007
Muscle	0.34 ± 0.007	0.51 ± 0.08	0.41 ± 0.005

Values were expressed as µ mole MDA/g wet wt. of the tissue.

Values are mean ±SE of 6 individual observations in each group.

P values <0.05*; <0.01**; <0.001***.

Comparisons were made between Group I & Group II & Group II Vs Group III.

Table5: The levels of reduced Glutathione (GSH) content in the tissues of *Labeorohita* treated with Cadmium and Cadmium in combination with Selenium for 30 days

Tissues	**Control (Group-I)**	**Treatments**	
		Cadmium (Group-II)	**Cadmium+Selenium (Group-II)**
Gill	4.24± 0.32	2.03 ±0.14***	3.43± 0.23***
Liver	7.26± 0.69	4.48± 0.31**	5.54± 0.41*
Kidney	6.48 ±0.43	4.12 ±0.21***	5.37 ±0.29*
Muscle	4.16± 0.30	3.05± 0.19***	3.96± 0.26*

Values were expressed as µ mole/mg wet wt. of the tissue.

Values are mean ±SE of 6 individual observations in each group.

P values <0.05*; <0.01**; <0.001***.

Comparisons were made between Group I & Group II & Group II Vs Group III

Table 6: The activity of Glutathione peroxidase (GP_X) in the tissues of *Labeorohita* treated with Cadmium and Cadmium in combination with Selenium for 30 days

Tissues	Control (Group-I)	Treatments	
		Cadmium (Group-II)	Cadmium+Selenium (Group-II)
Gill	16.37 + 1.42	10.27 ± 1.20*	14.85 ± 1.36*
Liver	29.23 + 2.16	21.52 ± 1.14 *	25.64 ± 1.52*
Kidney	23.57 ± 1.94	17.41 ± 1.50*	22.45 ± 1.24*
Muscle	17.91 ± 1.65	12.26 ± 0.96 *	15.38 ± 1.12 *

Table 7: The levels of total Sulfhydryl (TSH) content in the tissues of *Labeorohita*reated with Cadmium and Cadmium in combination with Selenium for 30 days

Tissues	Control (Group-I)	Treatments	
		Cadmium (Group-II)	Cadmium+Selenium (Group-II)
Gill	61.24 ± 2.56	40.76 ± 2.31***	56.41 ± 2.49***
Liver	81.47 ± 4.06	59.27 ± 3.10***	72.41 ± 3.49*
Kidney	72.64 ± 3.12	54.08 ± 2.76***	63.04 ± 2.94*
Muscle	50.19 ± 2.07	31.62 ± 1.91***	42.17 ± 2.11**

Values were expressed as µg/mg protein.

Values are mean ±SE for 6 observations in each group.

P values < 0.05*; < 0.01**; < 0.001***.

Comparisions were made between Group I Vs Group II Vs Group III.

Table 8: The levels of total protein content in the tissues of*Labeorohita* treated with Cadmium and Cadmium in combination with Selenium for 30 days

Tissues	Control (Group-I)	Treatments	
		Cadmium (Group-II)	Cadmium+Selenium (Group-II)
Gill	15.43±0.25	9.41±0.33***	11.18±0.29**
Liver	18.33±0.28	11.24±0.37***	14.02±0.26***
Kidney	16.27±10.24	10.54±0.31***	12.08±0.28**
Muscle	13.57±0.21	9.68±10.28***	11.02±0.23**

Values were expressed as mg/g wet wt. of the tissue.

Values are mean ± SE for 6 observations in each group.

P values < 0.05*; < 0.01**; < 0.001***.

Comparisions were made between Group I Vs Group II Vs Group III.

References:

1. ATSDR – 2008 Agency for toxic substance and disease registry. Toxicological profile for cadmium. US department of health and human service, public health service. Atlanta, GA: ATSDR.
2. Abdulkareem SI and Owolabi O.D. 2014. Toxicity of sub-lethal concentrations of monocrotophos (MCP) on the haematological, biochemical and growth responses of hybrid catfish, heteroclarias and contaminated. Heteroclariasfet rats. International journal of current microbiology and applied science: 3(6), 917-931.
3. Bock, A., Forchammer, J.H., Leinfelder, W., Sowers, G., Vepreck, B and Zinoni, F., 1991. Selenocysteine. The first amino acid. Mol. Microbial., 5: 515-520.
4. Shaffi., S.A.., Manohor, Y., Nandan, M.J. 2001. Influence of protective agents on metal induced respiratory distress in *Labeo rohita* (HAM). Bull. Environ. Contam. Toxical., 66(5): 611- 616.
5. Vineeta shukla, Pratimarathi., and K.V. Sastry. 2002. Effect of cadmium individually and in combination with other metals on the nutritive value of fresh water fish, channa punctatus. Journal of environmental biology 23(2): 105-110.
6. Finney, D.J. 1971. Probit analysis university press Cambridge.
7. Reitman, S., and Frankels., 1957. A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Amer. Journal clin . pathol., 28:56-63.
8. Lide D. 1992. CRC handbook of chemistry and physics. 73rd edition. Boca raton, FL: CRC press.

9. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193. 265-275.
10. Govindappa. S, and swami, K.S, 1965. Electrophoretic characteristics of subcellular components and their relation to enzymes activities in amphibian muscle fibres. Ind. J. Exp. Biol. 3, 209-212.
11. Nachlas, M.M., Margulies, S.I., Seligman, A.M. 1960. A colorimetric method for the estimation of succinate olehydrogenase activity. J, Biol, chem. 235,499.
12. Hogberg, J., Larsen, E.R., Kristogerson, A. and Ovrhenius, S. 1974. Biochem. Biophys. Res. Commun., 56, 836.
13. Rotruck J.T, Pope A.L, Ganther H.E, Swanson A.B, Hafeman D.G, and Hoekstra W.G, 1973:Science. 179:588-590.
14. Sedlak, J. and Lindsay, R.H. 1968. Estimation of total protein-bound and non-protein sulfhydryl groups in tissue with Ellman's. analytical biochemistry 25(1) 192-205.
15. Beutler, E and Kelley, B.M 1963. The effect of sodium nitrate on RBC glutathione. Experientia., 20:96-97.
16. Benett, C.A. and Franklin, N.L. 1967. Statistical analysis in chemistry and chemical industry. John wiley and sons, New York, PP. 105-135.
17. P. Manikandan, Jebitta M. Shirlin and J.M.V. Kalaiarasi. 2016: Studies on the biochemical changes in freshwater fish, *Channa punctalus* exposed to the heavy metal toxicant lead nitrate. International journal of novel trends in pharmaceutical science, 6(5):100-105.
18. Sobha, K., Poornima, A., Harini, P., Veeraian, K. 2007. A study on biochemical changes in the fresh water fish, catlacatla (hamilton) exposed to the heavy metal toxicant cadmium chloride. Kathmandu university journal of science, engineering and technology Vo 1(5):1-11.
19. Yiin, S.J., Chern, C.L., Sheu, J.Y., Tseng, w.ch. Lin, T. 1999. Cadmium induced renal lipid peroxidation in rats and protection by selenium. J. Toxicol. Environ. Health. Part A 57(6): 403-413.
20. Jamba, L., Nehru, B., Bansal, M.P. 1997. Redox modulation of selenium binding proteins by cadmium exposures in mice, Mol. Cell. Biochem., 177:169-175.
21. Ruangsomboon S and Wongrat L., 2006. Bioaccumulation of cadmium in an experimental aquatic food chain involving phytoplankton (Chlorella vulgaris), zooplankton (Moina macrocopa), and the predatory catfish Clarias macrocephalus x C. gariepinus. Aquat Toxicol. 2006 Jun 10;78(1):15-20. Michael K. Saiki, Barbara A. Martin1, Larry D. Thompson And Daniel Welsh, 2000. Copper,

22.Nordberg GF, Nogawa K, Nordberg M, Friedmann JM. Cadmium. In: Nordberg GF, Fowler BA, Nordberg M, Friberg L, editors. Handbook on the Toxicology of Metals. Amsterdam: Elsevier; 2007. pp. 445–486.