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ORIGINAL ARTICLE

Antagonistic effects of *Andrographis paniculata* methalonic extract on chromium-induced membrane damage in male Albino rats

S. K. Dey

Department of Physiology, Santal Bidroha Sardha Satabarshiki Mahavidyalaya (Affiliated to Vidyasagar University), Goaltore-721128, Paschim Medinipur, West Bengal, India

*E-Mail: ***sdeybiomed@gmail.com**

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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 (Cr) is one of the important heavy metal in both terrestrial and aquatic environments. Membrane damage is one of the vital consequences of Cr-induced cytotoxicity. *Andrographis paniculata* Nees, a membrane protectant may be used to reduce the Cr-induced membrane damage in liver and kidney. For the present study, male albino rats of the Wistar strain (80-100 g) were used. Rats were divided into three groups. 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. One of the Cr treated group serving as the supplemented groups injected methalonic extract of *Andrographis paniculata* at a dose of 500 mg per Kg body weight (ME-AP_{500}) daily for 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 shows that significant increases in membrane cholesterol level as well as significant decreases in membrane phospholipid level in Cr exposed animals. Alkaline phosphatase (ALP), total ATPase, and Na^+-K^+-ATPase activities of plasma membrane were significantly decreased after Cr treatment. Methalonic extract of *Andrographis paniculata* play an ameliorative role on Cr-induced membrane damage**.** These findings indicate that Cr treatment at the present dose and duration induces structural and functional alterations in the plasma membrane in both the liver and kidney. However, methalonic extract of *Andrographis paniculata* supplementation restored those alterations induced by Cr in plasma membrane of both liver and kidneys.

Key words: Chromium, Liver, Kidney, Plasma membrane, Andrographis paniculata Nees

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 (Chorvatovicová *et al.,* 1993). However, excessive intakes of Cr (VI) compounds are potent toxicants and carcinogens (De Flora *et al.,* 1990). Hepatic and renal toxicity is the most common toxicity found in Cr (VI)-exposed workers or animals (Hojo and Satomi 1991). This functional differentiation of Cr (III) and Cr (VI) is largely decided by the ionic permeability of the plasma membrane (De Flora and Watterhahn 1989). Thus, membrane damage is one of the crucial factors observed with Cr (VI) toxicity (Dey and Roy 2010).

Plants are attested medicinal wonders where their use in traditional medicine and healing date to ancient times. *Androg- raphis paniculata* (*A. paniculata*) (Burm.f.) Wall. Ex Nees is a medicinal plant with documented pharmacological and curative properties against infections and illnesses (Al-Bayati *et al.,* 2012; Saranya and Geetha 2011; Valdiani *et al*., 2017; Vakil and Mendhulkar 2013). Phytochemical compositions in *Andrographis paniculata* differ depending on the geographical location, season and time of harvesting (Hossain *et al.,* 2014). Leaves of *Andrographis paniculata* reportedly contain the highest phytochemical content compared to stems; roots and the whole plant also contain phytochemicals with pharmacological activities. *Andrographis Paniculata* contains principal compound andrographolide. Methanol extract of *Andrographis Paniculata* was more potent in antioxidant activities (Lin *et al.,* 2009). Methanolic extract of *Andrographis paniculata* showed highest antimicrobial activity against *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Escherichia coli* due to high andrographolide and neo-andrographolide contents (Valdiani *et al.,* 2017; Mohan *et al.,* 2013) and has been shown to kill drug-resistant Gram-positive bacteria (Mishra *et al.,* 2009). Our previous studies showed that antioxidants like vitamins and GSH were able to ameliorate Cr (VI)-induced membrane damage in the liver and kidneys (Dey *et al.,* 2001; Dey *et al.,* 2003a; Dey and Roy 2010).

However, to our knowledge no information is available regarding the role of *Andrographis Paniculata* methalonic extract 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 *Andrographis Paniculata* methalonic extract *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.

MATERIALS AND METHODS

Collection, identification and preservation of plant materials

Fresh plant part (Leaves) 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, Vidyasagar University. Specimen was labelled, numbered and noted with date of collection. Plant part was rinsed with sterile distilled water, air dried and stored in airtight bottle at 4^{o}C for further use.

Preparation of methalonic extract

Ten gram of grinded powder of *Andrographis Paniculata* was soaked in 30 ml of 70% methanol and was kept at 30^{0}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^{0}C. After that it was filtered through Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm for 10 min. Then the 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} (Dey *et al.,* 2011).

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

(Dey *et al.,* 2003b), with water *ad libitum*. 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 (Dey *et al.,* 2003b). The animals of one of the Cr-treated groups served as the supplemented group injected methalonic extract of *Andrographis paniculata* at a dose of 500 mg per Kg body weight (ME-AP_{500}) 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.

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^{0}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 Ghosh Chowdhuri *et al.,*(1995). 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^{0}C. The supernatants were collected and centrifuged again at 22,650×g for 20 min at 4^{0}C. The supernatants, thus obtained, were discarded and the pellets were suspended in 1mL 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.,* (1951) 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.,* (1953) and, Christopher and Ralph (1972), respectively.

Determination of alkaline phosphatase, total ATPase and Na^+-K^+- ATPase activities

Alkaline phosphatase (ALP) activity of the isolated membrane fractions were assayed using p-nitrophenyl phosphate (PNPP) as substrate according to the method of Linhardt and Walter (1963). Total ATPase and Na^+-K^+- ATPase activities were measured by the method of Sen *et al.,* (1981).

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 Students't-tests. The values for $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Phytochemical analyses that were performed previously on *Andrographis paniculata* extracts that were prepared using various extraction solvents including ethanol, methanol and acetic acid have proven andrographolide as the major bioactive component (Malahubban *et al.,* 2013; Roy *et al.,* 2010; Jarukamjorn and Nemoto 2008). In this study, methanolic leaves extract of *Andrographis paniculata* showed a promising protection against hepatotoxicity induced by paracetamol (Nagalekshmi *et al.,* 2011) and prevented thioacetamide-induced liver cirrhosis in rats (Bardi *et al*., 2014).

Based on a comparison of body weight gain on Cr exposed rat with that of control (Figure 1), it appears that weight gain was decreased in Cr-treated rats. The impact on body weight due to the direct effect of 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 ME-AP_{500} partially reversed the body weight fall to control levels.

The lowered body weight was not reflected in organ weight, as recorded just after sacrifice (Figure 2). Only the liver showed a significant increase in weight. Similar results were reported in our laboratory (Dey *et al.,* 2003b) 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 ME-

AP_{500} restored the changes in organ weights following metal exposure.

The Cr content of the liver and kidney tissues were increased significantly following Cr treatment (Figure 3). The increased levels of Cr in all tested organs studied following Cr treatment were found to be unaffected by supplementing Cr-treated rats with ME-AP_{500}. This shows that supplementation with ME-AP_{500} was not able to reduce the load of accumulated metal in the tissues. It was reported that protection with deferoxamine (DFO) against Cr was not attributed to either a reduced Cr uptake by the cells or alterations in Cr distribution within cells (Susa *et al.,* 1997a). It was reported also reported that pre-treatment with vitamin E and melatonin did not affect Cr uptake or distribution in cells after metal treatment (Susa *et al.,* 1996; *et al.,* 1997b). It was also demonstrated that the uptake of Na_2CrO_4 was not affected by pre-treatment with vitamin E (Sugiyama 1989). From the present study, it may be suggested that ME-AP_{500} 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 (Susa *et al.,* 1997a). It was suggested that an oxidative impact of Cr (VI) on membrane phospholipids indicates a probable structural alteration of the membrane (Ginter *et al.,* 1989). On the other hand, activation of the membrane bound enzyme indicates a functional alteration of the membrane (Bagchi *et al.,* 1997). 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 (Figure 4). This rise may be due to imbalance in cholesterol incorporation into the membrane. Thus Cr impaired the function of lecithin cholesterol acetyl transferase. On the other hand, decreased membrane phospholipids levels (Figure 5) 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 (Bagchi *et al.,* 1995). This enhanced catabolism of lipid may result in 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 membrane structure. The impact of Cr on membrane cholesterol and phospholipids contents was found to disappear when Cr was accompanied by ME-AP_{500}.

The report of impact of Cr on ALP activity of tissue membrane is contradictory (Kumar and Rana 1984; Chorvatovicová *et al.,* 1993; Susa *et al.,* 1997a). After Cr treatment, the activity of ALP in plasma membrane of both the liver and kidneys was found to be decreased (Figure 6) as observed in our earlier studies (Dey *et al.,* 2001; Dey *et al.,* 2003a; Dey and Roy 2010). This inhibition of ALP activity reflects selective damage of the plasma membrane (Kumar and Rana 1984), which is also supported by alterations in cholesterol and phospholipid contents (Figure 4 and Figure 5). In the present investigation, results indicate that the supplementation with ME-AP_{500} completely attenuated Cr-induced inhibition of membrane ALP activity of both liver and kidneys.

Total ATPase activity of membrane was reduced significantly in the Cr treated group in kidney but ME-AP_{500} supplementation cannot completely attenuated Cr induced inhibition of kidney membrane total ATPase activity (Figure 7). The inhibition of the energy production by cytotoxic concentration of Cr (Stohs and Bagchi 1995) 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 (Figure 8). The observed results are supported by findings on Cr induced reduction of membrane transport (Standeven and Wetterhahn 1991a; Standeven and Wetterhahn 1991b). When the Cr-treated group was supplemented with ME-AP_{500}, the Na^+-K^+ -ATPase activity was found to restore in kidney plasma membrane.

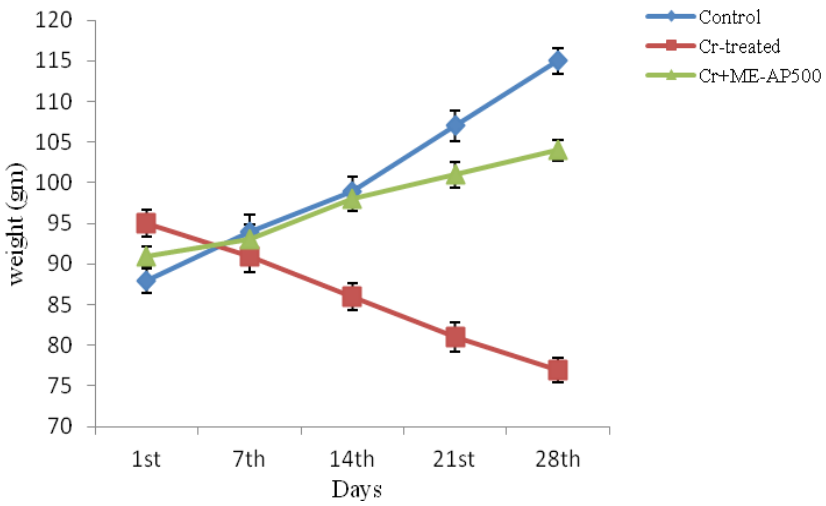

Figure 1. Changes in body weight after co-administration of ME-AP_{500} to Cr-treated rats.

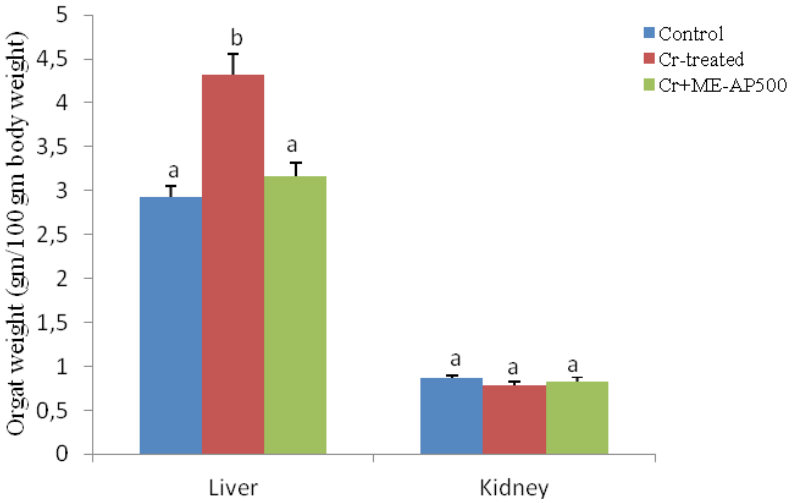

Figure 2. Changes in organ weight after co-administration of ME-AP_{500} to Cr-treated rats. Data represents mean ± SE, $p < 0.05$ and ANOVA followed by multiple comparisons Student's t-test. Same superscript in each vertical column did not differ from each other significantly.

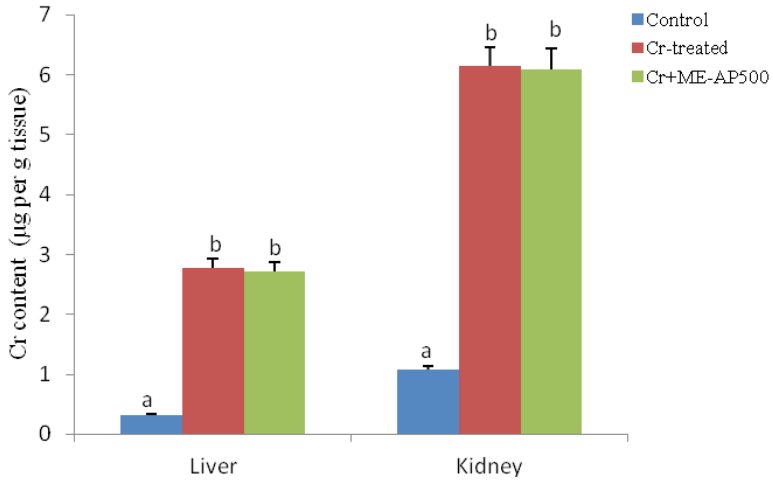

Figure 3. Changes in chromium content after co-administration of ME-AP_{500} to Cr-treated rats. Data represents mean ± SE, $p < 0.05$ and ANOVA followed by multiple comparisons Student's t-test. Same superscript in each vertical column did not differ from each other significantly.

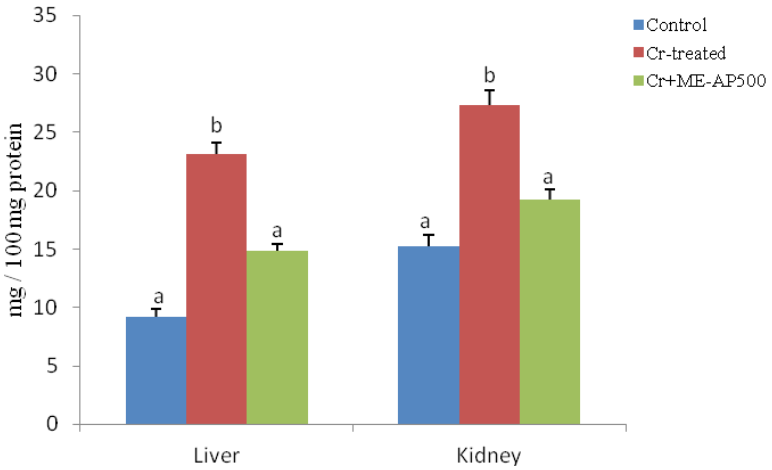

Figure 4. Changes in membrane cholesterol level after co-administration of ME-AP_{500} in Cr-treated rats. Data represents mean ± SE, $p < 0.05$ and ANOVA followed by multiple comparisons Student's t-test. Same superscript in each vertical column did not differ from each other significantly.

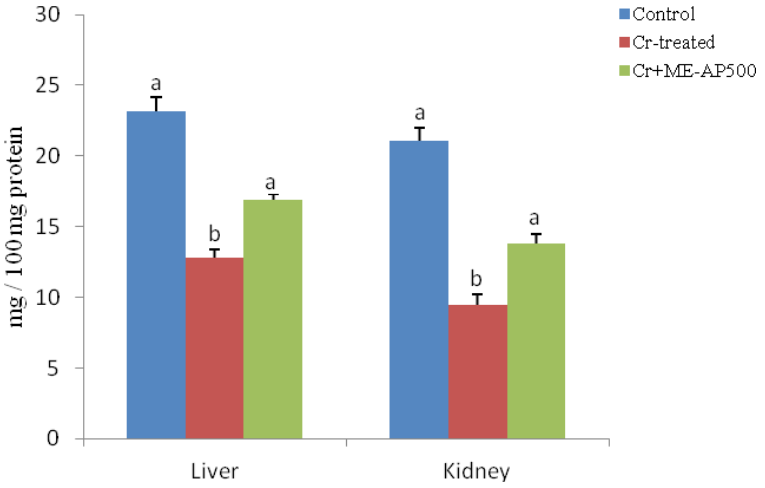

Figure 5. Changes in membrane phospholipid level after co-administration of ME-AP_{500} in Cr-treated rats. Data represents mean ± SE, $p < 0.05$ and ANOVA followed by multiple comparisons Student's t-test. Same superscript in each vertical column did not differ from each other significantly.

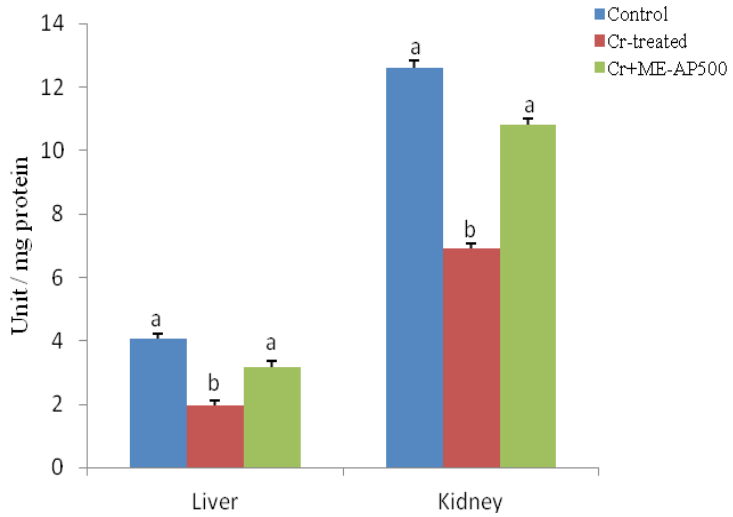

Figure 6. Changes in membrane ALP activity after co-administration of ME-AP_{500} in Cr-treated rats. Data represents mean ± SE, $p < 0.05$ and ANOVA followed by multiple comparisons Student's t-test. Same superscript in each vertical column did not differ from each other significantly.

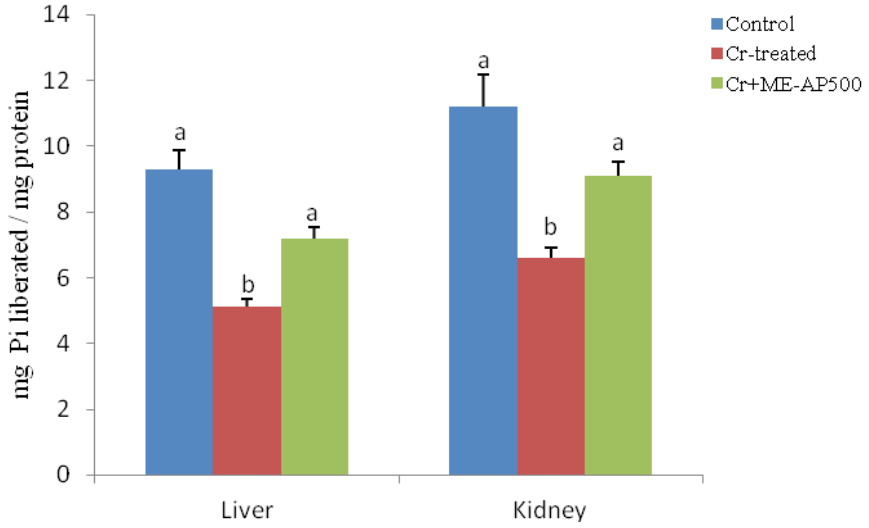

Figure 7. Changes in membrane ATPase activity after co-administration of ME-AP_{500} in Cr-treated rats. Data represents mean ± SE, $p < 0.05$ and ANOVA followed by multiple comparisons Student's t-test. Same superscript in each vertical column did not differ from each other significantly.

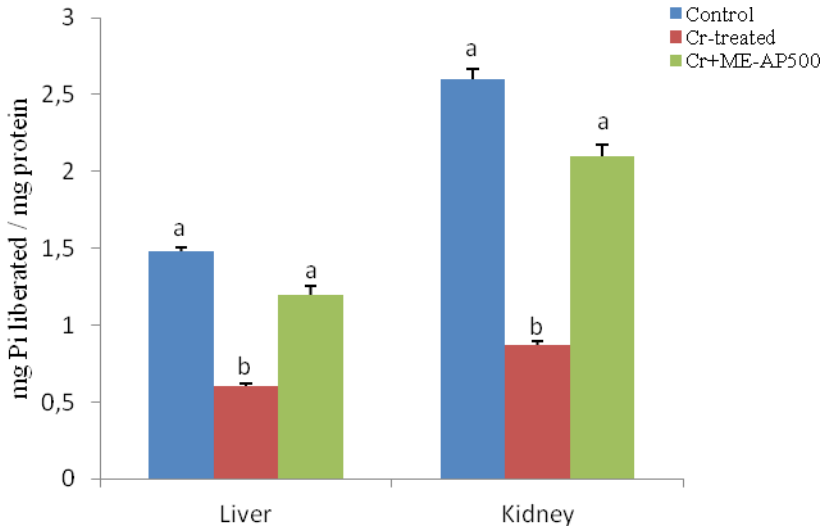

Figure 8. Changes in membrane Na^+-K^+-ATPase activity after co-administration of ME-AP_{500} in Cr-treated rats. Data represents mean ± SE, $p < 0.05$ and ANOVA followed by multiple comparisons Student's t-test. Same superscript in each vertical column did not differ from each other significantly.

CONCLUSION

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

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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