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Role of methanol extract of *Andrographis paniculata* nees on chromium-induced alteration of functional status of liver in male albino rats

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ABSTRACT

Occupational exposure to Chromium (Cr) compounds provides the primary source of information on Cr toxicity and carcinogenicity in humans. Methanol extract of *Andrographis paniculata*, a hepato protectant may be used to reduce the Cr-induced liver impairment in male Wistar rats. To know the functional status of liver, we determined the activities of ALP, SGOT and SGPT and the contents of total bilirubin, direct bilirubin, albumin, total protein and serum glucose. The ALP, SGOT and SGPT activities were significantly higher in response to chromium (0.8 mg per 100 g body weight, i.p., for a period of 7 days). On the other hand, the levels of total bilirubin, direct bilirubin, albumin, total protein and serum glucose were lower in Cr-treated rats. Alteration of these parameters indicates that continuous accumulation of chromium may cause the liver impairment by the generation of free radicals. However, Methanol extract of *Andrographis paniculata* (100mg per kg body weight daily at an interval of 3 h after injection of Cr for a period of 7 days) supplementation restored the alterations of liver impairment induced by Cr.

KEYWORDS

Chromium;
Serum;
Andrographis paniculata;
Liver function.

INTRODUCTION

Chromium (Cr) is a widely used industrial chemical, finding applications in steel, alloy cast irons, chrome, paints, metal finishes, wood treatments, and it is known to cause allergic dermatitis as well as to have toxic and carcinogenic effects on humans and animals[5]. Cr occurs in the workplace primarily in the valence states of Cr^{3+} and Cr^{6+}. Von Berg and Liu[26] have summarized the acute toxicity, chronic toxicity, neurotoxicity, reproductive toxicity, genotoxicity, carcinogenicity and environmental toxicity of Cr.

Cr^{6+}or chromate, the biologically active form of environmental Cr, is taken up by cells and reduced intracellularly to reactive Cr^{5+} and Cr^{4+} species and then to stable Cr^{3+}. In the process, several toxic effects are associated with exposure to Cr compounds, including increased incidence of certain cancers, toxic towards living cells, tissue and organisms serious damage to such major organs as lung, liver and kidneys[9,10]. Epidemiological studies on occupational exposure to Cr compounds provide the primary source of information on

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Cr toxicity and carcinogenicity in humans[10,19] and the route of exposure can influence which organs are most effected[10,30].

Plant products exert their anti-carcinogenic effects by scavenging free radicals, by modulating carcinogen detoxification, through the antioxidant defense system[16]. *Andrographis paniculata* (Burm F) Wall Ex Nees (Family Acanthaceae) is used in the Indian traditional system of medicine against various diseases[2] and as a natural remedy for gastrointestinal disorders, sluggish fever, jaundice, and malaria. The plant has been reported to possess anti-diabetic[29], anti-pyretic[20], and hepatoprotective[21] properties.

In light of these findings, the present study was conducted to evaluate the protective effect of methanol extract of *Andrographis paniculata* Nees on Cr-induced alteration of functional status of liver.

MATERIALS AND METHODS

Animals

Mass-matched (120-140 g) male albino rats of Wistar Strain were obtained, divided in different groups, housed in polypropylene cages under standard condition of temperature (25±2C) and humidity (60±5%), with alternating 12 h light : 12 h dark cycles, and fed standard diet and tap water *ad libitum*. Animals were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, and Hyderabad, India and approved by the ethics committee of Vidyasagar University (West Bengal, India).

Collection of plant materials

Aerial parts of the *Andrographis paniculata* Nees were collected from the campus of the Indian Institute of Technology, Kharagpur, West Bengal, India and air dried. A voucher specimen was deposited at the CAL herbarium, Botanical Survey of India, Howrah, West Bengal, India.

Preparation of methanol extract of *Andrographis paniculata* (MEAP)

Clean dry plant samples were collected in a cotton bags. The materials were homogenized to fine power with the help of mixer grinder. Then these powered materials were used for extraction.10 gm. of powered materials were soaked in 30 ml of 70% methanol and were kept at 30°C for 12 hours on a rotary shaker. After 12 hours 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 hours at 30^0C. After that, it was filtered by Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm. for 10 min. Then the supernatant was collected and the supernatant was allowed to evaporate until completely dry. The extracts were kept sterile bottles and stored at 4°C for further use.

Treatment of animals

Laboratory acclimatized rats were divided into three groups of almost equal average body weight. The animals of two groups were injected intraperitoneally (i.p.) with CrO_3 at a dose of 0.8 mg per 100 g body weight per day (20% LD_{50}) for 7 days. The animals of one of the Cr-treated groups served as the supplemented group was administered methanol extract of *Andrographis paniculata* (MEAP) by oral gavage at a dose of 100 mg per kg body weight daily at an interval of 3 h after injection of Cr for a period of 7 days. The animals of the remaining group received only the vehicle (0.9% NaCl), served as control.

Collection of blood samples and preparation of serum

After the experimental period, over-night fasting rats were sacrificed by cervical dislocation. Then blood samples were drawn from hepatic vein immediately. Serum was obtained by centrifugation at 1500×g for 15 min. of blood samples taken without anticoagulant.

Biochemical analysis of serum

Alkaline phosphatase (ALP) was measured in serum according to King and King (1954). In serum, the activities of transaminases (SGOT & SGPT) were estimated according to Reitman and Frankel (1957). Total and direct bilirubin was estimated in blood serum according to Lo and Wu[6]. The serum albumin was estimated according to Doumas[4]. Total protein was determined according to Lowry et al.[22] using bovine serum albumin as standard. Serum glucose level was estimated by glucose oxidase-peroxidase (GOD-POD) method[18].

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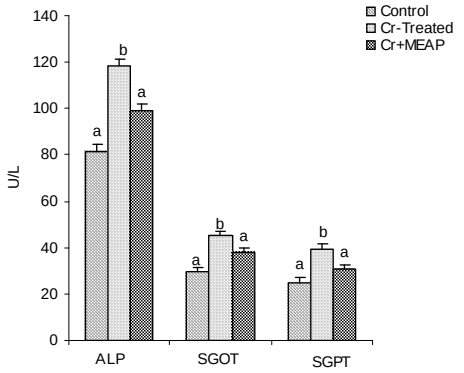

Figure 1 : Changes in serum ALP, SGOT, SGPT activities after co-administration of MEAP in Cr-treated rats. Data represents mean ± SE, N=6, p<0.05 and ANOVA followed by multiple comparisons Student's t-test. Note: Same superscript in each vertical column did not differ from each other

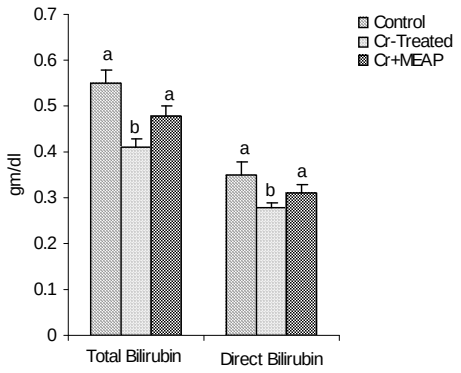

Figure 2 : Changes in serum total and direct bilirubin levels after co-administration of MEAP in Cr-treated rats. Data represents mean ± SE, N=6, p<0.05 and ANOVA followed by multiple comparisons Student's t-test. Note: Same superscript in each vertical column did not differ from each other

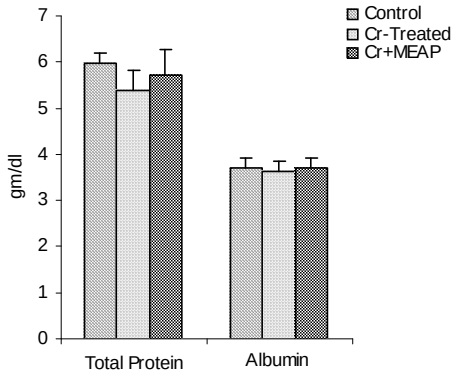

Figure 3 : Changes in serum total protein and albumin levels after co-administration of MEAP in Cr-treated rats. Data represents mean ± SE, N=6, p<0.05 and ANOVA followed by multiple comparisons Student's t-test. Note: Same superscript in each vertical column did not differ from each other

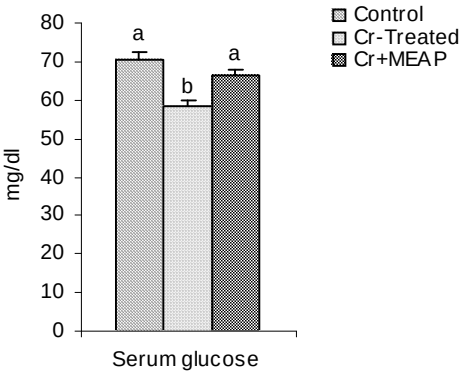

Figure 4 : Changes in serum glucose level after co-administration of MEAP in Cr-treated rats. Data represents mean ± SE, N=6, p<0.05 and ANOVA followed by multiple comparisons Student's t-test. Note: Same superscript in each vertical column did not differ from each other

Statistical analysis

Results were expressed in terms of mean and standard error of different groups. The differences between the mean values were evaluated by ANOVA followed by multiple Student's t-test[15]. The values for $p<0.05$ were considered significant.

RESULTS

To evaluate the functional status of liver in Cr-treated rats, we investigate some of the following parameters. In our experiment, results showed that ALP, SGOT and SGPT activities were significantly ($p<0.05$) increased in serum of Cr-treated rats when compared with control group (Figure 1). Significant decreased in the activities of ALP, SGOT and SGPT were found after supplementation with MEAP (Figure 1). Total and direct bilirubin levels of Cr-treated rats were significantly ($p<0.05$) decreased in serum (Figure 2) when compared to their respective control. Supplementation with MEAP to Cr-treated rats, the levels of total and direct bilirubin were nearly to control value (Figure 2). Total protein and albumin contents were not significantly decreased in Cr-treated rats than control (Figure 3). MEAP administered to Cr-treated rats, the levels of total protein and albumin were not significantly altered (Figure 3). On the other hand, it was found that the serum glucose level was significantly ($p<0.05$) decreased in response to chromium when compared with control (Figure 4). Significant increased in the level of serum glucose was found after supplementation with MEAP (Figure 4).

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DISCUSSION

Behari et al.[14] have reported that inhibition of acid phosphatase, adenosine triphosphatase and succinic dehydrogenase after administration of trivalent and hexavalent Cr. On the other hand, Nehru and Kaushal[3] reported significant increase in alkaline phosphatase activity due to lead intoxication. In the present study it was found that the ALP activity was significantly increased in Cr-treated rats but the supplementation with MEAP caused the significant recovery of ALP activity. The chromium and other heavy metals have been reported to raise the level of aminotransferases. Awadallah and Hanna[25] reported that the serum AST activity was significantly higher in animals injected with Cr than cobalt, zinc and manganese, while serum ALT activity were higher in cobalt than in chromium, zinc and manganese. Bavazzano et al.[23] reported that ALT and AST activities are higher in tannery workers as compared to workers in the shoe factory. In our study, the activities of SGOT and SGPT of Cr-induced rats were significantly increased. These results indicate that continuous accumulation of Cr can damage the hepatic membrane probably due to reduction of antioxidant status. MEAP administration caused a drastic fall in serum transaminases activity.

Bilirubin is the major end product of heamoglobin degradation. It also provides an exceedingly valuable tool for diagnosing both heamolytic blood diseases and various types of liver diseases[1]. Wu et al.[12] have reported that the 6-12 time increased dose of chromium picolinate resulted in anemia, hemolysis, liver dysfunction and renal failure. In the present study a significant decrease in the total and direct bilirubin were observed in Cr-treated group of rats. This result indicates that accumulation of chromium in the Cr-treated group may cause the liver impairment. MEAP supplementation in Cr-treated rats, the levels of total and direct bilirubin was reconverted to near normal level. Chen et al.[17] have not shown any significant effect on serum albumin and total protein after dietary Cr supplementation. Comparatively low level of albumin and total protein in Cr-treated rats may be due to decreased protein synthesis for chromium intoxication. The possible variation may relate to different levels of chromium exposure and liver toxicity[11,17]. MEAP administration to Cr-treated rats, the levels of total protein and albumin have not shown any significant alteration.

The effects of chromium in decreasing blood glucose have been reported in literature[7,8]. A similar decrease was evident in this study. Fujimoto[27] has reported that Cr, which is contained in glucose tolerance factor, showed lower blood concentration in patients with severe complications such as retinopathy or nephropathy. Therefore, it appears that Cr plays an important role in advancing diabetes mellitus. Vincent[13] has reported that Cr is an essential trace element for mammals and is required for maintenance of proper carbohydrate and lipid metabolism. This observation of lower glucose level directly correlates with the affects of Cr supplementation in improving glucose tolerance. MEAP has an important role to prevent the changes of glucose level in response to Cr.

Collectively these findings indicate that Cr treatment at the present dose and duration may cause the liver impairment and MEAP have an important role to counteract the liver damage in response to Cr. This hepatoprotective role of MEAP might be due to present of one or more principal components in *Andrographis paniculata* Nees.

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