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Effect of Trivalent and Hexavalent Chromium on Antioxidant Enzyme Activities and Lipid Peroxidation in a Freshwater Field Crab, *Barytelphusa guerini*

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Chromium is a heavy metal which is widely used in paint industry, electroplating industry pigments, dyes, tanning industry and in various other things. Trivalent and hexavalent Chromium compounds are predominent having various industrial applications (Dugan, 1972). There are many ways by which Chromium is released into the environment. It is one of the constiuents in effluents from a large number of industries, particularly the tanning industry (Dad and Qureshi, 1980; Rajamani and Madhav Krishna, 1982), and this creates potential threat to aquatic organisms.

Lipid peroxidation is one of the several mechanisms by which reactive oxygen species may be toxic to cells and tissues (Machlin and Bendich, 1987). It is a free radical phenomenon. Lipid peroxidation and free radical generation are complex and deleterious process which are closely related to toxicity (Harman *et al.,* 1981). It induces alteration in the structure and function of cellular membrane which could lead to cell injury. The disrupted tissues are known to undergo lipid peroxidation faster than healthy ones. (Meera and Kale, 1996). The lipid peroxidation process is considered to be an important indicator of membrane damage (Kale and Sitasawad, 1990) which leads to cell death (Kerr *et al.,* 1972).

Free radicals are continuously produced invivo (Halliwell and Gutteridge, 1989) and there are number of protective antioxidant enzymes for dealing with these toxic substances. The delicate balance between the production and catabolism of oxidants is critical for maintenance of the biological function. Chromium is known to induce many pathological disorders in animals (Tandon, 1983; Venugopal and Reddy, 1992). One of the main causes for this condition is suspected to be involvement of free radical generation. There are only a few reports about Cr induced oxidative damage on cell membranes due to free radical generation (Sengupta *et al.,* 1990; SuGiyama and Masayasu, 1992). Hence, the present investigation has been undertaken to study the lipid peroxidation and antioxidant defence system during Chromium (Cr^{+3} and Cr^{+6}) intoxication in *Barytelphusa guerini* a freshwater field crab of high edible importance for rural population that inhabit paddy field ecosystem. The crabs were collected from the ecosystem which was contaminated by Cr metal (Shivkumar *et al.,* 1997).

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MATERIALS AND METHODS

Freshwater field crabs were collected from paddy fields near Industrial area of Hyderabad (Patanchervu). The male crabs of uniform size (30-40 gms) in the intermoult stages were brought to the laboratory and were acclimated to laboratory conditions. They were fed with fish meat ad libitum. The crabs were transferred into plastic tubs containing dechlorinated tap water. The physico-chemical characters of water were as follows : pH 7.2 to 7.4; dissolved oxygen 7.8-8.0 mg/L; CO_2 2.08 mg/L; Salinity 0.19 gm/L; Alkalinity 1.02 mg/L; Hardness of water 112 mg/L; Oxygen saturation 8%. The temperature of water was 27 ± 2°C. The stocks of toxicant solutions $CrCl_3$ and $K_2Cr_2O_7$, (Merck) were prepared in deionized water and mixed in water in required dilutions. To determine LC_{50} value the crabs were exposed to six serialconcentration of Chromium ($CrCl_3$ and $K_2Cr_2O_7$). The bioassay experiment of each concentration was repeated six times with parallel controls and mortality was noted in each concentration at 30 days. The average mortality in each concentration was taken to determine LC_{50} by plotting a graph with concentration on 'X' axis and % mortality on 'Y' axis. The LC_{50} value for $CrCl_3$ (125 mg/L) and for $K_2Cr_2O_7$ (90.46 mg/L) was calculated by using the probit curve.

The crabs were divided into three groups of 24 each group I served as a control, Groups II and III were exposed to a sublethal concentration (13 of LC_{50} i.e.. 41.2 mg/L of $CrCl_3$; 1/3 LC_{50} i.e. 30.15 mg/L) of $K_2Cr_2O_7$ for a period of 30 days as suggested by Konar (1969). During treatment with Chromium (Cr^{+3} and Cr^{+6}) the crabs were fed every 24 hrs before renewing the toxicant water. The animals were starved for 24 hrs prior to experimentation to avoid metabolic differences, if any existed due to differential feeding. Six crabs from each group were sacrificed after 1, 7, 15 and 30 days of exposure and hepatopancreas and gill tissues were isolated and transfered to deep freezer at -10°C. Each biochemical parameter was assayed in six individual animals, in triplicate in both control and Chromium (Cr^{+3} and Cr^{+6}) treated groups.

Lipid peroxidation was determined by the Living stone *et al.* (1990) method; hepatopancreas and gills were homogenized in cold KCl buffer (1.15%) and the crude homogenate was mixed with an equal amount of 20% of TCA (Trichloro acetic acid) and boiled for 10 minutes at 70°C. The solution was centrifuged at 12000 g in cold centrifuge for 20 min. 1 ml of supernatant was collected and treated with 0.4 ml of TBA (Thiobarbituric acid) and boiled for 10 min at 70°C. The intensity of the colour was measured at 532 nm against reagent blank, 1,1,3,3-tetraethoxy propane (TEP). LPO was expressed as nanomoles of MDA.

Xauthine oxidase (XOD) acitivy was measured according to Govindappa and Swami (1965). The reaction mixture contained xanthine as substrate and NAD as coenzyme. The protein content was estimated by the Lowry *et al.* (1951) method using bovine serum albumen as a standard.

Glucose-6-phosphate dehydrogenase activity was measured according to Lohr (1951). The reaction mixture 2 ml contained 0.4 ml of phosphate buffer (pH 7.8), 0.4 ml of INT, 0.5 ml of glucose-6-phosphate, 0.1 ml of NADP, 0.1 ml of triethalamine, 0.5 ml of enzyme. Enzyme activity was assayed by measuring formazan formed and at 495 nm against toluene blank.

Superoxide dismutase (SOD) an antioxidant enzyme activity estimated indirectly by the ability of the enzyme to inhibit O_2 dependent autoxidation of pyrogallol according to Marklund and Markelund (1974). The reaction mixture (2 ml) contained 1.5 ml of Tris HCl buffer pH (8.2), 0.2 ml of DETPA (Diethylene triamine penta acetic acid) 0.2 ml of pyrogallol and 0.1 ml of enzyme. The reaction was intiated by addition of pyrogallol and the increase in absorbance was read at 420 nm against reagent blank (which had pyrogallol alone); SOD activity activity was expressed as micromoles of enzyme / mg protein.

RESULTS AND DISCUSSION

The present results show that trivalent and hexavalent Chromium induced oxidative stress (table 1-4). The alterations are more marked in crabs treated with Cr^{+6} than those treated with Cr^{+3} form of the metal. This can be explained on the basis of an anionic vs cationic entry (Eastin *et al.*, 1980) and their differential uptake that subsequently leads to toxic manifestations and the ability of hexavalent Chromium to penetrate cell membrane more rapidly than the trivalent Chromium (Mertz, 1969; Baetjer *et al.*, 1974).

Peroxidative damage in biological membrances is known to be deleterious to the membrane structure and functions (Pryor, 1977). Lipid peroxidation can be triggered by the superoxide anion radical and hydroxyl anion ($O_2^{\cdot-}$, $^{\cdot}OH$) formed in the Fenton reaction. During Fenton reaction H_2O_2 is broken down to ‘OH and hydroxyl anion in the presence of catalytic amounts of iron(II) salts as well as Cc(I). These redox metals, often play a role in intiating this type of peroxidation (Halliwell, 1978).

In the present study the LPO was measured in the form of MDA content, it was significantly increased upto 15 days and there after it has been decreased in hepatopancreas. The decrease in LPO could be due to the increased activity of SOD and due to organ specificity. In gills the MDA content was increased throughout the exposure period. Venugopal *et al.* (1997) have shown similar results in LPO during heavy metal toxicity in the crab *Barytelphusa guerini*. Recent studies have shown that reduction from hexavalent to trivalent form of Chromium generates free radical species such as active oxygen radicals, which are responsible for lipid peroxidation (SuGiyama and Masayasu, 1992).

Increased activity levels of xanthine oxidase indicates the superoxide radical generation (McCord, 1985). In the present study the enhanced activity of XOD

Table 1. Lipid Peroxidation (LPO) in the tissues of Freshwater field crab *Barytelphusa guerini* exposed to a trivalent and hexavalent chromium

Tissues	1 Day			7 Days			15 Days			30 Days		
	Control	$CrCl_3$	$K_2Cr_2O_7$	Control	$CrCl_3$	$K_2Cr_2O_7$	Control	$CrCl_3$	$K_2Cr_2O_7$	Control	$CrCl_3$	$K_2Cr_2O_7$
Hepato Pancreas	12.46 ±0.654 -	13.45 ±0.94 +7.94	14.05 ±1.12* +12.76	12.16 ±0.595 -	14.58 ±1.18** +19.90	16.82 ±1.24** +38.32	11.96 ±0.731 -	15.32 ±0.98** +28.09	18.15 ±1.25*** +51.75	12.38 ±1.04 -	14.45 ±0.92** +16.72	16.78 ±1.13** +35.54
Gill	6.02 ±0.540 -	6.71 ±0.61* +11.46	6.89 ±0.652* +14.45	5.85 ±0.543 -	7.06 ±0.660* +20.68	7.64 ±0.713** +30.59	5.98 ±0.538 -	7.15 ±0.616* +19.56	7.57 ±0.525** +26.58	5.81 ±0.501 -	7.65 ±0.661** +31.66	8.32 ±0.814** +43.20

Values expressed as η moles of MDA (Malondialdehyde) / gm tissue. Values are mean ± S.D. of six individual observations. Experimental values significant at $P < 0.1$*, $P < 0.01$**, $P < 0.001$***.

Table 2. Xanthine oxidase (XOD) activity in the tissues of freshwater crab *Barytelphusa guerini* exposed to trivalent and hexavalent Chromium

Tissues	1 Day			7 Days			15 Days			30 Days		
	Control	$CrCl_3$	$K_2Cr_2O_7$	Control	$CrCl_3$	$K_2Cr_2O_7$	Control	$CrCl_3$	$K_2Cr_2O_7$	Control	$CrCl_3$	$K_2Cr_2O_7$
Hepato Pancreas	1.112 ±0.186 -	1.194 ±0.242 +7.37	1.263 ±0.260* +13.57	1.131 ±0.256 -	1.271 ±0.291* +12.37	1.392 ±0.244* +23.07	1.123 ±0.142 -	1.356 ±0.231* +20.74	1.582 ±0.226* +40.87	1.118 ±0.270 -	1.330 ±0.232* +18.96	1.532 ±0.227** +37.03
Gill	0.562 ±0.038 -	0.597 ±0.047 +6.22	0.624 ±0.051* +9.93	0.550 ±0.048 -	0.611 ±0.050* +11.09	0.662 ±0.055** +20.36	0.538 ±0.042 -	0.595 ±0.025* +10.59	0.636 ±0.036** +18.21	0.554 ±0.032 -	0.649 ±0.038** +17.14	0.765 ±0.056*** +37.18

Values expressed as μ moles of formazan / mg protein / hr. Values are mean ± S.D. of six individual observations. Experimental values significant at $P < 0.1$*, $P < 0.01$**, $P < 0.001$***.

Table 3. Glucose-6-phosphate dehydrogenase (G-6-PDH) activity in the tissues of freshwater field crab *Barytelphusa guerini* exposed to a trivalent and hexavalent chromium

Tissues	1 Day			7 Days			15 Days			30 Days		
	Control	$CrCl_3$	$K_2Cr_2O_7$	Control	$CrCl_3$	$K_2Cr_2O_7$	Control	$CrCl_3$	$K_2Cr_2O_7$	Control	$CrCl_3$	$K_2Cr_2O_7$
Hepato Pancreas	0.284 ±0.016 -	0.348 ±0.024** +22.5	0.387 ±0.029 +36.26	0.293 ±0.018 -	0.402 ±0.02*** +37.20	0.451 ±0.026*** +53.92	0.306 ±0.024 -	0.405 ±0.018*** +32.35	0.406 ±0.038*** +45.75	0.309 ±0.026 -	0.398 ±0.035** +28.80	0.433 ±0.525** +40.12
Gill	0.198 ±0.013 -	0.218 ±0.017NS +10.10	0.225 ±0.018** +13.63	0.210 ±0.014 -	0.236 ±0.017* +12.38	0.246 ±0.020* +17.14	0.226 ±0.019 -	0.265 ±0.021* +17.25	0.276 ±0.024* +22.12	0.207 ±0.016 -	0.250 ±0.022* +2.25	0.273 ±0.023** +31.88

Values expressed as μ moles of Formazan / mg protein / hr. Values are mean ± S.D. of six individual observations. Experimental values significant at $P < 0.1$*, $P < 0.01$**, $P < 0.001$***.

Table 4. Superoxide dismutase (SOD) activity in the tissues of freshwater field crab *Barytelphusa guerini* exposed to a trivalent and hexavalent chromium

Tissues	1 Day			7 Days			15 Days			30 Days		
	Control	$CrCl_3$	$K_2Cr_2O_7$	Control	$CrCl_3$	$K_2Cr_2O_7$	Control	$CrCl_3$	$K_2Cr_2O_7$	Control	$CrCl_3$	$K_2Cr_2O_7$
Hepato Pancreas	1.935 ±0.141 -	2.356 ±0.217 +21.75	2.688 ±0.204** +38.91	2.224 ±0.152 -	3.044 ±0.245** +36.87	3.619 ±0.279*** +65.91	2.136 ±0.196 -	2.793 ±0.211** +30.75	3.208 ±0.256** +50.18	2.015 ±0.185 -	2.689 ±0.221** +33.44	3.145 ±0.285*** +56.07
Gill	2.112 ±0.152 -	2.374 ±0.210** +12.40	2.503 ±0.223** +18.51	2.182 ±0.186 -	2.637 ±0.205** +20.85	2.944 ±0.232** +34.92	2.054 ±0.118 -	2.412 ±0.245** +17.42	2.687 ±0.261** +30.81	2.245 ±0.186 -	3.484 ±0.286*** +55.18	3.945 ±0.277*** +75.72

Values expressed as μ moles / mg protein. Values are mean ± S.D. of six individual observations. Experimental values significant at $P < 0.1$*, $P < 0.01$**, $P < 0.001$***.

indicates the generation of superoxide radicals. Increased generation of superoxide radicals could lead to LPO. Xanthine oxidase activity also reflects the intensity of the stress due to toxic action. Heavy metals are known to increase the biochemical stress in the organisms due to deterioration of metabolic cascade (Hudercova and Ginter, 1992). Therefore, in the present study increased activity levels of XOD suggest stress as a result of free radical generation due to Chromium toxicity which inturn cause the onset of LPO.

Glucose-6-phosphate dehydrogenase a key enzyme involved in the supply of reduced nucleotide NADP, is found to be increased in hepatopancreas and gills. Enhancement in the activity indicates the supply of addition energy to the crabs under oxidative stress. Reddy and Baghyalaxmi (1994) reported an increase in G-6-PDH in crab exposed to heavy metals. Sengupta et al. (1990) have shown similar increase in G-6-PDH activity in rat intestinal epithelial cells exposed to Chromium ($K_2Cr_2O_7$).

Superoxide dismutase a metallic antioxidant enzyme plays an active role during appearance of stress as a result of free radical generation due to heavy metal toxicity was found increased in both tissues. This increased activity levels of enzymes could be to combat free radical generation during Chromium toxicity. Similar results were also observed by Sengupta et *al.* (1990) in rats exposed to hexavalent Chromium. We conclude that the presence of oxyradical generation cause free radical scavengers, antioxidant enzymes to arise specifically from oxidative stress induced by Chromium further studies to assess the mechanism of action and the inter-relationship between free radical scavengers are under progress.

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