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AMELIORATIVE ROLE OF MIXED HYDRO-METHANOL SOLVENT EXTRACT (60:40) OF *ANDROGRAPHIS PANICULATA* NEES ON CHROMIUM-INDUCED MEMBRANE DAMAGE AND ELECTRON TRANSPORT CHAIN COMPLEXES IN LIVER AND LUNGS

Durga Pada Dolai[1], Soumita Dey[2], Amit Nandi[2], Somenath Roy[1] and Sankar Kumar Dey[2]*

[1]Immunology and Microbiology Laboratory, Department of Human Physiology, Vidyasagar University, Midnapore-721 102, West Bengal, India.
[2]Department of Physiology, S.B.S.S.Mahavidyalaya (Affiliated to Vidyasagar University), Goaltore-721 128, Paschim Medinipur, West Bengal, India.

***Corresponding Author: Sankar Kumar Dey**
Department of Physiology, S.B.S.S.Mahavidyalaya (Affiliated to Vidyasagar University), Goaltore-721 128, Paschim Medinipur, West Bengal, India.

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ABASTRACT

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 ameliorative effects of mixed hydro-methanol solvent extract at the ratio of 60:40 *of Andrographis paniculata* Nees on chromium-induced membrane damage and electron transport chain (ETC) in liver and lungs mitochondria. In this investigation, a group of male Wistar rats (80-100 g) 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 mixed hydro-methanol solvent extract in the ratio of 60:40 at a dose of 500 mg/kg body weight daily at an interval of six hours after injection of $K_2Cr_2O_7$ for a period of 28 days. Significantly decreased the Mito ETC Complex-I, II and III of Cr (VI) treated rats in liver and lungs mitochondria. On the other hand, increased the membrane cholesterol level but decreased the membrane phospholipid, membrane total ATPase and membrane Na^+-K^+ ATPase activity in response to Cr (VI) toxicity in liver and lungs. The results of the present study suggest that the administration of mixed hydro-methanol solvent extract in the ratio of 60:40 significantly supplement the chromium-induced such alterations in rat's liver and lungs.

KEYWORDS: Chromium (VI), Liver, Lungs, Plasma membrane, Mitochondria, Andrographis Paniculata.

INTRODUCTION

Hepatic and renal toxicity is the most common toxicity found in Cr (VI)-exposed workers or animals (Dey and Roy, 2010). This functional differentiation of Cr (III) and Cr (VI) is largely decided by the ionic permeability of the plasma membrane (De Flora and Wetterhahn 1989). Thus, membrane damage is one of the crucial factors observed with Cr (VI) toxicity (Dey and Roy, 2010). On the other hand, a strong decrease in the ATP levels in cells exposed to Cr (VI) was detected in rat thymocytes (Lazzarini et al., 1985). 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.

Andrographis paniculata is used in the Indian traditional system of medicine against various diseases. There are more than 20 different active bio constituents like flavonoids, phenols, alkaloid, glycosides, saponins and tannins are present in the *Andrographis paniculata*. The *Andrographis paniculata* extract also exhibits good anticancer, anti-bacterial and anti-fungal activities (Singha et al, 2003). *Andrographis Paniculata* extracts contain principal compound andrographolide. Methanol extract of *Andrographis Paniculata* was more potent in antioxidant activities. Compared to andrographolide, aqueous extract also possessed potent antioedema and analgesic activities (Lin et al, 2009).

Therefore, the aim of this present investigation was an attempt to reduce the effects of Cr-induced cytotoxicity by using the most potent mixed hydro-methanol solvent extract (60:40) of *Andrographis paniculata in vivo* in

terms of certain structural and functional alteration of plasma membrane, and mitochondrial electron transport chain (Mito–ETC) complexes.

MATERIALS AND METHODS

Chemicals: Potassium dichromate and other fine chemicals were purchased from Sigma Chemical Company, USA. 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 of Wistar strain of body weight 80-100 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 one week and water *ad libitum*.

Preparation of Mixed solvent extract

Hydro-Methanol (60:40) mixed solvent extract prepared by using the aqueous and methanol extract from *Andrographis paniculata*.

Mode of treatment

Animals were divided into three groups of almost equal average body weight of twelve animals each. The animals of two 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 one of the chromium treated groups serving as the supplemented groups injected with mixed hydro-methanol solvent extract in the ratio of 60:40 at a dose of 500 mg/kg body weight 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 collection of blood samples and tissues

After completion of drug treatment the animals were kept in fasted overnight prior to sacrifice. The intact liver and lungs were dissected out and adhering blood and tissue fluid were blotted dry weighted. All the samples were kept at -20^0C for further 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 analytical assesment.

Isolation of crude plasma membrane

Membrane fractions of the liver and lungs were isolated according to the method described by Ghosh Chowdhuri et al, (1995).

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

Estimation of membrane cholesterol and phospholipid

Cholesterol and phospholipid levels of the isolated membrane fractions were estimated by the methods of Zlatkis et al, (1953) and Christopher and Ralph (1972), respectively.

Determination of total ATPase and Na–K+–ATPase activities

Total ATP-ase and Na–K+–ATPase activities were measured by the method of (Sen et al.; 1981).

Assay of Mito–ETC complexes

Measurement of enzyme activity of individual complexes was performed spectrophotometrically. DPNH-coenzyme Q reductase (complex I) activity was estimated using the method of Hatefi and Rieske (1967). Succinate dehydrogenase coenzyme Q reductase (complex II) activity was measured using the method of Ziegler and Rieske (1967). Coenzyme Q cytochrome *c* reductase (complex III) activity was assayed using the method of Rieske (1967).

Protein Estimation

Protein estimation was done according to (Lowry et al, 1951) taking BSA as a standard.

Statistical Analysis

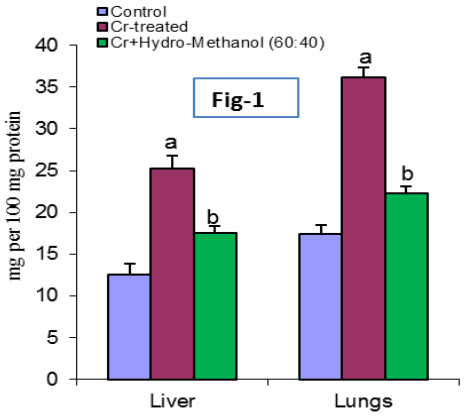
All the parameters were repeated at least three times. The data were presented as mean ± SEM. By performing ANOVA test (using a statistical package, Origin 6.1, Northampton, MA 01060, USA), the means of control and treated group were compared by multiple comparison t-test having $P<0.05$ as a limit of significance.

RESULTS

Alterations in membrane cholesterol and phospholipid levels, and the total ATPase, and Na^+–K^+– ATPase activities of the liver and lungs are presented in Figures 1-4. Significantly increase in membrane cholesterol and decrease in membrane phospholipid contents, total ATPase, and Na^+–K^+– ATPase activities in both the liver and lungs plasma membrane of the Cr-treated group. Hydro-Methanol (60:40) extract of *A. Paniculata* administered to Cr-mediated rats; the levels of cholesterol and phospholipid, and the total ATPase, and Na^+–K^+– ATPase activities in the liver and lungs plasma membrane were significantly recover when compared with chromium treated group.

The changes of specific activity of complexes I-III are presented in Figure 5-7. A significant decline in the specific activity of the enzyme complexes (I, II, III) was

observed in the chromium treated group. Supplementation with Hydro-Methanol (60:40) extract of ***A. Paniculata*** **showed significant recovery of the specific enzyme activity in liver and lungs mitochondria in response to chromium.**



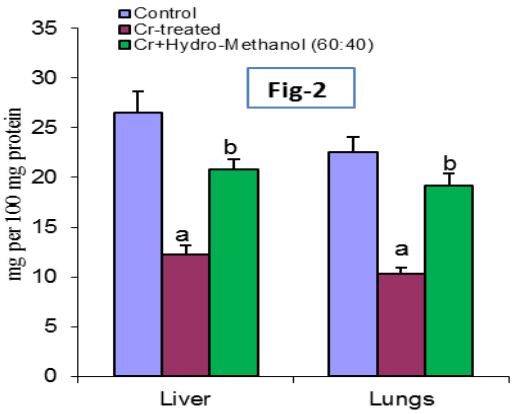
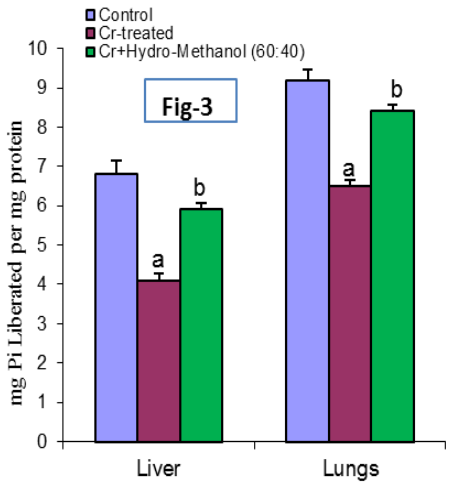


Figure 1 & 2: Changes in membrane cholesterol and phospholipids level after co-administration of Hydro-Methanol (60:40) extract of *A. paniculata* in Cr-treated rats. Data represents mean±SE, [a] *P* < 0.05 compared to control, [b] *P* < 0.05 compared to chromium.



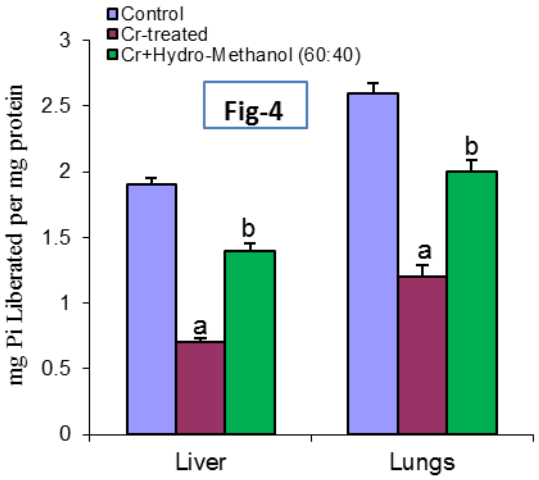
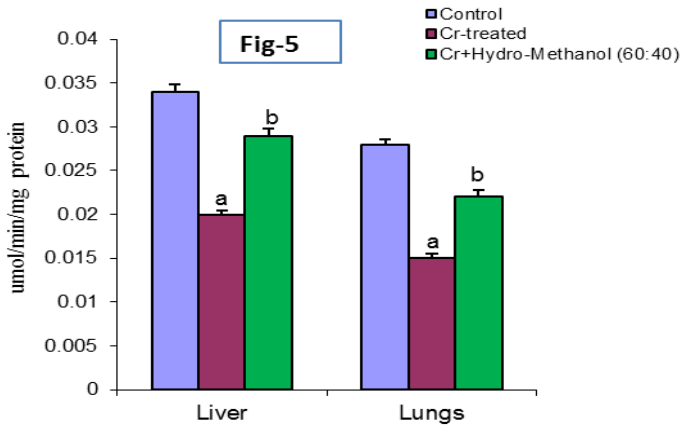
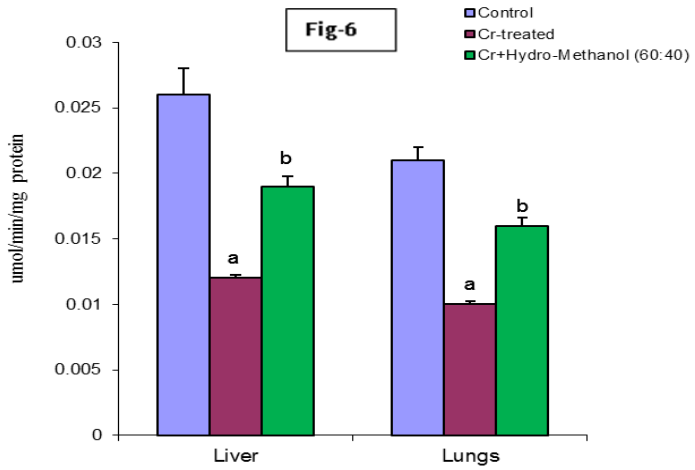


Figure 3 & 4: Changes in membrane Total ATPase and Na^+-K^+-ATPase activities after co-administration of Hydro-Methanol (60:40) extract of *A. paniculata* in Cr-treated rats. Data represents mean±SE, [a] *P* < 0.05 compared to control, [b] *P* < 0.05 compared to chromium.





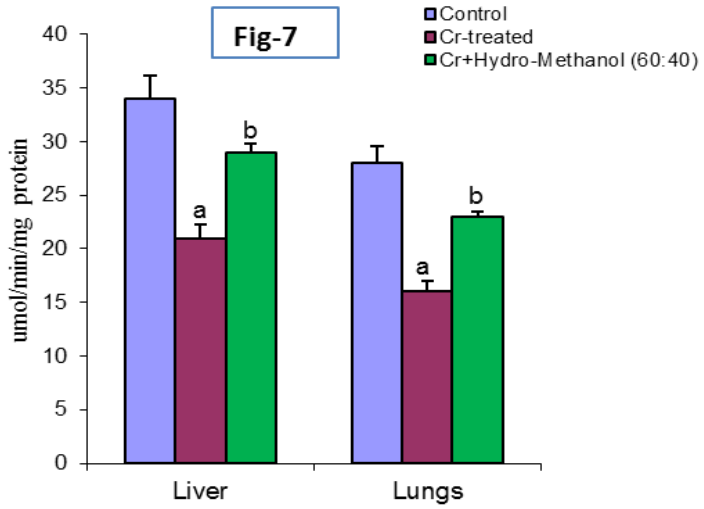


Figure-5, 6 & 7: Effect of Hydro-Methanol (60:40) extract of *A. paniculata* on Mito ETC Complex-I (DPNH-coenzyme Q reductase), Mito ETC Complex-II (succinate dehydrogenase coenzyme Q reductase) and Mito ETC Complex-III (coenzyme Q cytochrome c reductase) in chromium-induced rats. Data represents mean±SE, [a] *P* < 0.05 compared to control, [b] *P* < 0.05 compared to chromium.

DISCUSSION

In the present investigation, the chromium-induced membrane damage is clearly indicated by significant increases of membrane cholesterol content in both the liver and lungs (Figure-1). This rise may be due to imbalance in cholesterol incorporation into the membrane. Thus chromium may impair the function of lecithin cholesterol acetyl transferase. On the other hand, decreased membrane phospholipid levels (Figure 2) indicate the damage of the membrane structure of the cell. The probable impact of chromium on the lipid catabolising enzymes cannot be ruled out as evidenced by increased excretion of urinary lipid metabolites (Dey and Roy, 2010). This enhanced catabolism of lipids may result in accumulation of acetyl-CoA, which in turn may lead to increased synthesis of cholesterol in the tissues particularly in nonsteroid producing tissues. Thus, chromium by altering the relative proportion of cholesterol and phospholipid, may produce cellular damage to membrane structures. The impact of chromium on membrane cholesterol and phospholipid contents was found to disappear when chromium was accompanied by Hydro-Methanol (60:40) extract *of A. Paniculata*. Evidence indicates that these structural changes of the liver and lungs plasma membrane are attenuated by the Hydro-Methanol (60:40) extract *of A. Paniculata* supplementation.

Total ATPase activity of membrane was reduced significantly in the chromium-treated group in both the liver and lungs. Hydro-Methanol (60:40) extract *of A. Paniculata* supplementation exerted an effect on chromium-induced changes in total ATPase activity (Figure-3). The inhibition of the energy production by the cytotoxic concentration of chromium (Dey and Roy, 2010) may play some role in the Cr-induced changes of the ATPase activity. Na^+–K^+–ATPase activity was found to be reduced significantly in the chromium-treated group in both tested organs (Figure-4). The observed

results are supported by findings on chromium-induced reduction of membrane transport (Standeven and Wetterhahn 1991a, 1991b). When the chromium-treated group was supplemented with Hydro-Methanol (60:40) extract *of A. Paniculata*, the Na^+–K^+–ATPase activity was found to be restored in both the liver and lungs plasma membrane. The ATP hydrolyzing activity of Na^+–K^+–ATPase is inversely proportional with the cholesterol/phospholipid ratio of the membrane (Yeagle 1985). Thus, in the present investigation, decreased Na^+–K^+–ATPase activity may be related to altered cholesterol and phospholipid levels and may not be the direct effect of chromium itself.

Diminished activities of Mitochondrial ETC –Complex I, ll and III during Cr-VI treatment may be due to significant amount of leakage of electron through Mito-ETC, which may produce superoxide ions in liver and lung mitochondria. The formation of superoxide ions may increase in the presence of certain inhibitors, which cause the carriers upstream from the site of inhibition to become fully reduced (Genova et al, 2001 and Kushnareva et al, 2002). Mitochondrion plays a vital role in multi level regulatory process of apoptosis. Also, mitochondria are important site and major source of ROS generation (Boveris and Chance, 1973). ROS generated in mitochondria promotes release of cytochrome-C and pro-apoptic proteins which trigger caspase cascade and apoptosis (Ott et al, 2007). Some studies have suggested that loss of reduced glutathione will alter permeability of mitochondrial membrane and caspase activation (Cai and Jones, 1998). The Hydro-Methanol (60:40) extract *of A. Paniculata* supplementation increased the activity of Mitochondrial ETC –Complex I, ll and III (Figure-5, 6 & 7).

These findings indicate that Cr treatment at the present dose and duration induces structural and functional alterations in the plasma membrane in both the liver and kidney. These structural and functional changes may be attenuated by hydro-methanol (60:40) mixed solvent extract of *A. Paniculata* supplementation. These supplementary compounds act as one of the most important agents against chromium-induced mitochondrial dysfunction in rat liver and lungs. Hydro-methanol (60:40) mixed solvent extract of *Andrographis paniculata* may be used as potential therapeutic natural herbal product for amelioration of chromium-mediated organ dysfunction and diseases.

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