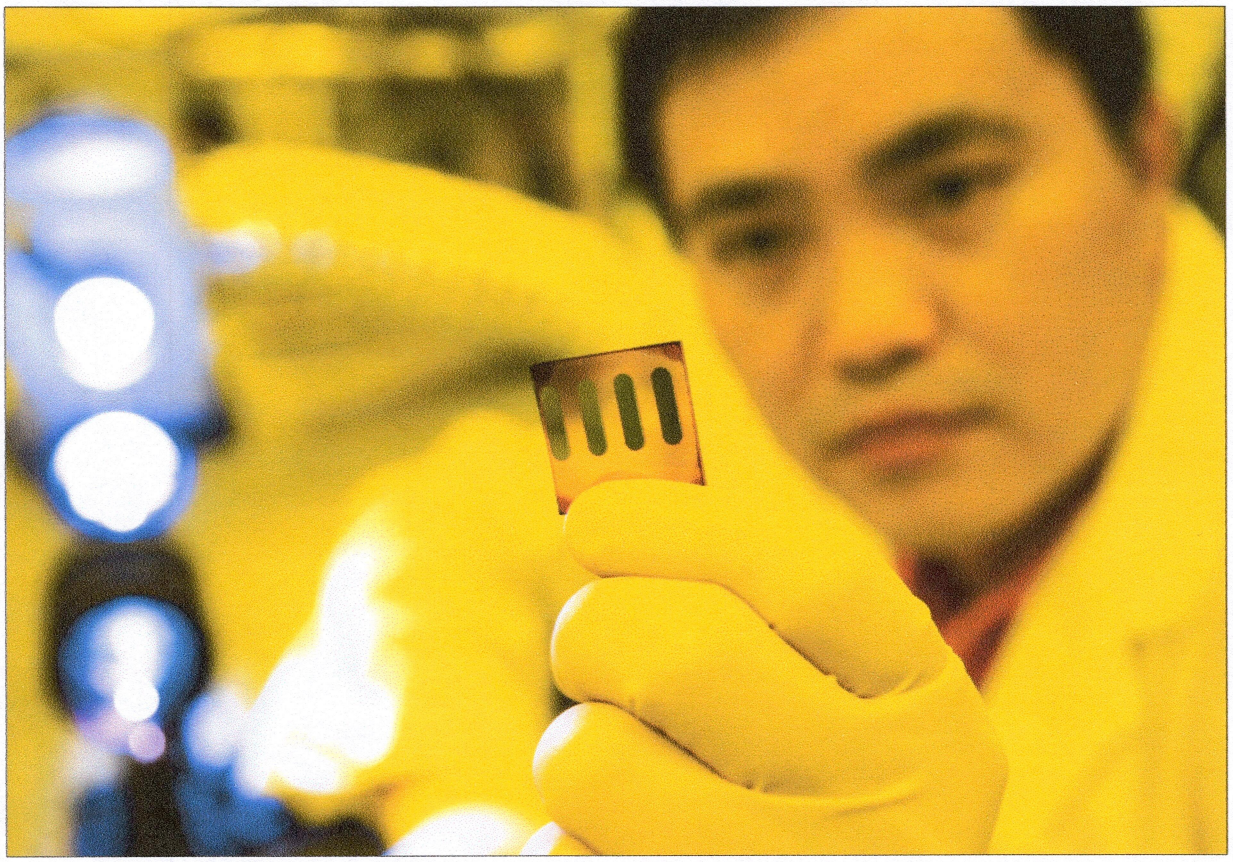

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Research Article
Ameliorative Role of *Andrographis paniculata* Nees Exract on Chromium-induced Oxidative Stress in Liver and Lungs Mitochondria

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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 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 $K_2Cr_2O_7$