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AMELIORATIVE ROLE OF ANDROGRAPHIS PANICULATA NEES ON CHROMIUM-INDUCED OXIDATIVE STRESS IN LIVER AND LUNGS MITOCHONDRIA

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ABSTRACT

Mitochondria are the crossroads of several crucial cellular activities; they produce considerable quantities of superoxide radical and hydrogen peroxide, which can damage important macromolecules. Potassium dichromate ($K_2Cr_2O_7$), a Cr (VI) compound, is the most toxic form of chromium and has been demonstrated to induce toxicity associated with oxidative stress in humans and animals. The aim of this study was to elucidate the protective effects of aqueous extract (AE-Ap) of *Andrographis paniculata* on chromium-induced oxidative stress in liver and lungs mitochondria. In this investigation, a group of male Wistar rats (100-120 gm) were induced by intraperitonial injection of vehicle (0.9% NaCl), $K_2Cr_2O_7$ (0.8 mg / 100 g body weight / day), $K_2Cr_2O_7$ plus AE-AP250 (250 mg/kg body weight/day) and $K_2Cr_2O_7$ plus AE-AP500 (500 mg/kg body weight/day) for the period of 28 days. Measurement of oxidative stress biomarkers like lipid peroxidation (MDA), conjugated dienes and nitric oxide contents were increased in both liver and lungs mitochondria. The decreased antioxidant marker enzymes like the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and glutathione-S-transferase (G-S-T) of Cr (VI) treated rats were accompanied by a significant decrease in the levels of glutathione (GSH and GSSG) in liver and lungs mitochondria. The results of the present study suggest that the administration of AE-AP significantly supplement the biochemical

marker enzyme, lipid peroxidation and enhanced the antioxidant status in rat's liver and lungs mitochondria.

KEYWORDS: Chromium, tissues, mitochondria, oxidative stress, *Andrographis paniculata* Nees.

INTRODUCTION

Potassium dichromate ($K_2Cr_2O_7$) is a chemical compound widely used in metallurgy, chrome plating, chemical industry, textile manufacture, wood preservation, photography and photoengraving, refractory and stainless steel industries and cooling systems (Barceloux, 1999). The oxidation state and solubility of chromium (Cr) compounds determine their toxicity. In contrast to Cr (III), which is a naturally occurring form and an essential trace element for humans and others mammals, Cr(VI) compounds are highly toxic (Wang et al., 2006). Dolai et al. (2016) have summarized the acute toxicity, chronic toxicity, neurotoxicity, reproductive toxicity, genotoxicity, carcinogenicity and environmental toxicity of chromium. Potassium dichromate is a hexavalent form of Cr and has been demonstrated to induce oxidative stress and carcinogenic in nature (Stohs and Bagchi, 1995; Bagchi et al., 2002a,b).

Mitochondria provide most of the cellular energy (ATP). A strong decrease in the ATP levels in cells exposed to Cr (VI) was detected in hamster fibroblasts (Debetto et al., 1982), human gingival fibroblasts (Messer and Lucas, 2000), and rat thymocytes (Lazzarini et al., 1985). This effect was closely correlated with the inhibition of cellular respiration in human and rat lymphocytes (Messer and Lucas, 2000), as well as rat hepatocytes (Ryberg and Alexander, 1984). The inhibitory action of Cr (VI) on mitochondrial respiration was detected in isolated rat liver (Ryberg and Alexander, 1984) mitochondria, and also in rat liver sub mitochondrial particles (Ryberg and Alexander, 1990). The mechanism by which Cr (VI) interferes with the mitochondrial bioenergetics was not clarified. It has been assigned to the oxidizing activity of Cr (VI), which shunts electrons from electron donors coupled to ATP production, and to the ability of Cr (III), derived from Cr (VI) reduction, to form stable complexes with ATP precursors and enzymes involved in the ATP synthesis (Debetto et al., 1982; Bianchi et al., 1982). Reduction of Cr (VI) induced the generation of hydroxyl radical ($^{\cdot}OH$) via the Fenton-mechanism (Shi et al., 1991). It is known that daily oral low-dose administration of Cr (VI) to rat's results in enhanced lipid peroxidation in liver and brain mitochondria (Travacio et al., 2000).

Medicinal plants and their active principles have received greater attention as potential anti-peroxidative agent (Lee and Park, 2002). *Andrographis paniculata* Nees, an important herbal drug has been widely used for centuries as an indigenous medicine. *Andrographis paniculata*, commonly known as 'Kalmegh', is a well known drug in the Ayurvedic system of medicine. It has been reported that *Andrographis paniculata* has a broad range of pharmacological activities such as analgesic, antipyretic, antiulcerogenic (Vedavathy and Rao, 1991) and choleretic (Shukla et al, 1991). Herbal products are known to exert their protective effects by scavenging free radicals and modulating carcinogen detoxification and antioxidant defense system.

The present study aimed to investigate the antiperoxidative role of different doses of AE-AP in chromium- induced oxidative damage in liver and lungs mitochondria.

MATERIALS AND METHODS

Chemicals: Potassium dichromate and other fine chemicals were purchased from Sigma Chemical Company, USA. Salt and vitamin mixtures were purchased from Merck, Germany. All other chemicals and reagents were purchased from Sisco Research Laboratory Pvt Ltd (SRL), India, and were of analytical grade.

Animals and diet: Adult male albino rats (n=48) of Wistar strain of body weight 100-120 *g* were obtained. They were maintained in accordance with the guidelines of the rule of Institutional Animal Ethics Committee of Vidyasagar University, Midnapore, and were housed in polypropylene cages and fed standard pellet diet (Hindusthan Lever Ltd, India) for 1 week and water *ad libitum*. Animals were divided into four groups and each group consisting 12 animals.

Collection, Identification and Preservation of Plant material: Fresh plant part was collected from the campus of IIT, Kharagpur, West Bengal, India. The taxonomic identity of this plant was determined by the expertise of the Department of Botany of our University. Specimen was labeled, numbered and noted with date of collection. Plant part was rinsed with sterilized distilled water, air dried and stored in airtight bottle at 4^{o}C for further use.

Preparation of aqueous extract: Clean dry plant sample was collected in a cotton bag. The material was grinded to fine powder with the help of mixer grinder. Then this powdered material was used for the preparation aqueous extract. 2 gm of powdered material was mixed

with 20 ml of sterile distilled water and kept on a rotary shaker for 12 hours at 38^{o}C. Thereafter, it was filtered with the help of Whatman No. 1 filter paper. The filtrate was then centrifuged at 2000 rpm for 10 min. Then the supernatant was collected and stored at 4^{o}C for further use (Zhang and Tan, 1996).

Mode of treatment: Animals were divided into four groups of almost equal average body weight of twelve animals each. The animals of three groups were induced by interperitonial injection with $K_2Cr_2O_7$ at a dose of 0.8 mg per 100 g body weight per day (20% LD_{50}) for 28 days, as described earlier (Dey et al., 2003). The animals of two of the chromium treated groups serving as the supplemented groups injected AE-AP250 (250 mg/kg body weight/day) and AE-AP500 (500 mg/kg body weight/day) daily at an interval of six hours after injection of $K_2Cr_2O_7$ for a period of 28 days. The animals of the remaining group received only the vehicle (0.9% physiological saline), served as control.

Animals sacrifice and sample preparation: After completion of drug treatment the animals were fasted overnight prior to sacrifice by the use of anesthesia. The intact liver and lungs were dissected out and adhering blood and tissue fluid were blotted dry weighted and kept at -20^{0}C prior to homogenization and analysis.

Homogenization of tissues

A weighted portion of different tissues was homogenized in an ice cold 0.2 M PBS (pH 7.4) using glass homogenizer. Homogenized tissues were used for biochemical assays.

Isolation of Mitochondria

Rat liver and lungs mitochondria were isolated from male albino rats by differential centrifugation according to conventional methods (Gazotti et al., 1979).

Analytical methods

Lipid peroxidation was measured according to the method of Ohkawa et al.(1979). Malondialdehyde (MDA) was determined from the absorbance of the pink coloured product (TBARS) of thiobarbituric acid-MDA reaction, at 530 mm. The reaction of MDA with TBA has been widely adopted as a sensitive method of lipid peroxidation in animal tissues. Conjugated dienes was measured according to the method of Slater (1980). NO release assays were done in liver and lungs according to the method of Sanai et al. (1998).

GSH (reduced glutathione) was measured according to the method of Griffith (1980). GSSG was also assayed after derivatization of GSH with 2 vinylpyridine. GSSG (oxidized glutathione) was measured by the method of Griffith (1980).

SOD activity was estimated by measuring the percentage inhibition of the pyrogallol auto-oxidation by SOD according to the method Marklund & Marklund (1974).

The rate of oxidation of reduced glutathione (GSH) by H_2O_2 as catalyzed by the glutathione peroxidase (GSH-Px) is assayed for the measurement of enzyme activity. Glutathione peroxidase activity was measured according to method of Pagila and Valentine (1967). The activity of glutathione reductase was measured by the method of Miwa (1972). Glutathione S-transferase activity was also measured according to the method of Habig et al. (1974).

Total protein of plasma and tissues was estimated according to the method of Lowry et al. (1951).

Statistical Analysis

The data were expressed as mean ± S.E.M. Comparisons of the means of control, chromium, chromium with AE-AP250 and chromium with AE-AP500 groups were made by two-way ANOVA with multiple comparison 't'-test, $P<0.05$ as a limit of significance.

RESULTS

The extent of lipid peroxidation and conjugated diene in liver and lungs mitochondria of control and experimental animals in each group are shown in figure 1 and 2. The level of MDA and conjugated diene were significantly increased in chromium treated rats in both liver and lungs mitochondria. Administration of aqueous extract of *Andrographis paniculata* to chromium treated rats significantly decreased the level of MDA and conjugated diene at the dose of AP250 and AP500 in liver and lungs mitochondria (Figure-1 and 2).

The level of NO was significantly higher in chromium treated rats compared with control. In chromium plus aqueous extract of *Andrographis paniculata* at the dose of AP250 and AP500, the level was significantly decreased in liver and lungs mitochondria (Figure 3).

Figure 4 shows that the activity of SOD was significantly reduced in liver and lungs mitochondria after chromium treatment when it compared with control. Administration of aqueous extract of *Andrographis paniculata* to chromium treated rats significantly increased

the activity of SOD at the dose of AP250 and AP500 in liver and lungs mitochondria (Figure-4).

Figure 5 and 6 indicates the level of GSH and GSSG and figure 7, 8 and 9 shows the activities of GPx, GR and GST in liver and lungs mitochondria. The level of GSH and GSSG, and activities of GPx, GR and GST in liver and lungs mitochondria were significantly decreased in chromium treated rats when compared to control. Administration of aqueous extract of *Andrographis paniculata* at the dose of AP250 and AP500 to chromium treated rats significantly increased the antioxidant status in liver and lungs mitochondria.

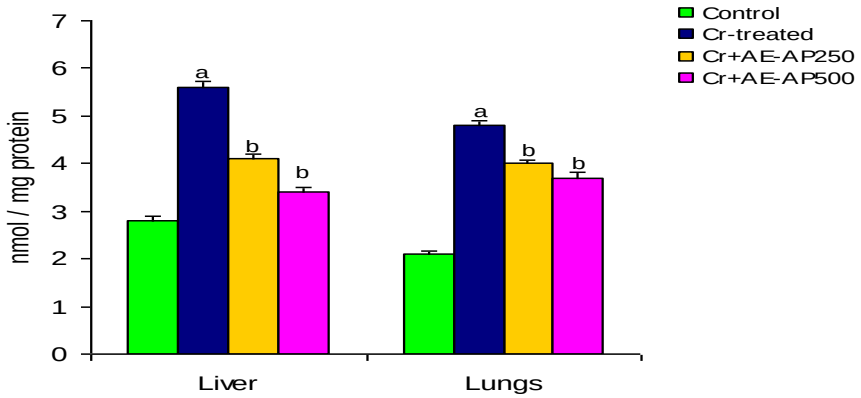

Figure 1: Changes the MDA concentration in liver and lungs mitochondria after co-administration of AE-AP250 and AE-AP500 in chromium treated rats. Data represents mean + SE, N=12. a P < 0.05 compared to control, b P < 0.05 compared to chromium.

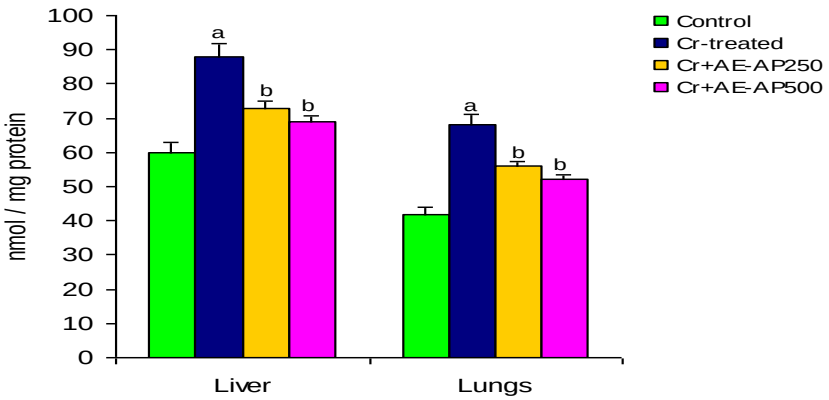

Figure 2: Changes the conjugated dienes concentration in liver and lungs mitochondria after co-administration of AE-AP250 and AE-AP500 in chromium treated rats. Data represents mean ± SE, N=12. [a] *P* < 0.05 compared to control, [b] *P* < 0.05 compared to chromium.

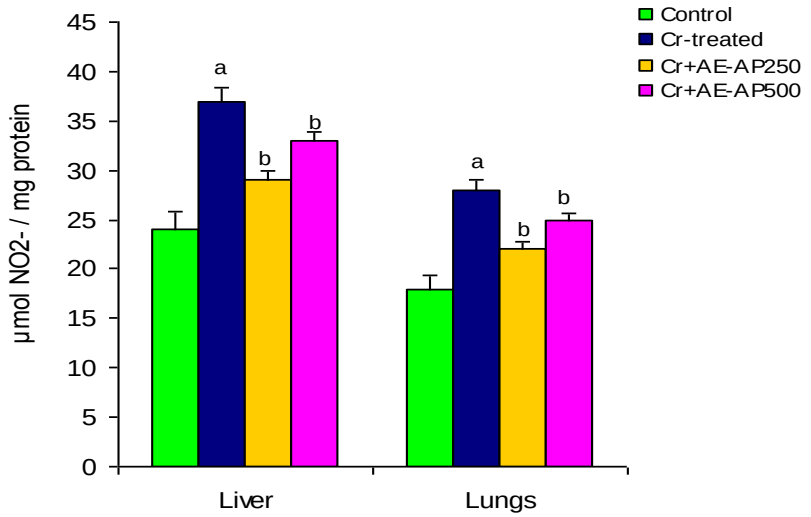

Figure 3: Changes the nitric oxide production (NO) in liver and lungs mitochondria after co-administration of AE-AP250 and AE-AP500 in chromium treated rats. Data represents mean ± SE, N=12. [a] *P* < 0.05 compared to control, [b] *P* < 0.05 compared to chromium.

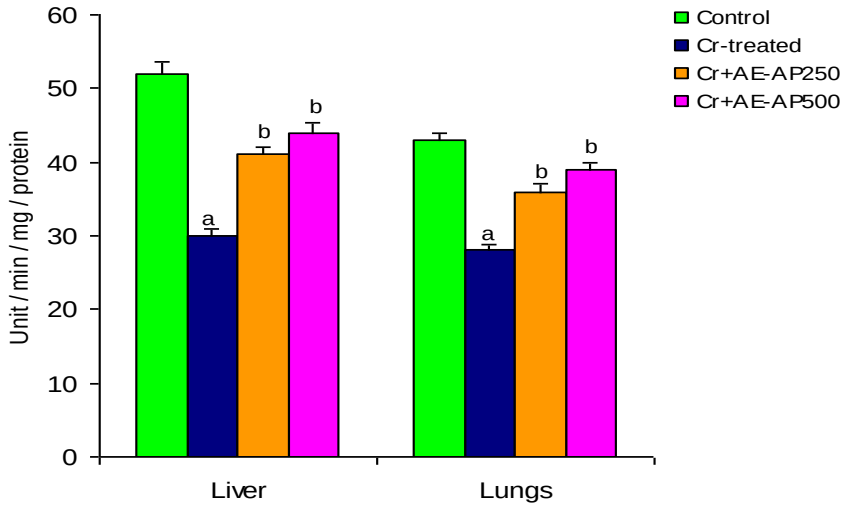

Figure 4: Changes the SOD activity in liver and lungs mitochondria after co-administration of AE-AP250 and AE-AP500 in chromium treated rats. Data represents mean ± SE, N=12. [a] *P* < 0.05 compared to control, [b] *P* < 0.05 compared to chromium.

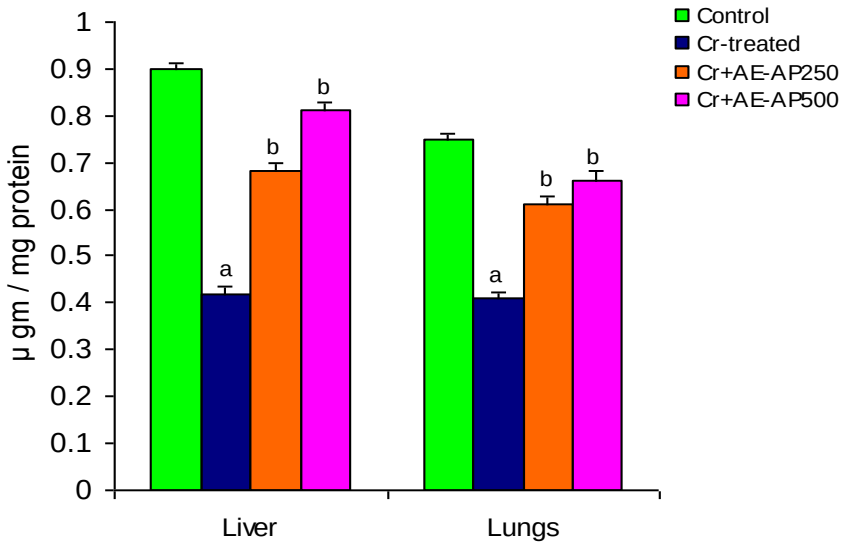

Figure 5: Changes the GSH level in liver and lungs mitochondria after co-administration of AE-AP250 and AE-AP500 in chromium treated rats. Data represents mean ± SE, N=12. [a] *P* < 0.05 compared to control, [b] *P* < 0.05 compared to chromium.

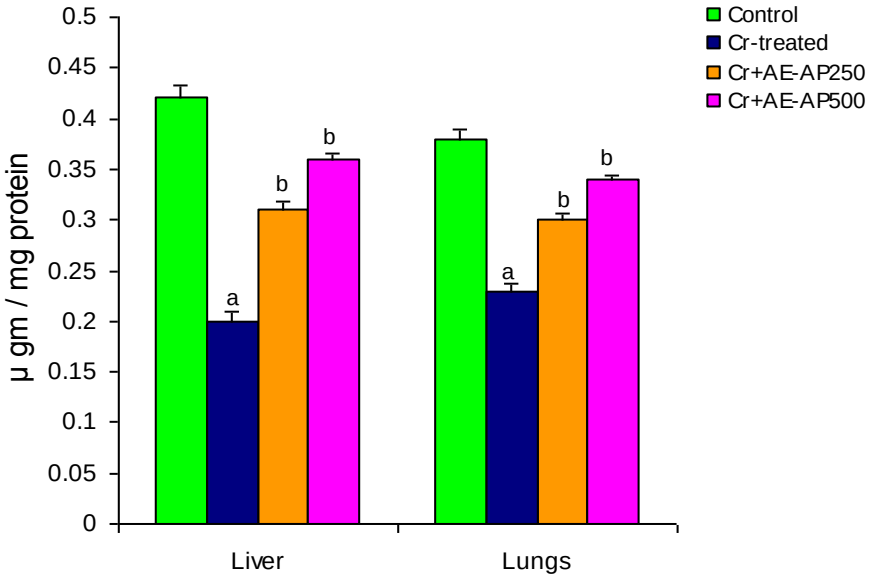

Figure 6: Changes the GSSG level in liver and lungs mitochondria after co-administration of AE-AP250 and AE-AP500 in chromium treated rats. Data represents mean ± SE, N=12. [a]*P* < 0.05 compared to control, [b] *P* < 0.05 compared to chromium.

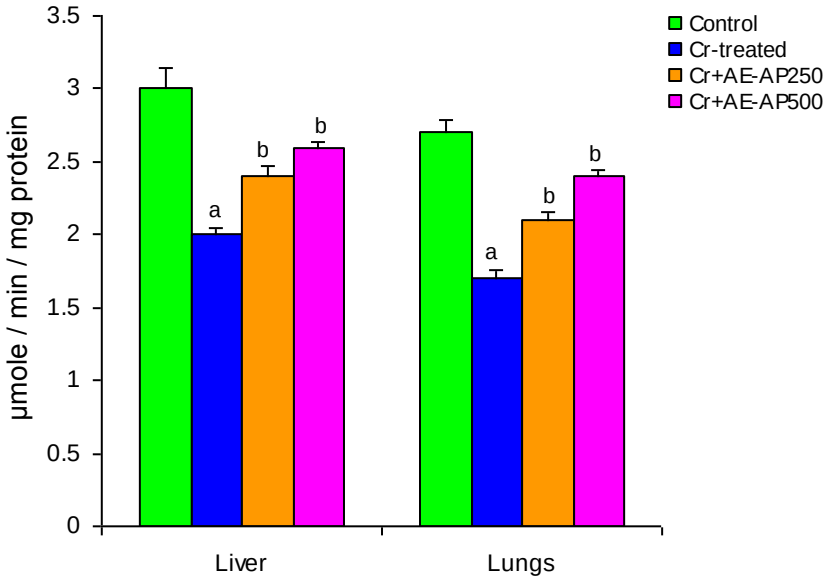

Figure 7: Changes the GPx activity in liver and lungs mitochondria after co-administration of AE-AP250 and AE-AP500 in chromium treated rats. Data represents mean ± SE, N=12. [a] *P* < 0.05 compared to control, [b] *P* < 0.05 compared to chromium.

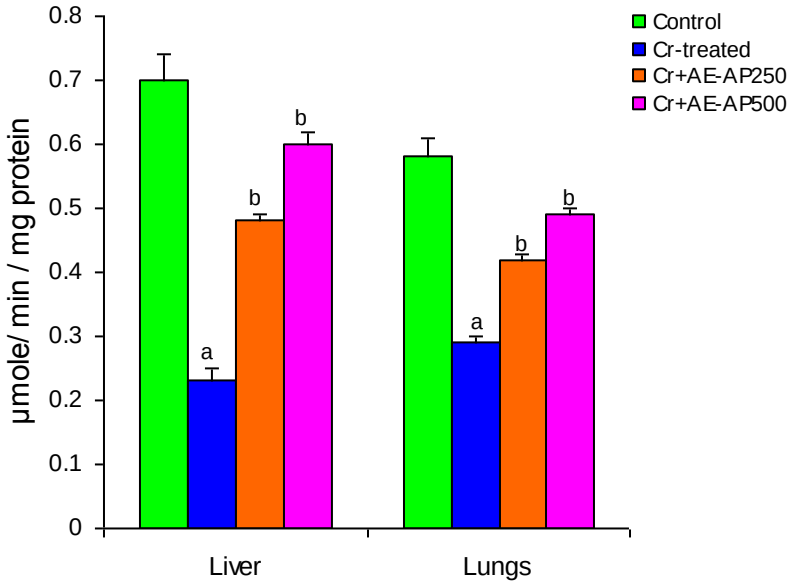

Figure 8: Changes the GR activity in liver and lungs mitochondria after co-administration of AE-AP250 and AE-AP500 in chromium treated rats. Data represents mean ± SE, N=12. [a] *P* < 0.05 compared to control, [b] *P* < 0.05 compared to chromium.

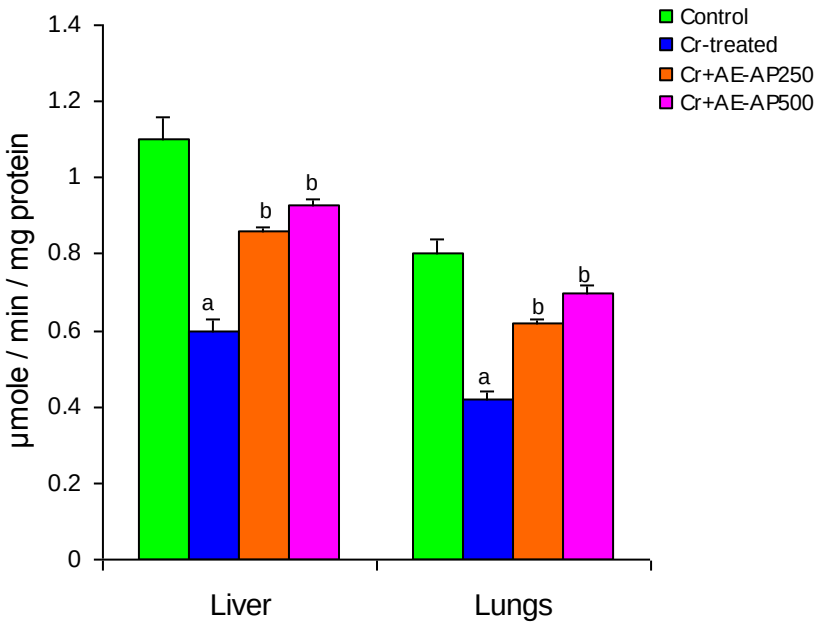

Figure 9: Changes the GST activity in liver and lungs mitochondria after co-administration of AE-AP250 and AE-AP500 in chromium treated rats. Data represents mean ± SE, N=12. [a] *P* < 0.05 compared to control, [b] *P* < 0.05 compared to chromium.

DISCUSSION

The mitochondrion appears to be an important cellular organelle playing a role in the metabolism of hexavalent Cr. Cr (VI) dramatically decreased oxygen consumption and NADH levels in isolated rat liver and heart mitochondria (Ryberg and Alexandar, 1990). Intact mitochondria take up and reduced chromium (VI), producing chromium (V) species and the chromium (V) generated efficiently oxidized NADH (Shi et al., 1991). Chromium (III), which does not penetrate into intact mitochondria, had no effect on the respiratory rats in sonicated mitochondria (Lazzarini et al., 1985). Chromium (VI) is a potent inhibitor of mitochondria, there enzymes (α-ketoglutarate dehydrogenase, pyruvate dehydrogenase and β-hydroxyl butarate dehydrogenase) may explain the observed inhibitory respiration in liver mitochondria and decreasing in cellular levels of ATP and GTP (Lazzarini et al., 1985). *Andrographis paniculata* Nees, an important herbal drug has been widely used for centuries as an indigenous medicine.

The aim of this study was to elucidate the protective effects of aqueous extract (AE-Ap) of *Andrographis paniculata* on chromium-induced oxidative stress in liver and lungs mitochondria. This study shows a significant increase the MDA and conjugated dienes levels

in liver and lungs mitochondria in chromium induced rats (Figure 1 & 2). Bagchi et al. (1995) showed that chromium (VI) induces increases in hepatic mitochondrial and microsomal lipid peroxidation. These results may be due to oxidative damage in inner mitochondrial membrane may also be involved. The generation of ROS is another cause to increase the MDA level in liver and lungs mitochondria due to chromium induced toxicity. Large amounts of ROS generated can bring on injury to cellular proteins, lipids, and DNA leading to oxidative stress (Nordbeg and Arner, 2001). The observed increase in MDA and conjugated dienes is a good evidence for oxidative stress. The study also showed a significant increase in NO production in liver and lungs (Figure 3). The simultaneous production of superoxide and NO produces peroxinitrite ($ONOO^-$), a very strong oxidant and nitrating agent. The formation of NO in liver and lungs mitochondria may have an important consequence, because this compound binds to the heam group from cytochromes (in particularly, cytochrome oxidase) and inhibits respiration (Poderoso et al., 1996). This may, in turn, stimulate $O_2^{\cdot -}$ formation which may react with more NO, forming peroxinitrite, an oxidant capable of inhibiting important enzymes and affecting mitochondrial integrity (Cassina and Radi, 1996; Radi et al., 2002).

SOD is believed to play a major role in the first line of antioxidant defense by catalyzing the dismutations of super oxide anion radicals to form H_2O_2 and [O_2^-]. In the present study, decreased SOD activity observed in chromium-induce group in liver and lungs mitochondria (Figure 4) could be explained by the massive production of super oxide anion. The production of such anions overrides enzymatic activity and leads to a fall in its concentration in tissues (Srinivasan et al., 2008). Pedraza-chaverri et al. (2005) indicated that most of the antioxidant enzymes become inactive after potassium dichromate exposure either due to the direct binding of heavy metals to enzyme active site if it contains SH group or to the displacement of metal co-factors from active sites. The production of such anions leads to a fall in its concentration in liver and lungs mitochondria.

It has been known that lipid peroxidation occurs as a result of the decrease in intracellular reduced glutathione (GSH) concentration. Ueno et al. (1988) reported that the content of intracellular GSH in isolated rat hepatocytes was diminished after chromium (VI) treatment. The levels of GSH and GSSG have significantly diminished in liver and lungs mitochondria (Figure 5 & 6). Glutathione is normally present in millimolar concentrations in cells and is known to protect the cellular system against the toxic effects of lipid peroxidation. It is very

important in maintaining cellular redox status (Rao and Shaha, 2001) and its depletion is considered as a marker of oxidative stress (Lu, 1999). These results suggested that super oxide anion and H_2O_2 are main source of chromium induced free radicals depleting the cellular antioxidant. GP_X is considered to biologically essential in the reduction of H_2O_2. The decline in the activity of GP_X in chromium – induce rats in our study (Figure 7) may be due to the intracellular accumulation of ROS with subsequent development of liver and lungs injury. Glutathione reductase (GR) is one of the most important enzymes detoxifying against oxidative stress because GR is considered biologically essential in reduction of oxidized glutathione to reduced glutathione. In our present study, the activity of GR in liver and lungs mitochondria declined in chromium- induces rats (Figure 8). This might have been triggered by low levels of NADPH, which is a cofactor of GR to convert GSSG to GSH. Glutathione-S- transferase (GST) is an enzyme that utilizes glutathione in reaction contributing to the transformation of a wide range of components including carcinogens, therapeutic drugs and products of oxidative stress. The decreased in the activity of GST in chromium – induced rat liver and lungs mitochondria (Figure 9) might be due to increased oxidative stress as these enzymes tend to deplete in the presence of ROS.

Administration of aqueous extract of *Andrographis paniculata* at the dose of AP250 and AP500 modulated the changes induced by chromium supporting the hypothesis that plant products are effective antioxidative agent. *Andrographis paniculata* treatment prevents BHC induced increase in the activity of enzyme δ - glutamyl transcriptase, glutathion, J-transferase and lipid peroxidation. It has protective effects on oxidative stress by increasing activity of antioxidant enzyme and decreases lipid peroxidation (Trivedi NP, Rawal VM 2001). Thus, *Andrographis paniculata* may stabilize the cell membrane and significantly reduce the activities of marker enzymes and decrease the extent of lipid peroxidation and conjugated diene in liver and kidney.

Andrographis paniculata significantly enhanced the antioxidant status in liver and lungs mitochondria of chromium treated rats and protect cells against the damaging effects. It is reported that vitamin C can completely prevent lipid peroxidation in human plasma exposed to cigarette smoke (Frei et al, 1991). Andrographolide were tested for a protective effect against liver toxicity produced in mice by giving them carbon tetrachloride (Kapil et al . 1993). This chemical damages the liver by causing lipid peroxidation. In another study, andrographolide was shown to produce a significant increase in bile flow (Shukla et al;

1992). Biochemical and histological evidences indicate that andrographolide was hepatoprotective against galactosamine or paracetamol induce rats (Handa & Sharma, 1990). The antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase and the levels of glutathione were decrease following BHC effect. Administration of *Andrographis paniculata* showed protective effects in the activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase as well as the level of glutathione. These results indicate antioxidant action of *Andrographis paniculata* (Trivedy and Rawal, 2001).Thus aqueous extract of *Andrographis paniculata* exerts its protective effect at the dose of AP250 and AP500 against chromium-induced toxicity in liver and lungs mitochondria by modulating the extent of lipid peroxidation and augmenting antioxidant defense system but the dose of AP500 is more protective than AP250. The results of the present study indicate that aqueous extract of *Andrographis paniculata* may emerge as a preventive agent against liver and lungs carcinogenesis.

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Competing interests: The authors declare there are no conflicts of interest.

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