Chapter-3

Observation of the Cr (VI) induced toxicity in liver and lungs Mitochondria

Contents

3.0 Introduction

One of the most important and available chemical salt of hexavalent chromium is Potassium dichromate ($K_2Cr_2O_7$). Commercially this compound is mainly utilised in numerous metal industries, metal wielding, metal chrome finishing and stainless steel factories, chemical paint industry, tannery, textile industry, wood processing and photography (Barceloux, 1999). Inadequately treated effluents from such industries carry highly reactive metal pollutants including chromium and potentially threaten healthy survival of living beings. Toxicity of usually available chromium compounds is dependent on their water solubility and valence state. Trivalent chromium is regarded one of the essential micronutrient for crucial physiological functioning of human beings and mammals. More soluble hexavalent chromium compounds have shown higher toxicity to occupational exposure in industries (Wang et al, 2006). Dolai et al, (2016) have reviewed literatures to elucidate the mode of short term (acute) and long-term (chronic) adverse effects of chromium exposure resulting carcinogenesis, cutaneous allergy, pulmonary injury, neurotoxicity, toxicity of reproductive organs, DNA damage and mutagenesis. Cytotoxicity and tissue damage related to oxidative stress and carcinogenesis are mainly due to hexavalent form of chromium (Bagchi et al, 2002b).

Mitochondria are principal provider of energy (ATP) produced in cellular oxidative process. ATP levels are decreased to great extent in fibroblast cells of hamster treated with Cr (VI) compound (Debetto et al, 1982), fibroblast cells of human gingiva (Messer and Lucas, 2000), and in cells of isolated rat thymus (Lazzarini et al, 1985). This observation has reasonably explained with the hindrance of oxidative intracellular mitochondrial respiration in lymphocytes of rat (Messer and Lucas,

2000), isolated cells of rat liver (Ryberg and Alexander, 1984). The inhibition of mitochondrial oxidative respiration has been demonstrated in isolated rat hepatocytes (Ryberg and Alexander, 1984) and also in sub mitochondrial microsomes of rat liver (Ryberg and Alexander, 1990). The precise mechanism of Cr (VI) induced interference of the mitochondrial oxidative phosphorylation for ATP production is not clearly explained. The reasonable explanation has been propounded that the strong oxidising property of hexavalent chromium halts the electron transport chain taking electron for intracellular reduction. Thus ATP production is reduced, as well as oxidative reactive stress is created inside the cell. Trivalent chromium produced in the process of hexavalent Cr (VI) reduction bind with mitochondrial core enzymes and decrease energy generation (Debetto et al, 1982). Cr (VI) reduction leads to generation of highly reactive hydroxyl radicals ($^{\cdot}OH^{-}$) from H_2O_2 through Fenton-reaction (Shi et al, 1991). Daily oral administration of low-dose Cr (VI) to experimental rats shows augmented lipid peroxidation of mitochondria in isolated brain and liver (Travacio et al, 2000).

In this present research investigation, hexavalent chromium-induced oxidative damage in liver and lungs mitochondria are evaluated.

3.1 Methodology

3.1.1Chemicals

Procurement of necessary chemicals, reagents and other articles was described earlier in chapter -II

3.1.2 Maintenance of animals

Animals' maintenance was described in chapter-II.

3.1.3 Mode of treatment

Rats were divided into experimental and control groups of almost matching equal average body weight about 80-100 gram. Experimental group were injected intraperitoneally (i.p.) with $K_2Cr_2O_7$ at a dose of 800 microgram per 100 gram of body weight per day (20% LD_{50}) for 28 days, as described earlier (Dey et al, 2003). Control group rats were injected intraperitoneally the vehicle (0.9% NaCl) only,

3.1.4 Tissue collection: Collection of tissues was described in chapter-II.

3.1.5 Homogenization of tissues: Tissue homogenization was described in chapter-II

3.1.6 Isolation of Mitochondria

Liver and lungs mitochondria of male albino rats were isolated according to differential centrifugation method (Gazotti et al, 1979).

3.1.7 Analytical methods

Following methods like Lipid peroxidation, Conjugated dienes, SOD, GSH, GSSG, GPx, GR, GST and protein were estimated, described in chapter-II. But NO production was tested in liver and lungs mitochondria as per the described method (Sanai *et al,* 1998).

3.1.8 Statistical Analysis

Data analysis was described in chapter-II.

3.2 Results

The MDA and conjugated dienes content are elevated to a significant level in liver and lungs mitochondria after chromium treatment in comparison with control group (Figure 1 & 2). Also it has been noticed that NO production is increased remarkably in liver and lungs mitochondria after chromium treatment when it is compared with control rat group (Figure 3).

The activity of SOD is noticeably decreased in selected tissue mitochondria in response to chromium when it is compared with control (Figure 4). But the activity of catalase in liver and lungs homogenate has remarkably diminished against control group (Figure 5).

The GSH and GSSG levels are remarkably decreased in liver and lungs mitochondria in response to chromium injection (Figure 6 & 7). GPx, GR and GST activities have been greatly decreased after chromium treatment in liver and lungs mitochondria (Figures 8, 9 & 10).

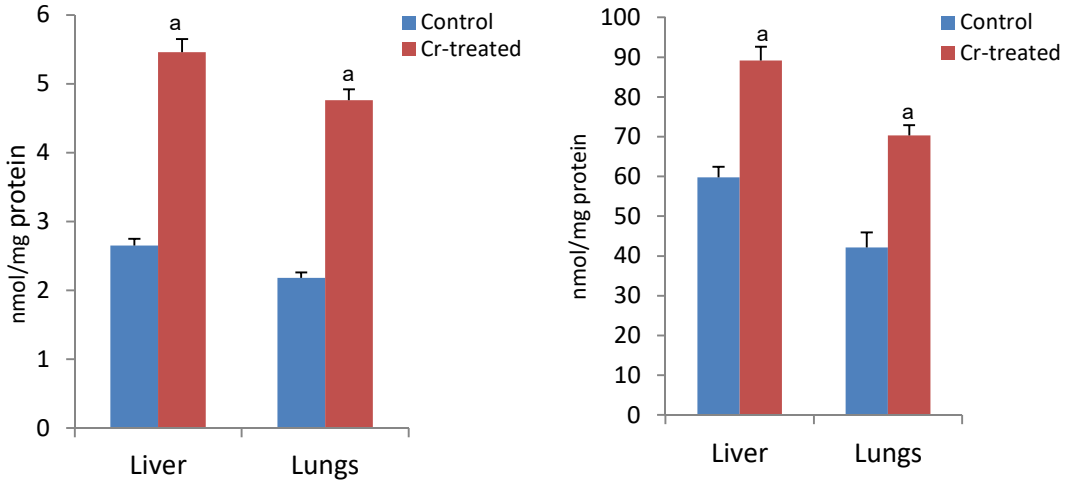

Figure 1 & 2: Changes the MDA and conjugated dienes content in liver and lungs mitochondria after chromium treatment. [a] noted significant difference ($P<0.05$).

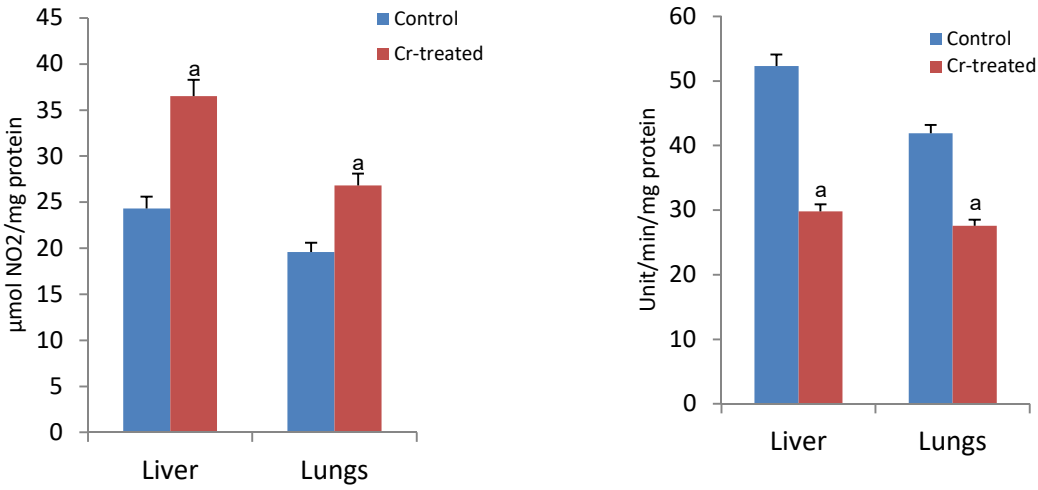

Figure 3 & 4: Changes the nitric oxide release (NO) and the activity of SOD in tissue mitochondria in response to chromium. [a] noted significant difference ($P<0.05$).

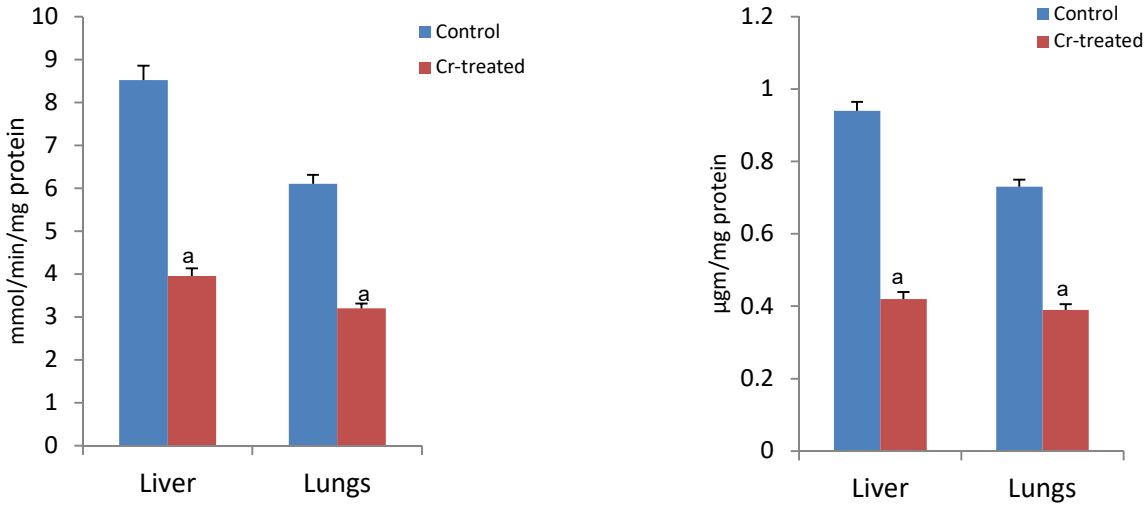

Figure 5 & 6: Shows variation of the activity of catalase in liver and lungs homogenate and the GSH level in liver and lungs mitochondria after exposure to chromium. [a] noted significant difference ($P<0.05$).

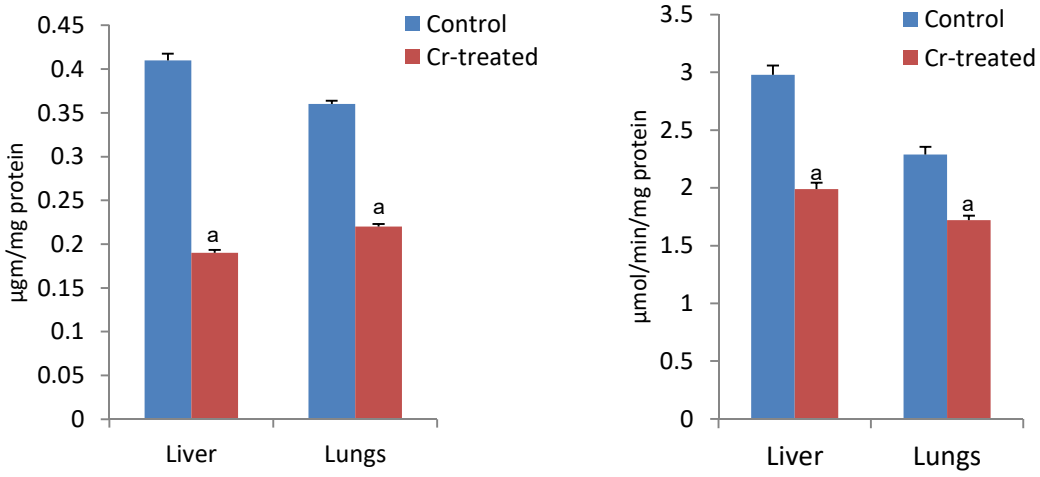

Figure 7 & 8: Changes the GSSG level and GPx activities in tissue mitochondria after chromium treatment. [a] noted significant difference ($P<0.05$).

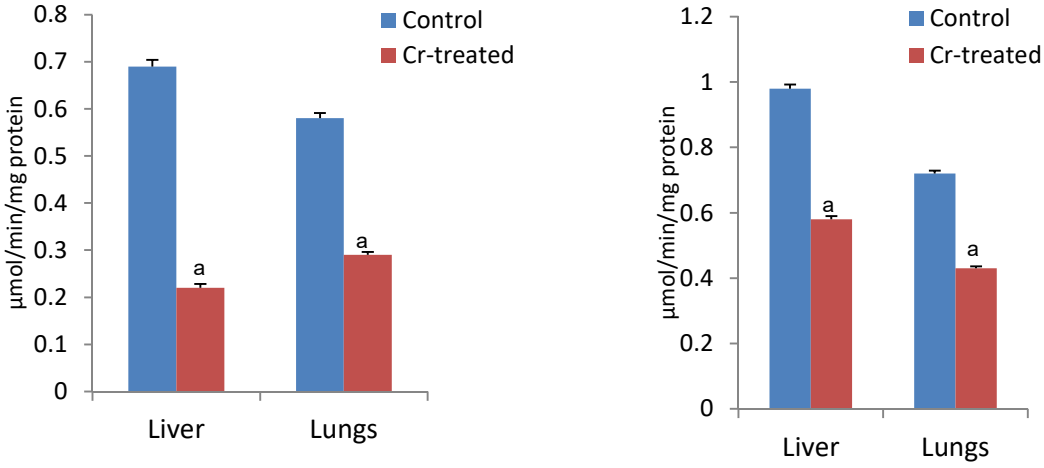

Figure 9 & 10: Variation of GR and GST activities in liver and lungs mitochondria after chromium exposure. [a] noted significant difference ($P<0.05$).

3.3 Discussion

The mitochondrion, an important cytoplasmic organelle, plays a major site for the metabolic transformation of hexavalent chromium. During metabolism of Chromium (VI), level of NADH and oxygen consumption is profoundly decreased in isolated rat liver and heart mitochondria (Ryberg and Alexandar, 1990). Hexavalent chromium can easily penetrate across the intact mitochondrial membrane and is reduced to chromium (V) which efficiently reduces NADH level (Shi et al, 1991). But, trivalent Chromium unable to penetrate so easily to enter inside mitochondria has no major effect on the respiratory transport chain in sonicated mitochondria of rats (Lazzarini et al, 1985). Chromium (VI) strongly inhibits few important mitochondrial respiratory enzymes like pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and β-hydroxy butyrate dehydrogenase that might explain the respiratory inhibition observed in hepatocyte mitochondria and reduced production of ATP and GTP (Lazzarini et al, 1985).

In the current experimental studies, the toxicity of hexavalent chromium in previously determined effective dose and duration has been contemplated in isolated liver and lungs mitochondria. The experiment displays a noticeable raised MDA level and CD content in liver and lungs tissue mitochondria of chromium treated rats (Figure 1 & 2). Bagchi et al, (1995) have revealed that hexavalent chromium enhances lipid peroxidation in liver mitochondria and microsomes. Those abnormalities might be due to damage of inner mitochondrial membrane as a result of either direct insult or oxidative stress from Chromium (VI) reduction. The generation of highly reactive chemical species may be one of the possible causes for raising the MDA in liver and lungs tissue mitochondria due to toxicity of chromium.

Excess quantity of reactive oxidative species of chemicals usually create huge cellular oxidative stress disrupting lipo- protein membrane architecture, intracellular proteins and alteration of DNA resulting tissue destruction (Nordbeg and Arner, 2001). The markedly increased MDA and CD level (Figure 1 & 2) and higher NO production (Figure 3) are good corroborative evidence of toxic damage in rat liver and lungs mitochondria due to potassium di-chromate induced oxidative stress. Excess production and release of NO in mitochondria of rat liver and lungs and simultaneous generation of strongly reactive oxidative and nitrating agents are toxic consequences of hexavalent chromium. Highly reactive superoxide ($O_2^{\cdot-}$) anions and NO form per-oxinitrite ($ONOO^-$) a very potent nitrating compound, binds with the heam containing cytochromes such as cytochrome oxidase and hinders aerobic cellular respiration (Poderoso et al, 1996); as well as disintegrates some important intracellular enzymes and affects mitochondrial structural integrity (Cassina and Radi, 1996; Radi et al, 2002).

SOD plays a major role in antioxidant defence role by catalysing the dismutation of superoxide($O_2^{\cdot-}$) radicals to less harmful H_2O_2.Present study depicts reduced SOD activity in rat mitochondria isolated from liver and lung tissues of chromium treated group (Figure 4).The explanation of such decreased SOD activity may be due to huge production of super oxide radical surpassing enzymatic detoxification of oxidants concentration in tissues (Srinivasan et al, 2008; Pedraza-chaverri et al, 2005).Diminution of most of the antioxidant enzyme level is probably either by directly combining of heavy metals to SH group containing active site of enzymes or inactivating enzymes by removal of co-factors from active sites of it after chromate administration. Catalase enzyme found in peroxisomes present in the cell cytoplasm

and in heart mitochondria of rabbit (Radi et al, 1991) is a principal H_2O_2 detoxifying intracellular enzymatic antioxidant. But this important enzyme is not available in other tissue mitochondria including skeletal muscles (Phung et al, 1994). Here catalase activity is measured in liver and lungs tissue homogenates (Figure 5). The activity of enzyme CAT (catalase) is suppressed probably due to the excess production of H_2O_2 in chromium exposure group of rats compared to control group.

It is well-known fact that decreased intracellular concentration of (GSH) will amplify lipid peroxidation. Intracellular GSH content has been diminished in isolated rat after heavy metal chromium (VI) exposure hepatocytes (Ueno et al, 1988). GSH and GSSG level have decreased remarkably in isolated liver and lungs mitochondria of hexavalent chromium treated rats (Figure 6 & 7). Glutathione protects the cells against the toxic vulnerability of lipid peroxidation by heavy metals like lead, chromium, cobalt, aluminium and cadmium. For optimal enzyme activity it maintains critical state of cellular redox potential (Rao and Shaha, 2001) and it is depleted markedly during oxidative stress (Lu, 1999). GPX activity has also declined to a great extent in chromium (VI) injected rats than control group in current study (Figure 8). These findings clearly suggest that chromium mediated super oxide anion and H_2O_2 are mainly depleting the cellular antioxidants. To reduce H_2O_2, GP_X is suggested to play an essential role against excess intracellular ROS accumulation and subsequent damage of liver and lung tissues. Important detoxifying enzyme, Glutathione reductase alleviates oxidative stress by reducing glutathione from oxidised form to reduced form. Present research study displays clear diminution of the GR activity in tissue mitochondria of chromium treated rats (Figure 9). Mitochondrial injury causes low NADPH which acts as an important

cofactor of Glutathione reductase (GR) for conversion of reduced glutathione (GSH) from oxidised glutathione (GSSG). Glutathione- S- transferase (GST) utilizes glutathione in various intracellular reduction reactions during metabolic transformation of heavy metals, carcinogen drugs, highly oxidative chemicals and xenobiotic. The observed diminished activity of GST in liver and lungs mitochondria (Figure 10) and other results of present experiment as discussed above clearly indicate augmentation of oxidative stress associated with depletion of those antioxidant and ROS scavenging enzymes in the presence of huge amount of highly reactive oxidant chemicals. Probable overall mechanism of complex interactions between ROS generation during intracellular Cr (VI) reduction and depletion of endogenous antioxidant enzyme for development of chromium toxicity is depicted below (Figure 11).

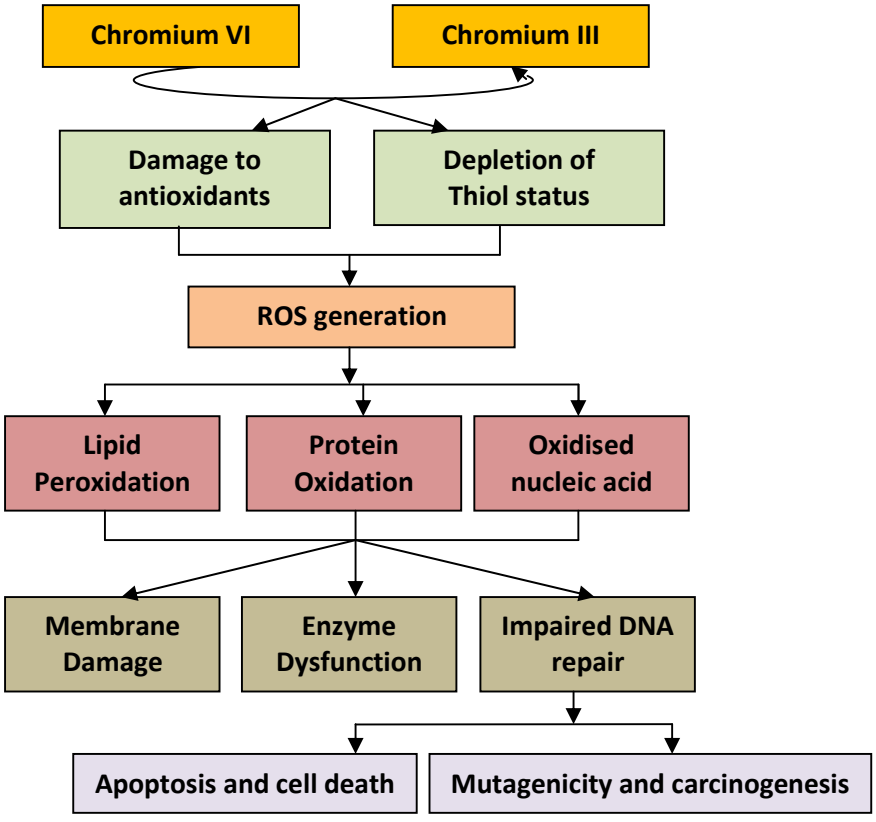

Figure11: Mechanism of chromium toxicity

3.4 Conclusion

The present experiment has highlighted the essential critical role of mitochondria in development of hexavalent chromium induced tissue toxicity led by huge oxidative stress. However, to explore further the exact underlying mechanism behind Cr (VI) challenged oxidative tissue mitochondrial damage, more in-depth studies are required.