ejpmr, 2020,7(4), 457-464

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

STUDY THE PROTECTIVE EFFICACY OF MIXED SOLVENT EXTRACT OF *ANDROGRAPHIS PANICULATA* NEES IN DIFFERENT PROPORTIONS AGAINST CHROMIUM (VI)-INDUCED TOXICITY

Durga Pada Dolai[1], 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.

Article Received on 29/01/2020 **Article Revised on 19/02/2020** **Article Accepted on 09/03/2020**

ABSTRACT

Potassium dichromate ($K_2Cr_2O_7$) has been demonstrated to induce oxidative stress and carcinogenic in nature. In the present study, the protective effect of aqueous and methanol *extract* of *Andrographis paniculata* in different proportions was studied against Cr (VI) induced toxicity of liver and lung mitochondria of male albino rat. A group of male albino rats (80-100 g) were obtained and divided into six groups. The animals of five 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 a period of 28 days. The animals of four of the chromium treated groups serving as the supplemented groups were injected with mixed hydro-methanol extract in the ratio of 70:30, 60:40; 50:50; 40:60 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. After completion of chromium-treatment the animals were sacrifice and intact liver and lungs were dissected out for further use. Measurement of lipid peroxidation (MDA), conjugated dienes and antioxidants were used to monitor the antiperoxidative effects of different solvent extract in liver and lungs mitochondria. The increased lipid peroxides, conjugated dienes and NO release in liver and lungs of chromium-treated rats was accompanied by a significant decrease in the levels of glutathione (GSH and GSSG) and the activities of glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione-S-transferase (G-S-T), superoxide dismutase (SOD) and catalase (CAT). The important findings in this study corroborated the facts that particular 60:40 mixed hydro-methanol solvent extract of *Andrographis paniculata* has greater potential benefit than other ratio of mixed hydro-methanol solvent extract like 70:30, 60:40, 50:50 and 40:60 in maintenance of oxidative equilibrium, scavenging of ROS and augmented anti-oxidant defense against chromium-induced toxicity in liver and lungs mitochondria.

KEYWORDS: Chromium, Animal, Liver, Lungs, Toxicity, Oxidative stress, *Andrographis paniculata* Nees.

INTRODUCTION

Oxidative stress refers to cellular alteration of function as a result of mismatch between production of reactive free radicals and cellular defence mechanism against it. In oxidative stress, reactive free radical generation increases, but scavenging of those free radicals diminishes or reduction of modified macromolecules to repair oxidative stress or both. Cr (VI) alters mitochondrial functions, responsible for oxidative stress and alteration of Immune function resulting in disorders of different organs and diseases.

In previous study, efficacy of different (Aqueous, Methanol, Petroleum-ether) solvent extract of *Andrographis paniculata* was observed against Cr (VI) induced toxicity in liver and lung mitochondria of male albino rat. Out of the three different solvent extracts of *Andrographis paniculata*, namely Aqueous, Methanol, Petroleum-ether group of supplementation; Methalonic extract of *Andrographis paniculata* resulted in significant prevention of superoxide generation, lipid peroxidation, NO-release and improvement of SOD activity as well as GSH concentration in liver and lung to protect Cr (VI) induced toxicity. On the other hand, aqueous extract of *Andrographis paniculata* showed also a potential effectiveness than petroleum-ether extract of *Andrographis paniculata* against chromium-induced toxicity in liver and lungs mitochondria (Dolai et al, 2020). It has been reported that *Andrographis paniculata* has a broad range of pharmacological activities such as analgesic, antipyretic, antiulcerogenic (Madav et al. 1995). Dey et al (2011) reported that methanol extract of

Andrographis paniculatare restored the alterations of liver impairment induced by hexavalent chromium. It was observed that aqueous extract of *Andrographis paniculata* acts as a preventive agent against nicotine induced oxidative stress in liver and kidney (Dey et al, 2016). Andrographolide can protect CCl-4 induced lipid peroxidation of rat liver (Kapil *et al*, 1993). So, the present investigation was intended to study the hydro-methanol extract in different ratio of *Andrographis paniculata* can be used as potential ameliorative agents against *in vivo* chromium-induced toxicity in male albino rat.

METHODOLOGY

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 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

Andrographis paniculata crude extract are prepared with mixed solvent aqueous and methanol in the ratio of 70:30, 60:40, 50:50, 40:60 as described earlier (Dolai et al, 2020).

Mode of treatment

Animals were divided into six groups of almost equal average body weight. The animals of five 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 four of the chromium treated groups serving as the supplemented groups were injected with mixed hydro-methanol extract in the ratio of 70:30, 60:40; 50:50; 40:60 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 sample preparation: After completion of drug treatment the animals were kept in fasted overnight prior to sacrifice by the use of anaesthesia. The intact liver and lungs were dissected out and adhering blood and tissue fluid were blotted dry weighted and kept at -20^0C.

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). Conjugated dienes was measured according to the method of Slater (1980). NO release assays were done in liver and lungs mitochondria according to the method of Sanai et al. (1998). SOD activity was estimated according to the method of Marklund & Marklund (1974). GSH was measured according to the method of Griffith (1980). GSSG was measured by the method of Griffith (1980). 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 ± standard error. The significance in the differences between the means were evaluated by student's 't' test, and probability levels of 5% or less were considered to be statistically significant.

RESULTS

Following chromium exposure, the MDA and conjugated dienes concentration increased significantly in all the organs (Figure-1 & 2). It was observed that the concentration of MDA and conjugated dienes were restored in liver and lungs mitochondria in chromium treated rats following supplementation with the different ratio of mixed hydro-methanol solvent extract and it is maximum in the ratio 60:40 of hydro-methanol mixed solvent extract rather than other ratio.

The nitric oxide (NO) production and SOD activity in liver and lungs mitochondria were also observed to be significantly increased and decreased respectively in response to chromium (Figure-3 & 4). It was observed that significantly decreased NO. production and increased the SOD in chromium-treated rats after supplementation with the different ratio of mixed solvent extract. Maximum counteraction was found in both NO production and SOD activity at the ratio 60:40 of mixed hydro-methanol solvent extract in liver and lungs mitochondria in response to chromium.

The levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) were found to be significantly diminished in liver and lungs mitochondria of rats when exposed to chromium (Figure-5 & 6). In hydro-methanol supplemented groups of rats, it was found that the level

of GSH and GSSG significantly increased in all mixed solvent ratio but was maximum at 60:40 in both liver and lungs mitochondria.

Figure – 7, 8 & 9 shows that the activities of glutathione peroxidise (GPx), glutathione reductase (GR) and glutathione-s-transferase (GST) were significantly decreased in liver and lungs mitochondria in chromium-treated rats. On the other hand, GPX, GR and GST activities were restored significantly in both the tested organs after supplementation with the different ratio of mixed hydro-methanol extract of *Andrographis paniculata*. But such supplementation was showed to be maximum at the ratio 60:40 of mixed hydro-methanol solvent extract in liver and lungs mitochondria in response to chromium.

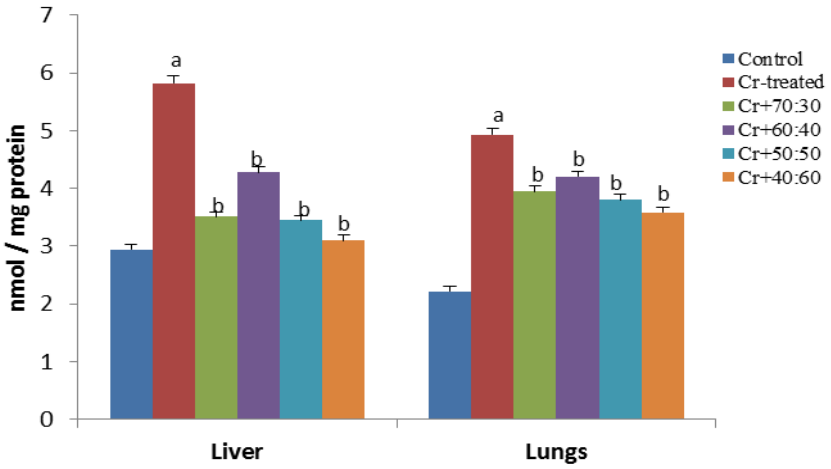

Figure 1: Changes the MDA concentration in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of 70:30, 60:40, 50:50 and 40:60 in chromium treated rats. Data represents mean + SE. a P < 0.05 compared to control, b P < 0.05 compared to chromium.

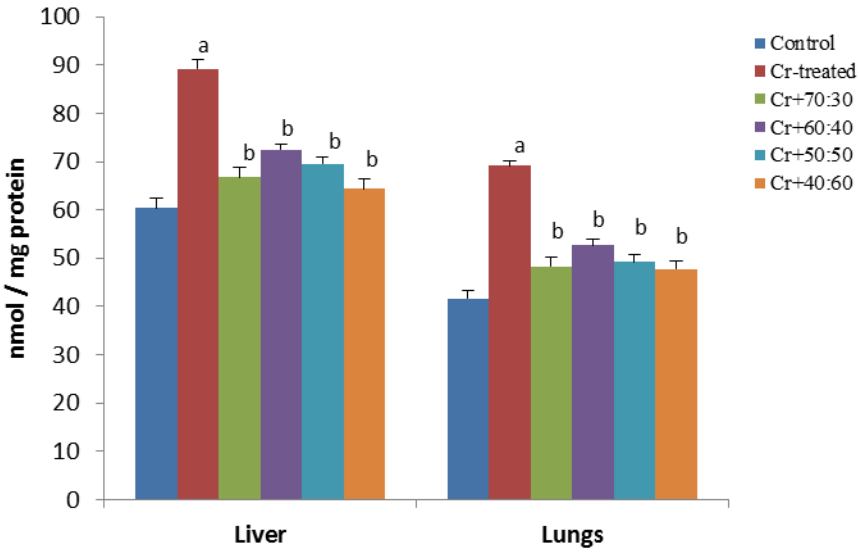

Figure 2: Changes the conjugated dienes concentration in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of 70:30, 60:40, 50:50 and 40:60 in chromium treated rats. Data represents mean + SE. a P < 0.05 compared to control, b P < 0.05 compared to chromium.

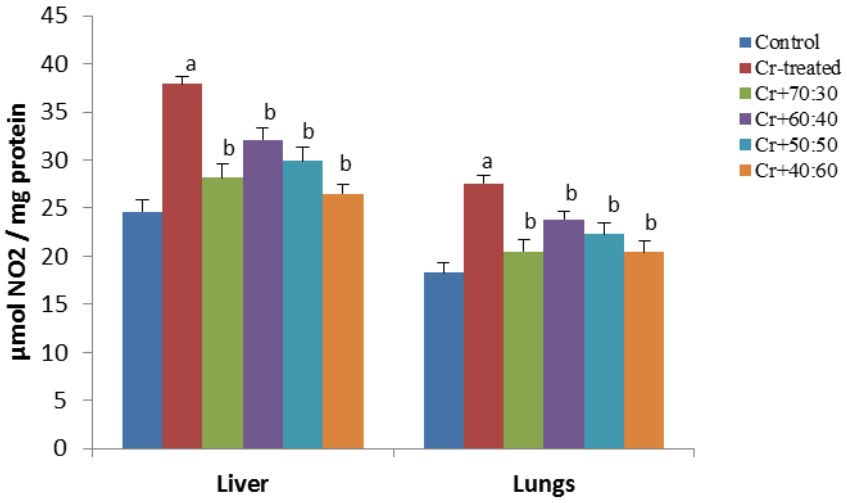

Figure 3: Changes the nitric oxide (NO) production in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of 70:30, 60:40, 50:50 and 40:60 in chromium treated rats. Data represents mean + SE. a P < 0.05 compared to control, b P < 0.05 compared to chromium.

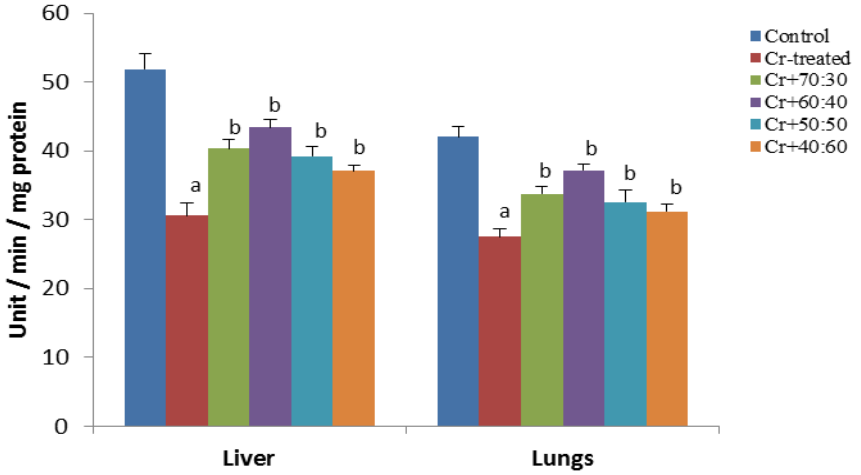

Figure 4: Changes the SOD activity in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of 70:30, 60:40, 50:50 and 40:60 in chromium treated rats. Data represents mean + SE. a P < 0.05 compared to control, b P < 0.05 compared to chromium.

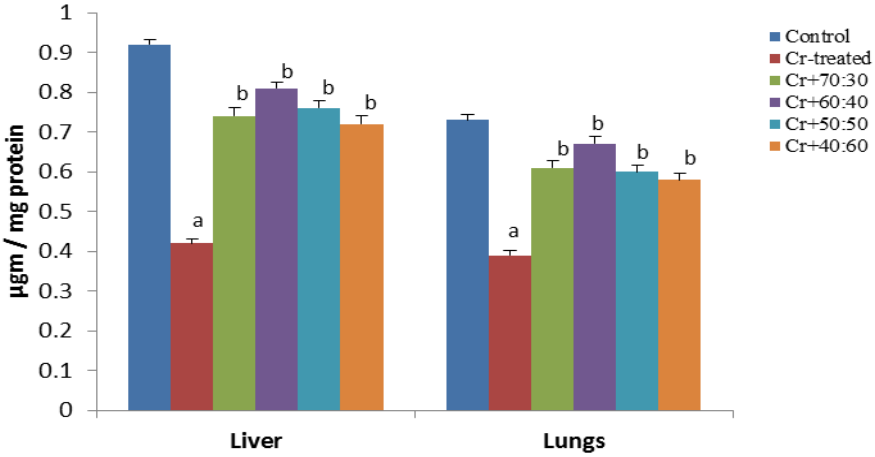

Figure 5: Changes the GSH level in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of 70:30, 60:40, 50:50 and 40:60 in chromium treated rats. Data represents mean + SE. a P < 0.05 compared to control, b P < 0.05 compared to chromium.

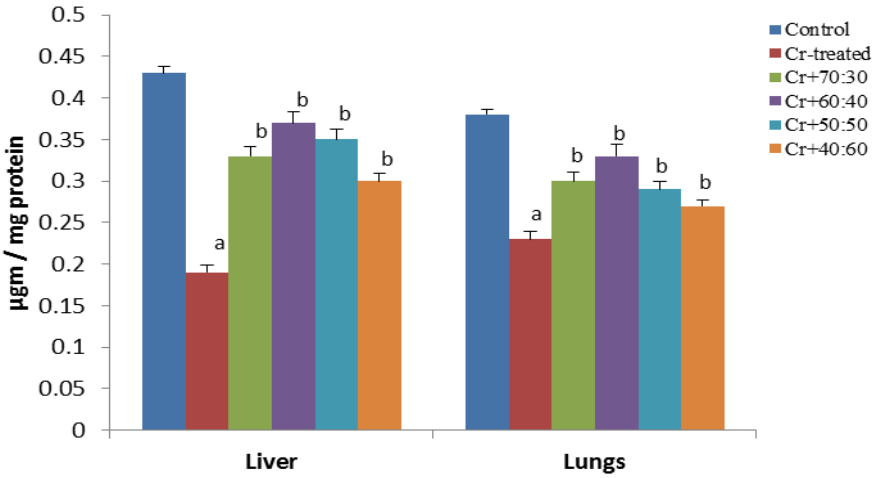

Figure 6: Changes the GSSG level in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of 70:30, 60:40, 50:50 and 40:60 in chromium treated rats. Data represents mean + SE. a P < 0.05 compared to control, b P < 0.05 compared to chromium.

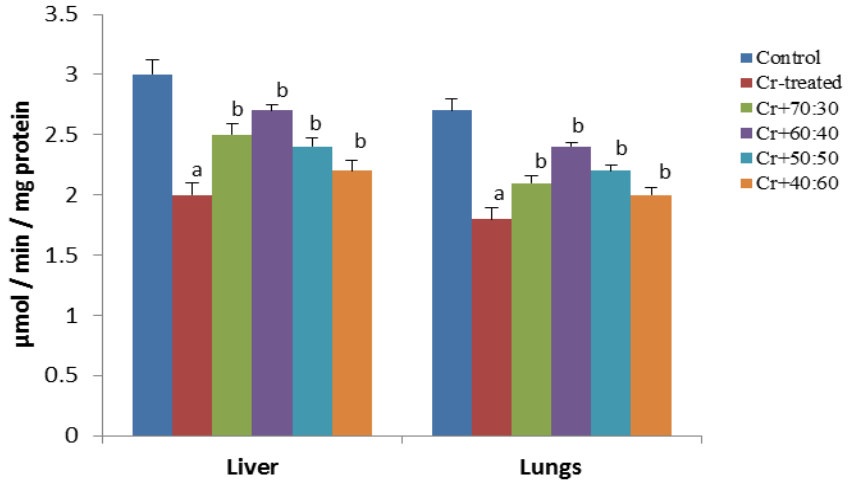

Figure 7: Changes the GPx activity in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of 70:30, 60:40, 50:50 and 40:60 in chromium treated rats. Data represents mean + SE. a P < 0.05 compared to control, b P < 0.05 compared to chromium.

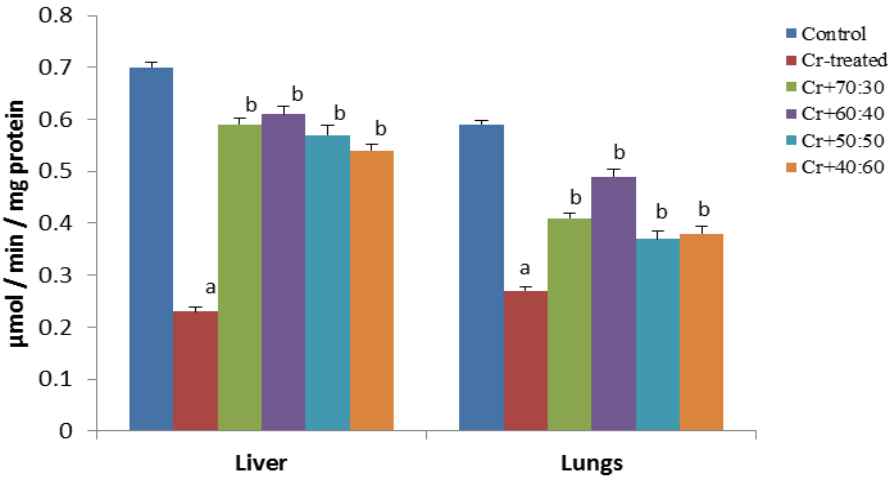

Figure 8: Changes the GR activity in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of 70:30, 60:40, 50:50 and 40:60 in chromium treated rats. Data represents mean + SE. a P < 0.05 compared to control, b P < 0.05 compared to chromium.

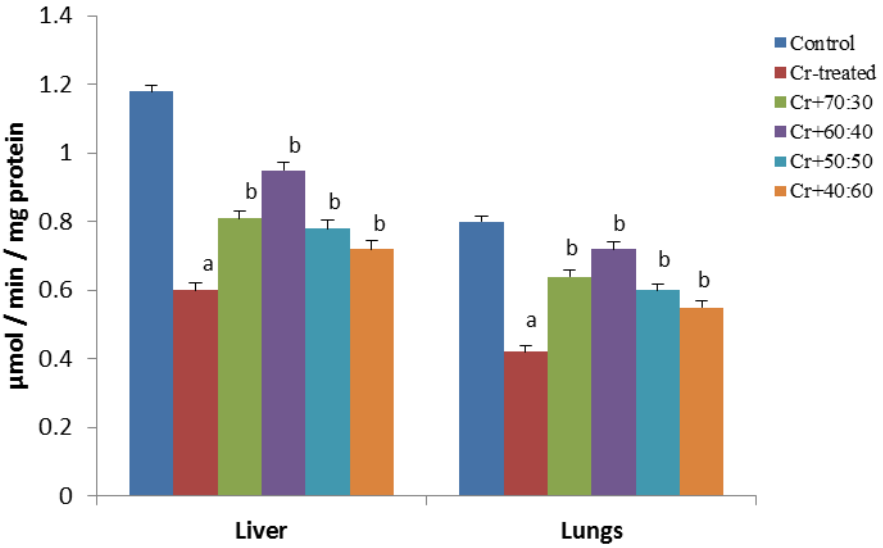

Figure 9: Changes the GST activity in liver and lungs mitochondria after co-administration of mixed solvent water and methanol in the ratio of 70:30, 60:40, 50:50 and 40:60 in chromium treated rats. Data represents mean + SE. a P < 0.05 compared to control, b P < 0.05 compared to chromium.

DISCUSSION

Oxidative stress is characterized by increased lipid peroxidation or altered nonenzymatic and enzymatic antioxidant system. However, initial formation of large amounts of oxygen and nitrogen species during stress may also initiate lipid peroxidation (Braughler and Hall 1989), as has been demonstrated in the liver and heart (Hu et al. 2000). From the present study, it was observed that MDA and conjugated dienes concentration were significantly increased in both liver and lungs mitochondria in response to chromium exposure (Figure-1 & 2). On the other hand, it was found that the NO production increased significantly in both tested organs (Figure-3). The formation of NO in brain mitochondria may have an important consequence, because this compound binds to the haem group from cytocromes (in particular, cytocrome oxidase) and inhibits respiration (Poderoso et al. 1996). This may, in turn, stimulate O_2^{*-} formation, which may react with more NO, forming peroxinitrite, an oxidant capable of inhibiting important enzymes and affecting mitochondrial integrity (Radi et al. 2002). Significant depletion in the concentration of MDA and CD, and NO production in liver and lungs mitochondria of mixed hydro-methanol solvent extract at different ratio supplemented rats indicate that the *Andrographis paniculata* has important properties to scavenging free radicals in response to chromium.

The deleterious effects resulting from the formation of ROS in the mitochondria are, to a large extent, prevented by various antioxidant systems. The first line of defense against chromium-induced ROS in the cell is provided by SOD. Superoxide is enzymatically converted to H_2O_2 by a family of metallo enzymes called SODs (Fridovich 1995). O_2^{*-} may reduce transition metals, which, in turn, can react with H_2O_2, produce $^*OH$, or spontaneously react with NO to produce peroxynitrite. It is important to maintain the steady state concentration of O_2^{*-} at the lowest possible level. Our study also supported the above findings. The mitochondrial SOD activity was very low during chromium treatment in liver and lungs mitochondria (Figure-4), which may be due to the overproduction of O_2^{*-} and H_2O_2, potent inhibitors of SOD activity. Administration of mixed solvent extract significantly increases the SOD activity in liver and lungs mitochondria. These results may suggest that mixed hydro-methanol solvent herbal extract of *Andrographis paniculata* attenuates oxidative stress in experimental tissues.

GSH is the main oxidant in the cell, and it directly scavenges free radicals and protects biomolecules from free radical attack. The significant decrease of GSH levels in this study was noted after chromium administration in liver and lungs mitochondria (Figure-5), and may indicate the inhibition of GSH synthesis and more utilization of GSH for detoxification of chromium-induced free radicals. A decrease in the GSSG (Figure-6) indicates that the conversion of GSSG to GSH has been severely affected (Singh et al. 2001). Moreover, a significant decrease in GPx, GR and GST activities has been observed in all the tested organs in response to chromium (Figure- 7, 8 & 9). This might have been triggered by low levels of NADPH, which is a co-factor of GR to convert GSSG to GSH. Hydrogen peroxide, the product of $O2^{*-}$ dismutation and the main precursor of $^*OH$ in the presence of reduced transition metals, is mostly decomposed by the enzyme GPx. In the liver, mitochondria account for about one third of the total GPx activity (Chance et al. 1979). A second GPx associated with the mitochondrial membrane, known as phospholipid–hydroperoxide GPx, is specifically involved in reducing lipid peroxides associated with the membrane (Nomura et al. 2000). On the other hand,

supplementation with different hydro-methanol solvent extract of *Andrographis paniculata* at different ratio recovered the GSH and GSSG level, and the activities of GPx, GR and GST in response to chromium in liver and lungs mitochondria. This depletion may result in the involvement of deleterious oxidative changes due to the accumulation of toxic product. So, the increased levels of antioxidant indicate the protective effect of *Andrographis paniculata* against chromium-induced oxidative stress. In the present study, the protective effect of aqueous and methanol *extract* of *Andrographis paniculata* in different proportions was studied against Cr (VI) induced toxicity of liver and lung mitochondria of male albino rat. For this experiment crude extract of *Andrographis paniculata* in the mixed hydro-methanol solvent ratios of 70:30, 60:40, 50:50 and 40:60 were successively used for the supplementation against Cr (VI) induced toxicity of liver and lung to find out most effective ratio of hydro-methanol extract of *Andrographis paniculata*. The important findings in this study corroborated the facts that particular 60:40 mixed hydro-methanol solvent extract of *Andrographis paniculata* has greater potential benefit than other ratio of mixed hydro-methanol solvent extract like 70:30, 60:40, 50:50 and 40:60 in maintenance of oxidative equilibrium, scavenging of ROS and augmented anti-oxidant defense against chromium-induced toxicity in liver and lungs mitochondria.

REFERENCES

1. Braughler JM and Hall ED. Central nervous system trauma and stroke. I. Biochemical considerations for free radical dormation and lipid peroxidation. Free Rad Biol Med, 1989; 6: 289–301.
2. Chance B, Sies H and Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev, 1979; 59: 527–605.
3. Dey SK, Dolai DP, Mandal D, Das B, Dash SK, Roy D and Roy S. Protective effect of Andrographis paniculata Nees and Vitamin-C in nicotine-induced oxidative stress in liver and kidney. World J Pharm Sci, 2016; 4(9): 345-352.
4. Dey SK, Karmakar BK, Jana S. Role of methanol extract of Andrographis paniculata nees on chromium induced alteration of functional status of liver in male albino rats. Bio Chem An Ind Jr, 2011; 5(1): 35-39.
5. Dey SK, Nayak P and Roy S. Alpha-tocopherol supplementation on chromium toxicity: a study on rat liver and kidney cell membrane. J Environ Sci, 2003; 15: 356-359.
6. Dolai DP, Roy S and Dey SK. Study the efficacy of different solvents extract of Andrographis paniculata Nees on chromium-induced oxidative stress in liver and lungs mitochondria. Singapore J of Sci Res, 2020; (In Press).
7. Fridovich I. Superoxide radical and superoxide dismutases. Annu Rev Biochem, 1995; 64: 97–112.
8. Gazotti P, Malmstron K and Crompton MA. Laboratory manual on transport and bioenergetics. In: Carofoli E, Semanza G, editors. Membrane Biochemistry. New York: Springer-Verlag, 1979; 62-69.
9. Grifith OW. Determination of glutathione and glutathione sulfide using glutathione reductase and 2-vinyl pyridine. Anal Biochem, 1980; 106: 207-212.
10. Habig WH, Pabst MJ and Jakoby WB. Glutathone S-transferases, the first enzymatic step in mercapturic acid formation. J Biol Chem., 1974; 249: 7130-7139.
11. Hu Y, Cardounel A, Gursoy E, Anderson P and Kalimi M. Anti-stress effects of dehydroepiandrosterone. Protection of rats against repeated immobilization stress-induced weight loss, glucocorticoid receptor production, and lipid peroxidation. Biochem Pharmacol, 2000; 59: 753–762.
12. Kapil A, Kou lIB, Banerjee SK and Gupta BD. Antihepatotoxic effects of major diterpenoid constituents of *Andrographis paniculata*. Biochemical Pharmacology, 1993; 46: 182-185.
13. Lowry OH, Roseborough NJ,. Farr AL and Randll AJ. Protein measurement with Folin's phenol reagent. J Biol Chem, 1951; 193: 265-275.
14. Madav S, TripathiC, Tandan SK and Mishra S. Analgesic, antipyretic, and antiulcerogenic effects of andrographolide. Ind. J. Pharm. Sci, 1995; 57: 121-125.
15. Marklund S and Marklund G. Involvement of superoxide anion radical in autoxidation of pyrogallol and a convenient assay of superoxide dismutase. Eur J Biochem, 1974; 47: 469-474.
16. Miwa S. Hematology In: Modern Medical Technology, 1972; 3: 306-310.
17. Nomura K, Imai H, Koumura T, Kobayashi T and Nakagawa Y. Mitochondrial phospholipid hydroperoxide glutathione peroxidase inhibits the release of cytochrome c from mitochondria by suppressing the peroxidation of cardiolipin in hypoglycaemia induced apoptosis. Biochem J, 2000; 51: 183–193.
18. Ohkawa H, Ohisi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 1979; 95: 351-358.
19. Paglia DE and Valentine WN. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med, 1967; 70: 158–169.
20. Poderoso JJ, Carreras MC, Lisder C, Riobo N, Schopfer F and Boveris A. Nitric oxide inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrialparticles. Arch Biochem Biophys, 1996; 328: 85-92.
21. Radi R, Cassina A and Hodara R. Nitric oxide and peroxynitrite interactions with mitochondria. Biol Chem, 2002; 383: 401-409.
22. Sanai S, Tomisato M, Shinsuka N, Mayoko Y, Mayoko H and Akio N. Protective role of nitric

oxide in S. aurues infection in mice. Infect Immun, 1998; 66: 1017–1028.
23. Singh SN, Vats P, Kumria MM, RanganthanS, Shyam R, AroraMP et al. Effect of high altitude (7,620 m) exposure on glutathione and related metabolism in rats. Eur J Appl Physiol, 2001; 84: 233–237.
24. Slater T I. Overview of methods used for detecting lipid peroxidation. Methods Enzymol, 1980; 105: 283-293.