

Antioxidants-rich Supplement Ameliorates Chromium-induced Oxidative Stress in Albino Wistar Rats

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ABSTRACT

Chromium (Cr), a naturally occurring element widely used in various industries, has recently been implicated to generate free radicals by its divalent and hexavalent forms inducing oxidative states. The carcinogenicity of Cr was suggested to be through inducing oxidative stress. Supplementation with antioxidants was reported to ameliorate oxidative stress in several studies. In this study, oxidative stress was induced using 20 mg/kg K₂Cr₂O₇ in albino Wistar rats' groups B, C and D. After 14 days, the rats in groups C and D were treated with 250 and 500 mg/kg/body weight antioxidant-rich supplements. The serum chromium level, activities of antioxidant enzymes (SOD, CAT, GPx) and GSH, antioxidant minerals (Se, Zn, Cu, Fe and Mn) and vitamins (A, C, and E) concentrations, and malondialdehyde (MDA) level were determined. The results showed significant ($p < 0.05$) weight gain in all the groups. Serum Cr level decreased significantly ($p < 0.05$) compared to control group (B). Significant ($p < 0.05$) increase in serum activity of enzymes (SOD, CAT and GPx), concentrations of GSH, vitamins, and minerals was observed in supplemented groups C and D compared to B. MDA levels were significantly ($p < 0.05$) reduced in group C compared to B. The results suggest that antioxidant-rich supplements could ameliorate chromium-induced oxidative stress.

Keywords: Oxidative stress, chromium, antioxidant-rich supplement

1.0

INTRODUCTION

Environmental pollution through water, air, and food has increased exposure to heavy metal (Timothy and Williams, 2019). Even though most of these metals are beneficial for the maintenance of biochemical and physiological processes, however, at high concentrations they exert toxic effects. Chromium (Cr) is a naturally occurring heavy metal capable of existing in oxidative states such as divalent (Cr²⁺), trivalent (Cr³⁺) and hexavalent (Cr⁶⁺). Cr³⁺ and Cr⁶⁺ are the most commonly occurring and toxic states (Sharma et al., 2022). Cr is present in rocks, plant and animal tissues, soil, water, volcanic dust and gases and serves as a contaminant in industrial effluents of leather-tanning, wood vanishing, metal plating, pigment manufacturing, cement production, welding and mining industries (Kořla & Kohnierzak 2019). The toxic states Cr³⁺ and Cr⁶⁺ when formed enter cell through the sulphate-anion channel and get reduced by reductants such as glutathione (GSH) and ascorbate, resulting in the release of superoxide anion ($\cdot\text{O}_2^-$), which is eventually converted to hydrogen peroxide (H₂O₂) and leads to generation of a spectrum of reactive oxygen species (ROS) (Singh et

al., 2022; Sharma et al., 2022). In this regard, Cr has been reported to induce hepatic, reproductive, biochemical, and hematological (Hossini et al., 2022), mutagenic (Sawicka et al., 2021), and behavioral (Garai et al., 2021) toxic effects. Generation of ROS is the leading cause of oxidative stress, an imbalance between pro-oxidants and antioxidants (Afzal et al., 2023). Oxidative stress is known to cause adverse health effect such as, kidney damage, liver damage, infertility, blood cell damage, cardiovascular diseases and many more, which may result to death (Kiran et al., 2023). It also leads to lipid peroxidation, DNA damage and protein oxidation which results in tissue damage (Kiran et al., 2023).

Antioxidants are molecules involved in scavenging reactive oxygen species to delay or inhibit oxidation of cells and other biological substances. The human body has inherent enzymatic (glutathione peroxidases, superoxide dismutase and catalase) and diet-derived non-enzymatic (vitamin C and E, β -carotene, uric acid and glutathione) antioxidants which fight and neutralize free radicals. The normal function and concentration of these antioxidants is majorly dependent on proper diet. Several

data showed that antioxidants and pro-antioxidants can counteract the effects of ROS, hence, combat oxidative stress (Engwa, 2018; Banerjee et al., 2017). Nutraceuticals, especially antioxidant-rich ones, have been explored in our center for the treatment and prevention of diseases associated with oxidative stress (Dama et al., 2024; Besora-Moreno et al., 2022; Saidu et al., 2012). Therefore, this study was undertaken to evaluate oxidative stress ameliorating effects of a formulated antioxidant-rich supplement against Cr-induced oxidative stress albino Wistar rats.

2.0 MATERIALS AND METHODS

2.1 Chemicals

All chemicals used in the study were of high purity analytical grade. Cr (K₂Cr₂O₇) was purchased from ABBACHEM Trading's Limited Nigeria; MDA, SOD, CAT, GPx, and GSH were obtained from Cayman Chemicals (Ann Arbor, MI, USA).

2.2 Preparation of antioxidant-rich supplement

The antioxidant-rich nutraceutical was prepared according to the method of Saidu et al., (2012). It was prepared locally using tomatoes, onions, garlic, ginger, lemon, melon seed and palm oil as ingredients in a ratio of 4:4:4:4:2:1:1 (w/w). The tomato, onion, garlic, ginger and melon seeds were dried at a temperature of 40°C, blended into fine powder. The palm oil was added, mixed thoroughly and lastly lemon juice mixed thoroughly and spread on a clean flat surface and allowed to dry at room temperature. This was stored in an airtight container at room temperature until needed.

2.3 Animals and experimental design

Twenty-four (24) 7-8-week-old adult male albino Wistar rats weighing 199-221 grams were used for the study. The rats were purchased from the animal house, Department of Biological Science, Usmanu Danfodiyo University, Sokoto and were handled according to the 2018 revised Department of Biochemistry animal handling guidelines. The rats were allowed to acclimatize for one week in the Biochemistry Department animal house. They were housed in polypropylene cages with wood shaving beddings, 6 rats per cage, 12/12-hour light/dark cycle at room temperature in a well-ventilated room and were allowed free access to rat chow feed clean tap water.

Six rats were maintained on a normal chow (group A) and the rest were given 20 mg/kg of K₂Cr₂O₇ (Banerjee et al., 2017) for 14 days by oral gavage. After 14 days, the rats were randomly divided into groups B, C, and D. Groups C and D were given 250 mg/kg and 500 mg/kg body weight respectively of the antioxidant-rich supplement for 14 days. Group B was given only distilled

water for the 14-day period. The animals were then allowed to fast overnight and then sacrificed in the morning by first placing them in a jar containing cotton wool soaked with chloroform. When the rats were in coma, jugular vein was located and sharply cut with a clean sterile scalpel blade to obtain blood which was collected into a clean centrifuge tube. The blood sample was allowed to clot for about 15 minutes and then centrifuged at 4000 revolution per minute (rpm) for 10 minutes. The serum was then aspirated and transferred into other sets of clean test tubes and stored at -4°C before use.

2.4 Biochemical parameters

2.3.1 Determination of serum chromium

Serum chromium was determined using atomic absorption spectrophotometer (AAS) according to the method of Nawrocka and Szkoda (2012). Sample (1 mL) was wet digested with equal volume of concentrated HNO₃ in a digestion chamber until white fumes evolution stopped. One mL of perchloric acid was then added and heated to a clear solution, then made up to 5 mL using distilled water and then used for Cr quantification. Using 1000 ppm standard solution for elements, the sample was analysed on AAS machine (Shimadzu AA-6300, Japan) and radiation absorbances were recorded which were used to quantify Cr in the sample.

2.4.2 Determination of antioxidant enzymes (SOD, CAT, and GPx) and Glutathione

The enzymes activities were determined using ELISA assay kit following the protocol for each on a microplate reader (Thermo Electron Corporation Multiskan Ex). SOD and CAT were determined at 450 nm while GPx and GSH at 340 nm wavelengths. The activities were calculated using the concentration of the standard for each assay kit.

2.4.3 Estimation of some antioxidant minerals (Cu, Mn, Zn and Se)

The concentrations of antioxidant minerals Cu, Mn, Zn and Se were determined using AAS as described for Cr above. The concentrations of these minerals were calculated.

2.4.4 Determination of serum Malondialdehyde (MDA) level

Serum MDA was determined according to previously described method of Buege and Aust (Buege and Aust, 1978). One mL of serum was added to 2 mL of mixture of tricarboxylic acid, thiobarbituric acid, and hydrochloric acid reagent (1:1:1 v/v). The mixture was shaken and incubated for 15 minutes in a hot water bath at 100°C. The tubes were allowed to cool at room temperature and centrifuged at 3000 rpm for 10 minutes.

The supernatant was aspirated, and absorbance was read at 535 nm against reagent blank. The concentration of MDA was calculated using beer-lamberts law using an extinction coefficient of $1.65 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

2.4.5 Determination of antioxidants vitamins (vitamin A, E and C)

2.4.5.1 Estimation of Serum level of Vitamin A

Vitamin A was determined colorimetric method of Rutkowski et al., (2006). One mL of the sample was placed in test-tube I (centrifugal) with a tight stopper and 1 mL of KOH solution was added, the tube was plugged and shaken vigorously for 1 minute. The tube was heated in a water bath (60°C , 20 minutes), then cooled down in cold water. One mL of xylene was added, the test-tubes were plugged and shaken vigorously again for 1 minute. The mixture was centrifuged at $1500 \times g$ for 10 minutes, the whole of the separated extract (upper layer) was collected and transferred into test tube II. The absorbance (A1) of the obtained extract at 335 nm against xylene was measured. The extract in test tube II was irradiated to UV light for 30 minutes, then the absorbance (A2) was measured. The concentration (Cx) of vitamin A (μM) in the sample was calculated using the formula:

$$C_x = (A_1 - A_2) \times 22.23$$

Where: 22.23 is the multiplier on basis of the absorption coefficient of 1% solution of vitamin A (as the retinol form) in xylene at 335 nm in a measuring cuvette about thickness = 1 cm.

2.4.5.2 Estimation of serum vitamin C

The level of vitamin C (ascorbic acid) in serum was estimated by the method of Rutkowski et al., (2006). To 0.5 mL of serum, 0.5 mL of distilled water and 1 mL of 5% TCA were added, mixed thoroughly and centrifuged at $3500 \times g$ for 20 minutes. To 1 mL of supernatant, 0.2 mL of DTC reagent (3 g of DNPH, 0.4 g thiourea and 0.05 g of copper sulphate dissolved in 100 mL of 9 N sulphuric acid) was added and incubated at 37°C for 3 hours. After incubation 1.5 mL of 85% sulphuric acid was added and colour developed. Standard was prepared along with the sample. Absorbance was measured at 530 nm after 30 minutes. Vitamin C concentration was calculated using the formula

$$\text{Vit C (mg/dl)} = \frac{\text{Absorbance of the Test}}{\text{Absorbance of the Standard}} \times \text{Concentration of the Standard}$$

2.4.5.2 Estimation of serum vitamin E

Vitamin E (tocopherol) was also estimated by the method of Rutkowski et al., (2006). Test tubes were labelled as standard, samples and blank into which standard solution, sample, and distilled water were pipetted respectively. The contents were mixed and centrifuged at 2500 rpm for 10 minutes, 1 mL of xylene layer was carefully pipetted

into another set of identically labelled test tubes and 1 mL of α -dipyridyl reagent was added to the test tubes. The contents of the test tubes were mixed, and the absorbance of samples and standard were read against blank at 460 nm. After 5 minutes 0.33 ml of ferric chloride was added to the test tubes, and another absorbance was taken at 520 nm. Concentration of vitamin E was calculated using the formula

$$\text{Vit E (mg/dl)} = \frac{\text{abs.of sample at 520nm} - \text{abs.of sample at 460nm} \times \text{conc.of std.} \times 0.29}{\text{Abs.of std.at 520nm}}$$

2.5 Data analysis

Data were analysed using IBM SPSS statistics 23, values were presented as mean \pm (SD). One-way ANOVA was used for comparison followed by Duncan multiple comparisons. A level of $p < 0.05$ was taken as statistically significant at 95% CI.

3.0 RESULTS AND DISCUSSION

3.1 Weight changes in antioxidant-rich supplement treated Cr-induced oxidative stress rats

The change in weights of experimental rats is depicted in Figure 1. The normal control group (group A) had a nonsignificant ($p > 0.05$) weight gain after the treatment period. On the contrary, untreated Cr-induced oxidative stress rats (group B) had reduction in body weight at the end of the treatment period. However, the reduction was not significant ($p > 0.05$) statistically. Both the antioxidant-rich supplement treated groups (groups C and D) showed a significant ($p < 0.05$) increase in weight gain and this is more profound in group D (500 mg/kg body weight antioxidant-rich supplement). Exposure to heavy metals can adversely affect the health status of animals and thus their ability to feed. This could explain the apparent reduction in body weight seen in group B. Similar decrease in body weight following administration of Cr was reported in rabbits (Chongsi et al., 2019). Surprisingly, they reported no change in organ weight, which should have been the main reason for the general body weight reduction. In another study, Ghosh and colleagues observed significant reduction in organ weight in addition to body weight after administration of Cr in Wistar rats. (Ghosh et al., 2023).

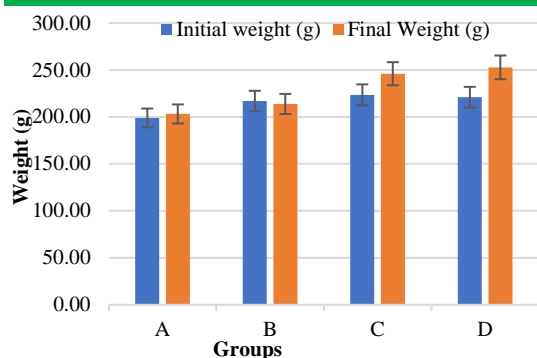


Figure 1. Weight changes in the treatment groups.

Rats' groups (n= 6 rats per group), A (normal control) administered distilled water; B (Negative control) administered 20 mg/kg body weight of $K_2Cr_2O_7$, for 2 weeks then distilled; groups C and D: administered 20 mg/kg body weight of $K_2Cr_2O_7$ for 2 weeks then followed by 250 and 500 mg/kg body weight respectively of antioxidants-rich supplement respectively for 2 weeks.

3.2 Serum Chromium Levels in Albino Rats

Serum Cr level in experimental animals is depicted in Table 1. There was significant ($p < 0.05$) reduction in serum Cr in antioxidant-rich supplement treated rats (C and D) compared to non-treated (group B). However, the reduction was more profound in the rats treated with 500 mg/kg antioxidant-rich supplement (group D). Similar significant reduction of Cr in rats after treatment with melatonin was reported by Benerjee *et al.* (2017), however, they used hepatic tissue homogenate and, in this study, serum was used. This suggests, apart from other effects, the antioxidant-rich supplement may increase excretion and or degradation of Cr in the experimental animals.

Table 1. Serum Chromium (Cr) Levels in Albino Rats

Rat Groups	Cr (mg/L)
A	ND
B	0.52 ± 0.26 ^a
C	0.40 ± 0.29 ^b
D	0.28 ± 0.08 ^c

Data are presented as mean ± SD. n= 6, A (normal control) administered distilled water; B (Negative control) administered 20 mg/kg body weight of $K_2Cr_2O_7$ for 2 weeks then distilled water; groups C and D: administered 20 mg/kg body weight of $K_2Cr_2O_7$ for 2 weeks then followed by 250 and 500 mg/kg body weight respectively of antioxidants-rich supplement respectively for 2 weeks.

Different superscript in column denotes statistically significant difference ($p < 0.05$).

3.3 Serum Malondialdehyde (MDA) Levels in Albino Rats

The serum MDA level in treatment groups is presented in Figure 2. There was significant ($p < 0.05$) reduction of MDA level in rats treated with the antioxidant-rich supplement. However, there was no difference between the doses of the supplement. The reduction in MDA level suggests ameliorating effect of the supplement on the lipid peroxidation. The increase in MDA level in untreated rats (group B) reaffirms previous report that Cr induces oxidative stress (Chongsi *et al.*, 2019). The reduction in MDA by the antioxidant-rich supplement corroborates previous studies reporting similar reduction due to treatment with vitamin E (Fang *et al.*, 2021), Melatonin (Benerjee *et al.*, 2017), vitamin C (Ibuki *et al.*, 2020), curcumin (Park *et al.*, 2021), vitamin E and bentonite, and extract of damsissa (Mohamed *et al.*, 2011) in Cr intoxicated rats.

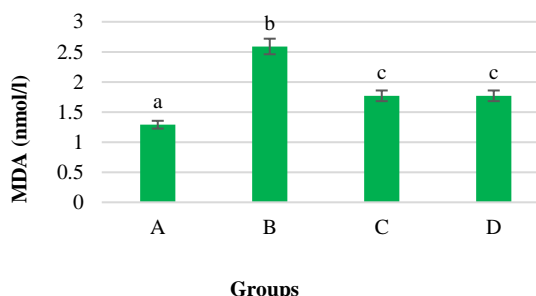


Figure 2. Serum level of malondialdehyde (MDA) in treatment groups.

Data presented as mean ± SD. A (normal control) administered distilled water; B (Negative control) administered 20 mg/kg body weight of $K_2Cr_2O_7$ for 2 weeks then distilled water; groups C and D: administered 20 mg/kg body weight of $K_2Cr_2O_7$ for 2 weeks then followed by 250 and 500 mg/kg body weight respectively of antioxidants-rich supplement respectively for 2 weeks. Different letter on the bars denotes significant difference ($p < 0.05$).

3.4 Activity of Serum antioxidant enzymes and GSH in albino rats

Table 2 depicts the activity of serum antioxidant enzymes and glutathione peroxidase. The activity of antioxidants enzymes (SOD, CAT and GPx) increase significantly ($p < 0.05$) in groups C and D following supplementation with antioxidant-rich supplement. The change in activity observed was significantly ($p < 0.05$) high compared to control (B). The increase in CAT and GSH was more profound in rats treated with 500 mg/kg body weight of the supplement (group D) than those treated with 250

mg/kg body weight (group C) indicating a dose dependence. Cr exposure leads to increase generation of more superoxide anions, which are quenched effectively in the presence of SOD (Singh et al., 2022). Furthermore, this Cr exposure was suggested to cause increased generation of hydroxyl radicals which are subsequently neutralized through the catalase-glutathione reductase and/or GPx reactions. GPx activity was also significantly ($p < 0.05$) increased in supplement treated groups (C and D) compared to nontreated (group B). Moreover, there was significant ($p < 0.05$) difference between the two groups with the supplement doses of 250 mg/kg and 500 mg/kg body weight. Hence, the effect of the antioxidant-

rich supplement on GPx was dose dependent. The lower levels of antioxidant enzymes and GSH in nontreated group (B) confirms that Cr inactivates the enzymes by either direct binding to their SH moiety or displacing their metal cofactor (Haidar et al., 2023; Kotyk et al., 2021). Similar increase in CAT, GPx, SOD, and GSH was reported by Banerjee and colleagues following administration of melatonin in Cr exposed rats (Banerjee et al., 2017). The level of GSH was reported to increase following treatment with vitamin C (Ibuki et al., 2023) and ginger (Jalali et al., 2021) in Cr exposed rats. Mashkoo et al. (2023) demonstrated that increased SOD activity following treatment with vitamin E in broilers.

Table 2. Activity of Serum Antioxidant Enzymes activities and GSH

Parameters	Animal groups			
	A	B	C	D
CAT (ng/mL)	0.27 ± 0.13 ^a	0.20 ± 0.09 ^b	0.39 ± 0.10 ^c	0.31 ± 0.05 ^d
GPx (μmol GSH utilized/min/mL)	0.004 ± 0.002 ^a	0.002 ± 0.002 ^b	0.003 ± 0.002 ^b	0.004 ± 0.003 ^a
GSH (mg/dl)	12.96 ± 0.05 ^a	5.94 ± 0.87 ^b	5.39 ± 1.18 ^c	6.74 ± 0.29 ^d
SOD (U/mL)	3.80 ± 0.54 ^a	2.17 ± 0.06 ^b	2.63 ± 0.89 ^c	2.40 ± 1.12 ^c

Different superscript in rows denotes statistically significant difference ($p < 0.05$). Data presented as mean ± SD. n= 6 A (normal control) administered distilled water; B (Negative control) administered 20 mg/kg body weight of K₂Cr₂O₇ for 2 weeks then distilled water; groups C and D: administered 20 mg/kg body weight of K₂Cr₂O₇ for 2 weeks then followed by 250 and 500 mg/kg body weight respectively of antioxidants-rich supplement respectively for 2 weeks

3.5 Serum Antioxidant Vitamins A, C, and E Concentrations

Table 3 depicts the concentrations of antioxidant vitamins (A, C, and E) in experimental rats. There was significant ($p < 0.05$) dose dependent increase in vitamin A and C in rats supplemented with antioxidant-rich supplement. However, the significant increase in vitamin E was not dose dependent. The decrease in these vitamins observed in Cr exposed nontreated rats (group B) could be due to suppression of antioxidant systems by chromium. The antioxidant-rich supplement might contain antioxidant vitamins based on the known components; hence, it is expected that it supplies the vitamins to replenish those consumed by Cr-induced oxidative stress. Furthermore, these vitamins alleviate the Cr-induced oxidative effects, for instance, vitamin C promoted the reduction of Cr⁶⁺ to less toxic Cr³⁺ in rats (Fatima et al., 2025) and reduced lipid peroxidation (Ibuki et al., 2020). Studies also have shown protective effect of vitamin E alone and in combination with other substances against Cr toxic effects (Fang et al., 2021; Mashkoo et al., 2023). To our knowledge, this may be the first report on decrease in antioxidant vitamins A, C, and E following Cr administration and their concomitant increase on treatment with antioxidant-rich supplement.

Table 3. Serum Antioxidant vitamins in experimental rats' groups

Parameters	Animal groups			
	A	B	C	D
Vitamin A (mg/dL)	50.64 ± 0.22 ^a	8.46 ± 0.09 ^b	10.68 ± 0.02 ^c	24.63 ± 0.03 ^d
Vitamin C (mg/dL)	73.52 ± 0.09 ^a	57.73 ± 0.13 ^b	74.85 ± 0.06 ^c	117.03 ± 0.12 ^d
Vitamin E (mg/dL)	0.23 ± 0.024 ^a	0.17 ± 0.006 ^b	0.30 ± 0.011 ^c	0.33 ± 0.018 ^c

Data presented as mean ± SD. n= 6, A (normal control) administered distilled water; B (Negative control) administered 20 mg/kg body weight of K₂Cr₂O₇ for 2 weeks then distilled water; groups C and D: administered 20 mg/kg body weight of K₂Cr₂O₇ for 2 weeks then followed by 250 and 500 mg/kg body weight respectively of antioxidants-rich supplement respectively for 2 weeks. Different superscript in rows denote statistically significant difference ($p < 0.05$).

3.6 Serum Antioxidant Minerals Levels Concentrations

The effect of antioxidant-rich supplements on serum antioxidant minerals Cu, Fe, Se, and Zn levels is as shown in Table 4. Reactive oxygen species (ROS) are known to decrease the antioxidant system including essential minerals. In particular, Fe homeostasis was reported to be altered, and its absorption is limited by Cr (Piskin et al., 2022).

Therefore, the significant increase in Cu, Fe, Se, and Zn in antioxidant-rich supplement treated rats could relate to the neutralization effect of the supplement on ROS. The increase in Cu and Zn correlates with the increase in SOD. These metals serve as cofactors for the enzymes and thus increase their activity and stability. Similarly, increase in Se and Zn correlate with the increase in GPx, since Se serves as its cofactor (Sharma et al., 2020) and Zn stimulator (Lopes-Pires et al., 2021). The present study reaffirms the findings of Besora-Moreno et al. (2022) on the effect of locally prepared antioxidant-rich supplements on antioxidant minerals.

Table 4. Serum Antioxidant Minerals Concentrations

Parameters	Animal groups			
	A	B	C	D
Cu (mg/L)	0.22 ± 0.015 ^a	0.10 ± 0.002 ^b	0.15 ± 0.010 ^c	0.16 ± 0.005 ^c
Fe (mg/L)	1.97 ± 0.59 ^a	1.27 ± 0.12 ^b	2.05 ± 0.10 ^a	1.37 ± 0.53 ^b
Se (µg/L)	0.50 ± 0.002 ^a	0.45 ± 0.053 ^a	0.51 ± 0.015 ^a	0.49 ± 0.010 ^a
Zn (mg/L)	0.13 ± 0.017 ^a	0.06 ± 0.007 ^b	0.46 ± 0.047 ^c	0.71 ± 0.015 ^d

Data presented as mean ± SD. n= 6, A (normal control) administered distilled water; B (Negative control) administered 20 mg/kg body weight of K₂Cr₂O₇ for 2 weeks then distilled water; groups C and D: administered 20 mg/kg body weight of K₂Cr₂O₇ for 2 weeks then followed by 250 and 500 mg/kg body weight respectively of antioxidants-rich supplement respectively for 2 weeks. Different superscript in rows denotes statistically significant difference (p < 0.05).

4.0 CONCLUSION

The results of this study suggest that the formulated antioxidant-rich supplement has an ameliorative effect on Cr-induced oxidative stress by modulating MDA levels, increasing the activities of antioxidant enzymes, and elevating the levels of antioxidant vitamins A, C, and E, as well as minerals (Cu, Fe, Se, and Zn) in a Cr-induced oxidative stress model using albino Wistar rats. Furthermore, this study suggests, for the first time, that Cr may reduce the levels of vitamins A, C, and E, likely by exerting oxidative stress.

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6.0 CONFLICT OF INTEREST

The authors declare no conflict of interest.

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