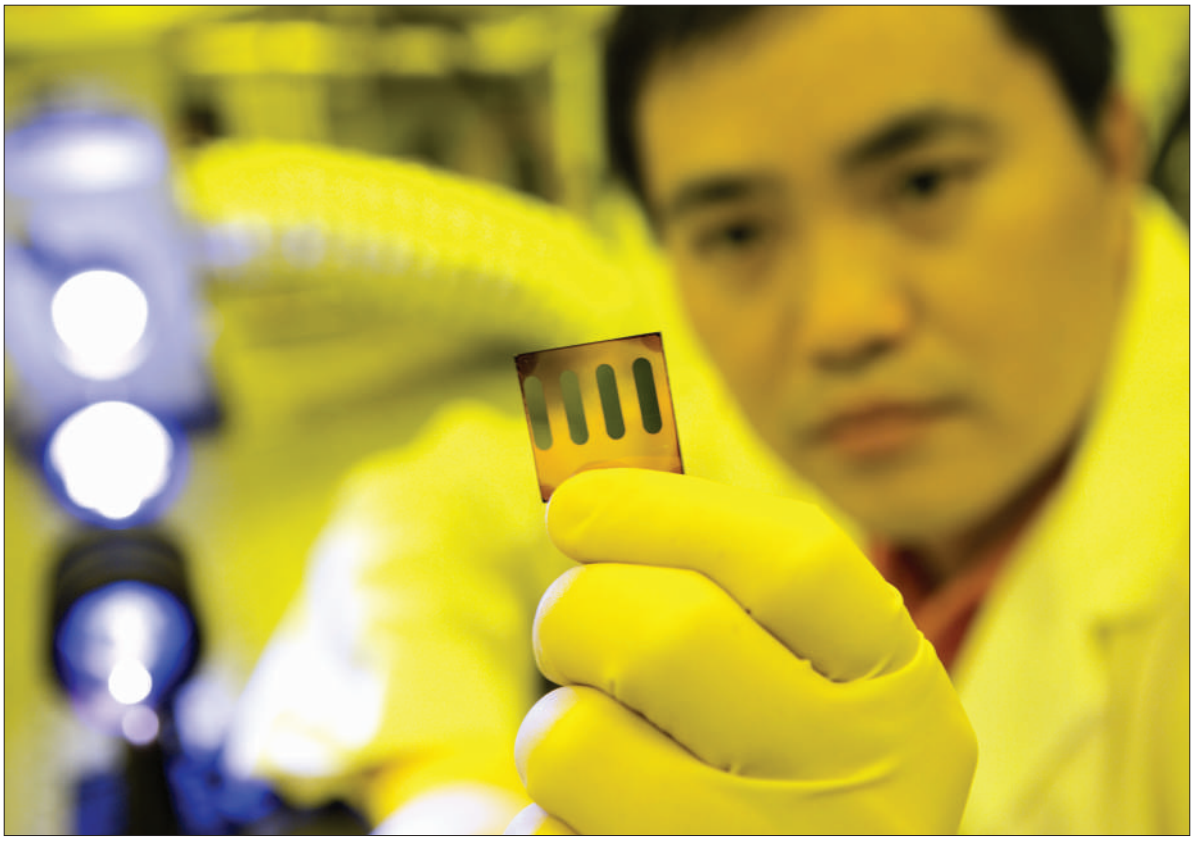

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Research Article
Ameliorative Role of *Andrographis paniculata* Nees Exract on Chromium-induced Oxidative Stress in Liver and Lungs Mitochondria

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Abstract

Background and Objective: Potassium dichromate ($K_2Cr_2O_7$) has been demonstrated to induce oxidative stress and carcinogenic in nature. This study was aimed to evaluate the protective effects of different solvent (aqueous, methanol and petroleum ether) extract of *Andrographis paniculata* on lipid peroxidation and antioxidants status against chromium-treated liver and lungs mitochondria. **Materials and Methods:** A group of male Wistar rats (80-100 g) were obtained and divided into eight groups. The animals of seven groups were induced $K_2Cr_2O_7$ at a dose of 0.8 mg per 100 g body weight per day (20% LD_{50}) for 28 days. The animals of six of the chromium treated groups injected different solvent extracts at a dose of 250 and 500 mg kg^{-1} b.wt., daily 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. **Results:** 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 and conjugated dienes 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). **Conclusion:** The results of the present study suggest that the administration of different solvent extract significantly supplement the lipid peroxidation and enhanced the antioxidant status.

Key words: Chromium, animal, liver, lungs, toxicity, oxidative stress, *Andrographis paniculata*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The occurrence of heavy metals in the environment and their enormous industrial use has led to an increase in the frequency of the human organ toxicity. Among different heavy metals chromium is one of the important heavy metal in both terrestrial and aquatic environments[1]. It is also a trace element which is extracts from chromate[2]. Chromium presents in environment in various oxidation states. Trivalent chromium is extensively used as supplement and also a good element for glucose/insulin homeostasis[3], where as hexavalent chromium is highly toxic for their easy permeation at physiological pH through the permease system[3]. Hepatic and renal toxicity is the most common toxicity observed in Cr (VI)-exposed workers or animals[2]. This functional differentiation of Cr (III) and Cr (VI) is largely decided by the ionic permeability of the plasma membrane[4]. Cr (VI) compounds are the most toxic since they can be easily absorbed and transported across membranes via non-specific anion carriers[5]. Thus, membrane damage is one of the crucial factors observed with Cr (VI) toxicity[6]. Inside the cells, Cr (VI) is reduced through reactive intermediates such as Cr (V) and Cr (IV) to the more stable Cr (III) by cellular reluctant[7]. This reduction process generates reactive oxygen species (ROS) and induces soft tissues' damage such as liver, pancreas, cerebellum and kidney[8].

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[9,10]. Reduction of Cr (VI) induced the generation of hydroxyl radical (.OH) via the Fenton mechanism[11]. 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[12].

Medicinal plants and their active principles have received greater attention as potential antiperoxidative agent[13]. *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[14] and choleretic[15]. 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 at different doses of different solvent extract in chromium-induced oxidative damage in liver and lungs mitochondria.

MATERIALS AND METHODS

Description of the study area: This experiment was conducted during the year 2017/18 and 2018/19 in the Department of Human Physiology, Vidyasagar University, Midnapore, West Bengal, India.

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 (n = 96) 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 1 week and water *ad libitum*. Animals were divided into eight groups and each group consisting 12 animals.

Collection, identification and preservation of plant materials and extract preparation: 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 Vidyasagar University. Specimen was labelled, numbered and noted with date of collection (Plate 1). Plant part was rinsed with sterilized distilled water, air dried and stored in airtight bottle at 4°C for further use (Petridish, Borosil, Ahmedabad, India).

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. Two gram of powdered material was

Kingdom:	Plantae
(unranked):	Angiosperm
(unranked):	Eudicots
(unranked):	Asrerids
Order:	Lamiales
Family:	Acanthaceae
Genus:	*Andrographis*
Species:	*A. paniculata*

Plate 1: *Andrographis paniculata* collected from Indian Institute of Technology Campus, Kharagpur, West Bengal, India (Aluminium Alloys Plate, Shanghai, China)

mixed with 20 mL of sterile distilled water and kept on a rotary shaker for 12 h at 38°C. Thereafter, it was filtered with the help of Whatman No. 1 filter paper (Whatman Clifton, NJ, USA). The filtrate was then centrifuged (Remi, Goregaon (East), Mumbai, India) at 2000 rpm for 10 min. Then the supernatant was collected and stored at 4°C for further use[16].

Preparation of methanol extract: Ten gram of grinded powder of *A. paniculata* plant materials were soaked in 30 mL of 70% methanol and were kept at 30°C for 12 h on a rotary shaker. After 12 h the previous portion of added methanol was evaporated so to make the same volume, methanol was added and then it was placed on a rotary shaker for another 12 h at 30°C. After that it was filtered through Whatman No. 1 filter paper (Whatman Clifton, NJ, USA). The filtrate was centrifuged (Remi, Goregaon (East), Mumbai, India) at 2000 rpm for 10 min. Then the supernatant was collected and stored at 4°C for further use. Then supernatant was collected and allowed to evaporate until completely dry. Then 30 mg of dry extract was re-suspended in 1 mL of 70% methanol. The final concentration of the extract was 30 mg mL^{-1} [17].

Preparation of petroleum-ether extract: Ten gram of grinded materials were mixed with 20 mL of petroleum-ether solvent and kept on a rotary shaker for 12 h at 30°C. Thereafter, it was filtered with the help of Whatman No. 1 filter paper (Whatman Clifton, NJ, USA). The filtrate was then centrifuged (Remi, Goregaon (East), Mumbai, India) at 2000 rpm for 10 min. Then the supernatant was collected and stored at 4°C for further use. Then supernatant was collected and allowed to evaporate until completely dry. Then, 30 mg of dry extract was re-suspended in 1 mL of 70% methanol. The final concentration of the extract was 30 mg mL^{-1} [18].

Mode of treatment: Animals were divided into eight groups of almost equal average body weight of twelve animals each. The animals of seven groups were induced by intraperitoneal injection with $K_2Cr_2O_7$ at a dose of 0.8 mg per 100 g b.wt. per day (20% LD_{50}) for 28 days, as described earlier[6]. The animals of six of the chromium treated groups serving as the supplemented groups injected AE-AP 250 (Aqueous Extract of *Andrographis paniculata* 250 mg kg^{-1} b.wt./day), AE-AP 500 (Aqueous Extract of *Andrographis paniculata* 500 mg kg^{-1} b.wt./day), ME-AP 250 (Methanol Extract of *Andrographis paniculata* 250 mg kg^{-1} b.wt./day), ME-AP 500 (Methanol Extract of *Andrographis paniculata* 500 mg kg^{-1} b.wt./day), PEE-AP 250 (Petroleum-ether Extract of *Andrographis paniculata* 250 mg kg^{-1} b.wt./day) and PEE-AP 500 (Petroleum-ether extract of *Andrographis paniculata* 500 mg kg^{-1} b.wt./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 cervical dislocation. The intact liver and lungs were dissected out and adhering blood and tissue fluid were blotted dry weighted and kept at -20°C.

Homogenization of tissues: A weighted portion of different tissues was homogenized (Royal Scientific, Tiruchirappalli, Tamilnadu, India) in an ice cold 0.2 M PBS (pH 7.4) using glass homogenizer (Borosil, Ahmedabad, India)[8]. 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[19].

Analytical methods: Lipid peroxidation was measured according to the method of Ohkawa *et al.*[20]. 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[21]. NO release assays were done in liver and lungs mitochondria according to the method of Sanai *et al.*[22]. SOD activity was estimated by measuring the percentage inhibition of the pyrogallol auto-oxidation by SOD according to the method Marklund and Marklund[23].

GSH (reduced glutathione) was measured according to the method of Griffith[24]. GSSG was also assayed after derivatization of GSH with 2 vinylpyridine. GSSG (oxidized glutathione) was measured by the method of Griffith[24].

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[25]. The activity of glutathione reductase was measured by the method of Miwa[26]. Glutathione S-transferase activity was also measured according to the method of Habig *et al.*[27]. Total protein of plasma and tissues was estimated according to the method of Lowry *et al.*[28].

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

The results found that the changes of MDA and conjugated dienes concentration were significantly increased in response to chromium but after the supplementation of different doses of aqueous, methanol and petroleum ether extracts of *A. paniculata* (Fig. 1-2), it was found that AP 500 of aqueous and methanol extract of *A. paniculata* have the potent role to counteract the chromium-induced toxicity in liver and lungs mitochondria.

The changes of Nitric oxide production (NO) and the SOD activity in response to chromium and the supplementation role of different doses of aqueous, methanol and petroleum ether extracts of *A. paniculata* was found in Fig. 3-4. The results showed that NO production increased and SOD activity decreased significantly in response to chromium in liver and lungs mitochondria. On the other hand, AP 500 of aqueous and methanol extract of *A. paniculata* have the potent role to counteract the chromium-induced toxicity.

The levels of GSH and GSSG were significantly diminished in liver and lungs mitochondria in response to chromium treatment (Fig. 5-6) but after supplementation with the different doses of aqueous, methanol and petroleum ether extracts of *A. paniculata,* it was found that AP 500 of aqueous and methanol extract of *A. paniculata* have the potent role to counteract the chromium-induced tissue toxicity.

The activities of GPx, GR and G-S-T were significantly decreased in response to chromium when compared with control in both liver and lungs mitochondria (Fig. 7-9). On the other hand, it was found that the supplementation role of AP 500 of aqueous and methanol extract of *A. paniculata* have the potent role to counteract the chromium-induced toxicity in liver and lungs mitochondria.

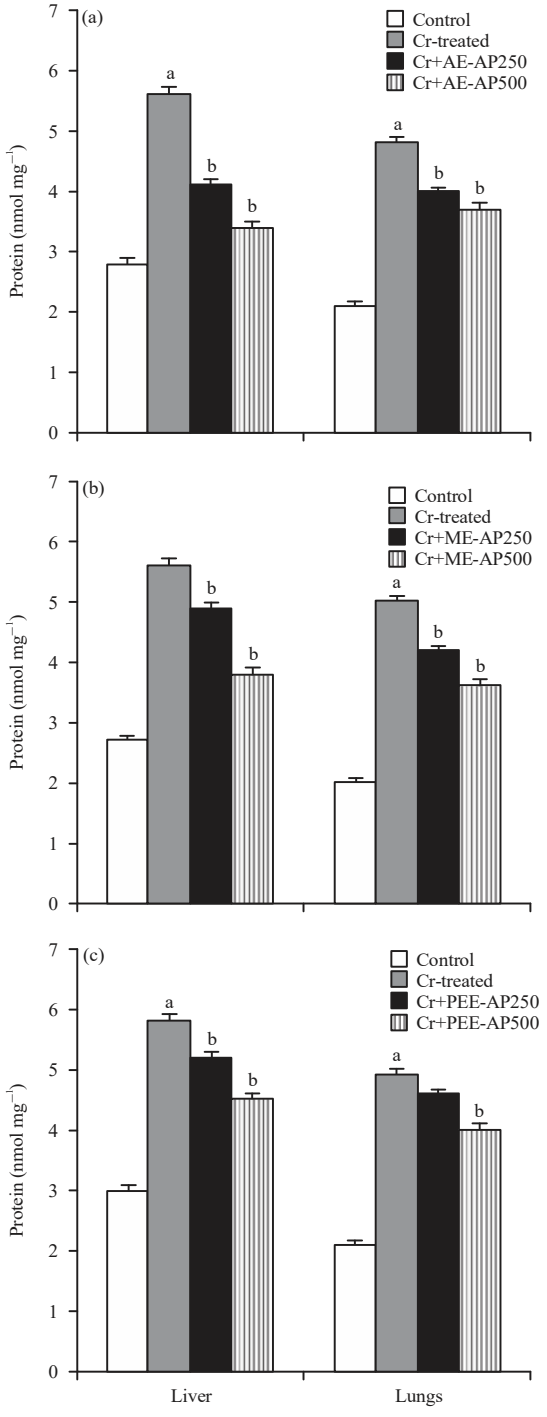

Fig. 1(a-c): Changes the malondialdehyde (MDA) concentration in liver and lungs mitochondria
After co-administration of AE-AP (Aqueous extract of *Andrographis paniculata*) 250 and 500, ME-AP (Methanol extract of *Andrographis paniculata*) 250 and 500 and PEE-AP (Petroleum-ether extract of *Andrographis paniculata*) 250 and 500 in chromium treated rats. Data represents mean±standard error, [a]p<0.05 compared to control, [b]p<0.05 compared to chromium

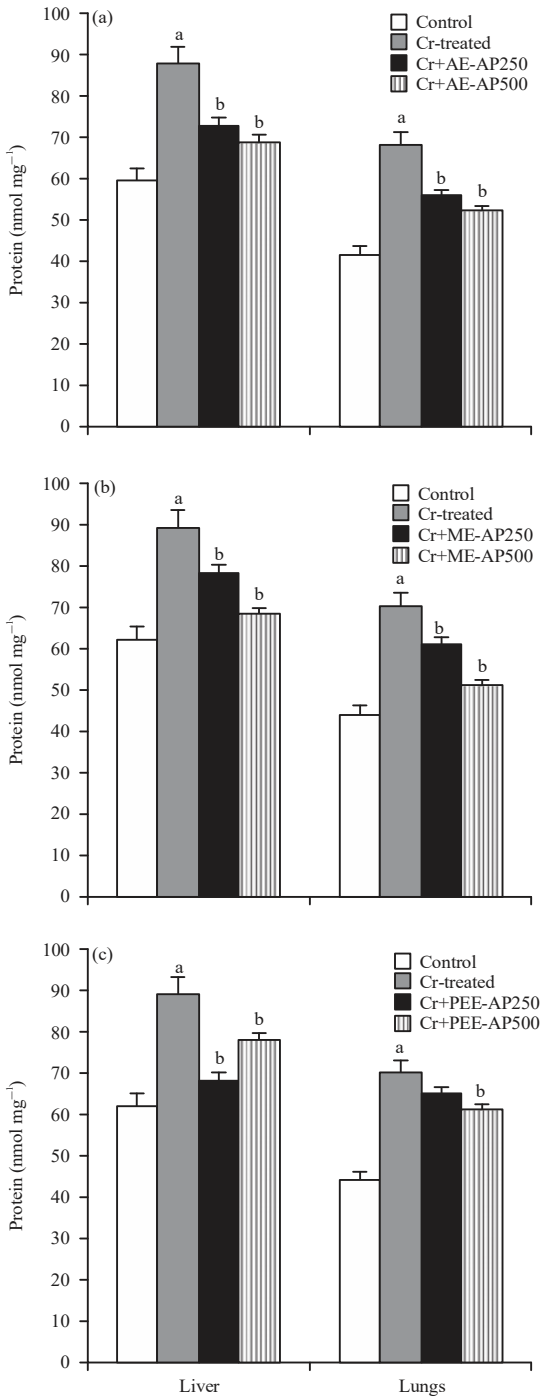

Fig. 2(a-c): Changes the conjugated dienes (CD) concentration in liver and lungs mitochondria
After co-administration of AE-AP (Aqueous extract of *Andrographis paniculata*) 250 and 500, ME-AP (Methanol extract of *Andrographis paniculata*) 250 and 500 and PEE-AP (Petroleum-ether extract of *Andrographis paniculata*) 250 and 500 in chromium treated rats. Data represents mean±standard error, [a]p<0.05 compared to control, [b]p<0.05 compared to chromium

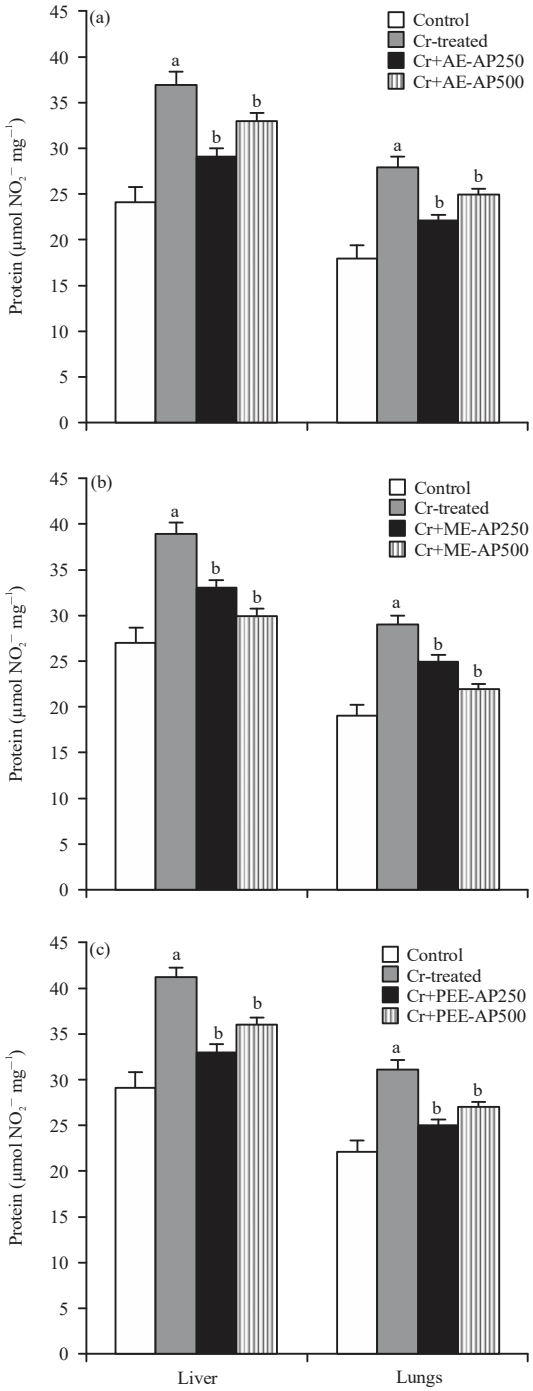

Fig. 3(a-c): Changes the nitric oxide production (NO) in liver and lungs mitochondria

After co-administration of AE-AP (Aqueous extract of *Andrographis paniculata*) 250 and 500, ME-AP (Methanol extract of *Andrographis paniculata*) 250 and 500 and PEE-AP (Petroleum-ether extract of *Andrographis paniculata*) 250 and 500 in chromium treated rats. Data represents mean±standard error, [a]p<0.05 compared to control, [b]p<0.05 compared to chromium

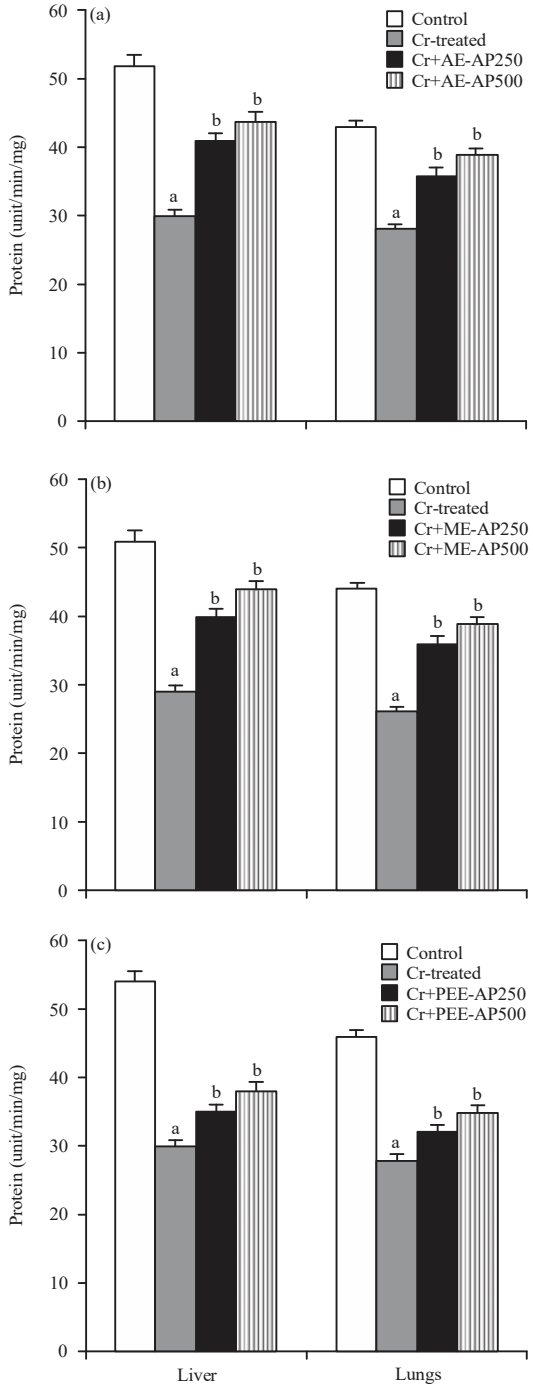

Fig. 4(a-c): Changes the super oxide dismutase (SOD) activity in liver and lungs mitochondria

After co-administration of AE-AP (Aqueous extract of *Andrographis paniculata*) 250 and 500, ME-AP (Methanol extract of *Andrographis paniculata*) 250 and 500 and PEE-AP (Petroleum-ether extract of *Andrographis paniculata*) 250 and 500 in chromium treated rats. Data represents mean±standard error, [a]p<0.05 compared to control, [b]p<0.05 compared to chromium

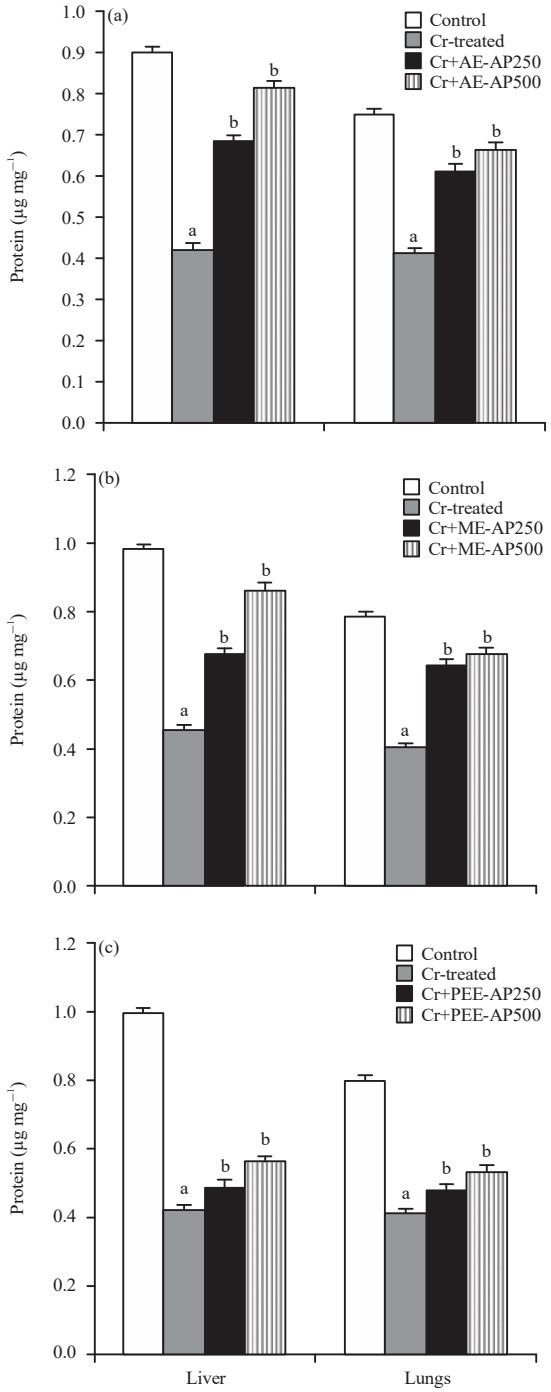

Fig. 5(a-c): Changes the reduced glutathione (GSH) level in liver and lungs mitochondria

After co-administration of AE-AP (Aqueous extract of *Andrographis paniculata*) 250 and 500, ME-AP (Methanol extract of *Andrographis paniculata*) 250 and 500 and PEE-AP (Petroleum-ether extract of *Andrographis paniculata*) 250 and 500 in chromium treated rats. Data represents mean±standard error, [a]p<0.05 compared to control, [b]p<0.05 compared to chromium

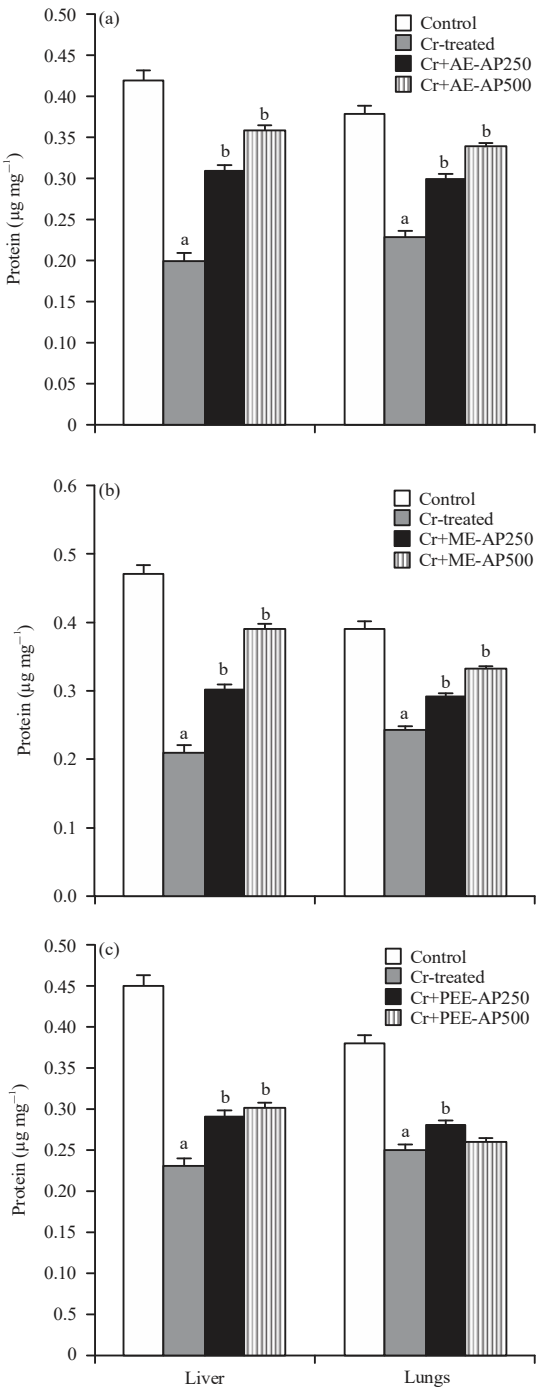

Fig. 6(a-c): Changes the oxidized glutathione (GSSG) level in liver and lungs mitochondria

After co-administration of AE-AP (Aqueous extract of *Andrographis paniculata*) 250 and 500, ME-AP (Methanol extract of *Andrographis paniculata*) 250 and 500 and PEE-AP (Petroleum-ether extract of *Andrographis paniculata*) 250 and 500 in chromium treated rats. Data represents mean±standard error, [a]p<0.05 compared to control, [b]p<0.05 compared to chromium

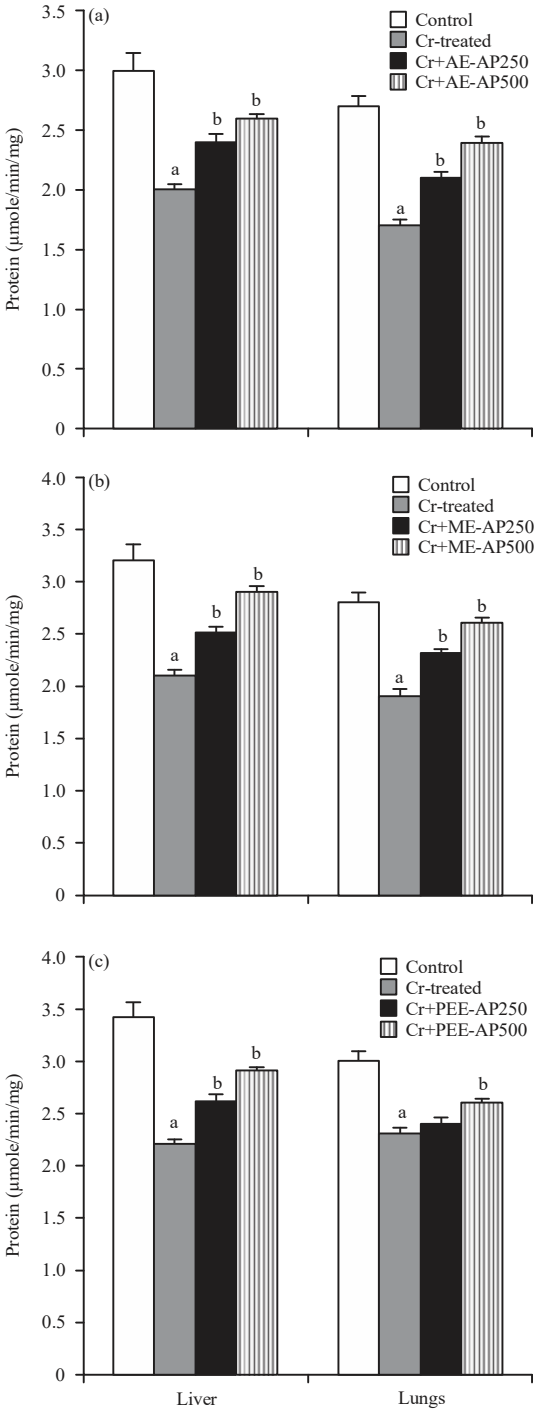

Fig. 7(a-c): Changes the glutathione peroxidase (GPx) activity in liver and lungs mitochondria

After co-administration of AE-AP (Aqueous extract of *Andrographis paniculata*) 250 and 500, ME-AP (Methanol extract of *Andrographis paniculata*) 250 and 500 and PEE-AP (Petroleum-ether extract of *Andrographis paniculata*) 250 and 500 in chromium treated rats. Data represents mean±standard error, [a]p<0.05 compared to control, [b]p<0.05 compared to chromium

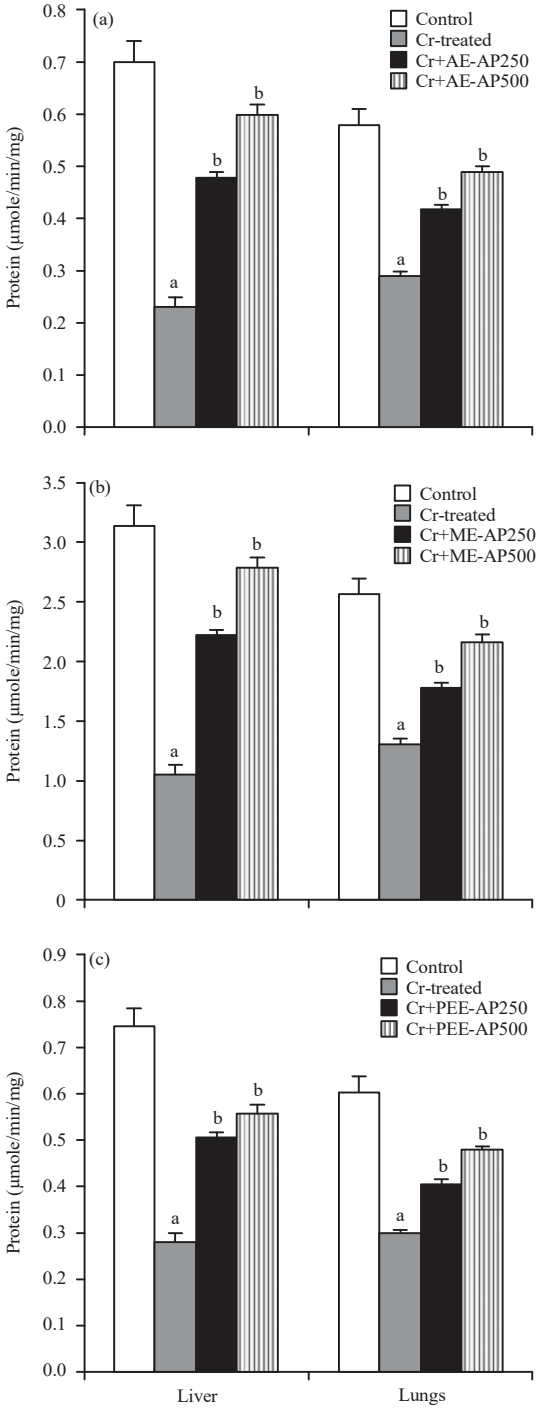

Fig. 8(a-c): Changes the glutathione reductase (GR) activity in liver and lungs mitochondria

After co-administration of AE-AP (Aqueous extract of *Andrographis paniculata*) 250 and 500, ME-AP (Methanol extract of *Andrographis paniculata*) 250 and 500 and PEE-AP (Petroleum-ether extract of *Andrographis paniculata*) 250 and 500 in chromium treated rats. Data represents mean±standard error, [a]p<0.05 compared to control, [b]p<0.05 compared to chromium

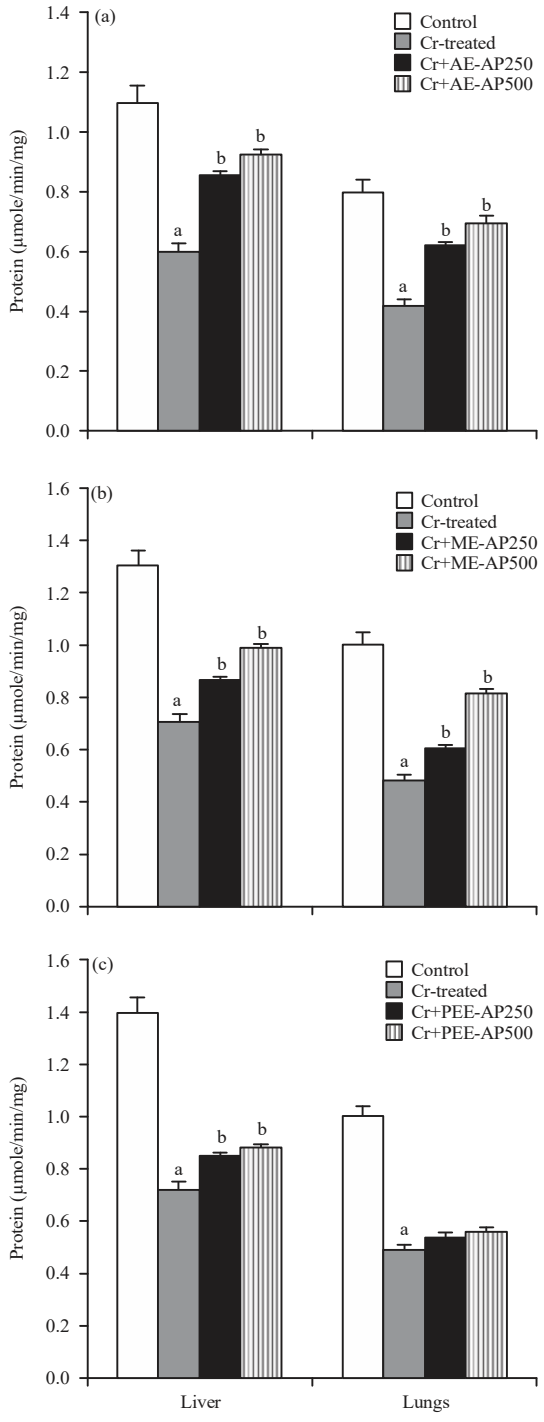

Fig. 9(a-c): Changes the glutathione-s-transferase (GST) activity in liver and lungs mitochondria

After co-administration of AE-AP (Aqueous extract of *Andrographis paniculata*) 250 and 500, ME-AP (Methanol extract of *Andrographis paniculata*) 250 and 500 and PEE-AP (Petroleum-ether extract of *Andrographis paniculata*) 250 and 500 in chromium treated rats. Data represents mean±standard error, [a]p<0.05 compared to control, [b]p<0.05 compared to chromium

DISCUSSION

Cr (VI) dramatically decreased oxygen consumption and NADH levels in isolated rat liver and heart mitochondria[29]. Intact mitochondria take up and reduced chromium (VI), producing chromium (V) species and the chromium (V) generated efficiently oxidized NADH[11]. Chromium (III), which does not penetrate into intact mitochondria, had no effect on the respiratory rats in sonicated mitochondria[30]. Chromium (VI) is a potent inhibitor of mitochondria, there enzymes (α-ketoglutarate dehydrogenase, pyruvate dehydrogenase and β-hydroxyl butyrate dehydrogenase) may explain the observed inhibitory respiration in liver mitochondria and decreasing in cellular levels of ATP and GTP[30]. *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), methanol extract (ME-AP) and petroleum ether extract (PEE-AP) at different doses (250 and 500 mg kg^{-1} b.wt./ day) 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 (Fig. 1-2). Bagchi *et al.*[31] 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 study also showed a significant increase in NO production in liver and lungs (Fig. 3a-c). The simultaneous production of superoxide and NO produces peroxynitrite ($ONOO^-$), 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 haem group from cytochromes (in particularly, cytochrome oxidase) and inhibits respiration[32]. This may, in turn, stimulate O_2^-. Formation, which may react with more NO, forming peroxynitrite, an oxidant capable of inhibiting important enzymes and affecting mitochondrial integrity[33]. 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 (Fig. 4a-c) could be explained by the massive production of super oxide anion. On the other hand, it was found that administration of solvent extracts 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.

Ueno *et al.*[34] 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 (Fig. 5-6). It is very important in maintaining cellular redox status[35] and its depletion is considered as a marker of oxidative stress[36]. 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 GPX in chromium-induce rats in this study (Fig. 7a-c) 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 present study, the activity of GR in liver and lungs mitochondria declined in chromium-induces rats (Fig. 8a-c). 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 (Fig. 9a-c) might be due to increased oxidative stress. But it was found that, after supplementation with different solvent extract play a vital role to counteract the oxidative stress in response to chromium in liver and lungs mitochondria.

Administration of solvent extracts 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-glutamyl transcriptase, glutathione, J-transferase induced increase in the activity of enzyme and lipid peroxidation. It has protective effects on oxidative stress by increasing activity of antioxidant enzyme and decreases lipid peroxidation[37]. 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. Andrographolide were tested for a protective effect against liver toxicity produced in mice by giving them carbon tetrachloride[38]. This chemical damages the liver by causing lipid peroxidation. In another study, andrographolide was shown to produce a significant increase in bile flow[15]. Biochemical and histological evidences indicate that andrographolide was hepatoprotective against galactosamine or paracetamol induce rats[39]. 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*[37]. Thus solvent extracts 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 in methanol and aqueous extract than petroleum ether is more protective. The results of the present study indicate that methanol and aqueous extract of *Andrographis paniculata* at the dose of AP500 may emerge as a preventive agent against liver and lungs carcinogenesis.

CONCLUSION

Present investigation suggested that chromium induced oxidative stress could be protected or minimized through the administration with solvent extracts of *A. paniculata*, Natural antioxidants strengthen the endogenous antioxidant defences from ROS and restored the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. In this context, *Andrographis paniculata* may rightly be mentioned as a plant with antioxidant activity. The protective action of this plant may be due to presence of andrographolide. It may suppress the chromium induced ROS generation and ROS mediated oxidative stress in different tissues. This finding may recommended that methanol and aqueous extract of *Andrographis paniculata* may have some important components, having the antioxidant property to diminish or prevent chromium induced toxicity.

SIGNIFICANCE STATEMENT

This study discovered the importance of evaluating the important solvent extract of *Andrographis paniculata* that can be beneficial for the supplementation of chromium-induced oxidative stress. This study will help the researchers to uncover the critical areas of the effective compound that many researchers were not able to explore. Thus a new theory on ethnopharmacology may be arrived at.

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