

Research Article



Meryem Şentürk*, Fatma Uyanık and Zuhale Hamurcu

Effects of chromium picolinate on the parameters of oxidative and chromosomal DNA damage in rabbits

Krom pikolinat'ın tavşanlarda oksidatif ve kromozomal DNA hasarı parametrelerine etkisi

<https://doi.org/10.1515/tjb-2016-0220>

Received November 1, 2016; accepted August 10, 2017; previously published online September 9, 2017

Abstract

Objectives: This study investigated the effects of chromium chloride ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$), chromium picolinate (CrPic) and picolinic acid on malondialdehyde (MDA), 8-hidroksi-2'-deoksiguanosine (8-OHdG) and genome damage in rabbits.

Material and methods: Forty, New Zealand rabbits were equally assigned to four groups which received either distilled water or 20 mg/day Cr ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$), 200 µg/day CrPic and 1400 µg/day picolinic acid for 50 days. On the 25th and 50th days, MDA and 8-OHdG levels and the apoptotic-necrotic cells, micronucleus (MN), micronuclear buds (NBUD) and nucleoplasmic bridges (NPB) frequencies and on the 50th day, serum Cr and liver MDA levels were determined.

Results: CrPic increased live weight and feed consumption. On the 25th day of treatment, decreases were detected in MDA levels and MN, NPB and NBUD frequencies in CrPic and picolinic acid groups, and in 8-OHdG levels in $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ and CrPic groups. Slight or significant differences were determined in all investigated parameters between the measurements of on days 25 and 50.

Conclusion: Improvements in of MDA and 8-OHdG levels and genome damage indicators due to CrPic and picolinic

acid on the 25th day of the treatment may show that short term of CrPic supplementation reduces oxidative and chromosomal DNA damage in rabbits.

Keywords: Apoptosis; Chromium picolinate; DNA damage; Micronucleus; Oxidative stress.

Özet

Amaç: Bu çalışma, tavşanlarda krom klorür ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$), krom pikolinat (CrPic) ve pikolinik asidin malondialdehit (MDA), 8-hidroksi-2'-deoksiguanozin (8-OHdG) ve genom hasarı üzerine etkilerinin belirlenmesi amacıyla gerçekleştirildi.

Gereç ve Yöntem: Çalışmada 40 Yeni Zelanda tavşanı, eşit sayıda olacak şekilde 4 gruba ayrılarak distile su veya $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ formunda 20 mg/gün Cr, 200 µg/gün CrPic ve 1400 µg/gün pikolinik asit 50 gün verildi. Denemenin 25. ve 50. günlerinde MDA ve 8-OHdG düzeyleri ve apoptotik -nekrotik hücre sayıları, mikronükleus (MN), nükleoplazmik köprü (NPK) ve nükleer bud (NBUD) sıklığı ile denemenin 50. gününde serum Cr ve karaciğer MDA düzeyleri belirlendi.

Bulgular: Chromium picolinate (CrPic) yem tüketimi ve canlı ağırlığı arttırdı. Çalışmanın 25. gününde, CrPic ve pikolinik asit verilen gruplarda MDA düzeyleri ile MN, NPK ve NBUD sıklığı, $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ve CrPic verilen gruplarda da 8-OHdG düzeyleri azaldı. İncelenen tüm parametreler yönünden 25. ve 50. günlerde yapılan ölçümler arasında önemsiz yada önemli farklılıklar saptandı.

Sonuç: Çalışmanın 25. gününde CrPic ve pikolinik asit; MDA, 8-OHdG ve genom hasarı göstergelerinde iyileşmeye neden olduğundan tavşanlara kısa süreli CrPic verilmesinin oksidatif ve kromozomal DNA hasarının önlenmesinde etkili olabileceği kanaatine varıldı.

Anahtar Kelimeler: Apoptozis; DNA hasarı; Krom pikolinat; Mikronükleus; Oksidatif stress.

*Corresponding author: Meryem Şentürk, Faculty of Veterinary Medicine, Department of Biochemistry, University of Erciyes, Kayseri 38039, Turkey, Tel.: 03522076666/29851, Fax: 03523372740, e-mail: meryemgltn@hotmail.com.

<http://orcid.org/0000-0002-3763-8310>

Fatma Uyanık: Graduate School of Health Sciences, University of Erciyes, Kayseri, Turkey, e-mail: fatmauyanik@hotmail.com

Zuhale Hamurcu: Faculty of Medicine, Department of Medical Biology, University of Erciyes, Kayseri, Turkey, e-mail: zhamurcu@erciyes.edu.tr

Introduction

Trivalent chromium (Cr^{+3}) is an essential trace element that is required for the regulation of carbohydrate, lipid, protein and nucleic acid metabolisms [1–3]. Several studies have claimed that chromium deficiency can lead to symptoms similar to type 2 diabetes, cardiovascular disease and glucose intolerance; growth retardation, lack of glucose and lipid homeostasis [2, 3] and these symptoms can be reversed with chromium supplementation [1–3].

Chromium compounds are found in organic and inorganic forms [4]. The absorption of inorganic chromium is poor due to the difficulty of passing through the membranes [5]. Solely 0.04%–2% of Cr^{+3} taken in the body can be absorbed and this absorption is affected by oxalate, iron and carbohydrates present in the nutrients [6, 7]. Therefore, several compounds were chelated with organic substances such as picolinic acid, nicotinic acid, propionic acid and methionine to increase the absorption of chromium [8] thus organic Cr compounds are more readily absorbed compare to the inorganic compounds [9].

Previous studies have shown the beneficial effects of chromium compounds on body development in human [10, 11] and performance [12–17], carcass traits [18, 19] and immunity [14, 20] in several animal species. Some previous studies suggested that CrCl_3 inhibits lipid peroxidation and ROT formation [21], reduced elevated malondialdehyde (MDA) level in quail exposed to heat stress [13, 22].

Chromium picolinate (CrPic) is commonly used as a dietary supplement particularly in human. In the form of CrPic, 5 and 10 mg/kg Cr reduced oxidative stress and inflammation in obese rats [23] and 100 and 250 mg/kg CrPic did not cause DNA damage in diabetic obese mice [24]. However, some studies investigating the effects of CrPic on people in details showed no beneficial effects on weight loss, fat loss and tissue size [25, 26] some other studies claimed even adverse effects such as cytotoxicity and genotoxic of this organic chromium compound [27–29]. Moreover, the safety of CrPic has been questioned recently. It has been claimed that CrPic causes chromosomal damage due to the picolinate content [30]. In physiological conditions, the CrPic is reduced to Cr^{+2} compounds by biological reductants such as ascorbic acid and thiol, which are air sensitive. These reductants create hydroxyl radicals that cause deoxyribonucleic acid (DNA) damage [31, 32]. Studies suggesting the cytotoxic and genotoxic effects of CrPic were generally carried out in vitro [24, 31, 33]. However, limited studies investigated the effects of CrPic on similar variables at in vivo conditions [29, 34].

In the previous studies investigating possible cytotoxic and genotoxic effects of CrPic, conflicting results have been reported [27, 29, 35, 36]. Therefore, the presented study was performed in rabbits to investigate the effects of trivalent chromium as an inorganic form, CrCl_3 , and a commonly used organic form especially in human, CrPic, along with the picolinic acid ($\text{C}_6\text{H}_5\text{NO}_2$), which was accused to have clastogenic effects, on serum and liver levels of (MDA), which is an indicator of oxidative stress, and plasma 8-OHdG, an indicator of oxidative DNA damage. In addition, it was investigated that whether these inorganic and organic chromium compounds cause genomic damage.

Materials and methods

Animals, management and experimental design

In the study, 40, 16 weeks old New Zealand rabbits obtained from the Hakan Çetinsaya Experimental and Clinical Research Center, Faculty of Medicine (DEKAM), University of Erciyes were used. Animals were maintained in polycarbonate caged sized $48 \times 61 \times 46$ cm (two rabbits in each) at this center that provides the highest possible health status and appropriate high standard conditions ($21^\circ\text{C} \pm 2^\circ\text{C}$ room temperature, $50\% \pm 5\%$ humidity, environmental ventilation systems providing air flow rotation of 12 per hour and 12 h light-12 h dark lightening schedule) for the animal welfare throughout the study.

A commercially available pellet diet for rabbits containing 2500 kcal/kg metabolizable energy (ME), 17% crude protein and 1648 ppm Cr by analysis that met the daily nutritional requirement of the rabbits and routinely used for the feeding of rabbit in DEKAM was provided throughout the experiment. Water and feed were supplied ad libitum during the study. In the beginning of the study, all animals were weighed to provide equal mean live weight of the groups, and they assigned into four groups containing 10 animals (five male, five female) in each. Rabbits in groups I (control), II, III and IV received either 2 mL distilled water or 20 mg/day Cr as $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, 200 $\mu\text{g}/\text{day}$ CrPic and 1400 $\mu\text{g}/\text{day}$ picolinic acid, which is the picolinic acid level in CrPic, respectively by the gavage method for 50 days. The above mentioned Cr levels were used in the present study because 20 mg/kg of CrCl_3 had been found to be effective in the studies [17, 18], the daily level of CrPic used in humans had been assumed to be 200 μg , 1400 μg picolinic acid corresponds to the level of picolinic acid contained in CrPic. This study was approved

by the Erciyes University Experimental Animal Ethics Committee with approval no. 10/67 dated 11.08.2010.

Data and sample collection

Live weights after 12 h fasting and feed consumption of the animals were recorded at weekly intervals. Blood samples (5–6 mL) were collected from V. auricularis caudalis of the animals on 25th and 50th days of the experiment into tubes with heparin for CBMN cyt and 8-OHdG analyses and without anticoagulants for MDA and Cr analyses. Plasmas were separated immediately and sera were separated by centrifugation at $1300\times g$ for 10 min following 1 h incubation at room temperature. Samples were aliquoted into cryovials, and plasma samples for 8-OHdG and serum samples for MDA were kept at -80°C whereas sera for Cr analyses were stored at -20°C until analyses.

At the end of the experiment, rabbits were euthanized with 45–50 mg/kg of ketamine/xylazine mixture. Collected liver samples into sterile plastic bags were transferred to the laboratory under cold chain, and immediately processed for MDA analysis. Liver samples weighing 25 mg were put into 1.5 mL of sterile cryovials. The RIPA buffer (Cayman, 10010263) containing 250 μL protease inhibitor was added to each vial, and sonicated at 40 V for 15 s under ice then centrifuged at 4°C at $1600\times g$ for 10 min. Separated tissue supernatants were kept at -80°C until the determination of MDA levels.

Food samples were collected from five different parts of the feed bulk, and these samples were combined and blended then chemical analysis of the food was performed according to A.O.A.C. [37]. ME was calculated according to Carpenter ve Clegg [38].

Measurement of the MDA and 8-OHdG levels

Serum and tissue MDA (TBARS ELISA Kit, Cayman, USA, Cat. no 10009055) and plasma 8-OHdG (Northwest Life Science Specialistand LLC, Washington, Code: NWK 8-OHdG 02) levels were determined by enzyme linked immunosorbent assay (ELISA) according to the instruction of manufacturers of commercially available kits using μQuant (Bio-Tek) ELISA reader.

Measurement of chromium in food and sera

Feed and serum chromium levels were determined at Erciyes University Technology Research and Application

Center. The food sample (250 mg) in Teflon cell was digested with 5 mL of 65% HNO_3 in microwave for 20 min then analyzed with ICP/MS (Agilent 7500a series). Standard solutions containing 0, 1, 5, 10, 20, 30, 40, 50 ppb chromium, and for correction of the bias in calibration curve, internal standards containing Be, Sc, Ra and Bi were used for determination of chromium in food and serum samples.

T-Lymphocyte cultures

Two sets of T-lymphocyte culture were prepared from each rabbit immediately. An amount of 0.4 mL whole blood sample taken from heparin containing tubes were inoculated into tubes containing 4 mL peripheral blood karyotyping medium (complete culture medium w/o phytohemagglutinin; Biological Industries, B-01-198-1B) then incubated at 37°C for 72 h for cytokinesis-block micronucleus cytome assay (CBMN cyt).

Cytochalasin-B (cyt-B) (Sigma-Aldrich Co., St. Louis, MO, USA Cat. no:6762) measuring 75 μL was added to the cultures providing a 3 $\mu\text{g}/\text{mL}$ final concentration at 44 h of incubation for determination of binucleated cells. Incubation was terminated at 72 h, the cultures were treated for 4 min with hypotonic 0.1 M KCl solution (Merck, 340TA611835), and fixed in two cycling of cold methanol:glacial acetic acid (3:1) (Merck, 247K18855556.). The fixed cells were spread onto glass slides and stained with 6% Giemsa for 8 min. Four slides from two parallel cultures of each rabbit were prepared. All of the slides were coded and blind red.

Examination of the slides

Slides were examined under an optical microscope (Zeiss Primo Star) at $\times 40$ magnification. The number of micronucleus (MN), micronuclear buds (NBUD) and NPB in 1000 binucleated cells were recorded to determine DNA damage. To determine the cell proliferation rate, 1000 mononuclear cells were counted, and binucleated and multinucleated cells were scored. Nuclear Division Index (NDI) was calculated using the following formula: $\text{NDI} = (\text{M1} + 2\text{M2} + 3\text{M3} + 4\text{M4})/\text{N}$. Membrane blebs and condensed and pyknotic nuclei in cells were considered as indicators of apoptosis. Swollen cells, lysis in cell membranes and leakage of cell content were accepted as necrosis. Apoptotic and necrotic cells were scored in 1000 mononuclear cells for cell death. The frequencies of MN, NBUD, NPB and NDI were calculated as the percentage for each rabbit.

Analysis of data

Statistical analysis of the data was performed with SPSS 15.0 software package. Number of total rabbits (40) and rabbits ($n=10$) in each group were determined with G-Power software (84%). The normality testing of the data were determined with Shapiro-Wilk test. The difference between the groups was detected with one-way analysis of variance (ANOVA). When F values were significant, Duncan Multiple Comparison Test was performed. The differences between groups and within groups were determined with repeated measures ANOVA. All data were expressed as means \pm standard deviation of means (SD) unless otherwise stated.

Results

At the end of the experiment, compare to control group, live weight increased significantly in $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ($p=0.003$) and picolinic acid given animals whereas the increase in CrPic group could not reach significance. However, overall live weight changes showed a significant increase only in animals given CrPic ($p=0.026$). The food consumption was also higher in CrPic group (Table 1).

MDA levels on 25th day of treatment of the study decreased ($p=0.007$) in CrPic and picolinic acid-treated groups compare to the control group, and no significant difference was determined between the $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ and the control groups. At the end of the study, there was no significant difference between control and treatment groups. Considering the differences between sampling times, MDA levels in control and $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ given groups were significantly lower (respectively $p=0.001$, $p=0.000$) on 50th day of treatment than 25 whereas no difference was determined in animals treated with CrPic

and picolinic acid. Chromium and picolinic acid treatments had no effects on tissue MDA and serum Cr levels (Table 2).

Plasma 8-OHdG levels decreased in $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ and CrPic ($p=0.000$) given groups but no difference was observed between control and picolinic acid groups on the 25th day of treatment. At the end of the study, compare to control, slight but not significant decreases were determined in 8-OHdG levels in all treatment groups. No difference was seen between the 8-OHdG levels determined at first and second sampling times in CrPic group whereas significant decreases occurred in control, picolinic acid ($p=0.000$) and $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ($p=0.028$) treated animals on the 50th day of treatment compare to 25th day of treatment (Table 2).

The frequencies of MN in CrPic and MN, NPK, NBUD in picolinic acid treated rabbits decreased ($p=0.007$), $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ had no effect on these parameters on the 25th day of treatment. However, none of the treatments affected the MN, NPK, NBUD frequencies on the 50th day of treatment (respectively $p=0.084$, $p=0.145$, $p=0.271$). The frequencies of MN ($p=0.021$) and NBUD ($p=0.000$) in control; MN ($p=0.035$), NPK ($p=0.000$) and NBUD ($p=0.002$) in $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$; NPK ($p=0.014$) in CrPic given animals were lower on the 50th day of treatment than the percentages determined on the 25th day of treatment. No time effect of picolinic acid on MN, NPK and NBUD frequencies was observed (Table 3).

No difference was detected between groups concerning the apoptotic cell percentage on both 25th and 50th days of treatment. Necrotic cell counts in all of the treatment groups significantly decreased ($p=0.000$) on the 25th day of treatment whereas the necrotic cell percentage was significantly lower ($p=0.000$), solely in picolinic acid-treated animals than controls on the 50th day of treatment. Apoptotic ($p=0.006$) and necrotic ($p=0.000$) cell counts in control, necrotic cell counts in $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ($p=0.012$) and in picolinic acid ($p=0.011$) treated groups

Table 1: Live weight and food consumption of rabbits receiving chromium (III) chloride, chromium picolinate and picolinic acid.

Variables	Control ($n=10$) $\bar{X} \pm \text{SD}$	$\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ (20 mg/day) ($n=10$) $\bar{X} \pm \text{SD}$	CrPic (200 $\mu\text{g/day}$) ($n=10$) $\bar{X} \pm \text{SD}$	Picolinic acid (1400 $\mu\text{g/day}$) ($n=10$) $\bar{X} \pm \text{SD}$	p-Value
Live weight (kg)					
At the beginning	2.61 ± 0.35^a	2.80 ± 0.40^a	2.50 ± 0.17^a	2.77 ± 0.21^a	0.061
At the end	$2.91 \pm 0.23^{b,d}$	$3.23 \pm 0.28^{b,c}$	$3.04 \pm 0.14^{b,c,d}$	$3.17 \pm 0.18^{b,c}$	0.003
p-Value	0.037	0.018	0.000	0.001	
Live weight changes (kg)	0.30 ± 0.21^d	$0.43 \pm 0.30^{c,d}$	0.54 ± 0.38^c	$0.40 \pm 0.28^{c,d}$	0.026
Total food consumption (kg)	4.77	4.87	5.17	4.52	

^{a,b}The values with different superscripts in the same column differ significantly. ^{c,d}The values with different superscripts in the same row differ significantly. $\bar{X} \pm \text{SD}$, means \pm standard deviation of means.

Table 2: The malondialdehyde, 8-hydroxy-2'-deoxyguanosine and chromium levels in rabbits receiving chromium (III) chloride, chromium picolinate and picolinic acid.

Parameters	Control $\bar{X} \pm SD$	$CrCl_3 \cdot 6H_2O$ (20 mg/day) $\bar{X} \pm SD$	CrPic (200 $\mu g/day$) $\bar{X} \pm SD$	Picolinic acid (1400 $\mu g/day$) $\bar{X} \pm SD$	p-Value
MDA ($\mu mol/L$)	n = 10	n = 10	n = 10	n = 10	
Day 25	$6.22 \pm 3.04^{a,c}$	$5.81 \pm 1.63^{a,c}$	4.31 ± 1.33^d	4.16 ± 1.10^d	0.000
Day 50	2.13 ± 0.58^b	2.41 ± 0.87^b	3.11 ± 1.79	3.18 ± 1.44	0.126
p-Value	0.001	0.000	0.063	0.065	
8-OHdG (ng/mL)	n = 10	n = 10	n = 10	n = 10	
Day 25	$4.82 \pm 1.20^{a,c}$	$3.70 \pm 1.32^{a,d}$	3.73 ± 0.80^d	$5.05 \pm 0.69^{a,c}$	0.000
Day 50	3.33 ± 0.95^b	2.85 ± 0.86^b	3.03 ± 0.66	2.52 ± 0.64^b	0.069
p-Value	0.010	0.028	0.047	0.000	
Liver MDA ($\mu mol/L$) (Day 50)	n = 6	n = 6	n = 6	n = 6	
	12.36 ± 3.63	12.98 ± 3.11	11.55 ± 2.14	10.76 ± 2.00	0.555
Serum Cr (ppm) (Day 50)	n = 6	n = 6	n = 6	n = 6	
	0.0495 ± 0.016	0.0762 ± 0.013	0.0568 ± 0.021	0.0507 ± 0.012	0.061

^{a,b}The values with different superscripts in the same column differ significantly. ^{c,d}The values with different superscripts in the same row differ significantly. $\bar{X} \pm SD$, means \pm standard deviation of means.

Table 3: The frequencies of micronuclei, micronuclear buds and nucleoplasmic bridges in cultured peripheral lymphocytes of rabbits receiving chromium (III) chloride, chromium picolinate and picolinic acid.

Parameters	Control $\bar{X} \pm SD$	$CrCl_3 \cdot 6H_2O$ (20 mg/day) $\bar{X} \pm SD$	CrPic (200 $\mu g/day$) $\bar{X} \pm SD$	Picolinic acid (1400 $\mu g/day$) $\bar{X} \pm SD$	p-Value
MN (%)	n = 6	n = 6	n = 6	n = 6	
Day 25	$1.78 \pm 0.82^{a,c}$	$1.20 \pm 0.49^{a,c,d}$	0.83 ± 0.37^d	0.92 ± 0.41^d	0.007
Day 50	0.77 ± 0.37^b	0.62 ± 0.31^b	0.75 ± 0.41	1.08 ± 0.28	0.084
p-Value	0.021	0.035	0.720	0.427	
NPK (%)	n = 6	n = 6	n = 6	n = 6	
Day 25	0.90 ± 0.93^c	$0.98 \pm 0.40^{a,c}$	$0.63 \pm 0.38^{a,c,d}$	0.10 ± 0.09^d	0.000
Day 50	0.13 ± 0.19	0.08 ± 0.13^b	0.17 ± 0.05^b	0.28 ± 0.17	0.145
p-Value	0.075	0.000	0.014	0.063	
NBUD (%)	n = 6	n = 6	n = 6	n = 6	
Day 25	$8.40 \pm 2.57^{a,c}$	$7.12 \pm 2.91^{a,c}$	6.85 ± 1.99^c	4.17 ± 0.47^d	0.001
Day 50	2.42 ± 0.69^b	3.40 ± 0.68^b	5.40 ± 6.29	3.83 ± 1.34	0.271
p-Value	0.000	0.002	0.558	0.577	

^{a,b}The values with different superscripts in the same column differ significantly. ^{c,d}The values with different superscripts in the same row differ significantly. $\bar{X} \pm SD$, means \pm standard deviation of means.

Table 4: The cell death percentages in cultured peripheral lymphocytes of rabbits receiving chromium (III) chloride, chromium picolinate and picolinic acid.

Parameters	Control $\bar{X} \pm SD$	$CrCl_3 \cdot 6H_2O$ (20 mg/day) $\bar{X} \pm SD$	CrPic (200 $\mu g/day$) $\bar{X} \pm SD$	Picolinic acid (1400 $\mu g/day$) $\bar{X} \pm SD$	p-Value
Apoptotic cells (%)	n = 6	n = 6	n = 6	n = 6	
Day 25	6.75 ± 1.98^a	4.77 ± 3.18	3.82 ± 0.92	8.35 ± 5.41	0.054
Day 50	3.62 ± 0.92^b	3.30 ± 0.99	5.67 ± 4.91	4.07 ± 1.87	0.368
p-Value	0.006	0.306	0.386	0.097	
Necrotic cells (%)	n = 6	n = 6	n = 6	n = 6	
Day 25	$30.28 \pm 9.93^{a,c}$	$12.33 \pm 4.99^{a,d}$	7.12 ± 3.82^d	$6.53 \pm 2.19^{a,d}$	0.000
Day 50	$6.12 \pm 1.62^{b,c}$	$5.92 \pm 1.09^{b,c}$	6.23 ± 0.94^c	$3.40 \pm 1.17^{b,d}$	0.000
p-Value	0.000	0.012	0.594	0.011	

^{a,b}The values with different superscripts in the same column differ significantly. ^{c,d}The values with different superscripts in the same row differ significantly. $\bar{X} \pm SD$, means \pm standard deviation of means.

were lower on the 50th day of treatment than the levels determined on the 25th day of treatment. No time effect was detected in CrPic group (Table 4).

Discussion

Previous studies have showed that dietary trivalent chromium is involved in carbohydrate, lipid, protein and nucleic acid metabolisms [2, 17], and positively affects performance [14, 16] and carcass traits in several animal species [18, 19]. No effects of chromium on the live weight of the broilers [12], and laying quail [17, 19] supplemented with 20 mg/kg Cr in the form of $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ have been reported. However, some other studies indicated increases in live weight of broilers with CrPic [13] and $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ [15] and in turkey poults with chromium nicotinate supplementation [39]. In the presented study, a 50 day treatment of rabbits with inorganic and organic chromium compounds and picolinic acid increased live weight of the animals. The increased live weight reached significance in solely CrPic treated rabbits ($p=0.023$) those food consumption were also higher than control and the other treated animals. The increases in live weight may either resulted from increased food intake or may be resulted from better absorption and availability of this organic chromium complex [7, 40].

In recent years, CrPic is widely used as a supplement by people in many countries in various forms such as chewing gum, sports drinks, nutritional bars and drug in addition to the chromium provided by food [41]. However, despite the studies indicating the beneficial impact of CrPic [14, 16, 18, 19], the use of the CrPic safely has been questioned [31, 34, 41] recently due to the presence of several studies claiming the deleterious effects of this synthetic chromium compound on cell structure and DNA [27, 29, 30].

It has been demonstrated that air sensitive biological reductants such as ascorbic acid and thiol, which create hydroxyl radicals (HO^\bullet) leading the damage of DNA, reduce the trivalent chromium in CrPic to Cr^{+2} [32]. Formation of HO^\bullet and increases in cell damage with high level of CrPic have been reported by Stearns et al. [30] and Bagchi et al. [31]. In addition, the physiological concentrations (50–260 ppb) of the CrPic were also accused for the reactive oxygen species (ROS) formation [34] whereas it has been suggested that CrCl_3 avoids the ROS formation by suppressing the lipid peroxidation [21].

The MDA, one of the most frequently synthesized lipid peroxidation products [42] can reach the detectable level in blood and urine under oxidative stress

conditions [43]. The increased MDA level due to oxidative stress has been shown to be decreased by chromium treatment supplementation [3, 17]. In patients with type 2 diabetes, the increased thiobarbituric acid-reactive substances (TBARS) level due to high plasma glucose and autooxidation of small molecules was reduced by 18.2% with 400 $\mu\text{g}/\text{day}$ chromium picolinate by Anderson et al. [3]. In a hamster study performed by Vinson et al. [22], the combined chromium and grape seed extract reduced TBARS level by 77%.

The reduction in MDA content of the CrPic and picolinic acid groups ($p=0.000$) on 25th day of treatment supports the decline in the elevated serum MDA levels exposed to heat stress and chromium treatment [17]. In a rat study of Preuss et al. [44], supplementation of diet with 5 mg/kg of chromium acetate and chromium nicotinate reduced TBARS whereas CrPic less effective. CrCl_3 had no effect on renal and hepatic TBARS. Contrary to the results of Preuss et al. [44], in the presented study, CrPic and picolinic acid significantly decreased serum MDA ($p=0.000$) levels. CrPic and picolinic acid treatments also resulted in a slight but not significant decrease in liver MDA level. However, no effect of $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ on serum and liver MDA levels confirms the findings of Preuss et al. [44] who did not determine any effect of CrCl_3 on renal and hepatic TBARS. Lack of the effect of chromium on liver MDA level may be due to short residential time of this element in the cells thus lower accumulation in the liver, and may also due to the separation of this chelated compound into its components by the modification occurred in hepatocyte microsomes [21]. Furthermore, absence of the effects of CrPic and $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ on serum chromium level may result from the maintenance of the chromium in blood stream for short-term because of rapid excretion of chromium from organism [45].

In the organism, 8-OHdG is generated by the insertion of a OH group to C8 atom of guanine molecule through ROS [46]. In a study conducted in the calf's thymus, no effect of the trace metals such as CHCl_3 on the 8-OHdG stimulation was reported [47]. Chang et al. [48] have determined a fall in liver 8-OHdG level in parallel with the decrease in oxygen free radical levels in New Zealand rabbits. Similarly, in the presented study, on 25th day of treatment, decrease in serum MDA levels in animals treated with CrPic ($p=0.000$) and $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ (slight but not significant) were in harmony with the decrease in plasma levels of 8-OHdG ($p=0.000$). As 8-OHdG is an important indicator of oxidative DNA damage induced by ROS [49], the declines both in MDA and 8-OHdG indicates no deleterious effect of CrPic on DNA, instead this organic chromium compound would be helpful in preventing ROS dependent DNA damage.

Morphological changes in the nucleus shape and structure of fragmentation is the most important indicator of cellular damage. The morphological changes include chromatin condensation, formation of apoptotic cells, aneuploid DNA and DNA fragmentation. In some of the previous studies carried out on animals and in vitro conditions investigating the toxic effects of chromium compounds, some Cr^{+3} complexes have caused genotoxic and cytotoxic damages [27], whereas no adverse effects has been reported in the others [35].

Stearns et al. [30] suggested that both soluble and particular forms of CrPic ranging from 50 μM to 1.0 mM doses and picolinic acid doses higher than 40 $\mu\text{g}/\text{cm}^2$ were clastogenic in Chinese hamster ovary. These authors showed that relatively high doses generated clastogenic effects, and cell cycling was retarded and metaphase was reduced due to the toxic effects of the high chromium levels. Komorowski et al. [33], investigated cytogenetic the effects of CrPic on bone marrow of Sprague-Dawley rats receiving diet supplemented with 33, 250 or 2000 mg/kg CrPic and showed no chromosomal abnormalities in male or female rats. High levels of dietary CrPic given to rats (2500 mg/kg) and mice (50,000 mg/kg) caused neither chromosomal aberrations nor increases in micronuclei frequency [50].

In a study by Whittaker et al. [36] who evaluated the cellular toxicity and mutagenicity of CrPic, CrCl_3 and picolinic acid in *Salmonella typhimurium* and in L5178Y mouse lymphoma mutation assay, no mutagenic effects due to CrPic and CrCl_3 in the bacteria were reported. However, these authors showed mutagenic response induced by relatively nontoxic doses of these chromium compounds in the L5178Y mouse. İmamoğlu et al. [29] also reported the increases in the frequency of MN in lymphocytes of calves receiving 400 μg CrPic daily but they determined no increase in calves receiving 200 μg CrPic. However, in the present study, the frequencies of MN and NBUD in chromium (III) chloride, CrPic and its moiety picolinic acid receiving rabbits was in the reduction trend. CrPic and picolinic acid reduced NPK frequency. The more pronounced declines were determined with CrPic and picolinic acid ($p=0.007$) considering the MN frequency whereas the decrease in NBUD ($p=0.001$) and NPK ($p=0.000$) were significant solely in picolinic acid receiving animals.

Manygoats et al. [27] reported CrPic-induced morphological changes in CHO AA8 cells. They found increases in apoptosis with the increasing CrPic levels which was consistent with the results of the study performed by İmamoğlu et al. [29] indicating a dose-dependent increase of the percentage of irregularly shaped and fragmented nuclei and apoptotic cells. Shirivastava et al. [28] have

claimed that the 1, 10, 50 ve 100 μM doses of trivalent chromium complexes such as CrPic, CrCl_3 , $[\text{Cr}(\text{salen})(\text{H}_2\text{O})_2]^+$, $[\text{Cr}(\text{EDTA})(\text{H}_2\text{O})]^-$, $[\text{Cr}(\text{en})_3]^{3+}$, and $[\text{Cr}(\text{ox})_3]^{3-}$ were cytotoxic at nuclear level in human dermal fibrocyt. On the other hand, in the present study, no difference was observed in apoptotic cell percentage on 25th day of treatment whereas the percentages of necrotic cell were reduced ($p=0.000$) by $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, CrPic and picolinic acid but the decrease was significant ($p=0.000$) only in picolinic acid receiving rabbits on 50th day of treatment. Lack of the influence of $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ and CrPic on apoptotic cell generation whereas causing reduction in necrotic cell formation confirms the findings of Komorowski et al. [33] who evaluated the cytogenetic effects of CrPic in bone marrow cells of Sprague-Dawley rats dosed orally with 33, 250 or 2000 mg/kg of CrPic and found no chromosomal anomalies.

Determination of inconsistent results with some previous studies indicating that chromium complexes produce genotoxic and cytotoxic effects may be due to the use of lower chromium doses than those studies [29, 30, 32, 36].

The results of this study have showed that supplementation of 20 mg/kg $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, 200 μg CrPic and 1400 μg picolinic acid for a short-term provided improvements in MDA and 8-OHdG levels instead of genotoxic and cytotoxic effects. CrPic can be used for reducing oxidative and chromosomal DNA damage.

Acknowledgements: This study was supported by the Scientific Research Projects Commission of Erciyes University, project number: TSD-10-3326, and summarized from PhD thesis., (Grant / Award Number: '16.409 Turkish Liras').

Conflict of interest statement: There are no conflicts of interest among the authors.

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