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Oxidative Damage in Erythrocytes of Rats after 1 and 2 Weeks of Exposure to Chromium (VI): Protective Effect of Selenium

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Abstract

Potassium dichromate (K2Cr2O7), an environmental pollutant, induces oxidative stress. The objective of this study was to evaluate the potential protective effect of Selenium (Se) against erythrocyte damages induced by K2Cr2O7 in adult rats at the end of the 1st and 2th weeks of treatment. Animals were randomly divided into four groups of six each: group I served as controls which received distilled water, group II received in drinking water K2Cr2O7 alone (700 ppm); group III received both K2Cr2O7 in drinking water and Se via diet (0.5 Na2SeO3 mg/kg of diet); group IV received Se (0.5 mg/kg of diet). All groups were killed after one and two weeks of treatment. Blood samples were collected in order to determinate some biochemical parameters. Rats exposed to K2Cr2O7 showed an increase in malondialdehyde levels, protein carbonyl and advanced oxidation products and a decrease of AChE activity, glutathione, non-protein thiol and vitamin C levels. While the activities of SOD and GPx increased. We noticed a decline in catalase activity after 1 and 2 weeks of bichromate exposure. Co-administration of Se restored the parameters indicated above to near-normal values. Therefore, our investigation revealed that selenium could be a useful element preventing K2Cr2O7-induced erythrocyte damages.

Keywords: Erythrocytes; Potassium dichromate; Selenium; Rats; Acetylcholinesterase; Oxidative stress

Introduction

Chromium (Cr) is listed by the Environmental Protective Agency as one of the 129 priority pollutants and one of 14 most noxious heavy metals. Cr can occur in several oxidation states: Trivalent chromium [Cr(III)] is accepted as an essential trace element whereas hexavalent chromium [Cr(VI)] compounds have been shown to be related with several toxic effects in human and animals [1]. Contamination of soils with Cr(VI) came from different industrial operations, smelting, tanning, electroplating etc. The transfer of this metal in the food chain from soil is of great importance, by the uptake process and accumulation by crop plants [2]. Human exposure to Cr(VI) salts such as potassium dichromate ($K_2Cr_2O_7$) can be made according to Pellerin and Booker [3] by three possible routes via inhalation, dermal contact or ingestion by the oral route.

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Indeed, once inside cells, intracellular reduction of Cr(VI) generates variable amounts of organic radicals, Cr(V), and Cr (IV) and finally a stable amount of stable Cr(III). Toxicity of Cr(VI) has been attributed to the formation of Cr(III)–DNA adducts [4] and oxidative damage by intermediate Cr forms and ROS [5]. Hexavalent chromium is a strong oxidizing agent. It causes dermatotoxicity, immunotoxicity, neurotoxicity, genotoxicity, cytotoxicity, mutagenesis and carcinogenicity [6]. Hematotoxicity is also accrued, because erythrocyte are permanently in contact with potentially damaging levels of oxygen. Their metabolic activity is able to reserve this injury under normal conditions. The reduced products, [Cr(V) and Cr(IV)], bind to hemoglobin and other intracellular proteins, resulting in the elevation of total Cr levels in the RBC fraction of blood for several weeks [7]. Erythrocytes are equipped with many defense systems representing their antioxidant capacity [8]. The hematological alterations like the elevated RBC count, the reduced MCV and PLT counts, have been reported by Ahsan [9] in occupants residing near the tanneries. Studies on chromium (VI) administrated to rats via their drinking water for 3 months [10] and 2 years [11], have reported hematological alterations.

Administration of trace elements and other antioxidants has been proposed as a therapeutic adjuvant to reduce the oxidative stress induced by metal exposure. Selenium considered as an essential element, plays an important role as antioxidant. The most important metabolic role of Se in mammalian cells occurs in the active site of selenoenzyme GSH-Px. The latter protects cells against damages induced by free radicals and also permits the regeneration of a membrane lipid molecule through reacylation [12]. In recent years, there has been a great deal of studies carried out on selenium metabolism [13]. Exogenous selenium was given to experimental animals as sodium selenite form [14]. Due to the health problems induced by many environmental pollutants including metals, previous studies have evaluated the relative antioxidant potential of Se [15].

Thereby, the present study was conducted on erythrocyte damages induced by dichromate exposure of adult rats during one or two weeks and the putative protective effects of selenium in adult rats.

Materials and Methods

Chemicals

Potassium dichromate ($K_2Cr_2O_7$) was obtained from Merck (Darmstadt, Germany). Sodium selenite (Na_2SeO_3) was purchased from Sigma (St. Louis, MO, USA). All other chemicals of analytical grade were provided from standard commercial suppliers.

Animals and treatment

Female Wistar rats with an initial mean body weight of 130 $\pm$ 10 g, obtained from the Central Pharmacy (SIPHAT. Tunis. Tunisia), were used in this study. They were maintained under standard laboratory conditions (temperature 22 $\pm$ 2°C; 12 h light–dark cycle). The animals had free access to water and commercial standard pellet diet (SICO, Sfax. Tunisia). The content of Se in standard diet (0.17 mg Na_2SeO_3/kg) was determined, after mineralization, by the Electrothermic Atomic Absorption Spectrometry technique (ETAAS). Measurements were performed on a Perkin-Elmer 5100/Zeeman Atomic Absorption Spectrometer with a 196-nm wavelength. The experimental procedures were carried out according to the National Health Institute Guidelines for Animal Care and approved by the Ethical Committee of the Sciences Faculty of Sfax.

One week after acclimatization, the rats were randomly divided into four groups of six animals each: group I serving as negative controls received standard diet; group II received via drinking water $K_2Cr_2O_7$ alone (700 ppm, orally); group III received both 700 ppm of $K_2Cr_2O_7$ via drinking water and Se via diet (0.5 mg/kg of diet) and group IV serving as positive controls received via diet Se (0.5mg/kg of diet) as sodium selenite (Na_2SeO_3). The present study was designed to investigate the toxicity of $K_2Cr_2O_7$. Animals were sacrificed after 1 or 2 weeks of treatment. The $K_2Cr_2O_7$ dose and the treatment period were selected on the basis of previous studies [16,17]. The selenium dose (0.5 mg/kg of diet), used in our experiment and in a previous study of our laboratory, gave high protection against stress conditions [18]. According to Hotz et *al*. [19], lower doses of selenium than that used in our experiment are of less protection while higher doses are not much effective.

At the end of the experimental periods (one and two weeks), the animals of different groups were killed by cervical decapitation to avoid stress.

Preparation of samples

Some blood samples were collected into EDTA tubes and centrifuged at 2200 × *g* for 15 min. Supernatants were then removed and the sediments containing erythrocytes were suspended in phosphate buffer saline solution (0.9% NaCl in 0.01M phosphate buffer, pH 7.4) and centrifuged as reported by Sinha et *al*. [20]. This process was repeated two fold. After removing cells' debris by centrifugation at 3000 × *g* for 15 min, the hemolysats were obtained and stored at −80°C until biochemical analysis.

Hematological study

Blood red cell (RBC) and white cell (WBC) counts, hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were quantified in an automatic hematological assay analyzer (Beckman Coulter, USA).

Determination of erythrocytes hemolysis

Erythrocytes hemolysis was determined by osmotic fragility behavior using grated NaCl solutions. A 100 µL aliquot of blood samples collected from all studied groups were added to tubes containing graded saline solutions (0.0 to 0.9% of NaCl). After gentle mixing and standing for 30 min at room temperature, the erythrocytes suspensions were centrifuged at 1270xg for 10 min. The absorbance of the released hemoglobin into the supernatant was measured at 540 nm [21].

Biochemical assays

Lipid peroxidation: The concentration of malondialdehyde (MDA) in tissues, an index of lipid peroxidation, was determined spectrophotometrically according to Draper and Hadley [22]. An aliquot of 0.5 ml of erythrocyte extract supernatant was mixed with 1 ml of trichloroacetic acid solution and centrifuged at 2500 xg for 10 min. One milliliter of a solution containing 0.67% thiobarbituric acid (TBA) and 0.5 ml of supernatant were incubated for 15 min at 90°C and cooled. Absorbance of TBA-MDA complex was determined at 532 nm using 1,1,3,3-tetra-ethoxypropane as standard. Results were expressed as µmol/g of tissue.

Protein quantification

Protein content in the erythrocytes was determined according to Lowry et *al*. [23] using Bovine serum albumin as a standard.

Determination of antioxidant enzyme activities

Catalase (CAT) activity was assayed by the method of Aebi [24]. Enzymatic reaction was initiated by adding an aliquot of 20 µl of the hemolysate and the substrate (H_2O_2) to a concentration of 0.5 M in a medium containing 100 mM phosphate buffer (pH 7.4). Changes in absorbance were recorded at 240 nm. CAT activity was calculated in terms of µmoles H_2O_2 consumed/min/mg of protein.

Superoxide dismutase (SOD) activity was estimated according to Beauchamp and Fridovich [25]. The reaction mixture contained 50 µl of hemolysate in potassium phosphate buffer (PH 7.8), 0.1 mM EDTA, 13 mM L-methionine, 2 µM riboflavin and 75 mM Nitro Blue Tetrazolium (NBT). The developed blue colour reaction was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was expressed as units/mg of protein.

Glutathione peroxidase (GPx) activity was measured according to Flohe and Gunzler [26]. The enzyme activity was expressed as nmol of GSH oxidized/min/mg protein.

Glutathione (GSH) content in erythrocytes

GSH in erythrocytes was determined by the method of Ellman [27] modified by Jollow et *al*. [28]. The method is based on the development of a yellow colour when DTNB (5,5-dithiobtis-2 nitro benzoic acid) is added to compounds containing sulfhydryl groups. A 500 µl aliquot of hemolysate in phosphate buffer was added to 3 ml of 4% sulfosalicylic acid. The mixture was centrifuged at 1600 x g for 15 min. Five hundred microliters of supernatants were taken and added to Ellman's reagent. The absorbance was measured at 412 nm after 10 min. Total GSH content was expressed as µg/mg protein.

Non-protein thiols (NPSH) content in erythrocytes

Erythrocyte NPSH levels were determined by the method of Ellman [27]. A 500 µl aliquot of supernatant was mixed with 10% trichloroacetic acid (1V/1V). After centrifugation, the protein pellet was discarded and free–SH groups were determined in a clear supernatant. A 100 µl aliquot of supernatant was added to 850 µl of 1M potassium phosphate buffer (pH=7.4) and 50 µl of DTNB (10 mM) 5,5-dithio-bis (2-nitrobenzoic acid). The colorimetric reaction was measured at 412 nm. Results were expressed as µmol/g tissue.

Sulfhydryl groups content in erythrocytes

This assay is based on the reduction of 5,5′-dithio-bis (2-nitro-benzoic acid) (DTNB) by thiols, generating a yellow derivative (TNB) whose absorption is measured spectrophotometrically at 412 nm [29]. Briefly, 0.1 mM DTNB was added to 120 µl of hemolysate and the mixture was incubated during 30 min in a dark at room temperature. The sulfhydryl groups content is inversely correlated to proteins oxidative damage. Results were reported as nmol of TNB/mg protein and represented as percent of control values.

Protein carbonyl content in erythrocytes

Protein carbonyl content is assayed by a method based on the reaction of carbonyls with dinitrophenylhydrazine (DNPH) forming dinitrophenylhydrazone, a yellow compound, measured spectrophotometrically at 370 nm [30]. Briefly, a 200 µL aliquot supernatant of sample was added to 400 µl of 10 mM DNPH in 2 M HCl, or to 2 M HCl (blank). The mixture was kept in dark room for 1 h and vortexed each 15 min. Then, 500 µL of 20% TCA were added to each tube. The mixture was vortexed and centrifuged at 14000 × g for 3 min. The supernatant obtained was discarded. The pellet was washed with 1 mL ethanol:ethyl acetate (1v/1v), vortexed and centrifuged at 14000 × g for 3 min to remove the free DNPH. The supernatant was discarded and the pellet was resuspended in 600 µl of 6 M guanidine (prepared in a 20 mM potassium phosphate pH 2.3), vortexed and incubated at 60°C for 15 min. Then, it was centrifuged at 14.000 × g for 3 min and the supernatant was used to measure absorbance in a spectrophotometer at 370 nm. The molar extinction coefficient of 22.000 for DNPH was used to calculate the concentration of carbonyls content in the erythrocytes and the results were reported as nmol/mg protein and represented as percent of control values.

Ascorbic acid levels in erythrocytes

Ascorbic acid determination was performed as described by Jacques-Silva et *al*. [31]. Proteins in the hemolysate were precipitated in 10 volumes of a cold 4% trichloroacetic acid solution. An aliquot of supernatent (300 µL) was adjusted to a final volume of 1 mL and incubated at 38°C for 3 h. Then 1 mL of H_2SO_4 65% (v/v) was added to the medium. The reaction product was determined using color reagent containing 4.5 mg/mL dinitrophenyl hydrazine and $CuSO_4$ (0.075 mg/mL). The data were expressed as µ mole of ascorbic acid/g tissue.

Acetylcholinesterase activity in erythrocytes

Acetylcholinesterase (AChE) activity was measured immediately in the hemolysate according to the method of Ellman et *al*. [32], using acetylthiocholine iodide as a substrate. The reaction mixture was composed as follows: phosphate buffer (0.1 M; pH 8) and 0.01 M DTNB. The hydrolysis rate of acetylthiocholine iodide was measured at 412 nm through the release of the thiol compound which, when reacted with DTNB, produces the colour-forming compound TNB. The reaction was initiated by adding 0.075 M acetylthiocholine iodide. Activities were expressed as micromoles of substrate/min/mg protein.

Statistical analysis

The data were analyzed using the statistical package program Stat view 5 Software for Windows (SAS Institute, Berkley, CA). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Fisher's Protected Least Significant Difference (PLSD) test as a post hoc test for comparison between groups. All values were expressed as means ± SEM. Differences were considered significant if $p<0.05$.

Results

Hematological parameters

Effects of $K_2Cr_2O_7$ on hematological parameters in adult rats exposed during 1 and 2 weeks to chromium (VI) are shown in Table 1. Compared with the control group, RBC counts and Hb concentration were reduced after one and two weeks of chromium exposure by (6 and 11,8%) and (8,4 and 10,7%) respectively. No changes were observed in MCV, MCH, and MCHC values. While, WBC increased by 19 and 55%, respectively. Co-treatment with Se restored all the parameters

indicated above to near normal values. Hematological parameters of rats which received only 0.5 mg of Se kg^{-1} of diet were not significantly changed, when compared to those of negative controls.

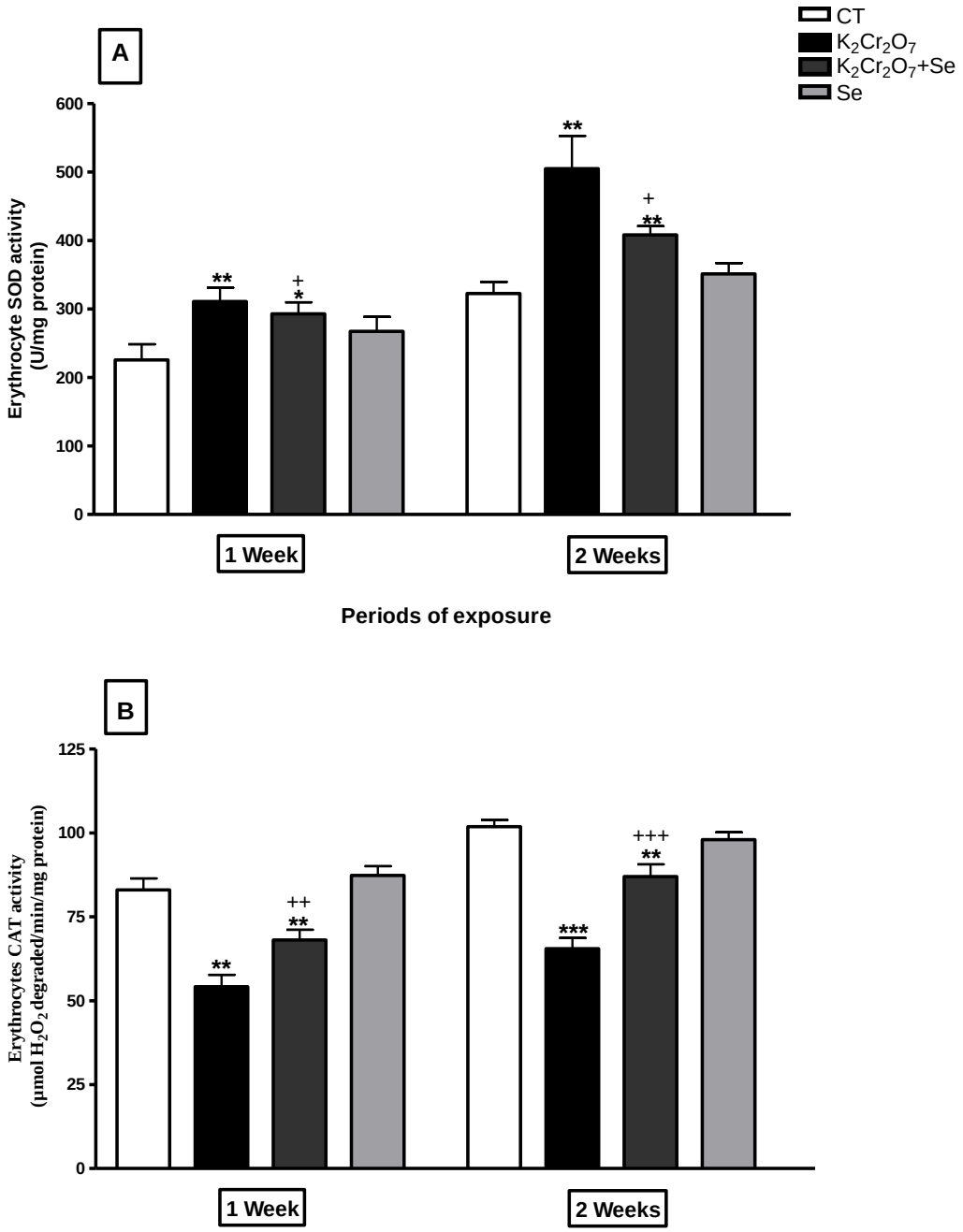
Table 1: Haematologic parameters [red blood cell (RBC), hemoglobin, hematocrit, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC)] and white blood cell (WBC) of control and treated rats with K2Cr2O7, K2Cr2O7+selenium (K2Cr2O7+Se) or selenium (Se) during 1 and 2 weeks.

Parameters & treatments	**Controls**	**$K_2Cr_2O_7$**	**$K_2Cr_2O_7$+Se**	**Se**
RBC count (10^6/mm^3)				
1 Week	8.36 ± 0.17	7.82 ± 0.11***	7.98 ± 0.31*+	8.15 ± 0.46
	9.97 ± 0.46	8.97 ± 0.24***	9.07 ± 0.30*++	9.35 ± 0.90
WBC count (10^3/mm^3)				
1 Week	11.38 ± 1.17	14.40 ± 0.67***	13.60 ± 1.38***	12.35 ± 0.30
2 Weeks	11.25 ± 1.07	17.45 ± 1.61**	14.60± 1.37 ***++	11.46 ± 1.33
	5	5	5	5
Hemoglobin (g/dL)				
1 Week	14.15 ± 0.25	12.95 ± 0.15***	13.61 ± 0.52**+	13.71 ± 0.70
2 Weeks	15.53 ± 0.61	13.86 ± 0.71***	15.26 ± 0.49*++	15.73 ± 0.52
Hematocrit (%)				
1 Week	44.35 ± 5.71	42.66 ± 1.30	43.50 ± 1.48	44.86 ± 2.09
	50.58 ± 3.69	48.37 ± 1.47		
MCV (mm^3/RBC)				
1 Week	54.68± 0.63	55.31 ± 1.59	54.26 ± 0.59	55.31 ± 0.84
		54.65 ± 0.66		56.03 ± 1.61
MCH (pg/RBC)				
1 Week	17.06 ± 0.39	16.78 ± 0.28	17.39 ± 0.65	17.06 ± 0.35
2 Weeks	17.31 ± 0.39	17.78 ± 0.58	17.65 ± 0.95	17.77 ± 0.79
	5			5
MCHC (g/dL)				
1 Week	31.06 ± 0.58	30.62 ± 0.23	31.51 ± 0.45	30.72 ± 0.49
2 Weeks	31.55 ± 0.99	32.37 ± 0.88	32.35 ± 0.69	31.62 ± 0.69
				5

	5	5	5	

Erythrocytes hemolysis

We observed an increase in OF of erythrocytes upon two week of $K_2Cr_2O_7$ treatment reflected by mean erythrocyte fragility (MEF) at 0.4% NaCl concentration of 0.80% as compared to MEF at 0.64%, 0.68%, and 0.58% NaCl in control, Se, and Se+$K_2Cr_2O_7$ -treated groups, respectively (Figure 1).



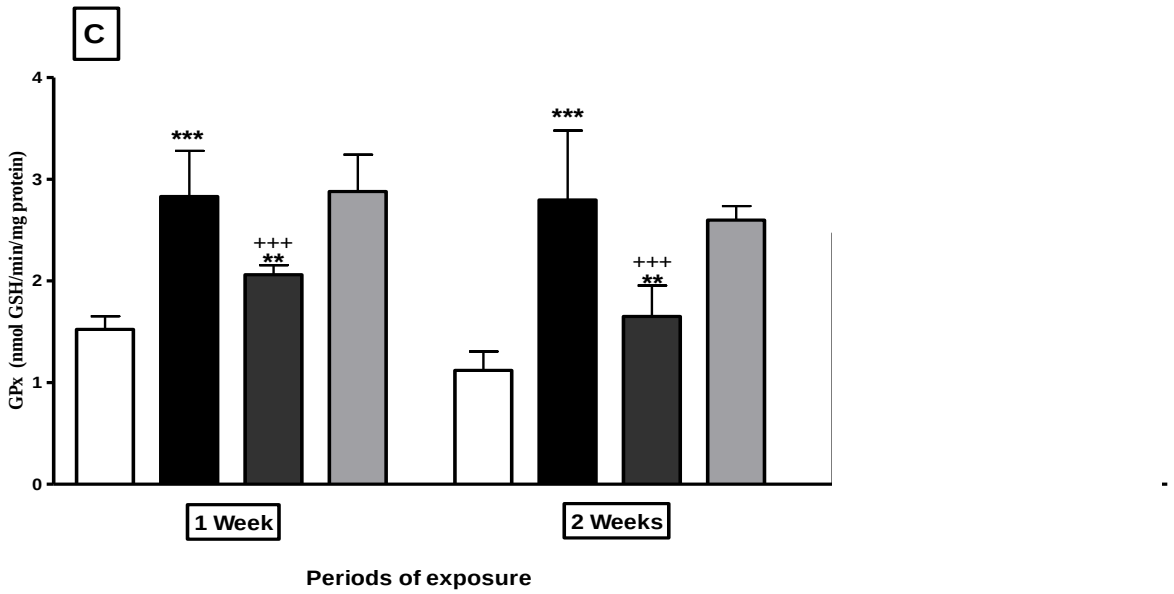


Figure 1: Effect of $K_2Cr_2O_7$ on mean erythrocyte osmotic fragility (MEOF) of rats. The MEOF of control and treated rats with $K_2Cr_2O_7$, selenium (Se) or their combination ($K_2Cr_2O_7$+Se) were determined as described in Materials and methods. MEOF has been denied as 50% of erythrocytes hemolysis. It has been extrapolated from the erythrocyte osmotic fragility curve of NaCl concentrations from 0.1% to 0.9%; however, The amount of lysis in each tube expressed in percent of hemolysis was compared with a 0.1% NaCl tube which corresponds to 100% of lysis.

d peroxidation and protein oxidative damage in erythrocytes

Our results (Table 2) revealed an increase of lipid peroxidation in $K_2Cr_2O_7$-treated group as evidenced by the enhanced malondialdehyde levels (+10 and +23%, respectively) after one and two weeks of exposure. The co-administration of Se significantly modulated erythrocyte malondialdehyde levels reaching normal values.

Protein oxidative damage

Table 2 shows that $K_2Cr_2O_7$ significantly increased carbonyl formation in erythrocyte during one and two week of exposure (+32 and +38, respectively), when compared to controls (Table 2). We also observed that $K_2Cr_2O_7$ induced a progressive sulfhydryl oxidation in the period of exposure ($p<0.001$), compared to those of control group (Table 2). Supplementation of Se ameliorated the levels of those parameters in ($K_2Cr_2O_7$+Se) group when compared to $K_2Cr_2O_7$ group.

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Table 2 : Erythrocyte MDA, PCO, SulC, GSH, NPSH and Vitamin C contents in adult rat controls or treated with K2Cr2O7, K2Cr2O7+Se, selenium (Se), during 1 and 2 weeks.

Parameters & treatments	Control	$K_2Cr_2O_7$	$K_2Cr_2O_7$+Se	*Se*
MDA[a]				
1 Week	16.93 ± 1.34	18.73 ± 1.79*	16.83 ± 1.66+	16.48 ± 1.72
	15.85 ± 1.73	19.58 ±	17.33 ± 0.71*+++	15.45 ± 1.33
PCO[b]				
1 Week	100± 4.62	132.02 ±	118.24 ± 8.31**++	104.86 ± 3.76
	100 ± 3.25	6.17	106.42 ±	99.73 ± 5.06
SulC[c]				
1 Week	100 ± 13.26	58.24 ± 3.88***	71.44 ±	100.78± 7.10
2 Weeks	100 ± 5.18	45.32 ±	4.72***+++	101.97±
GSH[d] **1 Week**	43.06 ± 3.39	28.76 ± 1.73***		42.49 ± 2.21
2 Weeks	49.31 ± 2.63	33.96 ± 2.02**		46.84 ± 4.49
NPSH[e]	0.82 ± 0.08	0.61 ±		0.83 ± 0.06
1 Week	0.88 ± 0.04	0.05***	0.72 ± 0.08*+	0.94 ± 0.07
		0.58 ± 0.04***	0.65 ± 0.06 *+++	
Vitamin C[f]				33.57 ± 2.52
1 Week	34.25 ± 2.17	22.81 ±	25.16 ± 2.81*+	35.33 ±5.39
		1.97***		

Table 2 showed that $K_2Cr_2O_7$ significantly increased protein carbonyl levels in erythrocytes during one and two weeks of exposure (+32 and +38, respectively), when compared to controls. We also observed that $K_2Cr_2O_7$ induced a progressive sulfhydryl oxidation during treatment exposure ($p<0.001$), compared to those of control group. Supplementation of Se ameliorated the levels of parameters indicated above in ($K_2Cr_2O_7$+Se) group when compared to $K_2Cr_2O_7$ group.

Protein oxidative damage

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sulfhydryl oxidation in the period of exposure ($p<0.001$), compared to those of control group (Table 2). Supplementation of Se ameliorated the levels of those parameters in ($K_2Cr_2O_7$+Se) group when compared to $K_2Cr_2O_7$ group.

Erythrocyte Glutathione (Gsh) levels

A decrease of glutathione (GSH) levels in erythrocytes was evident in $K_2Cr_2O_7$ group after one and two weeks of exposure (-33 and -32, respectively), when compared to controls (Table 2). Supplementation of Se in the diet of $K_2Cr_2O_7$ group ameliorated GSH levels when compared to $K_2Cr_2O_7$-group (Table 2).

Non Protein Thiol (Npsh) levels in erythrocytes

A decrease of NPSH levels by 26 and 34%, respectively, was evident in the erythrocytes of rats exposed one and two to $K_2Cr_2O_7$ (Table 2). Supplementation of Se ameliorated the levels of NPSH in ($K_2Cr_2O_7$+Se) group when compared to $K_2Cr_2O_7$ group.

Vitamin C level in erythrocytes

Exposure rats to $K_2Cr_2O_7$ during 1 and 2 weeks caused a gradual decrease of vitamin C levels when compared to controls. Supplementation of Selenium in the diet of the chromium-treated group restored vitamin C levels without reaching control values (Table 2).

Erythrocyte enzymatic antioxidants activities

SOD activity increased after 1 and 2 weeks of $K_2Cr_2O_7$ exposure by +18%, +56%. We noticed a decline in erythrocyte catalase activity ($p<0.001$). We also observed that $K_2Cr_2O_7$ induced a progressive increase in GPx activity ($p<0.001$). The co-administration of Se in the diet of $K_2Cr_2O_7$ group improved glutathione peroxidase, catalase and superoxide dismutase activities, without reaching control values (Figure 1).

Erythrocyte Acetylcholinesterase activity (AchE)

A gradual decrease acetylcholinesterase activity (AchE) iin erythrocytes was evident in $K_2Cr_2O_7$ group after one and two weeks of exposure (-42% and -54% respectively), when compared to controls (Figure 2). Supplementation of selenium in the diet of the chromium-treated group restored AchE activity without reaching control values.

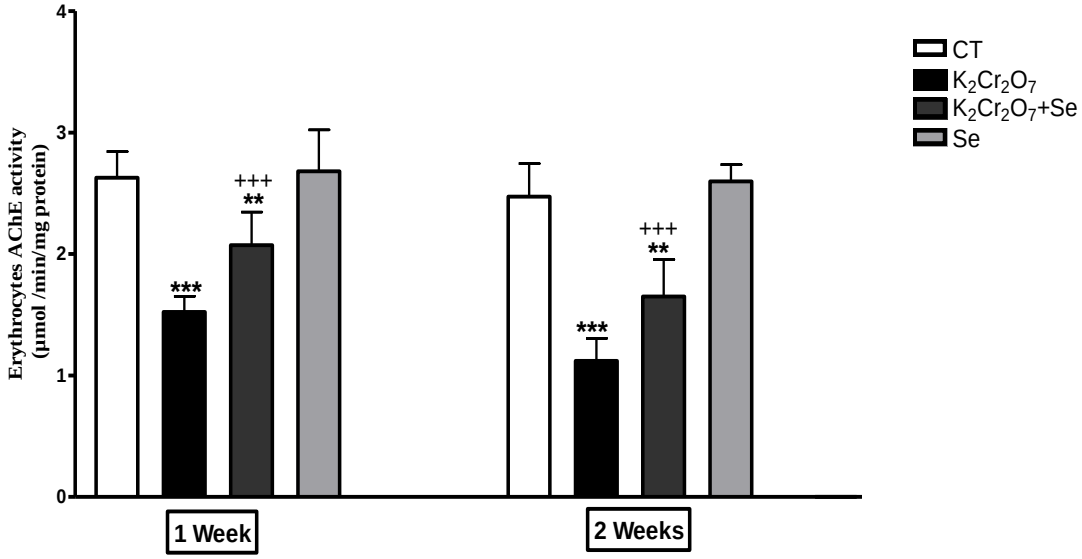

Figure 2: Acetylcholinesterase activity (AchE) in erythrocytes of control and treated rats with $K_2Cr_2O_7$, selenium (Se) or their combination ($K_2Cr_2O_7$+Se) during 1and 2 weeks. Values are means ± SE for six rats in each group. $K_2Cr_2O_7$; $K_2Cr_2O_7$+Se and Se treated groups vs. control group: $^{**}p<0.01$; $^{***}p<0.001$. ($K_2Cr_2O_7$+Se) group vs. $K_2Cr_2O_7$ group: $^{+++}p<0.001$.

Erythrocyte Osmotic Fragility (OF)

We observed an increase in erythrocyte osmotic fragility (OF) upon two week of $K_2Cr_2O_7$ treatment reflected by mean erythrocyte fragility (MEF) at 0.4% NaCl concentration of 0.80% as compared to MEF at 0.64%, 0.68%, and 0.58% NaCl in control, Se, and Se+$K_2Cr_2O_7$ -treated groups, respectively (Figure 3).

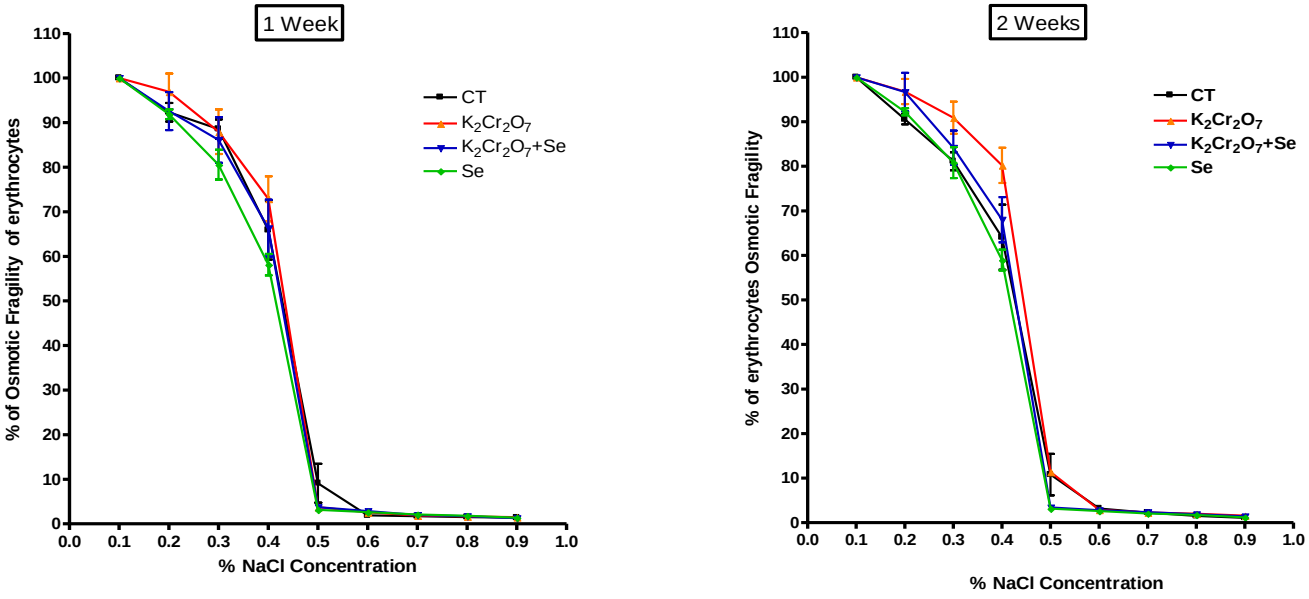

Figure 3: Erythrocyte enzymatic antioxidant activities of superoxide dismutase (A), catalase (B) and glutathione peroxidase (C), in control and treated rats with $K_2Cr_2O_7$, $K_2Cr_2O_7$+Se, selenium (Se), during 1 and 2 weeks. Values are means ± SE for six rats in each group. $K_2Cr_2O_7$; $K_2Cr_2O_7$+Se and Se treated groups vs. control group: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$. ($K_2Cr_2O_7$+Se) group vs. $K_2Cr_2O_7$ group: $^{+}p<0.05$; $^{++}p<0.01$; $^{+++}p<0.001$.

Discussion

Chromium is a toxic metal widely used in different industries [33]. It promotes an early oxidative stress and afterward contributes to the development of various pathological conditions due to its long retention in some tissues [34]. The use of antioxidants brings a novel option to chromium therapy. In this context, the present study investigated the potential protective effect of Se on erythrocytes toxicity induced by chromium in adult rats treated during one and two weeks.

Our results showed that $K_2Cr_2O_7$ induced oxidative damage in erythrocytes leading to hematotoxicity objectified by the reduction of RBC counts and Hb concentration. This could be the result of hematogenesis impairment by the inhibition of RBC production from pro-erythroblast and by erythrocyte destruction in hemopoietic organs as reported by the previous

findings of Kumar and Barthwa [35] and De Vizcaya-Ruiz et *al*. [36]. Indeed, a reduction in hemoglobin concentration found by us spoke in favour of erythrocytes breakdown as reported by De Vizcaya-Ruiz et *al*. [36]. Our findings represented a possible clinical picture of anemia following exposure to $K_2Cr_2O_7$. Co-administration of selenium through diet improved the parameters indicated above. According to Semba et *al*. [37] and Van Nhien et *al*. [38] a strong association of low serum selenium levels with anemia was observed in children living Vietnamese and adults living in the United States. They consider selenium as an essential trace element which may be used as a dietary supplement against anemia. In addition, this metal caused a significant increase in the level of WBC in our experimental study indicating an activation of the defense mechanism. The leucocytosis observed in the present work spoke in favour of rats protection by immune system against oxidative stress caused by chromium. Selenium, added in the diet of $K_2Cr_2O_7$-treated rats, decreased white blood cells number without reaching control values. In fact, previous reports of Smith et *al*. [39] and Van Nhien et *al*. [38] have suggested that selenium is a vital trace element for immune system function.

Osmotic fragility is used to display structural changes of the erythrocytes membrane when they are subjected to osmotic stress [40]. In the present study, the susceptibility of erythrocytes to hemolysis in presence of $K_2Cr_2O_7$ was greater than that of control. Our finding was similar to the previous study of Okwusid; [40] who has reported that exposure of heavy metals to high iron level found in the RBCs creates free radicals leading to erythrocyte membrane destruction. Furthermore, an increase of protein oxidation in erythrocytes as well as a significant decrease in sulfhydryl content found by us clearly indicated the presence of oxidative stress in erythrocytes membrane as reported by previous studies [41]. The increased oxidative stress may be responsible for the enhance of erythrocytes fragility in $K_2Cr_2O_7$-treated rats. Furthermore, according to Gutteridge [42], extensive lipid peroxidation in biological membranes causes disturbances of structural integrity, a loss of fluidity, a decreased membrane potential, and an increased permeability to ions. These changes lead to the rupture of membranes and the release of cell content [43]. The richness of erythrocyte membranes in polyunsaturated fatty acids may put the erythrocytes vulnerable to oxidative damage [44]. The present work showed a graded increase in erythrocytes MDA levels of $K_2Cr_2O_7$-treated during one and two weeks. Previous studies have also reported the increased levels of lipid peroxidation in plasma, blood and urine samples of workers exposed to chromium(VI) during chromium plating process [45]. Coadministration of selenium in the diet of chromium treated rats significantly decreased the extent of lipid peroxidation. This finding could be explained, according to Ognjanovic et *al*. [46], by the important role of Se in preventing hydroxyl radicals' formation and in protecting the integrity and the functions of tissues.

To defend themselves against oxidative stress, erythrocytes are equipped with an effective and complex antioxidant system, including protective enzymes and biological antioxidants such as SOD, CAT and GPx. In the present study, the increase in SOD activity is indicative of more production of $O^{\bullet-}$ and its increased dismutation to H_2O_2. While a high activity of GPx suggests more reduction in H_2O_2 to water in a GSH-dependent pathway. The over expression of these antioxidant enzymes indicated that the production of free radicals exceeded the capacity of detoxification mechanisms against $K_2Cr_2O_7$ induced oxidative stress. CAT was the only antioxidant enzyme whose activity was decreased, which implied a Fenton reaction-mediated conversion of more H_2O_2 to the ultimate toxicant, $OH^{\bullet}$ [47]. CAT and SOD constitute a mutually protective set of enzymes [48]. Because LPO was elevated in spite of an increase in SOD activity, an overproduction of $O^{\bullet-}$, which synergistically inhibited CAT [48], could be a reasonable explanation for the inhibition of the CAT activity. Although GPx and CAT share the substrate H_2O_2, the GSH redox cycle is a major protective mechanism against low levels of oxidant stress,

whereas CAT becomes more important in protecting against a severe oxidant stress [49]. In animal cells, especially in human erythrocytes, the principal enzyme for H_2O_2 detoxification is GPx. CAT has a much lower affinity for H_2O_2 than GPx [50] which speaks in favour of CAT depletion. We have observed the restoration of the of antioxidant enzyme activities in chromium rats co-treated with selenium particularly after two weeks of treatment. Indeed, El-Demerdash [15,51] have demonstrated in rats exposed to metals that selenium maintains the activities of antioxidant enzymes near normal levels, thus emphasizing its effects as an antioxidant.

Exposure to $K_2Cr_2O_7$ is also characterized by the depletion of non-enzymatic antioxidants including GSH, NPSH and vitamin C. This may be due to their consumption in quenching free radical probably generated by $K_2Cr_2O_7$. Indeed, Since GSH is involved in recycling vitamin C by mediating the reduction in dehydroascorbate [52], GSH deficiency would be expected to produce a deficiency of vitamin C in $K_2Cr_2O_7$ treated rats. Moreover it has been reported that supplementation of Se in the diet of chromium treated rats preserved the NPSH level from oxidative damage. These findings are in agreement with our previous study [53] where we have showed that antioxidants supplementation preserves cellular thiols and maintains the cells integrity.

It is well known that the impairment of blood parameters is also estimated by AChE activities in erythrocytes. In fact, the determination of AChE activity in the blood is of great consideration in the diagnosis of poisoning caused by reversible and irreversible inhibitors of this enzyme, including metals [54]. In the current study, ingestion of $K_2Cr_2O_7$ by rats provoked the inhibition of AChE activity. The inhibition of erythrocyte AChE activity observed in our study probably caused the accumulation of acetylcholine (ACh), which might cause, according to Malik and Summer [55], the stimulation of lymphocytes, an elevated concentration of cellular cGMP and an increased lymphocyte mobility and cytotoxicity.

In conclusion, the present study demonstrates that Se has a potential protective effect against $K_2Cr_2O_7$-induced erythrocyte damages. This element may be easily incorporated into the diet and thus may have a protective effect against chromium-induced hematotoxicity.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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