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Protective effects of selenium against potassium dichromate-induced hematotoxicity in female and male Wistar albino rats

Effets protecteurs du sélénium contre l'hématotoxicité induite par le dichromate de potassium chez les rats femelles et mâles albinos Wistar

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Abstract – Objective: Potassium dichromate ($K_2Cr_2O_7$) is a potent pollutant for human and animal health. The purpose of the current work is to compare the effect of $K_2Cr_2O_7$ using variations in the dose, route of administration and duration of exposure in male and female Wistar albino rats and to research the interaction of chromium and selenium with a special focus on hematopoiesis. **Materials and methods:** $K_2Cr_2O_7$ was subcutaneously administered alone (10, 50 and 100 mg/kg body weight) or $K_2Cr_2O_7$ (10 mg/kg) in association with selenium (0.3 mg/kg) was administered to female Wistar albino rats. Male rats received in their drinking water $K_2Cr_2O_7$ (30 mg/L/day) alone or in association with Se (0.3 mg/L/day) for 20 consecutive days. The hematological parameters were evaluated on days 3, 6 and 21 after subcutaneous (sc.) treatment in female rats and on days 10 and 20 after oral administration in male rats. **Results:** $K_2Cr_2O_{7-}$ induced during the first three days a significant ($p < 0.05$) dose-dependent decrease in the number of erythrocytes, platelets, leucocytes, lymphocytes and the hematocrit levels, and a dose-dependent increase in the number of granulocytes and monocytes. In the drinking water, chromium sc. significantly decreased the number of leucocytes and lymphocytes on day 10 after treatment and elevated the number of granulocytes and monocytes 20 days later. Selenium sc. counterbalanced the hematotoxic effects of chromium in female rats. **Conclusion:** These results suggest that the selenium has a protective role against the hematotoxicity of subcutaneous chromium in female Wistar rats.

Key words: Chromium, rat, selenium, hematotoxicity

Résumé – Objectif : Le dichromate de potassium ($K_2Cr_2O_7$) est un polluant potentiellement néfaste pour la santé humaine et animale. Cette étude a été entreprise afin de rechercher une éventuelle interaction entre le $K_2Cr_2O_7$ et le sélénium (Se) sur l'hématopoïèse chez les rats albinos Wistar mâles et femelles. **Matériel et Méthodes :** Le $K_2Cr_2O_7$ est administré seul (10, 50 et 100 mg/kg) par voie sous-cutanée (sc) ou en association (10 mg/kg) avec le sélénium (0,3 mg/kg). Les rats mâles reçoivent dans l'eau de boisson le $K_2Cr_2O_7$ (30 mg/L/jour) seul ou en association avec le sélénium (0,3 mg/L/jour) pendant 20 jours consécutifs. Les variations des paramètres hématologiques sont évaluées au 3^e, 6^e et 21^e jour chez les rattes et au 10^e et 20^e jour chez les mâles. **Résultats :** Les résultats montrent que le chrome engendre dès le 3^e jour après son administration par voie sc chez la femelle une diminution notable et dose-dépendante ($p < 0,05$) du nombre d'érythrocytes, du taux d'hématocrite, du nombre de plaquettes sanguines, de leucocytes, de lymphocytes et une augmentation dose-dépendante du nombre de granulocytes et de monocytes. Le chrome administré par voie orale diminue le nombre de leucocytes et de lymphocytes dès le 10^e jour du traitement et augmente celui des monocytes et des granulocytes 20 jours plus tard. La présence du sélénium par voie sous-cutanée contrebalance les effets hématotoxiques du chrome chez la ratte. **Conclusion :** Ces résultats suggèrent que le sélénium a un rôle protecteur contre l'hématotoxicité induite par le chrome administré par voie sc chez la ratte Wistar.

Mots clés : Chrome, rat, sélénium, hématotoxicité

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1 Introduction

Hexavalent chromium Cr (VI) is a major terrestrial pollutant. It is widely used in various industries, including pigments for manufacturing and painting, metal plating and leather tanning. Cr (VI) ingested with food such as vegetables or meat and water is reduced to Cr (III) before entering the bloodstream [1, 2]. Chromium enters the body through the lungs, gastrointestinal tract and, to a lower extent, through skin [3–5]. It is known that oral intake including food and water is the major route of exposure to chromium for the general population. Regardless of the route of exposure, Cr (III) is poorly absorbed, whereas Cr (VI) is more readily absorbed [6, 7]. Cr (VI) can easily enter the cell through SO_4^{2-} and HPO_4^{2-} channels [8] and remains there for the life of the cell [9]. After entering the cell, Cr (VI) undergoes a chain reaction with production of Cr intermediates such as Cr (V) and Cr (IV) by cellular reductants such as ascorbic acid and riboflavin, glutathione and serum protein [10]. The reduced product binds to intracellular proteins, resulting in an elevation of total chromium in the blood cell for several weeks [9]. During this reduction process, Cr produces reactive oxygen species (ROS) [11], and generates oxidative stress. This in turn is responsible for defective hematopoiesis [12]. It was established that Cr (VI) is a strong oxidant which causes cellular dysfunction and cell death [4, 6, 13–15]. The routes of excretion of chromium are via the kidney/urine and bile/faeces [17]. Selenium is of fundamental importance to human and animal health at low concentration. It is an essential component of several major metabolic pathways, including thyroid hormone metabolism [18]. But it is toxic to many systems at higher concentrations [19].

The purpose of the current work is to compare the effect of potassium dichromate ($K_2Cr_2O_7$) using variations in the dose, route of administration and duration of exposure in male and female Wistar albino rats and to research the interaction of chromium and selenium with a special focus on erythropoiesis and the immune system.

2 Materials and methods

2.1 Animals

Adult female and male Wistar albino rats (Charles River Labs, St-Aubin-lès-Elbeuf, France) were kept in a lighting schedule of 12 h light: 12 h darkness at 22 ± 1 °C with free access to food and water. Rats were housed at five rats per cage.

2.2 Chemicals

Potassium dichromate ($K_2Cr_2O_7$,H_2O) and selenium (Se) were purchased from Sigma-Aldrich (Chemie Gmbh., Taufkirchen, Germany). $K_2Cr_2O_7$,H_2O and Se were dissolved in sterile saline (NaCl 0.9%) and the pH was adjusted when necessary to 7.5.

2.3 Experiments

Each animal was anaesthetized with diethyl ether sc., and was weighed before each experiment. The control groups were injected sc. with a single dose of 0.3 mL/rat of NaCl 0.9%, or given distilled drinking water.

$K_2Cr_2O_7$ was given alone as a single sc. at 10, 50 and 100 mg/kg body weight to female rats alone or 30 mg/L/day for 20 consecutive days in distilled drinking water to male rats. Se was given sc. with a dose of 0.3 mg/kg body weight in association with the lower dose of $K_2Cr_2O_7$ (10 mg/kg body weight) or in the drinking water (0.3 mg/L/day) with $K_2Cr_2O_7$ (30 mg/L/day). Selenium was used to block the effects of the potassium dichromate. Blood samples were collected on EDTA from the jugular vein for hematological study on days 3, 6 and 21 after subcutaneous injection and on days 10 and 20 for the oral route. The determination of hematological parameters was performed by Coulter Erma Inc. PCE-21-ON.

2.4 Statistical analysis

Data for each group of experiments (n = 6) were statistically analyzed by analysis of variance and expressed as mean ±S.E.M. Significant differences between the treated group mean and its control group were performed by Student's "*t*" test. Differences were considered to be significant if $p < 0.05$. Data were analyzed with Excel for Windows, version 5.1, USA.

3 Results

3.1 Effects of $K_2Cr_2O_7$ alone or in combination with selenium on erythropoiesis in female and male Wistar albino rats

3.1.1 Effects on erythrocytic counts

In the female Wistar albino rats, 10 mg/kg body weight of subcutaneous chromium induced a slight but significant decrease ($p < 0.05$) in the erythrocytic counts in comparison with control. This decrease became not significant from 6 to 21 days after treatment, while 50 mg/kg body weight progressively decreased the erythrocytic counts from 3 to 6 days by 10% and 22%, respectively, and reached a maximum of 40% on day 21 after exposure (Tab. I). 100 mg/kg body weight of Cr induced a significant diminution in erythrocytic counts during the experimental period of 20%, 32% and 10%, respectively, in comparison with control. Pretreatment of females with selenium counteracted the effect of chromium on the number of erythrocytes during the first three days compared with chromium alone ($p < 0.05$).

On the contrary, 30 mg/L/day of $K_2Cr_2O_7$ alone or in association with selenium (0.3 mg/L/day) in the drinking water had no effect on the number of erythrocytes in male rats (Tab. II).

Table I. Effects of subcutaneous $K_2Cr_2O_7$ erythropoiesis in female Wistar albino.

Parameters		Vehicle controls 0.3 mL/rat (sc.)	$K_2Cr_2O_7$ 10 mg/kg (sc.)	$K_2Cr_2O_7$ 50 mg/kg (sc.)	$K_2Cr_2O_7$ 100 mg/kg (sc.)	$K_2Cr_2O_7$ + Se Cr (10 mg/kg) + Se 0.3 mg/kg)
Erythrocyte counts ($\times 10^6$/mm^3)	Day 3	7.29 ± 0.38	6.84 ± 0.043*	6.53 ± 0.53	5.86 ± 0.42*	7.40 ± 0.21#
	Day 6	7.39 ± 0.28	5.74. ± 1.37	5.79 ± 0.10*	5.03 ± 0.45*	6.54 ± 0.72
	Day 21	7.22 ± 0.25	6.74. ± 1.31	4.26 ± 0.551**	6.46 ± 0.57	6.72 ± 0.81
Hematocrit values (%)	Day 3	39.88 ± 4.18	34.04 ± 1.04	36.5 ± 1	36.16 ± 3.2	37.11 ± 1.22
	Day 6	38.36 ± 1.63	30.54 ± 0.48*	35.07 ± 0.4	31.98 ± 2.8*	32.25 ± 0.91
	Day 21	38.4 ± 1.42	37.44 ± 0.6	24.55 ± 4.6**	34.95 ± 1.97*	35.11 ± 1.23
Hemoglobin concentrations (dl)	Day 3	16.40 ± 0.98	14.46 ± 1.98	15.15 ± 1.65	15.03 ± 0.83	16.62 ± 0.20#
	Day 6	16.30 ± 0.67	10.34 ± 0.16*	12.90 ± 0.81*	16.40 ± 0.98	11.50 ± 0.59
	Day 21	16.20 ± 0.42	12.20 ± 0.21*	8.92 ± 1.27**	12.46 ± 0.73*	12.70 ± 0.93
Platelet counts /$\times 10^3$ mm^3	Day 3	1457.0 ± 102.3	758.40 ± 316.8*	895.5 ± 65.32*	1160.60 ± 13.02*	1054.01 ± 395
	Day 6	1683.33 ± 45.65	1297.01 ± 83.8*	1598.25 ± 494.8	1489.66 ± 181*	1310.30 ± 112
	Day 21	1114.02 ± 27.9	1191.8 ± 83	701.5 ± 271.67*	1164.83 ± 88	1200.21 ± 79

Each value for erythropoiesis represents the mean ± SEM of 6 rats per group.
** $p < 0.01$, * $p < 0.05$ compared with control value, or * $p < 0.05$ compared with control value.
# $p < 0.05$, $K_2Cr_2O_7$ (10 mg/kg, sc.) + Se (0.3 mg/kg, sc.) with $K_2Cr_2O_7$ (10 mg/kg, sc.) (Student's *t* test).

Table II. Effects of oral $K_2Cr_2O_7$ on erythropoiesis in male Wistar albino rats.

Parameters		Vehicle controls Distilled water	$K_2Cr_2O_7$ 30 (mg/L/d)	$K_2Cr_2O_7$ + Se $K_2Cr_2O_7$ (0.3 mg/L/d) + Se (0.3 mg/L/d)
Erythrocyte counts $\times 10^6$/mm^3)	Day 10	6.95 ± 0.26	6.85 ± 0.41	6.91 ± 0.18
	Day 20	7.04 ± 0.33	7.06 ± 0.24	7.12 ± 0.31
Hematocrit values (%)	Day 10	38.51 ± 1.15	38.15 ± 1.12	38.44 ± 0.52
	Day 20	38.16 ± 1.3	37.52 ± 0.91	37.82 ± 0.43
Hemoglobin concentrations (dl)	Day 10	13.33 ± 0.39	13.2 ± 0.37	13.12 ± 0.45
	Day 20	13.6 ± 0.43	12.66 ± 0.35	13.75 ± 1.20
Platelet counts $\times 10^3$/mm^3	Day 10	842.33 ± 123	993.83 ± 82.62	1021 ± 77.32
	Day 20	1080.83 ± 123	1309.33 ± 80*	1298 ± 0.26

Each value for erythropoiesis represents the mean ± SEM of 6 rats per group.
* $p < 0.05$ compared with control value, student's *t* test.

3.1.2 Effects on hematocrit values

On the other hand, the subcutaneous administration of $K_2Cr_2O_7$ at graduated doses (10, 50 and 100 mg/kg body weight) had no effect on the hematocrit values during the first three days after treatment while, on day 6 after exposure, 10 and 100 mg/kg body weight doses significantly decreased the hematocrit values by 20% and 16%, respectively, in comparison with the control, whereas, on day 21 after treatment, the hematocrit values were significantly reduced by 23% only with 50 mg/kg body weight in comparison with control (Tab. I). 30 mg/L/day of oral $K_2Cr_2O_7$ had no effect on the hematocrit values in male Wistar albino rats compared with the control (Tab. II). Selenium combined with chromium had no effect on the hematocrit values.

3.1.3 Effects on hemoglobin concentrations

Similarly, the concentration of hemoglobin was slightly but not significantly decreased during the first three days after exposure to the graduated doses of subcutaneous $K_2Cr_2O_7$. The decrease was highly significant from 6 to 21 days after treatment for the lower dose by 37% and middle dose by 24%, respectively, compared with control, while the higher dose markedly decreased the hemoglobin concentration by 23% only on day 21 after treatment compared with control (Tab. I). In male Wistar albino rats the oral route induced a negligible decrease in the hemoglobin concentration only on day 20 of treatment (Tab. II). Pretreatment of females with selenium sc. significantly ($p < 0.05$) increased the concentrations of hemoglobin on day 3 of the experiment compared with 10 mg/kg chromium alone.

3.1.4 Effects on blood platelets

The graduated doses of chromium induced a significant decrease in the number of blood platelets during the first three days after treatment of about 48%, 38% and 20%, respectively, and on day 21 with 50 mg/kg body weight, of chromium sc., compared with the control group in female Wistar albino rats. On day 6 the chromium induced a slight increase in platelet counts with the lower dose (+11%) and middle dose (+37%). This elevation in the number of platelets was highly significant

Table III. Effects of subcutaneous $K_2Cr_2O_7$ on leucopoiesis in female Wistar albino rats.

Parameters		Vehicle controls 0.3 mL/rat	$K_2Cr_2O_7$ 10 mg/kg (sc.)	$K_2Cr_2O_7$ 50 mg/kg (sc.)	$K_2Cr_2O_7$ 100 mg/kg (sc.)	$K_2Cr_2O_7$ + Se Cr (10 mg) + Se (0.3 mg)
Leucocyte counts/(mm^3)	Day 3	10 433.33 ± 2157	7320 ± 894.02*	4650 ± 851*	4700 ± 596.93*	7665.21 ± 434
	Day 6	11 566.66 ± 96.45	6180. ± 995	9225 ± 551*	2730 ± 246.41*	7151 ± 465
	Day 21	11 400 ± 1137.24	9520 ± 1073.19	15 492 ± 1888*	14 836.6 ± 2153.9*	11 142 ± 89.31#
Lymphocytes/(mm^3)	Day 3	8905 ± 515.96	8580 ± 1177.4	3850 ± 526.6*	3633.33 ± 348.8*	8452.12 ± 634
	Day 6	8153.33 ± 403.55	6020 ± 815.78*	7750 ± 526.6	5950 ± 1285	6423.51 ± 415
	Day 21	8026.66 ± 588	8920 ± 960.72	8275 ± 994	8133 ± 92.62	9141.14 ± 354
Monocytes/(mm^3)	Day 3	276.5 ± 76.77	376 ± 101.58	565.5 ± 184.3*	433.33 ± 123*	383.661 ± 98.52
	Day 6	288.5 ± 144.31	420 ± 167.33	1295 ± 505.25*	866.66 ± 66.66*	490.62 ± 83.21
	Day 21	288.5 ± 144.31	266 ± 102.81	575 ± 280.7	633.33 ± 36.6*	279.56 ± 100.1
Granulocytes/(mm^3)	Day 3	151.66 ± 11.83	340 ± 75.03*	462.5 ± 77.89*	403.33 ± 107.51*	386.23 ± 65.31
	Day 6	165 ± 98.8	400 ± 50*	497.5 ± 2.89*	551.66 ± 58.97*	482.13 ± 41.41
	Day 21	153.33 ± 45.52	224 ± 122.3	160 ± 38.63	226.66 ± 26.39	312.21 ± 124.23

Each value for leucopoiesis represents the mean ± SEM of 6 rats per group
* $p < 0.05$ compared with control value.
# $p < 0.05$, $K_2Cr_2O_7$ (10 mg/kg, sc.) + Se (0.3 mg/kg, sc.) with $K_2Cr_2O_7$ (10 mg/kg, sc.) (Student's *t* test).

Table IV. Effects of oral $K_2Cr_2O_7$ on leucopoiesis in male Wistar albino rats.

Parameters		Vehicle controls Distilled water	$K_2Cr_2O_7$ 30 (mg/L/d)	$K_2Cr_2O_7$ + Se Cr (0.3 mg/L/d) + Se (0.3 mg/L/d)
Leucocytes/(mm^3)	Day 10	11 013 ± 482	9633 ± 1096	10 021.53 ± 963
	Day 20	11 233 ± 1364	7066.66 ± 803.51*	7935.15 ± 837
Lymphocytes/(mm^3)	Day 10	9283.33 ± 487.46	7700 ± 1025.32	8154.30 ± 421
	Day 20	9216.66 ± 709.26	5816 ± 505.28*	6015.25 ± 489.62
Monocytes/(mm^3)	Day 10	250 ± 68.13	1311 ± 82.21**	1132.54 ± 102
	Day 20	231.66 ± 40.14	250 ± 37.51	240.53 ± 21.30
Granulocytes/(mm^3)	Day 10	506.83 ± 3.59	616.66 ± 11.35*	586.41 ± 18.61
	Day 20	466.66 ± 73.22	533.33 ± 119.2	492.61 ± 31.42

Each value for leucocytes represents the mean ± SEM of 6 rats per group
** $p < 0.01$. * $p < 0.05$ compared with control value, student's *t* test.

(+27%) on day 21 after subcutaneous administration in comparison with control, as shown in Table I. Similarly, in male Wistar albino rats, 30 mg/L/day of chromium added to drinking water progressively increased the platelet counts (+21%) on day 20 compared with control, as shown in Table II. Pretreatment of rats with selenium had no effect on the number of blood platelets compared with chromium administered alone sc. or in the drinking water.

3.2 Effects of $K_2Cr_2O_7$ alone or in combination with selenium on leucopoiesis in female and male Wistar albino rats

3.2.1 Effects on total leucocyte counts

A significant decrease in the number of leucocytes was immediately observed during the first three days after exposure to graduated doses of subcutaneous $K_2Cr_2O_7$ (10, 50, 100 mg/kg body weight) of 6%, 55% and 55%, respectively, in comparison with control. This decrease was maintained on day 6 with the middle and high doses at 47%, 20% and 76%, respectively, while on day 21 a marked increase in the number of leucocytes was observed with the middle dose of 39% and the highest of 30% compared with 6 days after treatment (Tab. III). In drinking water at 30 mg/L/day $K_2Cr_2O_7$ significantly decreased the leucocyte counts from 10 to 20 days after treatment (Tab. IV). Selenium sc. counteracted the effect of chromium on the number of leucocytes on day 21 after sc. treatment in female rats (Tab. III).

3.2.2 Effects on lymphocyte counts

10 mg/kg body weight of $K_2Cr_2O_7$ induced a significant decrease in the lymphocyte counts of 47% only on day 6 after subcutaneous treatment in female rats, while 50 and 100 mg/kg body weight sc. immediately provoked a significant decrease on day 3 after exposure of 57% and 59%, respectively. This diminution was only maintained with the high dose; 27% on day 21 after treatment in comparison with control (Tab. III). Male rats having received 30 mg/L/day of $K_2Cr_2O_7$ orally in drinking water showed a slight but not significant decrease in the lymphocyte numbers on day 10 after treatment: this

decrease became significant on day 20 and attained 37% in comparison with control (Tab. IV). Pretreatment of rats with selenium had no effect on lymphocyte counts either sc. or in the drinking water compared with $K_2Cr_2O_7$ alone.

3.2.3 Effects on monocyte counts

In female rats the monocyte counts augmented slightly but not significantly during the exposure period with the lower dose of $K_2Cr_2O_7$ sc., while 50 and 100 mg/kg body weight sc. immediately induced a progressive increase in the number of monocytes of 104% and 56%, respectively, on day 3 and of 349% and 200% on day 6 after treatment, while on day 21 this increase was only maintained with 100 mg/kg body weight at 119% in comparison with control (Tab. III). In male rats 30 mg/L/day of $K_2Cr_2O_7$ in drinking water induced a marked increase in the monocyte counts of 424% on day 10 after treatment which disappeared on day 20 in comparison with control (Tab. IV). Pretreatment of rats with selenium had no effect on monocyte counts either sc. or in the drinking water compared with $K_2Cr_2O_7$ alone.

3.2.4 Effects on granulocyte counts

The number of granulocytes immediately augmented during the first three days after treatment with the graduated doses of chromium administered subcutaneously to female rats by 124% (10 mg/kg body weight), 204% (50 mg) and 166% (100 mg), respectively. This increase was maintained from day 6 and was about 142%, 201% and 234%, respectively, until day 21 with 10 mg/kg at about 46% and the higher dose at 48% compared with control values (Tab. III). In drinking water, 30 mg/L/day of chromium induced a marked increase of 22% in the granulocyte counts on day 10 after treatment. This effect disappeared on day 20 after exposure compared with control values (Tab. IV). Pretreatment of rats with selenium had no effect on granulocyte counts either sc. or in the drinking water compared with $K_2Cr_2O_7$ alone.

4 Discussion

The present study demonstrated that in female Wistar albino rats, the lower subcutaneous dose of hexavalent chromium immediately affected the erythropoietic parameters indicating anemia. In fact, the reduction in the number of erythrocytes, the hematocrit values and platelet counts was immediately observed during the first three days after exposure to the lower dose of chromium, while hemoglobin concentrations decreased between day 6 and day 21. The middle dose, on the contrary, later decreased the number of erythrocytes, hematocrit values and hemoglobin concentrations between day 6 and day 21, while the platelet number decreased only during the first three days after subcutaneous exposure. The higher dose immediately decreased the number of erythrocytes during the first six days, and the hematocrit values decreased only on day 6, while hemoglobin concentrations diminished at the end of exposure and platelet counts only on day 3 after exposure to subcutaneous treatment. We also observed that on day 6, the graduated doses of chromium used in the present study tended to augment progressively the number of platelets.

Short-term exposure to low concentrations of chromium inducing a decrease in erythropoietic indices were reported in fish [20] and in mice [21]. This anemia could be due to iron deficiency and consequently to its reduced use for hemoglobin synthesis. Red blood cell chromium is currently considered the best indicator of hexavalent chromium exposure [9]. It was reported earlier that Cr (VI) can rapidly penetrate the membrane of erythrocytes and enter the cell and accumulates in erythrocytes of exposed workers [22–24]. The accumulation of Cr (VI) induced micronucleus frequency in erythrocytes of adult mice and their foetuses after intraperitoneal injection of Cr (VI) [4, 25] and caused DNA-protein crosslink formation in erythrocytes of fish [26]. Furthermore, in the erythrocyte, Cr (VI) was bound to a beta-chain of hemoglobin [17] which could explain the depletion of hemoglobin concentrations observed in the present study. On the other hand, the diminution in hemoglobin concentrations could be due to structural alteration of heme which disturbs hemoglobin synthesis, and also to the inhibition of the enzyme system involved in the synthesis of hemoglobin, as suggested earlier with other heavy metals [27, 28]. Potassium dichromate in drinking water had no effect on the number of erythrocytes or hematocrit levels in male Wistar albino rats. This is in accordance with a study on mice, in which Cr (VI) with drinking water did not induce any clastogenic effect on hematopoietic cells of adult mice and their foetuses [4]. This route of exposure is widely believed to cause much less toxicity than other exposure routes, because ingested Cr (VI) is converted into inactive trivalent chromium in the stomach [4, 29]. The diminution in platelet counts induced with graduated doses of chromium subcutaneously on day 3 after exposure could be due to the presence of infection, as observed in mice after inoculation with dengue virus [21]. On the contrary, the augmentation in platelet values induced by chromium on day 6 subcutaneously or at the end of the experiment in drinking water also reported in mice [21] suggested the presence of inflammation. Furthermore, our results demonstrated that chromium dichromate in drinking water or administered subcutaneously to male or female rats is susceptible to perturbing the immune response. Indeed, the leucopenia and lymphopenia observed on days 3 and 6 after subcutaneous Cr (VI) administration or on day 20 in drinking water were also observed in mice [21] and in fish [31]. It was reported that Cr (VI) easily enters physiological membranes and is actively transported into cells and remains there for the life of the cell. In persons occupationally exposed to Cr (VI), the determination of Cr (VI) showed a significant increase in chromium levels in the lymphocytes [32]. Furthermore, the depletion of lymphocytes has also been reported *in vivo* in patients with metallic prostheses and has been correlated with elevated chromium levels in blood [33]. Cr (VI) induced in humans exposed to it an apoptosis of blood lymphocytes [13] and significantly reduced the lymphocyte size [34]. Cr (VI) in contact with biological compounds may lead to peroxidation of biological compounds that are present in the cell or on its surface. In effect, some negative changes such as cell

membranes damaged due to peroxidation of unsaturated fatty acids or inhibition of mitochondrial transmembrane potential in rat lymphocytes [34] may occur and could explain the reduced lymphocyte and leucocyte counts. On the other hand, it was reported that Cr (VI) is genotoxic. Several studies reported that the one major lesion associated with Cr (VI) is the DNA damage in the intact lymphocytes [9]. Incubation of human lymphocytes with Cr (VI) resulted in a dose-dependent increase in DNA strand break. This was also detected in rat peripheral lymphocytes [35]. Furthermore, the decrease in the lymphocyte counts in our rats, which received Cr (VI) in drinking water for three weeks, could be due to the increase in the formation of DNA-protein crosslinks reported in the rat blood lymphocytes [36] and in the exposed population [37] or during *in vitro* or *in vivo* exposure [11]. The formation of DNA lesions induced by Cr (VI) may result from the implication of the enhanced reactive oxygen species (ROS) and hydrogen peroxide in human lymphocytes [34,38] and the decrease in glutathione levels and inhibition of proliferation of lymphocytes [39]. On the contrary, the present study showed that subcutaneous administration of potassium dichromate in female rats or in males 10 days after exposure in drinking water augmented the number of monocytes and granulocytes. Similar findings have been reported in fish [31] and in mice [21] exposed to Cr (VI) in drinking water, and in rats exposed to an atmosphere containing Cr (VI) [40]. Moreover, chronic exposure to these low clinically relevant concentrations of Cr (VI) induced a potent adaptive response with elevated glutathione-S-transferase expression and increased activities and expression of reactive oxygen scavengers, superoxide dismutases, catalase and glutathione peroxidase, and temporal increases in reduced glutathione levels, glutathione reductase activity, and glutamate cysteine ligase expression [41]. Monocytes were more susceptible to the toxicity of the metal. Indeed, chromium used in prostheses enhanced human blood monocyte/macrophage proliferation and significantly increased the level of interleukin-1α, interleukin-1β and TNF-α [42,43].

Selenium combined with the lowest dose of $K_2Cr_2O_7$ improved the decreased number of erythrocytes, leucocytes and hemoglobin concentrations induced by $K_2Cr_2O_7$ alone. The mechanism by which selenium acts has been primarily attributed to inhibition of oxidative stress induced by heavy metals of the formation of a complex between selenium and heavy metal [44].

As far as we know, there are no reports on the interaction of chromium and selenium in rats. However, in mice exposed to cadmium, selenium supplements have been found to restore the decreased levels of glutathione and prevent lipid peroxidation in the tests [45]. Recent studies in rats [46] with mercury have shown that selenium binds with sulfhydryl groups of proteins and mercury is then bound to this complex in the selenium/mercury ratio of 1:1 [47]. Consequently, mercury is retained in the tissues but is also withdrawn from active metabolism and the toxic effects are decreased. If selenium could also bind $K_2Cr_2O_7$ in a protein complex, this could remove selenium from other essential physiological functions in the body.

In the present study, the ameliorating effect of selenium sc. on the number of erythrocytes and leucocytes, and the concentration of hemoglobin might be due to an interaction of selenium with $K_2Cr_2O_7$ forming biologically inactive chromium-selenium and maintenance of membrane integrity by producing endogenous sulfhydryl groups.

5 Conclusion

The interesting finding in the present study is that short-term exposure to a low dose of $K_2Cr_2O_7$, sc. induces in female Wistar albino rats erythrocytopenia, thrombocytopenia, leucopenia, lymphopenia, granulocytosis, monocytosis and a decrease in hematocrit values and hemoglobin concentrations; on the other hand, in drinking water chromium is susceptible to affecting the immune response and induces leucopenia, lymphopenia, monocytosis and granulocytosis. In male Wistar albino rats, the oral route of exposure had no effect on erythropoietic parameters.

Selenium seemed to ameliorate the erythrotoxic and immunotoxic effect of potassium dichromate in female Wistar albino rats.

Conflict of interest

The author declares that there are no conflicts of interest.

References

1. Richelmi P, Baldi C. Blood levels of hexavalent chromium in rats. "*In vitro*" and "*in vivo*" experiments. Int J Environ Anal Chem. 1984; 17(3-4): 181–186.
2. Kerger BD, Finley BL, Corbett GE, Dodge DG, Paustenbach DJ. Ingestion of chromium (VI) in drinking water by human volunteers: absorption, distribution, and excretion of single and repeated doses. J Toxicol Environ Health. 1997; 50(1): 67–95.
3. Corbett GE, Finley BL, Paustenbach DJ, Kerger BD. Systemic uptake of chromium in human volunteers following dermal contact with hexavalent chromium (22 mg/L). J Expo Anal Environ Epidemiol. 1997; 7(2): 179–189.
4. De Flora S, Iltcheva M, Balansky RM. Oral chromium (VI) does not affect the frequency of micronuclei in hematopoietic cells of adult mice and of transplacentally exposed fetuses. Mutat Res. 2006; 610(1-2): 38–47.
5. Antonini JM, Stone S, Roberts JR, Chen B, Schwegler-Berry D, Afshari AA, Frazer DG. Effect of short-term stainless steel welding fume inhalation exposure on lung inflammation, injury, and defense responses in rats. Toxicol Appl Pharmacol. 2007; 223(3): 234–245.
6. O'Flaherty EJ, Kerger BD, Hayes SM, Paustenbach DJ. A physiologically based model for the ingestion of chromium (III) and chromium (VI) by humans. Toxicol Sci. 2001; 60(2): 196–213.
7. Cavalleri A, Minoia C. Serum and erythrocyte chromium distribution and urinary elimination in persons occupationally exposed to chromium (VI) and chromium (III). G Ital Med Lav. 1985; 7(1): 35–38.

8. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006; 160(1): 1−40.
9. Costa M, Zhitkovich A, Toniolo P, Taioli E, Popov T, Lukanova A. Monitoring human lymphocytic DNA-protein cross-links as biomarkers of biologically active doses of chromate. Environ Health Perspect. 1996; 104(5): 917−919.
10. Standeven AM, Watterhahn KE. Ascorbate is the principal reductant of chromium (VI) in rat lung ultrafiltrats and cytosols, and mediates chromium-DNA binding in vitro. Carcinogenesis. 1992; 13(8): 1319−1324.
11. Manerikar RS, Apte AA, Ghole VS. In vitro and in vivo genotoxicity assessment of Cr (VI) using comet assay in earthworm coelomocytes. Environ Toxicol Pharmacol. 2008; 25(1): 63−68.
12. Bainy ACD, Saito E, Carvalho PSM, Junqueira VBC. Oxidative stress in gill, erythrocytes, liver and kidney of Nile Tilapia (orechromis niloticus) from a polluted site. Aquat Toxicol. 1996; 34(2): 151−162.
13. Vasant C, Balamurugan K, Rajaram R, Ramasami T. Apoptosis of lymphocytes in the presence of Cr (V) complexes: role in Cr (VI)-induced toxicity. Biochem Biophys Res Com. 2001; 285(5): 1354−1360.
14. Wang XF, Xing ML, Shen Y, Zhu X, Xu LH. Oral administration of Cr (VI) induced oxidative stress, DNA damage and apoptotic cell death in mice. Toxicology. 2006; 228(1): 16−23.
15. Lei T, He QY, Cai Z, Zhou Y, Wang YL, Si LS, Cai Z, Chiu JF. Proteomic analysis of chromium cytotoxicity in cultured rat lung epithelial cells. Proteomics. 2008; 8(12): 2420−2429.
16. Myers JM, Antholine WE, Myers CR. Hexavalent chromium causes the oxidation of thioredoxin in human bronchial epithelial cells. Toxicology. 2008; 246(2-3): 222−233.
17. Barceloux DG. Chromium. Clin Toxicol. 1999; 37: 173−194.
18. Eder K, Kralik A, Kirchgessner M. Influence of deficient to subtoxic selenium intake on metabolism of thyroid hormones. Zeitschrift für Ernährungswissenschaft. 1995; 34(4): 277−283.
19. Hoffman DJ. Role of selenium toxicity and oxidative stress in aquatic birds. Aquat Toxicol. 2002; 57(1-2): 11−26.
20. Vutukuru SS. Acute effects of hexavalent chromium on survival oxygen consumption hematological parameters and some biochemical profiles of Indian major carp, Labeo rohita. Int J Res Public Health. 2005; 2(3): 456−462.
21. Shrivastava R, Srivastava S, Upreti RK, Chaturvedi UC. Effects of dengue virus infection on peripheral blood cells of mice exposed to hexavalent chromium with drinking water. Indian J Med Res. 2005; 122(2): 111−119.
22. Lewalter J, Korallu U, Hardorf C, Weidmann H. Chromium bound detection in isolated erythrocytes: a new principle of biological monitoring of exposure to hexavalent chromium. Int Arch Occup Environ Health. 1985; 55(4): 305−318.
23. Minoia C, Cavalleri A. Chromium in urine, serum and red blood cells in the biological monitoring of workers exposed to different chromium valency states. Sci Total Environ. 1988; 71(3): 323−327.
24. Stridsklev IC, Schaller KH, Langard S. Monitoring of chromium and nickel in biological fluids of stainless steel welders using the flux-cored-wire (FCW) welding method. Int Arch Occup Environ Health. 2004; 77(8): 587−591.
25. De Flora S, D'Agostini F, Balansky R, Micale R, Baluce B, Izzotti A. Lack of genotoxic effects in hematopoietic and gastrointestinal cells of mice receiving chromium (VI) with the drinking water. Mutat Res. 2008; 659(1-2): 60−67.
26. Kuykendall JR, Miller KL, Mellinger KN, Cain AV. Waterborne and dietary hexavalent chromium exposure causes DNA-protein crosslink (DPX) formation in erythrocytes of largemouth bass Micropterus salmoides. Aquat Toxicol. 2006; 78(1): 27−31.
27. Burden VM, Sandheinrich MB, Caldwell CA. Effects of lead on the growth and delta-aminolevulinic acid deshydratase activity of juvenile rainbow trout. Oncorhynchus mykiss. Environ Pollut. 1998; 101(2): 285−289.
28. Gurer H, Ozgune H, Neal R, Spitz DR, Erçal N. Antioxidant effects of N-acetylcysteine and succimer in red blood cells from lead-exposed rats. Toxicology. 1998; 128(3): 181−189.
29. Paustenbach DJ, Finley BL, Mowat FS, Kerger BD. Human health risk and exposure assessment of chromium (VI) in tap water. J Toxicol Environ Health A. 2003; 66(14): 1295−1339.
30. Steinhagen D, Helmus T, Maurer S, Dinakaran Michael R, Leibold W, Scharsack JP, Skouras A, Schuberth HJ. Effect of hexavalent carcinogenic chromium on carp cyprinus carpio immune cells. Dis Aquat Org. 2004; 62: 155−161.
31. Arunkumar RI, Rajasekaran P, Michael RD. Differential effect of chromium compounds on the immune response of the African mouth breeder Oreochromis mossambicus (Peters). Fish Shellfish Immunol. 2000; 10: 667−676.
32. Lukanova A, Toniolo P, Zhitkovich A, Nikolova V, Panev T, Popov T, Taioli E, Costa M. Occupational exposure to Cr (VI): comparison between chromium levels in lymphocytes, erythrocytes, and urine. Int Arch Occup Environ Health. 1996; 69(1): 39−44.
33. VK Raghunathan, Ellis EM, Grant MH. Response to chronic exposure to hexavalent chromium in human monocytes. Toxicol In Vitro. 2009; 23(4): 647−652.
34. Geetha S, Singh V, Ram MS, IIavazhagan G, Banerjee PK, Sawhney RC. Immunomodulatory effects of seabuckthorn (Hippophae rhamnoides L.) against chromium (VI) induced immunosuppression. Mol Cell Biochem. 2005; 278(1-2): 101−109.
35. Gao M, Binks SP, Chipman JK, Levy LS, Braithwaite RA, Brown SS. Induction of DNA strand breaks in peripheral lymphocytes by soluble chromium compounds. Hum Exp Toxicol. 1992; 11: 77−82.
36. Coogan TP, Snyder MCA, Squib KS, Costa M, Differential DNA-protein crosslinking in lymphocytes and liver following chronic drinking water exposure of rats to potassium chromate. Toxicol Appl Pharmacol. 1991; 109: 60−72.
37. Taiola E, Zhitkovich A, Kinney P, Udasin I, Toniolo P, Costa M. Increased DNA-protein crosslinks in lymphocytes of residents living in chromium-contaminated areas. Biol trace Elem Res. 1995; 50(3): 175−180.
38. Asiak JB, Kowalik J. A comparison of the in vitro genotoxicity of tri- and hexavalent chromium. Gen Toxicol Environ Mut. 2000; 469: 135−145.
39. Geetha S, Sai Ram M, Singh V, Ilavazhagan G, Sawhney RC. Anti-oxidant and immunomodulatory properties of seabuckthorn (Hippophae rhamnoides): an-in vitro study. J Ethnopharmacol. 2002; 79: 373−378.

40. Cohen MD, Zelikoff JT, Chen LC, Schlesinger RB. Immunotoxicologic effects of inhaled chromium: role of particle solubility and co-exposure to ozone. Toxicol Appl Pharmacol. 1998; 152(1): 30−40.
41. Raghunathan VK, Tettey JN, Ellis EM, Grant MH. Comparative chronic in vitro toxicity of hexavalent chromium to osteoblasts and monocytes. J Biomed Mater Res A. 2009; 88(2): 543−550.
42. Lee SH, Brenan R, Jacobs JJ, Urban RM, Ragasa DR, Glant TT. Human monocyte/macrophage response to cobalt-chromium corrosion products and titanium particles in patients with total joint replacements. J Orthop Res. 1997; 15(1): 40−49.
43. Wang JY, Wicklund BH, Gustilo RB, Tsukayama DT. Titanium, chromium and cobalt ions modulate the release of bone-associated cytokines by human monocytes/macrophages in vitro. Biomaterials. 1996; 17(23): 2233−2240.
44. Santos FW, Zeni G., Rocha JBT, do Nascimento PC, Marques MS, Nogueira CW. Efficacy of 2, 3-dimercapto-1-propanesulfonic acid (DMPS) and diphenyl diselenide on cadmium induced testicular damage in mice. Food Chem Toxicol. 2005; 43: 1723−1730.
45. Sugawara N, Hirohata Y, Sugawara C. Testicular dysfunction induced by cadmium and its improvement caused by selenium in the mouse. J Environ Pathol Toxicol Oncol. 1989; 9(1): 53−64.
46. Yiin SJ, Cem CL, Sheu JY, Lin TH. Cadmium-induced liver, heart and spleen lipid peroxidation in rats and protection by selenium. Biol Trace Elem Res. 2000; 78(1-3): 219−230.
47. Chen RW, Lacy VL, Whanger PD. Effect of selenium on methyl mercury binding to subcellular and soluble proteins in rat tissues. Res Commun Chem Pathol Pharmacol. 1975; 12: 55−65.