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Modulatory Effect of Cadmium Injection on Endosulfan-Induced Oxidative Stress in the Freshwater Fish, *Channa punctata* Bloch

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Aquatic organisms are constantly exposed to mixture of chemicals and a myriad of physical stressors that may result in behavioral, physiological and biochemical changes. The resultant toxic effect of exposure to the mixture of chemicals may be additive, antagonistic or synergistic (Fent 2003). Interactive toxicity of two or more toxicants has been reported in various animal species (Hurk et al. 1998; Stuijfzand et al. 2000). Metalloids are common aquatic pollutants which are detected along with persistent organic pollutants in the pollutant mixtures and industrial effluents. Cadmium is one of the major metalloids for which there has been concern due to its toxicity in a wide range of animal models and also in humans (Pinot et al. 2000; Satoh et al. 2002). Cadmium is reported to interact with other toxicants and also elicit protective mechanisms mainly due to its ability to induce formation of metallothioneins, MT (Klaassen et al. 1999; Sato and Kondoh 2002). Endosulfan, a broad-spectrum chlorinated insecticide is widely used in agriculture to control invertebrate pests. Though its mechanism of action is attributed to its attack on gamma aminobutyric acid receptor complex in the central nervous system (Hassall 1990), endosulfan has been shown to elicit a range of toxic effects in laboratory animals and in the non-target organisms in field (Kalender et al. 2004; Park et al. 2004; You et al. 2004). Endosulfan residue, especially its ∝-isomer is considered a major environmental problem (Sutherland et al. 2004). In India also, cadmium and endosulfan are amongst the major pollutants in Yamuna river reported in a study conducted by the Central Pollution Control Board (CPCB 1994). Since cadmium and endosulfan contamination is quite widespread in aquatic environment, we were interested to study their interactive effect, especially the modulatory effect of cadmium on endosulfan-induced oxidative stress and toxicity to antioxidants in a freshwater fish model. The present study reports results of that study.

MATERIALS AND METHODS

Channa punctata Bloch (spotted snake-head) weighing 50-75 g maintained in 60-litre glass aquaria and acclimatized for 15 days at ambient temperature (25 ± 1^0C) were used in this study. A group of 30 acclimatized fish was exposed to cadmium chloride (Sigma Aldrich Corp., India, dissolved in sterilized saline solution) at the

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dose of 0.2 mg/kg intraperitoneally, i.p. (based on Wu et al. 1999) on every second day for 7 days (total 3 injections). On day 8, half of the fish (n=15) from this group were exposed to endosulfan (Hoescht, India, suspended in ethanol and mixed directly with tank water) at a concentration of 5 ppb for 24 hr (based on Pandey et al. 2001). Another group (n=15) was exposed to endosulfan at the same concentration for 24 hours. Control group (n=15) was maintained in tap water. After the completion of exposure schedule, fish were dissected to remove various tissues for preparation of homogenates in chilled phosphate buffer (0.1 M, pH 7.4) and post-mitochondrial supernatant (PMS) was prepared from each homogenate. All the chemicals and reagents were of high purity either from Sigma Aldrich or from the local sources.

Lipid peroxidation (LPO) was measured by the procedure of Uchiyam and Mihara (1978) as nanomoles of thiobarbituric acid reactive substance (TBARS) formed/hr/g of tissue using a molar extinction coefficient (ϵ) of 1.56×10^5 M^{-1} cm^{-1}. Antioxidants viz., catalase EC 1.11.1.6 (CAT), glutathione peroxidase EC 1.11.1.9 (GP), glutathione reductase EC 1.6.4.2 (GR), glutathione *S*-transferase EC 2.5.1.18 (GST), and reduced glutathione (GSH) were measured using the methods as described in one of our recent publications (Pandey et al. 2003). CAT activity was measured as nanomoles H_2O_2 consumed/min/mg protein and GP activity was calculated as nanomoles of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidized/min/mg of protein, at ϵ 6.22×10^3 $M^{-1}cm^{-1}$. GR activity was measured as nanomoles of NADPH oxidized/min/mg protein at ϵ 6.22×10^3 M^{-1} cm^{-1} and GST as nanomoles 1-chloro 2,4-dinitro benzene (CDNB) conjugates/min/mg protein using a ϵ of 9.6×10^3 M^{-1} cm^{-1}. Reduced glutathione was measured as nanomoles GSH/g tissue. Protein carbonyls were measured by the method of Floor and Wetzel (1998) as nanomoles of 2,4-dinitrophenayl hydrazine (DNPH) incorporated/mg protein using ϵ of 21×10^2 M^{-1} cm^{-1}. Metallothioneins were measured by the spectrophotometric method of Viarengo et al. (1997). Additionally, SDS-PAGE analysis of MT was done using the method as adopted by Ahmad et al. (2000). Cadmium content of the identified band in the gel was measured by graphite furnace atomic absorption spectrophotometer (AAS, Video 11, USA). Protein was estimated by the method of Lowry et al. (1951). Data were analyzed for significance of difference using two-factor analysis of variance (ANOVA) followed by the least square difference (LSD) analysis and the significance of results was ascertained at $p < 0.05$.

RESULTS AND DISCUSSION

Cadmium exposure (0.2 mg/kg b.wt., i.p., 3 injections) showed modulatory effect on endosulfan-induced oxidative stress as measured by LPO in gills and liver in *C. punctata*. However, in case of kidney no such effect was observed and LPO remain elevated. Cadmium alone at this dose level did not induce LPO in any of the tissues.

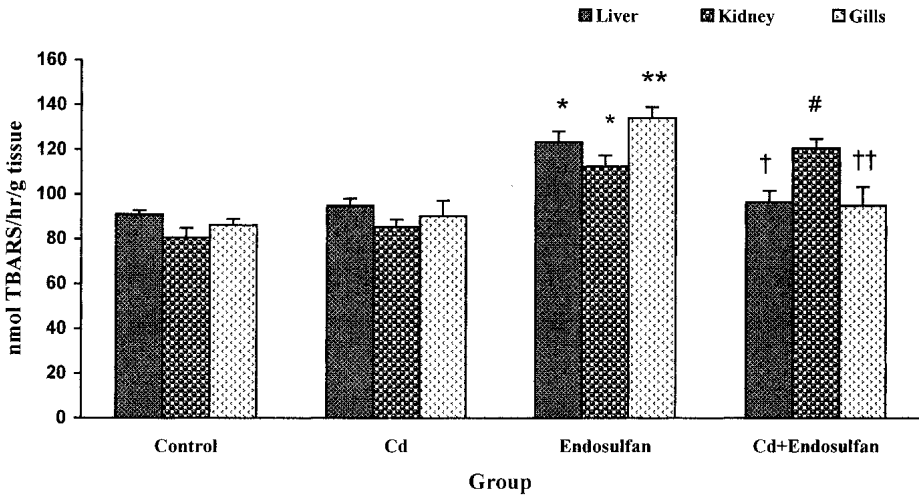

Figure 1. LPO values (means±S.E., n=6) in liver, kidney and gills of *Channa punctata*. Significant change is shown as *$p < 0.01$ and **$p < 0.001$ and $^{\#}p < 0.001$ when compared with control values, †$p < 0.05$ and ††$p < 0.01$ when compared with endosulfan-exposed group.

Oxidative stress-inducing effect of cadmium and its impact on antioxidants has been studied in different fish species and findings are contradictory. Romeo et al. (2000) while evaluating the relative effect of cadmium and copper in sea bass at the dose of 735ng/g and 127 ng/g, respectively showed that copper had more pronounced effect than cadmium on LPO in liver, kidney and muscles. Basha and Rani (2003) reported that cadmium (as cadmium chloride; 5ppm) had inducing effect on xanthine oxidase, superoxide dismutase, CAT, GST and GP in liver and kidney in *Oreochromis mosambicus* (tilapia). On the other hand, Tort et al. (1996) showed no significant effect of cadmium (as cadmium chloride; 1mg/kg, i.p.) on LPO or GSH in liver of *Onchorhynchus mykiss* (rainbow trout). Fish exposed to endosulfan showed a significant increase in LPO (Figure 2). The increase in gills was more pronounced as compared to other organs ($p < 0.001$). LPO inducing effect of endosulfan has been reported previously by some workers (Pandey et al. 2001, Dorval et al. 2003; Sohn et al. 2004). However, cadmium injection significantly ($p < 0.05$-0.01) reduced level of LPO in liver and gills in endosulfan-exposed fish when compared with endosulfan only exposed group (Figure 1). In addition to induction of antioxidant enzymes, endosulfan exposure significantly increased the formation of protein carbonyls in liver ($p < 0.01$), kidney ($p < 0.05$) and gills ($p < 0.05$) which demonstrates direct damage to proteins or chemical modification of amino acids in as a result of oxidative stress (Stadtman 1986). Cadmium pretreated endosulfan-exposed fish showed significant decrease in protein carbonyl levels in liver only ($p < 0.01$).

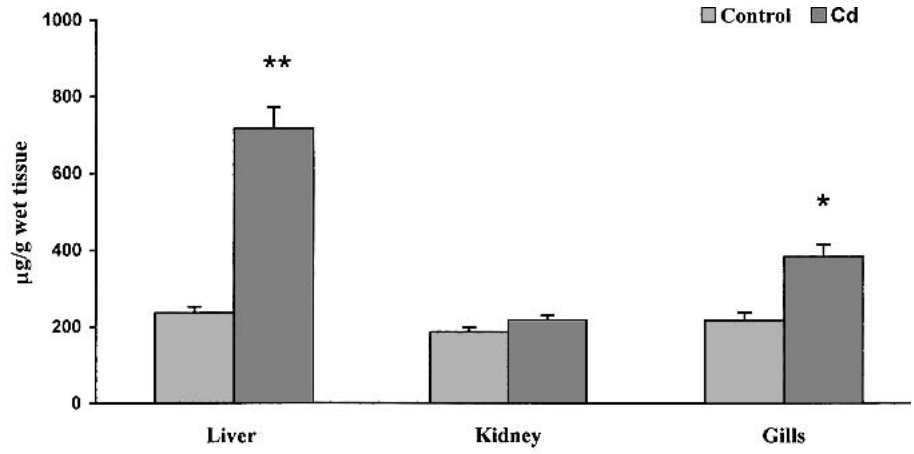

Figure 2. MT level in liver, kidney and gills of *Channa punctata* Bloch. Significant difference of values of control vs. cadmium chloride injected is indicated by *$p < 0.01$ and **$p < 0.001$ (mean ± S.E, n=6).

Gills and kidney showed no such response. This demonstrates that cadmium pre-treatment reduced oxidation of proteins in liver and thereby helping to restore the original protein configuration. In all the organs, endosulfan exposure caused a significant ($p < 0.05$-0.01) increase in the activities of GP, GST and GR as compared to control group, while CAT activity was significantly decreased in liver ($p < 0.001$), kidney ($p < 0.01$) and gills ($p < 0.05$). The significance levels for CAT indicate more pronounced effect in liver than other two organs. Increase in the activities of antioxidants is a general response of fish when exposed to environmental contaminants. As far as decrease in catalase activity is concerned, it could be due to the flux of superoxide radicals, which have been reported to inhibit catalase activity (Pandey et al. 2001). Cadmium pretreatment modulated the activities of antioxidant enzymes in liver and gills in endosulfan exposed group as compared to endosulfan alone group. The values returned to almost normal levels. A significant increase in GSH level in liver ($p < 0.001$) and gills ($p < 0.001$) was observed in endosulfan group (Table 1). In this case, kidney recorded a significant ($p < 0.001$) decrease over control values (Table 1). The increase in GSH demonstrates an adaptive response in fish exposed to an oxidative stress-inducing endosulfan in gills and liver only while in kidney it appears that antioxidant response has been overwhelmed by the ROS.

Endosulfan induced oxidative stress in fish and its effect on various antioxidants including GSH have been reported in recent studies (Dorval et al. 2003; Sohn et

Table 1. Antioxidant profile and other biochemical parameters in liver, kidney and gills of *Channa punctata* Bloch

Parameter	Liver				Kidney				Gills			
	Control	Cd	ES	Cd+ES	Control	Cd	ES	Cd+ES	Control	Cd	ES	Cd+ES
GP	214.3 ± 14.7	232.7 ± 20.6	410.3 ± 37.8^{b}	241.0 ± 22.6^{x}	298.9 ± 22.5	318.1 ± 13.9	443.4 ± 18.3^{b}	471.4 ± 25.0^{q}	279.8 ± 16.6	286.0 ± 13.5	357.9 ± 14.9^{a}	289.8 ± 10.5^{x}
GST	318.8 ± 22.8	335.6 ± 24.5	432.7 ± 20.4^{a}	349.8 ± 21.5^{x}	345.1 ± 24.8	355.8 ± 27.2	481.9 ± 17.9^{b}	504.2 ± 32.4^{p}	182.4 ± 13.6	190.5 ± 15.6	262.8 ± 16.3^{a}	205.2 ± 4.7^{x}
GR	112.4 ± 1.0	115.6 ± 2.0	130.1 ± 2.9^{b}	114.4 ± 1.4^{y}	114.9 ± 1.7	116.8 ± 1.8	124.6 ± 2.5^{a}	135.2 ± 3.0^{xq}	134.4 ± 2.9	137.2 ± 2.3	150.1 ± 5.2^{a}	147.1 ± 8.6
CAT	208.5 ± 9.7	217.3 ± 9.7	106.8 ± 5.5^{c}	205.4 ± 13.8^{y}	175.2 ± 9.8	181.6 ± 7.0	91.4 ± 7.7^{b}	82.2 ± 6.2^{r}	242.2 ± 12.0	269.3 ± 24.3	165.5 ± 13.9^{a}	269.2 ± 22.0^{x}
Pr. carb.	1.5 ± 0.1	1.5 ± 0.2	2.4 ± 0.1^{b}	1.6 ± 0.1^{y}	1.9 ± 0.03	2.0 ± 0.16	2.5 ± 0.14^{a}	2.5 ± 0.13	2.0 ± 0.06	2.1 ± 0.17	2.5 ± 0.17^{a}	2.1 ± 0.2
GSH	1.2 ± 0.03	1.3 ± 0.03	3.6 ± 0.17^{c}	1.3 ± 0.03^{z}	0.31 ± 0.003	0.31 ± 0.004	1.4 ± 0.03^{c}	1.5 ± 0.04^{r}	0.2 ± 0.004	0.21 ± 0.003	0.76 ± 0.02^{c}	0.24 ± 0.02^{z}

Values are expressed as mean ± SE (n = 6). Significant difference is indicated by $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ when compared with control values and $^{x}p < 0.05$, $^{y}p < 0.01$, $^{z}p < 0.001$ when compared with endosulfan group; $^{p}p < 0.05$, $^{q}p < 0.01$, $^{r}p < 0.001$ when compared with controls. Unit of expression of each parameter is given in Materials and Methods section. Abbreviations used: ES= endosulfan, Pr. carb.= protein carbonyls. Other abbreviations are defined in text.

al. 2004). Cadmium exhibited a restorative modulatory effect on endosulfan induced oxidative damage in gills and liver as assessed by LPO and other antioxidants. Cadmium is reported to elicit protective mechanisms mainly by its ability to induce MT (Klaassen et al. 1999). In this study, SDS-PAGE analysis showed that cadmium pre-treatment caused induction of MT-like protein in liver. AAS analysis also showed presence of cadmium in the identified bands on gel. The molecular weight of this MT-like protein was found to be 24 kDa. Furthermore, quantification of MT by spectrophotometric analysis data as shown in Figure 2 shows significant increase in MT concentrations in gills ($p < 0.01$) as well as in liver ($p < 0.001$). Exposure of fish to cadmium is known to induce the synthesis of MT in several fish species (Wu et al. 1999). Protection afforded by MT appears to be related to the ability of MT to scavenge free radicals and bind metals capable of eliciting oxidative stress (Sato and Kondoh 2002). However, in case of kidney, cadmium pretreatment had no effect or enhanced the toxicity of endosulfan as reflected in various biomarkers studied whereas cadmium alone had no toxicity. The spectrophtometric analysis showed MT in kidney but the levels in control and cadmium injected fish were almost same (Figure 2). It is concluded that the observed protection against the endosulfan-induced oxidative stress in gills and liver may in part be due to the induction of MT. It is also supported by the differences in MT–inducing potential of different tissues reflected in differential protection. Since in natural habitat a mixture of pollutants are released and persist, this study offers some insight into the likely impact of their interactions.

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REFERENCES

Ahmad I, Hamid T, Fatima M, Chand HS, Jain SK, Athar M, Raisuddin S (2000) Induction of hepatic antioxidants in freshwater fish (*Channa punctatus* Bloch) is a biomarker of paper mill effluent exposure. Biochim Biophys Acta 1523: 37-48

Basha PS, Rani AU (2003) Cadmium-induced antioxidant defense mechanism in freshwater teleost *Oreochromis massambicus* (Tilapia). Ecotoxicol Environ Saf 56: 218-221

CPCB, Central Pollution Control Board, (1994) Water quality status and statistics, Delhi, India

Dorval J, Leblond VS, Hontela A (2003) Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) exposed in vitro to endosulfan, an organochlorine pesticide. Aquat Toxicol 63: 229-241

Fent K (2003) Ecotoxicological problems associated with contaminated sites. Toxicol Lett 140-141: 353-365

Floor E, Wetzel G (1998) Increased protein oxidation in human substantia nigra pars compacta in comparison with basal ganglia and prefrontal cortex

measured with an improved dinitrophenylhydrazine assay. J Neurochem 70: 268-275

Hassall KA (1990) The biochemistry and uses of pesticides structure, metabolism, mode of action and uses in crop protection, 2nd ed., MacMillan, Houndsmill, England

Hurk P, Faisal M, Roberts Jr MH (1998) Interaction of cadmium and benzo (a) pyrene in mummichog (*Fundulus heteroclitus*): Effect on acute mortality. Mar Environ Res 46: 525-528

Kalender S, Kalender Y, Ogutcu A, Uzunhisarcikli M, Durak D, Acikgoz F (2004) Endosulfan-induced cardiotoxicity and free radical metabolism in rats: the protective effect of vitamin E. Toxicology 202: 227-235

Klaassen CD, Liu J, Choudhuri S (1999) Metallothionein: An intracellular protein to protect against cadmium toxicity. Ann Rev Pharmacol and Toxicol 39: 267-294

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193: 265-275

Pandey S, Ahmad I, Parvez S, Bin-Hafeez B, Haque R, Raisuddin S (2001) Effect of endosulfan on antioxidants of freshwater fish *Channa punctatus* Bloch: Protection against lipid peroxidation in liver by copper preexposure. Arch Environ Contam Toxicol 41: 345-352

Pandey S, Parvez S, Sayeed I, Haque R, Bin-Hafeez B, Raisuddin S. (2003) Biomarkers of oxidative stress: a comparative study of river Yamuna fish *Wallago attu* (Bl. & Schn.). Sci Total Environ 309: 105-115

Park D, Minor MD, Propper CR (2004) Toxic response of endosulfan to breeding and non-breeding female mosquitofish. J Environ Biol 25: 119-124

Pinot F, Kreps SE, Bachelet M, Hainaut P, Bakonyi M, Polla BS (2000) Cadmium in the environment: sources, mechanisms of biotoxicity, and biomarkers. Rev Environ Health 15: 299-323

Romeo M, Bennani N, Gnassia-Barelli M, Lafaurie M, Girard JP (2000) Cadmium and copper display different responses towards oxidative stress in the kidney of the sea bass *Dicentrarchus labrax*. Aquat Toxicol 48: 185-194

Satoh M, Koyama H, Kaji T, Kito H, Tohyama C (2002) Perspectives on cadmium toxicity research. Tohoku J Exp Med 196: 23-32

Sato M and Kondoh M (2002) Recent studies on metallothionein: protection against toxicity of heavy metals and oxygen free radicals. Tohoku J Exp Med 196: 9-22

Sohn HY, Kwon CS, Kwon GS, Lee JB, Kim E (2004) Induction of oxidative stress by endosulfan and protective effect of lipid-soluble antioxidants against endosulfan-induced oxidative damage. Toxicol Lett 151: 357-365

Stadtman ER (1986) Oxidation of proteins by mixed function oxidation systems: implication in protein turnover, aging and neutrophil function. Trends Biochem Sci 11: 11-12

Stuijfzand SC, Helms M, Kraak MH, Admiraal W (2000) Interacting effects of toxicants and organic matter on the midge *Chironomus riparius* in polluted river water. Ecotoxicol Environ Saf 46: 351-356

Sutherland TD, Home I, Weir KM, Russell RJ, Oakeshott JG (2004) Toxicity and residues of endosulfan isomers. Rev Environ Contam Toxicol 183: 99-113

Tort L, Kargacin B, Torres P, Giralt M, Hidalgo J (1996) The effect of cadmium exposure and stress on plasma cortisol, metallothionein levels and oxidative status in rainbow trout (*Oncorhynchus mykiss*) liver. Comp Biochem Physiol C 114: 29-34

Uchiyama M, Mihara M (1978) Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86: 271–278

Viarengo A, Ponzano E, Dondero F, Fabbri R (1997) A simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to Mediterranean and Antarctic molluscs. Mar Environ Res 44: 69-84

Wu SM, Weng CF, Yu MJ, Lin CC, Chen ST, Hwang JC (1999) Cadmium inducible metallothionein in tilapia (*Orechromis massambicus*). Bull Environ Contam Toxicol 62: 758-768

You J, Schuler LJ, Lydy MJ (2004) Acute toxicity of sediment-sorbed endrin, methoxychlor, and endosulfan to *Hyalella azteca* and *Chironomus tentans*. Bull Environ Contam Toxicol 73: 457-464