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Research Article

AMELIORATIVE ROLE OF GARLIC (*ALLIUM SATIVUM*) ON CHROMIUM (VI) - INDUCED MEMBRANE DAMAGE IN MALE ALBINO RATS

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ABSTRACT

Objective: Membrane damage is one of the most important consequences of chromium (Cr) induced cytotoxicity. Garlic possesses antioxidant property to scavenge the toxic radicals and cytoprotective activity. The aim of the present investigation is to evaluate the ameliorative role of aqueous extract of garlic (AEG) on Cr-induced membrane damage of both liver and kidneys in male albino rats.

Methods: Male albino rats of Wistar strain (80–100 g) were used for the present study. Rats were divided into three groups of almost equal average body weight. The animals of two groups were injected $K_2Cr_2O_7$ at a dose of 0.8 mg per 100 g body weight per day for 28 days. The animals of one of the Cr-treated groups served as the supplemented group supplied AEG (250 mg per kg body weight daily at an interval of 6 h after injection of Cr for a period of 28 days). The animals of the remaining group received only the vehicle (0.9% NaCl), served as control. The body weights of the animals were taken in each day of treatment schedule.

Results: The results indicated that significant increases in membrane cholesterol, as well as significant decreases in membrane phospholipid in Cr, exposed animals suggest structural alterations in both liver and kidneys plasma membrane. Alkaline phosphatase (ALP), total ATPase, and Na^+-K^+- ATPase activities of the plasma membrane were significantly decreased in both liver and kidneys after Cr treatment. On the other hand, AEG supplementation plays a vital role to restore such alterations induced by Cr in the plasma membrane of both liver and kidney.

Conclusion: These findings indicate that Cr treatment at the present dose and duration induces structural and functional alterations in the plasma membrane in both liver and kidney. However, AEG supplementation restored those alterations induced by Cr in the plasma membrane of both liver and kidneys but was not able to eliminate the deposited Cr from the liver and kidney tissues.

Keywords: Chromium, Liver, Kidney, Plasma membrane, Aqueous extract of garlic.

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INTRODUCTION

Chromium (Cr) is a naturally occurring heavy metal found commonly in the environment in two valence states: Trivalent Cr (III) and hexavalent Cr (VI). Cr (VI) is widely used in steel, alloy cast iron, chrome plating, leather tanning, paints, metal finishes, and wood treatment. Cr plays a dual role in nature with Cr (III) essential for glucose and lipid metabolism [1]. However, excessive intakes of Cr (VI) compounds are potent toxicants and carcinogens [2]. Hepatic and renal toxicity is the most common toxicity found in Cr (VI)-exposed workers or animals [3]. This functional differentiation of Cr (III) and Cr (VI) is largely decided by the ionic permeability of the plasma membrane [4]. Thus, membrane damage is one of the crucial factors observed with Cr (VI) toxicity [5].

Garlic (*Allium sativum*) is an herb used for the treatment of variety of diseases [6]. The anti-carcinogenic and anti-inflammatory properties of garlic extract and its derivatives also have recently been reported by several investigators [7]. Garlic compounds are having tremendous antioxidant property which exerts action by scavenging ROS, enhancing cellular antioxidant enzymes, and increasing glutathione in the cells [8]. Our previous studies showed that antioxidants such as Vitamins and GSH were able to ameliorate Cr (VI)-induced membrane damage in the liver and kidneys [5,9,10].

However, to the best of our knowledge no information is available regarding the role of garlic in Cr (VI)-mediated cell membrane damage. Therefore, the aim of this present investigation was an attempt to reduce the effects of Cr-induced cytotoxicity using garlic *in vivo* in terms of certain structural and functional components like cholesterol and phospholipids levels as well as alkaline phosphatase (ALP), total ATPase, and Na^+-K^+- ATPase activities of the liver and kidneys plasma membrane.

METHODS

Collection of plant materials

Plant parts, i.e., the fresh bulb of garlic were collected from the market. Each specimen was labeled with date of collection. Plant parts were cleaned and peeled off. Then, plant parts were dried in incubator <40°C.

Preparation of aqueous extract of garlic (AEG)

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 of aqueous extract. 2 g of powdered material was 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. The filtrate was then centrifuged at 2000 rpm for 10 min. Then the supernatant was collected and stored at 4°C for further use.

Maintenance and treatment of animals

Male albino rats of the Wister strain (80–100 g) were fed with a lab-prepared diet, as described elsewhere [11], with water *ad libitum*. They were maintained in accordance with the guidelines of the rule of the Institutional Animal Ethics Committee. 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 Cr as $K_2Cr_2O_7$ at a dose of 0.8 mg per 100 g body weight per day (20% LD_{50}) for

28 days, as described earlier [11]. The animals of one of the Cr-treated groups served as the supplemented group injected AEG orally at a dose of 250 mg per kg body weight daily at an interval of 6 h after injection of Cr for a period of 28 days. The animals of the remaining group received only the vehicle (0.9% NaCl), which served as control.

Tissue collection

After the experimental period, overnight fasting rats were sacrificed by cervical dislocation. The liver and kidneys were immediately dissected out of the body and weighed. The tissues were then quickly stored at −20°C. The concentration of Cr was measured in the liver and kidneys by atomic absorption spectrometry.

Isolation of crude membrane fraction

Membrane fractions of the liver and kidneys were isolated according to the method described by Chaudhuri *et al*. [12]. Tissues were homogenized with a glass homogenizer in 0.25 mol L^{-1} cold sucrose solutions. The homogenates were then centrifuged at 15,000 × g for 15 min at 4°C. The supernatants were collected and centrifuged again at 22,650 × g for 20 min at 4°C. The supernatants, thus obtained, were discarded and the pellets were suspended in 1 mL chilled Tris buffer (pH 7) after three washings with the same buffer.

Assay of membrane protein

Membrane protein was estimated using Folin–Ciocalteau reagent according to the method of Lowry *et al*. [13] using bovine serum albumin as the standard.

Estimation of membrane cholesterol and phospholipid

Cholesterol and phospholipid levels of the isolated membrane fractions were estimated by the methods of Zlatkis *et al*. [14] and Christopher and Ralph [15], respectively.

Measurement of ALP activity

ALP activity of the isolated membrane fractions were assayed using p-nitrophenyl phosphate as substrate according to the method of Linhardt and Walter [16].

Determination of total ATPase and Na^+-K^+- ATPase activities

Total ATPase and Na^+-K^+- ATPase activities were measured by the method of Sen *et al*. [17].

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-tests. The values for $p<0.05$ were considered significant.

RESULTS

Changes in the rat's body weight during the period of treatment are depicted in Fig. 1. It was found that the body weights of Cr treated rats are significantly decreased when compared with control. Further, bodyweight of Cr treated rats remained increased when supplemented with AEG.

Upon exposure to Cr, the liver and kidneys showed significant increases in metal content. Supplementation of Cr-treated rats with AEG did not prevent the elevation of Cr content in the liver and kidneys (Table 1). Whereas the weight of the liver significantly increased but the kidney weights remained unaltered. Supplementation with AEG prevented changes in the liver weight (Table 1).

Alterations in membrane cholesterol and phospholipids levels of liver and kidney were presented in Figs. 2 and 3. Significant increased in membrane cholesterol and decreased in membrane phospholipids contents in response to Cr-treated group were noted. AEG administered to Cr-treated rats plays a vital role to counteract the levels of cholesterol and phospholipids in the liver and kidney plasma membrane.

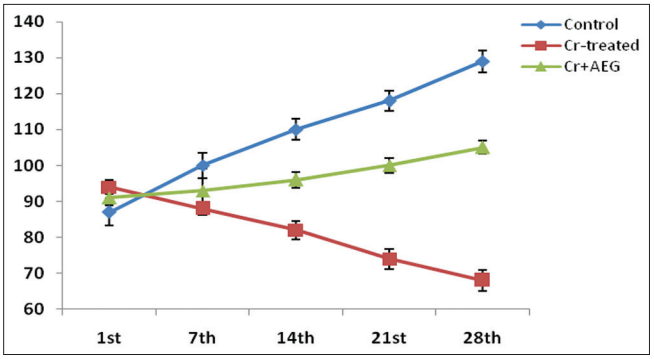

Fig. 1: Changes in body weight after co-administration of aqueous extract of garlic to chromium-treated rats

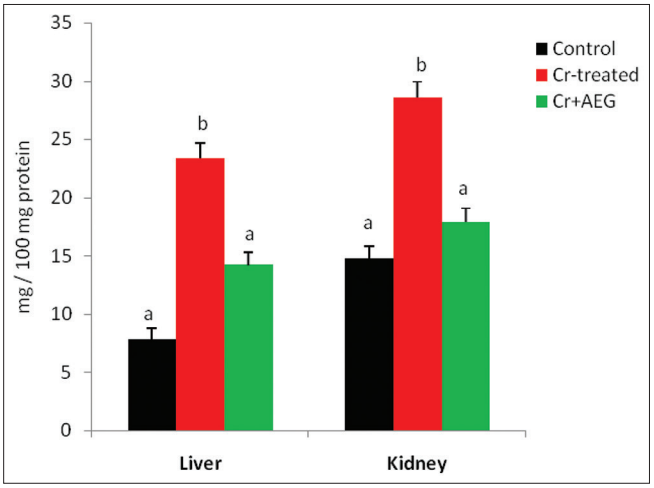

Fig. 2: Changes in membrane cholesterol level after co-administration of aqueous extract of garlic in chromium-treated rats. Data represent mean±SE, $p<0.05$, and ANOVA followed by multiple comparisons Student's t-test. Same superscript in each vertical column did not differ from each other significantly

Table 1: Tissue weight and Cr content of organs after co-administration of AEG in Cr-treated rats

Tissues	Groups of animals	Organ weight (g per 100 g bw)	Cr content (µg per g tissue)
Liver	Control	2.99±0.10^a	0.31±0.03^a
	Cr+treated	4.23±0.22^b	2.89±0.12^b
	Cr+AEG	3.16±0.26^a	2.83±0.18^b
Kidney	Control	0.81±0.06^a	1.09±0.08^a
	Cr+treated	0.79±0.05^a	6.32±0.34^b
	Cr+AEG	0.78±0.03^a	6.25±0.39^b

Data represent mean±SE, $p<0.05$, and ANOVA followed by multiple comparisons, Student's t-test. Same superscript in each vertical column did not differ from each other significantly, AEG: Aqueous extract of garlic, Cr: Chromium

Data represented Figs. 4-6 that ALP, total ATPase, and Na^+-K^+-ATPase activities in the plasma membrane of the liver and kidney were significantly decreased in Cr-treated group. With the simultaneous administration of AEG, ALP, total ATPase, and Na^+-K^+ -ATPase activities returned to nearly normal level but not reversed completely.

DISCUSSION

Based on a comparison of body weight gain on Cr exposed rat with that of control (Fig. 1), it appears that weight gain was decreased in Cr-treated rats. The impact on body weight due to the direct effect of

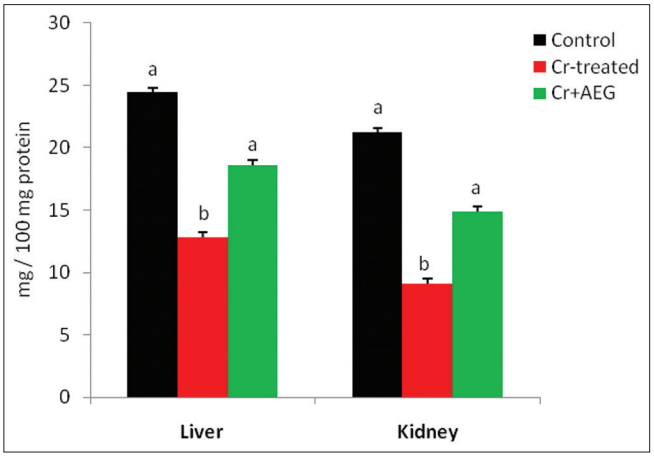

Fig. 3: Changes in membrane phospholipid level after co-administration of aqueous extract of garlic in chromium-treated rats. Data represents mean±SE, p<0.05 and ANOVA followed by multiple comparisons Student's t-test. Same superscript in each vertical column did not differ from each other significantly

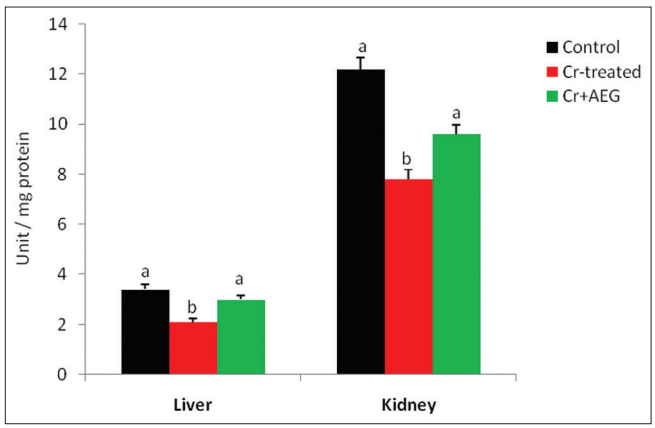

Fig. 4: Changes in membrane ALP activity after co-administration of aqueous extract of garlic in chromium-treated rats. Data represents mean±SE, p<0.05 and ANOVA followed by multiple comparisons Student's t-test. Same superscript in each vertical column did not differ from each other significantly

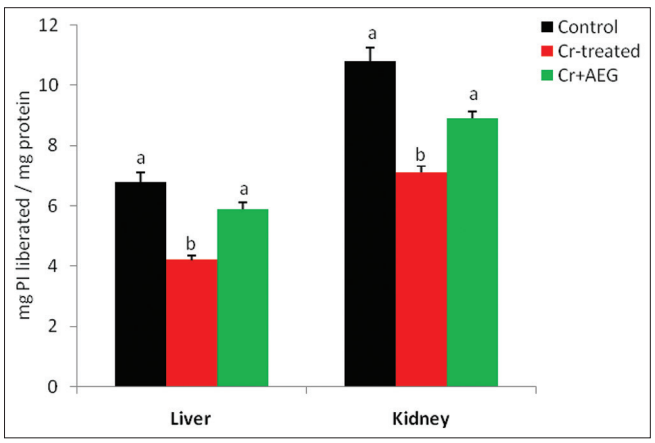

Fig. 5: Changes in membrane ATPase activity after co-administration of aqueous extract of garlic in chromium-treated rats. Data represents mean±SE, p<0.05 and ANOVA followed by multiple comparisons Student's t-test. Same superscript in each vertical column did not differ from each other significantly

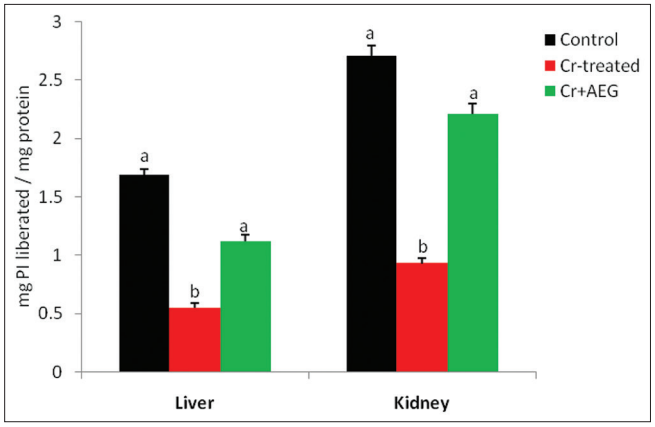

Fig. 6: Changes in membrane Na+-K+-ATPase activity after co-administration of aqueous extract of garlic in chromium-treated rats. Data represent mean±SE, p<0.05, and ANOVA followed by multiple comparisons Student's t-test. Same superscript in each vertical column did not differ from each other significantly

Cr and not due to reduce food intake as control rats were pair-fed with the Cr-treated rat. Supplementation of Cr-treated animals with AEG partially reversed the body weight fall to control levels.

The lowered body weight was not reflected in organ weight, as recorded just after sacrifice (Table 1). Only the liver showed a significant increase in weight. Similar results were reported in our laboratory [11] suggesting that Cr treatment at the given dose and duration increased the liver weight but the kidneys remain unaltered. Thus, Cr appears to have a differential impact on organ size but after supplementation with AEG restored the changes in organ weights following metal exposure.

The Cr content of the liver and kidney tissues was increased significantly following Cr treatment (Table 1). The increased levels of Cr in all tested organs studied following Cr treatment were found to be unaffected by supplementing Cr-treated rats with AEG. This shows that supplementation with AEG was not able to reduce the load of accumulated metal in the tissues. It was reported that protection with deferoxamine against Cr was not attributed to either a reduced Cr uptake by the cells or alterations in Cr distribution within cells [18]. It was also reported that pre-treatment with vitamin E and melatonin did not affect Cr uptake or distribution in cells after metal treatment [19,20]. Sugiyama [21] also demonstrated that the uptake of Na_2CrO_4 was not affected by pre-treatment with vitamin E. From the present study, it may be suggested that AEG exerted no effect on Cr uptake and distribution in different organs after metal treatment. Whether such supplementation has any impact on the distribution of different forms of Cr within the cells remains to be ascertained by further studies.

Various studies indicated that both hexavalent and trivalent Cr are biologically active oxidation states [18]. It was suggested that an oxidative impact of Cr (VI) on membrane phospholipids indicates a probable structural alteration of the membrane [22]. On the other hand, activation of the membrane-bound enzyme indicates a functional alteration of the membrane [23].

In the present investigation, the Cr-induced membrane damaged was clearly indicated by significantly increases of the membrane cholesterol content in the both liver and kidneys (Fig. 2). This rise may be due to imbalance in cholesterol incorporation into the membrane. Thus, Cr impaired the function of lecithin cholesterol acetyltransferase. On the other hand, decreased membrane phospholipids levels (Fig. 3) indicated that the damage of membrane structure of the cell. The probable impact of Cr on the lipid catabolizing enzymes cannot be ruled out as evidenced by increased excretion of urinary lipid metabolites [24]. This

enhanced catabolism of lipid may result in the accumulation of acetyl Co-A, which in term may lead to increased synthesis of cholesterol in the tissues particularly in nonsteroid producing tissues. Thus, Cr by altering the relative proportion of cholesterol and phospholipids may produce cellular damaged to the membrane structure. The impact of Cr on membrane cholesterol and phospholipids contents was found to disappear when Cr was accompanied by AEG. It was reported that serum cholesterol level is increased in response to Cr, but the simultaneous treatment of garlic extract relatively improved serum cholesterol level [25]. Evidence indicates that this structural change of both liver and kidneys plasma membrane is attenuated by the AEG supplementation.

This report of impact of Cr on ALP activity of tissue membrane is contradictory [1,18,26]. After Cr treatment, the activity of ALP in the plasma membrane of both the liver and kidneys was found to be decreased (Fig. 4) as observed in our earlier studies [5,9,10]. This inhibition of ALP activity reflects selective damage of the plasma membrane [26], which is also supported by alterations in cholesterol and phospholipid contents (Figs. 2 and 3). In the present investigation, results indicate that the supplementation with AEG completely attenuated Cr-induced inhibition of membrane ALP activity of both the liver and kidneys.

Total ATPase activity of membrane was reduced significantly in the Cr treated group in kidney but AEG supplementation cannot completely attenuated Cr-induced inhibition of kidney membrane total ATPase activity (Fig. 5). The inhibition of the energy production by cytotoxic concentration of Cr [27] may play some role in Cr-induced changes of the ATPase activity. Na^+-K^+ -ATPase activity was found to be reduced significantly in Cr treated organ (Fig. 6). The observed results are supported by findings on Cr-induced reduction of membrane transport [28,29]. When the Cr-treated group was supplemented with AEG, the Na^+-K^+ -ATPase activity was found to restore in the kidney plasma membrane.

CONCLUSION

These findings indicate that Cr treatment at the present dose and duration induces structural and functional alteration in the kidney plasma membrane. The structural and functional changes may be attenuated by AEG supplementation. The protective action of AEG might be due to the presence of one or more principal component in garlic. However, more detailed studies are needed to elucidated the exact mechanism underlying Cr induced membrane damaged.

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AUTHORS' CONTRIBUTIONS

Both authors contributed equally.

CONFLICTS OF INTEREST

There is no conflict of interest.

AUTHORS' FUNDING

Self.

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