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Ameliorative effect of selenium on cadmium induced biochemical alterations in *Cirrhinus mrigala* (Ham.)

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Heavy metal pollutants are generally discharged in the aquatic environment as a result of industrial processes and are causing major problems in the food chain. Pollution due to heavy metals is of serious concern and among them cadmium deserves special attention. Cadmium, a heavy metal has been recognized as one of the most toxic environmental and industrial pollutant due to the ability to induce severe alterations in various organs following either acute or chronic exposure. The present study was carried out to evaluate the protective influence of selenium against cadmium induced biochemical alterations in lipid peroxidation (LPO), reduced glutathione (GSH), glutathione peroxidase (GPx), total sulfhydryl group (TSH) and total protein in gill, liver, kidney and muscle tissues of *Cirrhinus mrigala* fingerlings for 30 days. A significant increase in the level of lipid peroxidation and concominant decrease in the activity of GSH, GPx, TSH and total protein levels were observed in all the tissues of cadmium (0.85 mg/l) treated group. Administration of cadmium along with the equimolar dose of selenium (0.34 mg/l), the above mentioned biochemical parameters were reverted near to their normal levels that showed the protective effect of selenium against cadmium induced toxicity.

Key words: Cadmium, Fish, Selenium, Enzymes, Metabolism.

INTRODUCTION

Aquatic environment in general harbours a lot of organisms many of which are used as food by man and these are likely to be subjected to the hazardous heavy metal pollution. Heavy metal contaminants in aquatic ecosystem pose a serious environmental hazard because of their persistence and toxicity. Unrestrained release of heavy metals into environment *viz.,* discharge of industrial effluents, sewage and agro chemicals into the water resources has not only rendered it unusable but at the same time has produced great harm to the non-target fauna such as fish (Vineetha Shukla *et al.,* 2002).

Cadmium compounds are used in the metal-plating, cadmium-nickel battery industry and as a stabilizing agents in many polyvinyl chloride products. Furthermore cadmium is widely used in solar cells, television tubes, radio sets, telephone wires, photography, lithography, calico printing, dyeing screens, scintillation counters and fertilizer industries (ATSDR, 1993).

Cadmium increases the generation of free radicals, promotes lipid peroxidation and depletes antioxidants and is carcinogenic. Cadmium affects the ionic transport through membranes, energy availability through mitochondrial function, detoxification through microsomal enzymes, intercellular communication by affecting cell adhesion in epithelial cells and many cell signaling functions by affecting intracellular calcium, inositol phosphate and protein kinase C (Rana *et al.,* 1996).

The metabolism and excretion of cadmium depends on the presence of antioxidants and thiols that aid cadmium metallothionein binding. Some of the specific changes that lead to tissue damage in chronic exposure of cadmium have been related to oxidative stress and thiol depletion. Cellular damage results from cadmium binding with sulfhydryl groups in tissues, production of lipid peroxides and the depletion of reduced glutathione (Ercal *et al.,* 2001). Cadmium has the ability to generate free radicals that leads to the expression of inflammatory chemokines and cytokines (Dong *et al.,* 1998).

Selenium is an essential dietary trace element that plays a crucial role in enzyme glutathione peroxidase (GPx), phospholipid hydroperoxide GPx and 5¢-deiodinase in the form of selenocysteine (Bock *et al.,* 1991). Inspite of various findings on the toxic effect of cadmium and the neutralizing effect of selenium in cadmium induced toxicity on changes in certain biochemical parameters, there is still a lacuna on its effect on the antioxidant enzymes, lipid peroxidation in various organs of the fingerlings of *Cirrhinus mrigala*.

Therefore, the present study has been designed to investigate the effect of chronic exposure of cadmium

on certain biochemical parameters such as (LPO, GSH, GPx, TSH) and total protein levels in some selected organs (gill, liver, kidney and muscle) of the fresh water fingerlings of *Cirrhinus mrigala* and their recovery through the supplementation with equimolar dose of selenium.

MATERIALS AND METHODS

The fingerlings of *Cirrhinus mrigala* of size 12 ± 2 cm in length and 15 ± 2 g in weight were procured locally from a fish farm and acclimatized at 28 ± 2 ºC in the laboratory conditions for 30 days. Acclimatized fishes were divided into 3 groups with 20 in each well aerated aquaria. The physico-chemical parameters of the fresh water maintained its optimum level (pH 7.2, alkalinity 88 mg/l as $CaCO_3$; DO - 8.0 ± 2 mg/l and hardness 178 mg/l as $CaCO_3$). Exposure of *Cirrhinus mrigala* to 8.5 mg/l of $CdCl_2$ for 96 hours resulted in 50% mortality. Thus 0.85 mg/l was determined as sublethal concentration of cadmium chloride by probit analysis method (Finney, 1971). The first group of *Cirrhinus mrigala* was kept in metal free water and treated as control. The second group was subjected to chronic exposure (30 days) of sublethal concentration of (0.85 mg/l) $CdCl_2$. Then the third group of fishes were treated with the sublethal dose of cadmium (0.85 mg/l) along with the equimolar concentration of selenium (0.34 mg/l) as sodium selenite for 30 days. At the end of the experimental period the fingerlings in each group were sacrificed and the vital tissues *viz.*, gill, liver, kidney and muscle were dissected out carefully and kept in ice cold phosphate buffered saline for the estimation of lipid peroxidation (LPO) reduced glutathione (GSH), glutathione peroxidase (GPx), total sulfhydryl group (TSH) and total protein levels following the standard methods of Hogberg *et al.* (1974) for LPO, Beutler and Kelley (1963) for GSH, Rotruck *et al.* (1973) for GPx, Sedlack and Lindsay (1968) for TSH and total protein level was quantified as per Lowery *et al.* (1951). Statistical analysis was performed by one way analysis of variance (ANOVA). Critical difference (CD) was calculated at 1% level according to the method Gomez and Gomez (1984) and the results were expressed as mean ± SD of six observations in each group.

RESULTS AND DISCUSSION

Lipid peroxidation (LPO) :

Chronic treatment with the sublethal concentration of cadmium to *Cirrhinus mrigala* resulted in a statistically significant ($p < 0.01$) increase of lipid peroxidation in all the selected tissues when compared with control group. Concominant treatment of cadmium with selenium showed a significant ($p < 0.01$) decrease in the level of lipid peroxidation when compared with cadmium treated group that implies the protective nature of selenium against cadmium induced toxicity (Table 1).

Lipid peroxidation is one of the important measure to understand the extent of metal toxicity and tissue damage in animals. Cadmium induced oxidative stress has been associated with the production of reactive oxygen species (ROS) comprising mainly of superoxide radical anion ($O_2^{\cdot -}$) hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot -}$). Reaction of these reactive oxygen species with cellular molecules has been shown to lead to peroxidation of membrane lipids and DNA damage (Dally and Hartwig, 1997).

Increased lipid peroxidation is generally believed to be an important underlying cause of initiation of oxidative stress related to various tissue injury, cell death and further progression of many acute and chronic diseases (Basu, 2003). The present results were clearly suggested that the ability of cadmium to induce oxidative stress in gill, liver, kidney and muscle tissues of *Cirrhinus mrigala* which was evidenced by increased lipid peroxidation following chronic exposure. This finding is in agreement with several reports demonstrating that cadmium induces the oxidative stress in tissues by increasing lipid peroxidation and by altering antioxidant status in experimental animals (El-Demerdash *et al.*, 2004). The elevated lipid peroxidation after cadmium intoxication indicating the possibility of more free electron leakage which could enhanced the toxicity with increased production of H_2O_2 (Halliwell and Gutteridge, 1986).

Reduced glutathione (GSH) :

In the present study a significant ($p < 0.01$) depletion of cellular antioxidants *i.e.* reduced glutathione content in all the tissues of cadmium treated fingerlings in comparison with that of the control group. The depleted levels of GSH were reverted significantly ($p < 0.01$) near to their normal levels in cadmium along with selenium treated group in comparison with cadmium treated group that showed a neutralizing effects of selenium against cadmium toxicity (Table 1).

GSH (L-glutamyl-L-cysteinylglycine) a tripeptide involved in the prevention of cellular oxidative stress, detoxification of electrophiles and maintenance of intracellular thiol status (Meister and Anderson, 1983). GSH is an important intracellular reductant that helps to maintain the essential sulfhydryl (SH) groups of an enzymes in their reduced state. GSH with its SH group becomes oxidized and forms a disulphide linkage with electrophiles (Gul *et al.*, 1999). In the present study a sharp decline in the level of GSH were observed in the

Table 1 : The levels of LPO and GSH activities in the tissues of *Cirrhinus mrigala* treated with cadmium and cadmium in combination with selenium for 30 days

Tissue	Enzyme	Control	Cadmium	Cadmium + Selenium
Gill	LPO	0.31 ± 0.02^a	0.50 ± 0.03^b	0.39 ± 0.02^a
	GSH	4.24 ± 0.32^a	2.03 ± 0.14^b	3.43 ± 0.23^c
Liver	LPO	0.54 ± 0.03^a	0.98 ± 0.04^b	0.72 ± 0.03^c
	GSH	7.26 ± 0.69^a	4.48 ± 0.31^b	5.54 ± 0.41^c
Kidney	LPO	0.47 ± 0.03^a	0.76 ± 0.04^b	0.61 ± 0.03^c
	GSH	6.48 ± 0.43^a	4.12 ± 0.21^b	5.37 ± 0.29^c
Muscle	LPO	0.34 ± 0.02^a	0.51 ± 0.03^b	0.41 ± 0.02^c
	GSH	4.16 ± 0.30^a	3.05 ± 0.10^b	3.96 ± 0.26^c

Values are expressed as n moles of MDA/g wet wt. of the tissue for LPO and ? moles/mg wet wt. of the tissue for reduced GSH.

Values are mean ± SD for six observations in each group.
Values are not sharing a common superscript letter differ significantly at $p<0.01$.

tissues of *Cirrhinus mrigala* treated with cadmium. The reduced levels of GSH represents, an indication of the severity of cadmium induced oxidative stress. Cadmium inhibits the expression of g-glutamyl cysteinyl synthase, an enzyme that catalyses the rate-limiting step in the biosynthesis of GSH could account in part for the decline in the level of GSH (Casalino *et al.*, 2002).

Cellular damage results from the binding of cadmium to sulfhydryl groups in tissues and the production of lipid peroxides are the major causes for the cadmium induced depletion of glutathione levels. Cadmium also has a very high affinity for glutathione and can form a complex with glutathione that is eliminated through bile (Casalino *et al.*, 2002). The depletion of cellular GSH due to the binding with free cadmium ions have also been reported by several investigators in various experimental animals (Beytut and Aksakal, 2002; Maracine *et al.*, 2002; Ikediobi *et al.*, 2004).

Glutathione Peroxidase (GPx) :

A perusal decrease ($p<0.01$) in the activity of glutathione peroxidase was recorded in all the tissues of cadmium treated fingerlings when compared with control group. Animals exposed with cadmium along with selenium showed a significant increase ($p < 0.01$) of GPx activity when compared with cadmium intoxicated group (Table 2).

Glutathione peroxidase is a selenoenzyme that provides an efficient means of destroying hydrogen peroxide at the expense of reduced GSH. GPx is a well known first line of defence against oxidative stress which in turn requires glutathione as a cofactor (Bruce *et al.*, 1982). In the present study fingerlings treated with cadmium showed a decreased level of GPx in all the selected tissues may be due to the generation of excess amount of free radicals by cadmium or may be due to the binding of cadmium with GSH that lowered the activities of GPx which is GSH dependent (El-Demerdash and Elagamy, 1999).

A considerable decline in the GSH contents in tissues would seem to enhance the risk of oxidative stress and reduces the activity of GPx (Almeida *et al.*, 2002). Reduced activity of GPx in cadmium treated starlings was reported by Congiu *et al.* (2000). These results have been interpreted as related to the formation of cadmium-selenium complexes, which could lower the selenium availability required for selenium dependent GPx (Sindhu *et al.*, 2004).

Total sulfhydryl group (TSH) :

Cadmium intoxicated fingerlings showed a marked decline ($p < 0.01$) in the level of total sulfhydryl groups in all the selected tissues when compared with control group. A significant ($p < 0.01$) reversal of TSH levels in cadmium along with selenium treated group that showed the protective nature of selenium against cadmium toxicity (Table 2).

Cysteine with sulfhydryl group, methionine and cysteine are the important cellular antioxidants plays a vital role in the metal detoxification. Cadmium and mercury shows a high affinity towards the sulfhydryl groups. Free Cd^{2+} and Hg^{2+} ions directly conjugates with SH groups and detoxified as mercapturic acid and then excreted.In the present investigation total sulfhydryl

groups in the tissues of *C. mrigala* intoxicated with cadmium showed a drastic decrease in their level. This may be due to the increased conjugation of Cd^{2+} ions directly with SH groups (Khandelwal *et al.,* 2002).

When the fingerlings were exposed to heavy metals like cadmium in some tissues this sulfhydryl rich protein increases through induction and exerts its protection by competing with free metal ions and partitioning them away from potential sites of toxic action (Kapila Manoj and Ragothaman, 1998). It has been convincingly established that metallothionein acts as a heavy metal sink or scavenger to bind with free Cd^{2+} and thus protect other important biomolecules from the deleterious effect of direct action of free Cd^{2+} ions. Toxicity occurs when the amount of free Cd^{2+} in a given sensitive organ exceeds the binding capacity of metallothionein for the given metal (Nolon and Shaikh, 1992).

Total protein :

Intoxication with the sub-lethal concentration of cadmium in *C. mrigala* fingerlings showed a significant ($p < 0.01$) decrease in the level of total protein content in all the selected tissues when compared with control group. In cadmium along with selenium treated group the declined protein contents were reverted significantly ($p < 0.01$) near to their normal levels when compared with cadmium treated group showed a protective efficacy of selenium against cadmium toxicity (Table 2).

Tissue proteins in aquatic animals under metal stress are known to play a pivotal role in the activation of compensatory mechanisms. In the present study relative to the control group the total protein contents of all the tissues of cadmium treated *C. mrigala* fingerlings showed a drastic decrease at the end of 30 days. This indicates that cadmium induces the proteolysis under toxic stress. The impairment of protein synthesis due to cadmium was reported by many investigators in various experimental animals (Richard *et al.,* 1998; Almeida *et al.,* 2002)

Significant decline in the tissue protein level in cadmium exposed *C. mrigala* clearly indicates that the protein catabolism as a source of energy under metal stress. Cadmium intoxication resulted in stimulated anaerobic metabolism and a trend in favour of protein utilization (Kapila Manoj and Ragothaman, 1999). Marked decrease in the total protein contents in these tissues indicates the rapid initiation of the breakdown of protein and mobilization of protein to meet the energy demand during cadmium stress. The decline in the total protein suggests an intensive proteolysis in the respective tissues which in turn could contribute to the increase of free amino acids to be fed into the TCA cycle as keto acids (Palanichamy *et al.,* 1986). Another reason for the

Table 2 : The levels of GPx, TSH and total protein content in the tissues of *Cirrhinus mrigala* treated with cadmium and cadmium in combination with selenium for 30 days

Tissue	Enzyme	Control	Cadmium	Cadmium + Selenium
Gill	GPx	16.37 ± 1.42^a	10.27 ± 1.20^b	14.85 ± 1.36^c
	TSH	61.24 ± 2.56^a	40.76 ± 2.31^b	56.41 ± 2.49^c
	Total protein	15.43 ± 0.25^a	9.41 ± 0.33^b	11.18 ± 0.29^c
Liver	GPx	29.23 ± 2.16^a	21.52 ± 1.14^b	25.64 ± 1.52^c
	TSH	81.47 ± 4.06^a	59.27 ± 3.10^b	72.41 ± 3.49^c
	Total protein	18.33 ± 0.28^a	11.24 ± 0.37^b	14.02 ± 0.26^c
Kidney	GPx	23.57 ± 1.94^a	17.41 ± 1.50^b	22.45 ± 1.24^c
	TSH	72.64 ± 3.12^a	54.08 ± 2.76^b	63.04 ± 2.94^c
	Total protein	16.27 ± 0.24^a	10.54 ± 0.31^b	12.08 ± 0.28^c
Muscle	GPx	17.91 ± 1.65^a	12.26 ± 0.96^b	15.38 ± 1.12^c
	TSH	50.19 ± 2.07^a	31.62 ± 1.91^b	42.17 ± 2.11^c
	Total protein	13.57 ± 0.21^a	9.68 ± 0.28^b	11.02 ± 0.23^c

Values are expressed as ? g of GSH consumed/min/mg protein for GPx, ? g/mg protein for TSH and mg/g wet wt. of the tissue for total protein

Values are mean ± SD for six observations in each group.

Values are not sharing a common superscript letter differ significantly at $p<0.01$.

depletion of total protein level may be due to the higher affinity of free Cd^{2+} ions towards different sulfhydryl amino acid residues of protein.

Treatment of cadmium along with selenium lowers the cadmium toxicity because selenium being less toxic metal occupy the SH groups which acts as receptor sites on plasma membrane. The partial occupation of these receptor sites blocked by selenium that prevents the occupation by cadmium (Shaffi *et al.,* 2001). Treatment of selenium along with cadmium reduced the cadmium induced lipid peroxidation and enhanced the antioxidant status in the present study suggested that selenium attenuates the cadmium toxicity. Yiin *et al.* (1999) have also reported that selenium was found to provide protection against cadmium induced lipid peroxidation in rats.

The mechanism of protection of selenium may be due to the depression of non-protein sulfhydryl groups in tissue. Selenium protect cells from toxic effect of cadmium by maintaining the availability of antioxidant non-protein sulfhydryl groups. Because cadmium has the high binding affinity towards SH groups that may reduces the free Cd^{2+} levels in tissue, thus attenuating the toxic effect of cadmium. Selenium may also detoxifies the cadmium through direct interactions not just by preventing the accumulation of peroxides (Yinn *et al.,* 1999; Shaffi *et al.,* 2001).

The work reported here in thus, clearly demonstrated the detoxifying effect of selenium in cadmium intoxicated fingerlings of *C. mrigala* by taking biochemical, antioxidant and tissue lipid peroxidation as an index. Therefore, detoxifying effect of selenium provides the first step towards selenite being considered favourably as a potential trace elements against cadmium intoxication. As selenium is already known to be a dietary requirement in micro-quantities, it is worth investing further the use of this element, as an antidote against heavy metal toxicities.

REFERENCES

Almeida, J.A., Diniz, Y.S., Marques, S.F.G., Faine, L.A., Ribas, B.O., Burneiko, R.C. and Novelli, E.L.B. (2002). The use of the oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus)* exposed to in vivo cadmium contamination. *Environ. Int.,* **27:** 673-679.

ATSDR (1993). Toxicological profile for cadmium and references cited. US Department of Health and Human Services.

Basu, S. (2003). Carbon tetrachloride induced lipid peroxidation: eicosanoid formation and their regulation by antioxidant nutrients. *Toxicol.,* **189:** 113-127.

Bennett, C. A. and Franklin, N.L. (1967). Statistical analysis in chemistry and chemical industry. John Wiley and Sons, New York, pp. 105-135.

Beutler, E. and Kelley, B.M. (1963). The effect of sodium nitrate on RBC glutathione. *Experientia,* **29:** 96-97.

Beytut, E. and Aksakal, M. (2002). The effect of long-term supplemental dietary cadmium on lipid peroxidation and the antioxidant system in the liver and kidneys of rabbits. *Turk. J. Vet. Anim. Sci.,* **26:** 1055-1060.

Bock, A., Forchammer, J.H., Leinfelder, W., Sowers G., Vepreck, B. and Zinoni, F. (1991). Selenocysteine: The 1st amino acid. *Mol. Microbiol.,* **5:** 515-520.

Bruce, A., Freeman, J. and James, D.L. (1982). *Lab. Invest.,* **47(5)**: 412-426.

Casalino, E., Calzsaratti, G., Sblano, C. and Landriscina, C. (2002). Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. *Toxicol.,* **179:** 37-50.

Congiu, L., Chicca, M., Pilastro, A., Turchetto, M. and Talandini, L. (2000). Effects of chronic dietary cadmium of hepatic glutathione levels and glutathione peroxidase activity in starlings (*Sturnus vulgaris*). *Arch. Environ. Contam. Toxicol.,* **38:** 357-361.

Dally, H. and Hartwig, A. (1997). Induction and repair inhibition of oxidative DNA damage by nickel(II) and cadmium(II) in mammalian cells. *Carcinogenesis,* **18:** 1021-1026.

Dong, W., Simeonova, P.R. and Gallucci, R. (1998). Toxic metals stimulate inflammatory cytokines in hepatocytes through oxidative stress mechanisms. *Toxicol. Appl. Pharmacol.,* **151:** 359-366.

El-Demerdash, F.M. and Elagamy, E.I. (1999). Biological effects in *Tilapia nilotica* fish as indicators of pollution by cadmium and mercury. *Int. J. Environ. Hlth. Res.,* **9:** 143-156.

Ercal, N., Gurer-Orhan, H. and Aykinburns, N. (2001). Toxic metals and oxidative stress part I. Mechanisms involved in metal-induced oxidative damage. *Curr. Top. Med. Chem.,* **1:** 529-539.

Finney, D.J. (1971). Statistical methods of Biological Assay, Probit analysis. Cambridge University press London, UK.

Gomez, K.A. and Gomez, A.A. (1984). Statistical procedure for agricultural research. Second Edition, Wiley Publishers, New York.

Gul, M., Atalay, M., Temocin, S., Janninen, O. and Sen, C.K. (1999). Poster presented to the 2nd national congress of the Turkish research society for free radicals and antioxidants, Eskisehir, Turkey, pp. 19-22.

Halliwell, B. and Gutteridge, J.M.C. (1986). Oxygen free radicals and iron in relation to biology and medicine. Some problems and concepts. *Arch. Biochem. Biophys.,* **246 :** 501-514.

Hogberg, J., Larson, R.E., Kristoferon, A. and Orrenius S. (1974). NADPH-dependent reductase solubilised from microsome by peroxidation and its activity. *Biochem. Biophys. Res. Commun.,* **56 :** 836-842.

Ikediobi, C.O., Badisa, V.L., Ayuk-Tarkem, L.T., Latinwo, L.M. and West, J. (2004). Response of antioxidant enzymes and redox metabolites to cadmium-induced oxidative stress in CRL-1439 normal rat liver cell. *Int. J. Mol. Med.,* **14:** 87-92.

Kapila Manoj and Ragothaman, G. (1998). Mercury, copper and cadmium induced changes in the total protein levels in muscle tissue of an edible estuarine fish, *Boleophthalmus dussumieri* (Cuv). *J. Environ. Biol.,* **20(3):** 231-234.

Khandelwal, S., Shukla, L.J. and Shankar, R. (2002). Modulation of acute cadmium toxicity in *Emblica officinalis* fruit in rat. *Indian J. Exp. Biol.,* **40 :** 564-570.

Lowry, O.H., Rosenbrough, N.J Farr, A.L. and Randall, R.J. (1951). Protein measurement with Folin-phenol reagent. *J. Biol. Chem.,* **193 :** 265-75.

Maracine, M., Lange A. and Segner, H. (2002). Time and concentration dependent alterations of the glutathione concentrations in RTG-2cells exposed to divalent metal cations (Manuscript Submitted for Publication in Cell Biol. Toxicol.).

Meister, A. and Anderson, M.E. (1983). Glutathione. *Ann. Rev. Biochem.,* **52 :** 711-760.

Nolan, C.V. and Shaikh, Z.A. (1992). Lead nephrotoxicity and associated disorders: biochemical mechanisms. *Toxicol.,* **73:** 127-146.

Palanichamy, S., Baskaran, P. and Balasubramanian, M.P. (1986). Sublethal effects of selected pesticides on protein, carbohydrate and lipid content of different tissues of *Oreochromis mossambicus. Proc. Sym. Pest. Resid. Env. Pollu.,* pp. 97-102.

Pope, A. and Rail, D.E. (1995). Environmental medicine. Integrating a missing element into medical education, Washington, DC.

Rana, S.V.S., Singh, R. and Verma, S. (1996). Protective effects of few antioxidants on liver function in rat treated with cadmium and mercury. *Indian J. Exp. Biol.,* **34:** 177-179.

Richard, A.C., Daniel, C., Anderson, P. and Hontela, A. (1998). Effects of subchronic Exposure of cadmium chloride on endocrine and metabolic functions in rainbow trout *Oncorhynchus* Mykiss, Arch. Environ. Contamination and *Toxicology,* **34:** 377-381.

Rotruck, J.T., Pope, A.L. and Ganter, H.E. (1973). Biochemical role as a component of glutathione peroxidase. *Science,* **179:** 588-590.

Sedlack, J. and Lindsay (1968). Estimation of total protein sulfhydryl groups in tissues with Ellman's reagent. *Anal. Biochem.,* **25**: 192-205.

Shaffi, S.A., Manohar, Y.R. and Nandan, M.J. (2001). Influence of protective agents on metal induced respiratory distress in *Labeo rohita* (Ham.). *Bull. Environ. Contam. Toxicol.,* **66:** 611-616.

Sindhu, R.K., Koo, J.R. Roberts, C.K. and Vaziri, N.D. (2004). Dysregulation of hepatic superoxide dismutase, catalase and glutathione peroxidase, in diabetes: response to insulin and antioxidant therapies. *Clin. Exp. Hypertension,* **26:** 43-53.

Vineetha Shukla, Pratima Rathi and Sastry, K.V. (2002). Effect of cadmium individually and in combination with other metals on the nutritive value of freshwater fish, *Channa punctatus*. *J. Environ. Biol.,* **23(2):** 105-110.

Yiin, S.J., Chern, C.L., Sheu, J.Y., Tseng, W.Ch. and Lin, T.H. (1999). Cadmium induced renal lipid peroxidation in rats and protection by selenium. *J. Toxicol. Environ. Hlth.,* **56 :** 101-111.