Effect of chromium on certain aspects of cellular toxicity

Sankar Kumar Dey[1],Somenath Roy [2]

ABSTRACT:

Background: The impact of chromium exposure was studied on liver, kidney, testis, spleen, cerebrum and cerebellum of male Wistar rats (80-100 g body weight).

Methods: It was observed that treatments of rats with chromium (i.p. at a dose of 0.8 mg / 100 g body weight per day) for a period of 28 days caused significant increase in chromium content while declining the body weight along with the organ weight, except liver.

Results: Decreased acid phosphatase (ACP) and alkaline phosphatase (ALP) activities were observed in most of the organs. Significant increases in the cholesterol contents of all the organs were associated with the significant decreases in the level of phospholipids. Lipid peroxidation decreased in liver and kidney while it increased in testis, cerebrum and cerebellum. Reduced glutathione (GSH) level was found to be increased in liver, spleen and cerebrum, and decreased in kidney and testis. Catalase activity became elevated in liver, kidney, spleen and cerebellum while it decreased in testis.

Conclusion: It is suggested that chromium treatment at the present dose and duration induces general tissue toxicity by causing membrane damage due to changes in the relative proportion of cholesterol and phospholipids in the membrane structure. In addition, tissue specific toxicity is affected by lipid peroxidation in testis, cerebrum and cerebellum, and in other tissues increased GSH level or enhanced catalase activity prevents lipid peroxidation to occur due to reactive oxygen species produced from chromium transformation.

Key words: Acid phosphatase,Alkaline phosphatase, Cholesterol,Chromium,Lipid peroxidation,Phospholipids, Reactive oxygen species.

INTRODUCTION

Chromium (VI) compounds exert their genotoxicity and mutagenicity by complex metabolic pathways that generate a variety of reactive forms of chromium and free radicals. Recent reports regarding chromium-induced carcinogenicity and apoptosis (1)have added a great deal of knowledge in the study of toxicological impact of chromium. In contrast to chromium (III), chromium (VI) is easily transported into cells at physiological pH through the permease system which transports phosphate and sulfate anions (2). Inside the cells chromium (VI) is reduced to reactive intermediates such as chromium (V), (IV) and finally to the more stable chromium (III) by cellular reductants including glutathione (GSH), cysteine, ascorbic acid and riboflavin as well as NADPH-dependent flavoenzymes such as microsomal cytochrome P_{450}(2,3). The reduction process generates free radicals such as active oxygen radicalsalthough itsgenotoxic and other noxious effects can be modified by antioxidants. In recent years, muchattention has been paid to the role of antioxidants, in

[1]Department of Bio-Medical Lab. Science & ManagementVidyasagar University, Midnapore West Bengal, India.
[2] Department of Human Physiology with Community Health, Vidyasagar University, Midnapore West Bengal, India.
*Corresponding Author:Sankar Kumar Dey
Email: sankar_dey@yahoo.co.in

particular active O_2 scavengers, on chromate-induced injuries such as DNA damage, lipid peroxidation, enzyme inhibition, cytotoxicity, mutationsetc(4).

Hepatic and renal toxicities have been reported in workers and in animals exposed to chromium (VI) (5,6). Lipid peroxidation *in vivo* has been considered to cause membrane damage and to play an important role in the induction of tissue injuries by chromium and some metals (7,8). On the other hand, Staceyand Klaassenhavesuggested that the increase in lipid peroxidation in isolated hepatocytes induced by heavy metals is not necessarily responsible for cell injury (9). Other studies have also revealed that lipid peroxidation induced by chromium is not the only factor for tissue damage and cytotoxicity (10, 11). Accordingly, the present investigation was intended to study the cellular toxicity following chromium exposure in terms of acid phosphatase (ACP), alkaline phosphatase (ALP) and catalase activities as well as cholesterol, phospholipid and reduced glutathione (GSH) contents along with lipid peroxidation.

MATERIALS AND METHODS

Chemicals: Cholesterol and catalase were purchased from Sigma Chemicals Company (St. Louis, MO, USA). Thiobarbituric acid, reduced glutathione, 5,5-dithiobis trinitrobenzoic acid and P-nitrophenyl phosphate were purchased from SRL, Bombay, India. Other chemicals used throughout the investigation were of analytical grade.

Animals and diets: Male albino rats of the Wistar strain (80–100 g) were fed with a lab-prepared diet, as described by Dey(12)with water ad libitum. Laboratory acclimatized rats were divided into two groups of almost equal average body weight. The animals of one of the groups were injected intra-peritoneally (*i.p.*) with chromium as CrO_3 at a dose of 0.8 mg / 100 g body weight per day (20% LD_{50}) for 28 days, as described earlier (12). The animals of the other group serving as controls received only the vehicle (0.9% NaCl).

Tissue collection: After the experiment period, the rats were sacrificed by cervical dislocation and their livers, kidneys, testes, spleens, cerebrums and cerebellums were immediately dissected out of the body, wiped off the blood and weighed. The recovered tissues were then stored at - 20^0C until analysis.

Estimation of chromium: Homogenates of tissues were digested with an acid mixture containing nitric acid, sulfuric acid and perchloric acid in a ratio of 6:1:1 over a regulated heater. After digestion, the acid mixture was evaporated with occasional additions of triple distilled water and the solution thus obtained was used for chromium estimation by Atomic Absorption Spectrophotometer.

Measurement of acid and alkaline phosphatase activities: Tissues were homogenized in ice-cold 0.25 M sucrose. The 2% (W/V) homogenates were used for the measurement of acid and alkaline phosphatase activities using p-nitrophenyl phosphate (PNPP) as substrate according to the method proposed by Linhardt and Walter (13).

Estimation of cholesterol and phospholipid: Cholesterol and phospholipid contents of tissues were estimated by methods stated by Zlatkis(14) and Christopher (15), respectively.

Assay for lipid peroxidation: Lipid peroxidation was measured by means of estimating the thiobarbituric acid reactive substances (16).

Measurement of GSH: The GSH contents of tissues were determined by the method of Beutler(17).

Determination of catalase activity: Catalase activity of tissues was assayed using hydrogen peroxide as substrate (18).

Statistical analysis: The significance in the differences between the means was evaluated by Student's t-test, and probability levels of 5% or less were considered to be statistically significant(19).

RESULTS

Changes in the body weight during the period of treatment are depicted in Figure-1

and it can be observed that the body weight of chromium-treated rats significantly decreased as compared to that of the control group.

Upon exposure to chromium, all the organs tested showed significant increases in chromium contents and while the mean weight of testis and spleen significantly decreased, mean livermass significantly increased (Table-1).

Following chromium exposure, ACP and ALP activities decreased significantly in all the organs tested except for ALP activity in liver, which remained unaltered (Figure-2).

Figure-3 shows alterations in cholesterol and phospholipid contents of organs in response to chromium exposure. Cholesterol content increased and phospholipid content decreased significantly in all the organs studied in chromium-treated rats (Figure-3).

The data presented in Figure-4 reveal that the lipid peroxidation significantly increased in testis, cerebrum and cerebellum, whereas it was reduced significantly in liver and kidney following exposure to chromium. The level of reduced glutathione was found to be significantly diminished in kidneys and testes, and increased in spleens and cerebrums of rats when exposed to chromium (Figure-4). On the other hand, the catalase activity showed significant increases in all the organs, except for cerebrumand especially testis following chromium treatment. Testis showed a significant decrease in catalase activity (Figure-5).

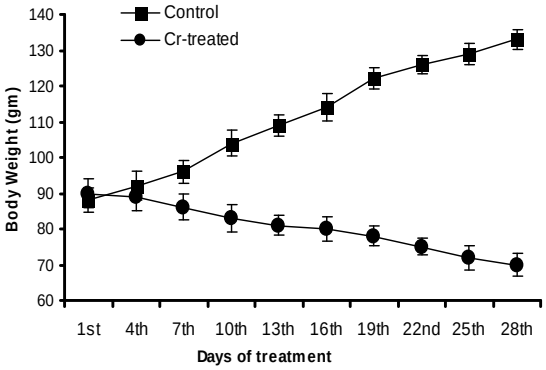


Figure 1: Changes in body weight of rats during the period of intra-peritoneal administration of chromium

Table 1: Mean organ weight and chromium content following CrO_3 administration†

Tissues	Groups of animals	Organ weight (g/100g bw)	Chromium content (µg/g tissue)
Liver	Control	3.07 ± 0.10	0.34 ± 0.03
	Chromium treated	4.66 ± 0.22*	2.97 ± 0.14*
Kidney	Control	0.79 ± 0.03	1.03 ± 0.03
	Chromium treated	0.78 ± 0.04	6.55 ± 0.17*
Testes	Control	1.46 ± 0.15	0.13 ± 0.01
	Chromium treated	1.04 ± 0.05*	1.40 ± 0.04*
Spleen	Control	0.94 ± 0.08	0.56 ± 0.04
	Chromium treated	0.57 ± 0.09*	3.74 ± 0.08*
Cerebrum	Control	0.90 ± 0.04	0.13 ± 0.01
	Chromium treated	0.86 ± 0.03	1.33 ± 0.04*
Cerebellum	Control	0.19 ± 0.01	0.12 ± 0.01
	Chromium treated	0.19 ± 0.01	1.14 ± 0.07*

† Values are means of six observations ± SEM. Indicates significant difference between the two groups (P<0.05).*

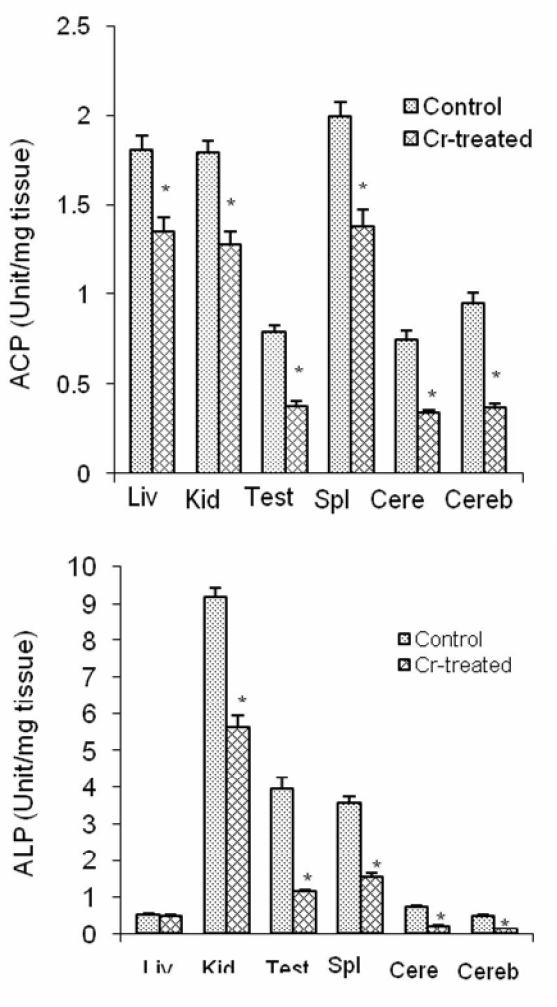
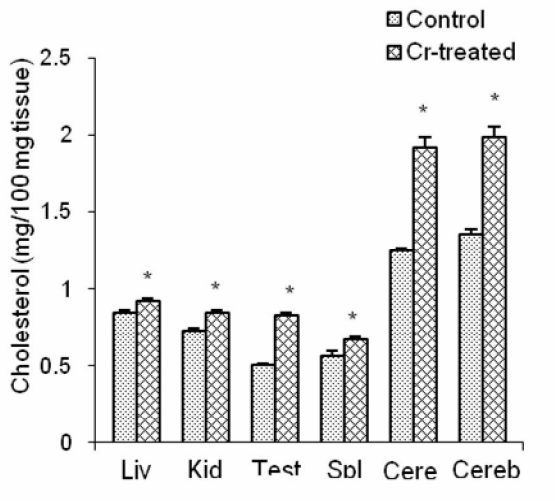


Figure 2: Acid phosphatase and alkaline phosphatase activities of different tissues following exposure to chromium.

Values are means of six observations ± SEM.

** Indicates significant difference between the two groups (P<0.05).*



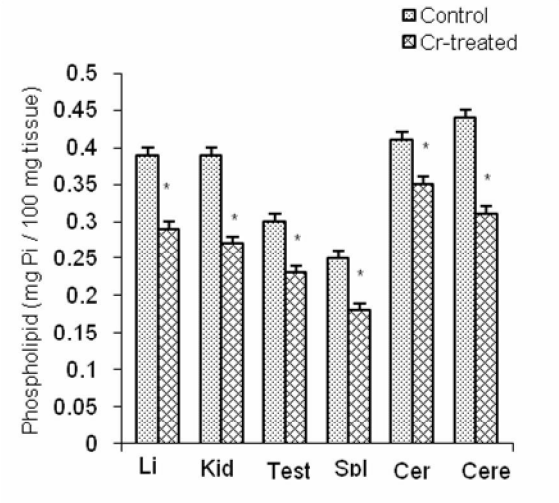


Figure 3: Cholesterol and Phospholipid content of different tissuesfollowing exposure to chromium.

Values are means of six observations ± SEM.

**Indicates significant difference between the two*

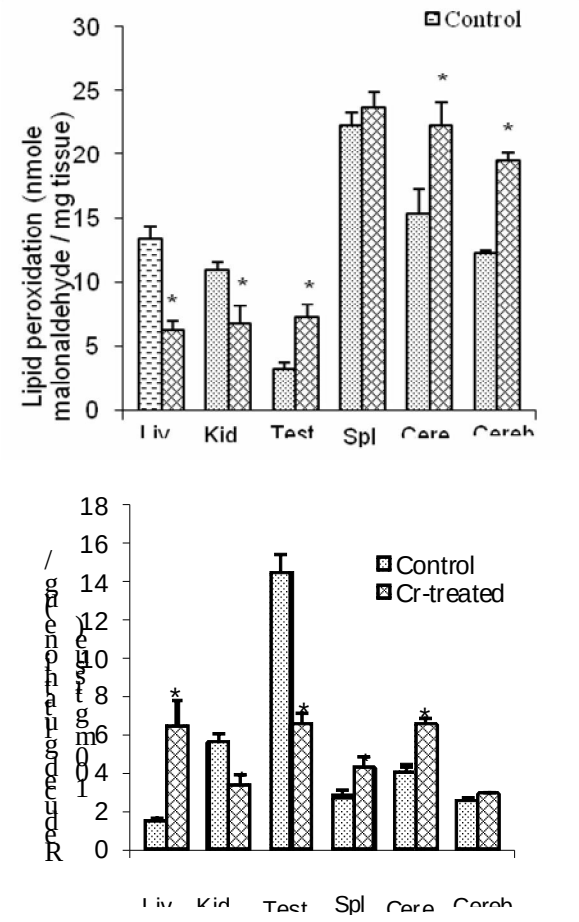


Figure 4: Lipid peroxidation and reducedglutathione content of different tissues following exposure to chromium.

Values are means of six observations ± SEM.

**Indicates significant difference between the two groups (P<0.05).*

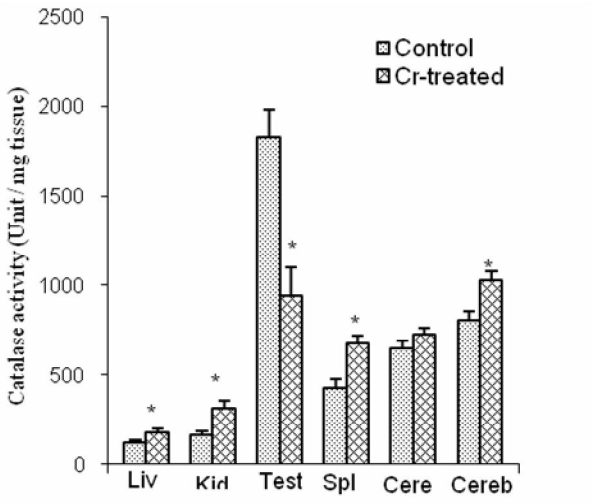


Figure 5: Catalase activity of different tissues following exposure to chromium.
Values are means of six observations ± SEM.
** Indicates significant difference between the two groups (P<0.05).*

DISCUSSION

During the study,chromium-exposed ratsloosed weight(Figure-1). This impact on body weight might be due to direct effects of chromium and not as a result of reduced food intake as the control rats were pair-fed to chromium-treated rats. This lowered body weight was not reflected in the individual organ weights, except for testis and spleen (Table-1). Among ass organs only liver showed a significant increase in its weight despite the increased chromium content. Other organs demonstratedeither decreased or unaltered weight in response to increased chromium content (Table-1). These findings suggest that chromium have a differential impact on organs size.

ACP mainly remains within the lysosomes which are primary responding organelles of cells to metal toxicity. A decrease in ACP activity in response to chromium exposure was observed in all organs tested (Figure-2) whichmight be due to the direct effect of chromium on lysosomes. Conversely,Susa reported that lysosome was the more stable portion of the cell when exposed to chromium (10) and Cr^{3+}has been describedto have possible role in stabilization of the cell membrane (20). Therefore the Cr^{3+} produced from the injected Cr^{6+}could have caused interruption in the release of ACP,leading to the observed decrease in its activity. Like ACP, ALP activity was also decreased significantly in all the organs, except for liver (Figure-2). These observations appear to be in conformity with earlier reports (21) and as ALP is a membrane-bound enzyme, the chromium-induced decrease in its activity indicates significant damage to the plasma membrane. Similar findingswere reported earlier in the kidney (7), and liver (22) of animals.

After chromium exposure, a significant increase in cholesterol and a significant decrease in phospholipid levels were observed in all studied organs (Figure-3). The increased cholesterol level could be due to its decreased utilization or increased synthesis. Chromium has inhibitory effects on steroidogenesis and can decreased utilization of cholesterol particularly in steroid-producing tissues such as testis (23). On the other hand, the decreased phospholipid level indicates damageto the membrane structure of the cell as a result of chromium impact on the lipid-catabolising enzymes whichhas been confirmed by increased excretion of urinary lipid metabolites following chromium exposure(24). The increase in lipid catabolism may result in accumulation of acetyl-coA, which in turn could lead to increased synthesis of cholesterol particularly in non-steroid producing tissues. Thus, by changing the proportion of cholesterol and phospholipids, chromium may cause alteration in membrane fluidity and damage the cell membrane structure and function.

Rana and Kumar (25) reported enhancement of lipid peroxidation in rat liver after heavy metal poisoning with mercury, molybdenum, copper, chromium and manganese. It has been demonstrated that the chromium (V) complexes which are produced following reduction of chromium (VI) by cellular biological reductants, react with hydrogen peroxide to generate hydroxyl radicals which in turn act as the initiators of primary events in chromium (VI) cytotoxicity (26,27). Bagchi(28) showed that

chromium (VI) induces increases in hepatic mitochondrial and microsomal lipid peroxidation. Additionally, dose-dependent dual role of chromium (VI) has been described. Hexavalent chromium of 1000 μM/l had an augmentative effect on thiobarbituric acid reactive substance formation in isolated rat hepatocytes, whereas the lower concentration (125 μM/l) of it showed an inhibitory effect on lipid peroxidation (29). We observed significant decrease in malonaldehyde production in liver and kidney while other organs, namely testis, cerebrum and cerebellum showed significant increases in the thiobarbituric acid reactive substance production (Figure-4). Yonaha(30) reported that both hexavalent and trivalent chromium, at lower concentrations in the range of 1-100 μM/l inhibited lipid peroxidation induced by ascorbate and NADPH in rat liver microsomes. Contrary to the above studies, Bagchireported treatment with chromium (VI) increases hepatic and brain mitochondrial and microsomal lipid peroxidation that reaches to maximum at approximately 60-75 days and interestingly no more significant escalationwas observed beyond 75 days(31). Additionally Ueno (29)had earlier showed that both hexavalent and trivalent chromium induces a peak rise in lipid peroxidation at 12 h in liver and at 48 h in kidney. Hence, it is clear that the dose and duration of exposure to chromium as well as the recovery capacity or adaptability of the individual influence the extent of lipid peroxidation.

It has been known that lipid peroxidation occurs as a result of the decrease in intracellular reduced glutathione (GSH) concentration (32,33). Ueno (29) reported that the content of intracellular GSH in isolated rat hepatocytes was diminished after chromium (VI) treatment. In the present investigation, a significant decrease in GSH level has been observed only in kidney and testis (Figure-4) whileit increased significantly in liver, spleen, and cerebrum and the GSH level in cerebellum remained unaltered (Figure-4). Furthermore, the catalase activity was found to be significantly increased in liver, kidney, spleen and cerebellum whereas it decreased in testis (Figure-5). Sengupta (34) showed that oral administration of chromium (VI) to rats led to a depression in nonenzymatic (including GSH) and enzymatic antioxidants (catalase). It was reported that *in vitro* chromium-induced lipid peroxidation was prevented by the addition of catalase which plays an important role in neutralizing hydrogen peroxide (29). Our data suggest that in liver reactive oxygen species produced from transformation of chromium are handled with increased amounts of GSH and enhanced activity of catalase and as a result lipid peroxidation was decreased. Consequently, lipid peroxidation might not be the cause of liver damage caused by chromium at the present dose and duration. In kidney the decrease in lipid peroxidation suggested that itwas not responsible for tissue damage. Although the GSH level in kidney was diminished, the accumulation of H_2O_2 due to reactive oxygen species originating from metabolic reduction of chromium was probably prevented by a greater increase in catalase activity. The diminution in GSH level and catalase activity of testis following exposure to chromium suggests accumulation of reactive oxygen species which in turn might have led to increased lipid peroxidation, causing testicular tissuedamage. The unaltered lipid peroxidation in chromium-exposed spleen tissue suggests that the increased GSH level and catalase activity observed were adequate in eliminating reactive oxygen species and thereby preventing lipid peroxidation in spleen. The enhanced lipid peroxidation with increased GSH content in chromium-exposed cerebrum indicates that the increase in GSH content alone is unable to counteract the lipid peroxidation by the reactive oxygen species produced from chromium transformation. In cerebellum, on the other hand, increased lipid peroxidation in spite of enhanced catalase activity suggests that increased catalase activity alone is incapableof preventing lipid peroxidation.

CONCLUSION

Thus, ourstudy indicated that chromium administration at the mentioned dose and duration induces general tissue toxicity due to membrane damage as a result ofalterations in the relative proportions of cholesterol and phospholipids in the membrane structure. Cellular damage due to lipid peroxidation appears to be restricted to testis, cerebrum, and cerebellum while in other tissues the increased GSH levels or catalase activity can prevent its propagation. Therefore; it appearsthat testis and brain are more vulnerable to the chromium exposure.

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