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The Effects of High Dietary Doses of Chromium(III) Complex with Propionic Acid on Nutritional and Selected Blood Indices in Healthy Female Rats

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Abstract People taking dietary supplements are usually determined to lose weight, supplement nutrition or reduce the risk of illness and negative effects of their state of health. Chromium(III) supplementation influence body composition and mass, glucose and lipid metabolism and it enhance insulin action. This fact could be of general interest because diabetes mellitus is an increasing health problem in many countries. The study describes the effects of high dietary doses of chromium(III) complex with propionic acid [Cr3] (from 100 to 1000 mg $Cr \cdot kg^{-1}$ diet) on the organisms of healthy female rats, with special regard to overall nutritional, carbohydrate, lipid and blood biochemical and morphological and haematological indices. The study was carried out on 30 10-week-old female Wistar rats, which were divided into five equal groups (six animals in each): the control group and four groups of tested animals which had free access to the diet supplemented with 100, 200, 500 and 1000 mg $Cr \cdot kg^{-1}$ (equivalent of 10, 20, 50 and 100 mg Cr·kg body weight (b.w.)·day^{-1}), given as $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]\cdot NO_3$, also known as Cr3, for 4 weeks. There were no significant differences in body mass gains, feeding efficiency ratio, internal organ masses or blood serum glucose concentrations, except for some changes in the serum triglycerides concentration, which decreased in the rats that received 500 and 1000 mg $Cr \cdot kg^{-1}$ diet, as opposed to the group treated with 200 mg $Cr \cdot kg^{-1}$ diet. The dietary supplementation of Cr3 for 4 weeks at doses of 100 to 1000 mg $Cr \cdot kg^{-1}$ diet did not affect overall nutritional indices and most blood biochemical, morphological and haematological indices.

Keywords Chromium(III) propionate complex · Supplementation rats

Introduction

Many studies have shown that Cr(III) plays an important role in normal carbohydrate, fat and protein metabolism and it improves insulin sensitivity [1–3]. However, the molecular mechanism of chromium action has not been thoroughly investigated. For this reason, the essentiality of chromium(III) has been greatly debated, as well as the effects of nutritional and pharmacological chromium(III) supplementation, especially on healthy subjects [4–7].

In the last two decades, trivalent chromium has become very popular as a nutritional supplement [8, 9]. It is used for body mass loss, building lean muscle mass and improving glucose and lipid metabolism [6]. The latest study by Ulas et al. [10] indicates the beneficial effects of CrHis supplementation on rats with diabetic retinopathy. Chromium is the second best-selling mineral supplement in the USA after calcium and before iron [11]. The Food and Nutrition Board of the US National Academy of Science set the adequate intake (AI) of chromium at 25 μg·day^{-1} for adult women and 35 μg·day^{-1} for men [12]. Trivalent chromium, the form found in food and dietary supplements, is considered to be safe [13]. The absorption rate of Cr(III) is very low, between 0.5 and 2 %. However, the organic forms are more absorbable than the inorganic ones [2, 14–18]. Due to the low absorption rate of chromium salts, it has become necessary to design and develop new organic chromium compounds

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[19, 20]. Many organic chromium complexes, including chromium picolinate, chromium nicotinate [21, 22], chromium histidinate [23, 24], chromium complex of D-phenylalanine [19, 25], chromium propionate complex [26, 27] and chromium glycinate complex [28] have been synthesised and demonstrated to be biologically effective. The form of trivalent chromium compound has been observed to affect its biological efficacy and toxicity [20, 29]. Chromium picolinate [$Cr(pic)_3$] is the most popular commercial chromium nutritional supplement. However, the safety of $Cr(pic)_3$ supplementation remains controversial [30].

The $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ cation, known as Cr3, has been studied and proposed as an alternative supplemental source of trivalent chromium. In a study conducted by Clodfedler et al. in 2004 [31, 32], the trinuclear cation $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ was found in vitro to imitate the chromodulin ability to stimulate the tyrosine kinase activity of the insulin receptor and increase insulin sensitivity, decrease plasma total cholesterol, LDL cholesterol as well as triglycerides concentration, as was proved on healthy and type-2 diabetic rat models.

Previous research has suggested that chromium propionate complex is absorbed with very high efficiency of 40–60 %, while popular Cr supplements such as: $CrCl_3$, Cr(III) nicotinate or $Cr(pic)_3$ are absorbed at only 0.5–1.3 % of the gavage dose [27].

The biological activity and safety of Cr3 has been studied on various experimental models. In a previous study, Staniek et al. [33, 34], Cr3 was demonstrated to exhibit low acute toxicity (LD_{50}>2000 mg·kg^{-1} body mass, the fourth class in the EU classification system), low genotoxic potential [34] and low teratogenic effect on female healthy rats [35]. Moreover, Cr3 was shown to improve carbohydrate and lipid metabolism in healthy male Wistar rats [36] as well as insulin sensitivity in male Wistar rats fed with a high-fructose diet [37]. Cr3 given at doses of 10 and 50 mg·kg^{-1} diet (equals to 1 and 5 mg Cr·kg^{-1} body mass per day) for 8 weeks was able to restore insulin sensitivity and normalise the β cell function almost to the level of the healthy Wistar rats in the insulin-resistant rat model [37].

In this study, we focused on the effects of high dietary doses of Cr3 on nutritional and selected blood indices in female rats.

Material and Methods

Test Chemicals

Chromium(III) propionate (Cr3) in the form of nitrate salt (chemical formula $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+NO_3^-$ was obtained in a laboratory at the Department of Technology and Instrumental Analysis, Poznań University of Economics, with the method described by Earnshaw et al. [38]. The contents of elemental Cr (21 %) was determined with the AAS method (spectrometer AAS-3 with BC correction, Zeiss, Germany).

Animals and Diets

Thirty female Wistar rats (n=30 females, age: 10 weeks old) were received from the Department of Toxicology, Medical University of Poznań, Poland. The animals were housed in the university-approved animal facility, in rooms maintained at 22±1 °C, with 55–60 % humidity and 12-h photoperiod (12-h light/dark cycle). After 5-day adaptation to the laboratory conditions, the rats were divided into five equal groups (the control group and groups treated with chromium(III) complex with propionic acid—six animals in each group, equal body mass 180 g). All the groups were fed with a commercial diet for maintenance of adult rodents (*Labofeed H*), enriched with 0, 100, 200, 500 and 1000 mg Cr(III)·kg^{-1} of diet (ca. 0, 10, 20, 50 and 100 mg Cr·kg body weight (b.w.)·day^{-1}) given as Cr3 for 4 weeks (Table 1). The Cr content in the basic diet was 0.5±0.06 mg·kg^{-1} (control group – C). The following contents of Cr(III) were measured in individual experimental diets: 107.5±6.5 mg·kg^{-1} A; 224.8±32.4 mg·kg^{-1} B; 535.5±26.22 mg·kg^{-1} C and 1049.5±17.6 mg·kg^{-1} D, respectively. The recommended level of dietary Cr for rats is around 1 mg·kg^{-1} diet (AIN-93). In the experiment, we used supranutritional doses of Cr, which were 100, 200, 500 and 1000 times greater than the reference one.

The rats were allowed free access to food and distilled water throughout the experiment period. The feed intake was measured daily, while body weight gains were monitored weekly. At the end of the experiment, after 12-h starvation, the rats were euthanised by intraperitoneal injection of thiopental (40 mg·kg^{-1} body weight). Blood was collected into tubes, and tissue samples (liver, kidneys, heart, spleen, pancreas, ovaries) were collected and weighed. All the procedures applied to animals had been approved by the Local Bioethical Commission in Poznań (no. 12/2005).

Laboratory Analysis

Blood serum indices were determined with the following methods: glucose concentration with the UV photometric method [39] and total, LDL, HDL and triglycerides (triacylglycerol) concentrations (TAG) with the colorimetric methods [40–42], using Olympus AU 560 equipment. The activity of ALT, AST and AP enzymes was measured with the kinetic methods [43, 44] and the urea concentration was measured with the kinetic method, using urease and glutamine dehydrogenase [45]. The total protein concentration was measured with the colorimetric method, using Cu^{2+} ions [46] and the creatinine concentration was measured with the Jaffe kinetic method with picric acid [45].

Table 1 Composition of the basic *Labofeed H* diet used in experiment (mean±SD)

Component	Unit	Content of compound
Energy	MJ·100 g^{-1}	1.69±0.03
Fat	%	3.16±0.07
Protein	%	24.10±0.21
Carbohydrates	%	54.96
Dry mass	%	88.73±0.05
Ash	%	6.51±0.11
Ca	g·kg^{-1}	13.41±1.61
Mg	g·kg^{-1}	2.24±0.06
Fe	mg·kg^{-1}	239.49±46.34
Zn	mg·kg^{-1}	133.19±42.31
Cu	mg·kg^{-1}	20.42±2.91

The blood haemoglobin (Hb) level was determined with the Drabkin cyanohaemoglobin method. The red blood cell count (RBC) and other blood morphology indices (haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), platelets (PLT), lymphocytes (LYMPH), granulocytes (GRAN), minimum inhibitory dilution (MID), platelet distribution width (PDW), mean platelet volume (MPV), red cell distribution width based on standard deviation (RDW) were obtained by means of the CELLDYN-1700 analytical haematology system [47].

For mineral analyses, diet samples were digested with concentrated 65 % spectra pure HNO_3 (Merck) in a Microwave Digestion System (MARS-5, CEM, USA). The concentrations of copper (Cu), zinc (Zn), iron (Fe), magnesium (Mg) and calcium (Ca) in mineralised samples were determined with the flame atomic absorption spectrometry method (F-AAS; Zeiss AAS-3, with BC, Germany). The Cr concentration was measured with a graphite furnace atomic absorption spectrometer (AAS EA 5, with BC, Jenoptic, Germany).

Statistical Analysis

The results are presented as mean±SEM. The data were analysed by means of one-way analysis of variance (ANOVA/MANOVA), followed by the Tukey's test to determine specific significant differences ($p<0.05$) using Statistica ver. 7.0 software (StatSoft, Tulsa, USA).

Results

The effects of Cr3 on overall nutritional indices are presented in Table 2. As can be seen, the high dietary doses of Cr3 (100 to 1000 mg Cr·kg^{-1} diet) did not affect the feed intake, body mass gain, feeding efficiency ratio and body mass or inner organs masses (absolute and relative) in the healthy female rats. No clinical signs of toxicity were observed. Table 3 presents serum biochemical indices, including glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides (TAG), urea, creatinine and total protein serum concentrations, as well as enzyme activities (ALT, AST and AP) in the treated and control female rats. The blood biochemical indices were not different in the Cr3-supplemented groups, except for the serum triglycerides concentration, which decreased by 32 % in the rats which received 500 and 1000 mg Cr kg^{-1} diet, as opposed to the group treated with 200 mg Cr kg^{-1} diet.

Tables 4 and 5 show blood morphological and haematological indices, including total white blood cell (WBC), red blood cell (RBC), lymphocytes (LYMPH), granulocytes (GRAN), platelet count (PLT), mid-cell (MID), mean platelet volume (MPV), platelet distribution width (PDW), haemoglobin (Hb), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and red cell distribution width (RDW) values in the treated and control rats. Most of these indices were not significantly different in the animals treated with Cr3, except the higher relative LYMPH (% L) (by 3.9 %) and lower MID values (% M) (by 32.6 %) in the group which received 500 mg Cr·kg^{-1} diet, as compared with the control rats. However, these changes did not go beyond the physiological ranges established for healthy rats.

Discussion

Previous studies indicated that organic forms of Cr(III) were more bioactive than inorganic forms (e.g., $CrCl_3$), probably due to the low absorption rate of inorganic chromium(III) compounds (0.5–2 %) [14–18]. In order to recognise the therapeutic potentials of Cr(III) supplementation, many organic chromium complexes were synthesised and their bioactivity was demonstrated.

Clodfelder et al. [27] reported that $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ was absorbed with very high efficiency of 40–60 %, better than popular Cr supplements, such as: $CrCl_3$, Cr(III) nicotinate or CrPic. The biological activity and safety of Cr3 has been studied on various experimental models [27, 31, 32, 34, 35, 48].

The ligand of the trivalent chromium compound has been proved to affect its absorption and toxicity [11, 20, 29]. The study of Staniek et al. [33] showed that LD_{50} of Cr3 was greater than 2,000 mg·kg^{-1} body mass when administered orally to rats. Other chromium (III) complexes did not exhibit acute toxic effects [3, 20–22, 28, 49–51].

It is suggested that supplementation with Cr(III) may have beneficial effects on the body composition and can be used as an adjuvant to weight loss [52, 53]. By improving cell sensitivity to

Table 2 The effect high dietary doses of Cr3 on nutritional indices in female rat (mean±SEM)

Index	Experimental groups					ANOVA
	Control (1 mg·kg^{-1})	A (100 mg·kg^{-1})	B (200 mg·kg^{-1})	C (500 mg·kg^{-1})	D (1000 mg·kg^{-1})	
Food intake (g·day^{-1})	18.3±0.5	18.3±0.4	18.5±0.4	19.2±0.5	20.1±0.2	NS
Body mass gain (g·day^{-1})	1.46±0.08	1.39±0.09	1.46±0.06	1.46±0.13	1.59±0.12	NS
Relative body mass gain (%)	0.81±0.04	0.76±0.05	0.81±0.03	0.81±0.06	0.88±0.07	NS
Feeding efficiency ratio (g·MJ^{-1})	4.72±0.24	4.47±0.23	4.68±0.22	4.50±0.33	4.64±0.32	NS
Final body mass (g)	205±10	200±9	202±7	203±6	205±6	NS
Liver mass (g)	5.644±0.148	5.595±0.290	5.730±0.299	5.276±0.204	5.631±0.264	NS
Relative liver mass (% b.w.)	2.76±0.06	2.80±0.10	2.83±0.08	2.60±0.04	2.75±0.08	NS
Kidneys mass (g)	1.431±0.051	1.395±0.050	1.468±0.066	1.411±0.052	1.414±0.033	NS
Relative kidneys mass (% b.w.)	0.70±0.02	0.70±0.02	0.72±0.02	0.70±0.01	0.69±0.02	NS
Spleen mass (g)	0.440±0.016	0.428±0.020	0.459±0.022	0.454±0.022	0.463±0.026	NS
Relative spleen mass (% b.w.)	0.22±0.01	0.21±0.00	0.23±0.01	0.22±0.01	0.22±0.01	NS
Heart mass (g)	0.664±0.029	0.626±0.017	0.658±0.018	0.620±0.020	0.659±0.023	NS
Relative heart mass (% b.w.)	0.32±0.01	0.31±0.01	0.33±0.01	0.31±0.00	0.32±0.00	NS
Pancreas mass (g)	0.630±0.020	0.724±0.066	0.702±0.040	0.657±0.028	0.634±0.045	NS
Relative pancreas mass (%)	0.31±0.02	0.37±0.05	0.35±0.02	0.33±0.02	0.31±0.03	NS
Ovaries mass (g)	0.097±0.005	0.093±0.005	0.093±0.006	0.099±0.006	0.100±0.004	NS
Relative ovaries mass (%)	0.047±0.001	0.047±0.003	0.046±0.004	0.048±0.003	0.049±0.002	NS

K control group, *A* supplemented group with 100 mg Cr(III)·kg^{-1} diet, *B* supplemented group with 200 mg Cr(III)·kg^{-1} diet, *C* supplemented group with 500 mg Cr(III)·kg^{-1} diet, *D* supplemented group with 1000 mg Cr(III)·kg^{-1} diet, *NS* differences statistically non-significant

insulin, Cr(III) may increase the utilisation of energy components and affect protein metabolism by stimulating the uptake of amino acids and thus, it may increase protein synthesis [54]. It is also proposed that Cr(III) increases the storage of glucose in muscle glycogen synthesis, thereby helping to reduce the deposition of fat and preventing obesity [53].

Onakpoya et al. [55] reported a clinically small but statistically significant weight loss in patients treated with Cr(III). Anton et al. [56] suggested that $Cr(pic)_3$ plays a role in food intake regulation, which may be mediated by direct effect on the brain, both in humans and animals. The authors indicate that by affecting the central nervous system Cr(III) can regulate food

Table 3 The effect high dietary doses of Cr3 on blood glucose concentration, lipid profile, hepatic enzymes activity and selected poisoning indices in female rat (mean±SEM)

Index	Experimental groups					ANOVA
	Control (1 mg·kg^{-1})	A (100 mg·kg^{-1})	B (200 mg·kg^{-1})	C (500 mg·kg^{-1})	D (1000 mg·kg^{-1})	
Glucose concentration (mg·dl^{-1})	105.7±4.9	98.7±8.6	98.8±4.1	92.5±2.5	94.8±3.9	NS
Total cholesterol concentration (mg·dl^{-1})	61.5±8.4	72.2±2.8	67.0±5.5	63.3±4.6	64.3±2.6	NS
LDL-cholesterol concentration (mg·dl^{-1})	4.67±0.49	5.17±0.54	5.17±0.54	5.00±0.26	5.17±0.87	NS
HDL cholesterol concentration (mg·dl^{-1})	39.8±3.6	41.5±1.3	39.8±1.8	37.2±3.5	38.8±1.0	NS
TAG - triglycerides concentration (mg·dl^{-1})	30.7±1.9^{ab}	32.2±2.8^{ab}	37.0±3.0^{b}	25.2±2.4^{a}	25.3±2.6^{a}	$p<0.05$
ALT (U·dm^{-3})	54.3±8.4	40.5±5.2	38.2±4.0	36.5±1.5	37.0±4.5	NS
AST (U·dm^{-3})	133.5±14.5	118.0±8.3	149.0±9.8	138.0±8.2	132.7±8.9	NS
Total protein concentration (g·dl^{-1})	5.10±0.08	5.10±0.15	5.15±0.13	5.15±0.13	4.92±0.06	NS
Creatinine concentration (mg·dl^{-1})	0.383±0.017	0.417±0.017	0.433±0.033	0.383±0.017	0.417±0.040	NS
Urea concentration (mg·dl^{-1})	45.8±2.0	49.3±2.1	57.5±5.3	50.2±1.3	47.7±7.2	NS

Different letter superscripts indicate a statistically significant difference at $p<0.05$

NS differences statistically non-significant

Table 4 The effect high dietary doses of Cr3 on blood morphological and hematological indices in female rat (mean±SEM)

Index	Experimental groups					ANOVA
	Control (1 mg·kg^{-1})	A (100 mg·kg^{-1})	B (200 mg·kg^{-1})	C (500 mg·kg^{-1})	D (1000 mg·kg^{-1})	
WBC (10^9·dm^{-3})	2.58±0.56	2.17±0.19	2.23±0.16	2.85±0.32	2.06±0.17	NS
RBC (10^{12}·dm^{-3})	7.34±0.22	7.88±0.38	7.62±0.07	7.63±0.15	7.47±0.30	NS
LYMPH (10^9·dm^{-3})	2.27±0.48	1.96±0.20	2.06±0.16	2.64±0.32	1.83±0.16	NS
LYMPH (% L)	88.4±0.5^{a}	90.6±1.0^{ab}	90.6±0.8^{ab}	91.9±0.7^{b}	89.1±0.6^{ab}	$p<0.05$
MID (10^9·dm^{-3})	0.183±0.044	0.140±0.019	0.125±0.011	0.140±0.010	0.128±0.012	NS
MID (% M)	5.58±0.30^{b}	4.35±0.40^{ab}	4.25±0.42^{ab}	3.76±0.36^{a}	4.93±0.28^{ab}	$p<0.05$
GRAN (10^9·dm^{-3})	0.175±0.036	0.150±0.000	0.150±0.000	0.160±0.010	0.160±0.010	NS
GRAN (% G)	5.98±0.47	5.01±0.56	5.12±0.52	4.34±0.44	5.98±0.52	NS
PLT (10^9·dm^{-3})	1006±33	1042±32	1057±19	1124±33	1040±20	NS
MPV (fl)	5.62±0.18	5.72±0.32	5.82±0.14	5.36±0.11	5.30±0.00	NS

Different letter superscripts indicate a statistically significant difference at $p<0.05$

NS differences statistically non-significant

intake. They found that 8-week-$Cr(pic)_3$ supplementation at a dose of 1,000 μg·day^{-1} significantly reduced the food intake and satiety and tended to decrease the body weight in healthy, overweight women. In addition, the group supplemented with $Cr(pic)_3$ were less hungry, as subjectively assessed with visual analogue scales (VAS), and rarely consumed high-fat products. Further study indicated that the direct injection of $Cr(pic)_3$ at 0.4, 4 and 40 ng into the Sprague-Dawley rats' cerebral ventricle decreased the dietary intake, as compared with the control group [56]. There have been suggestions that $Cr(pic)_3$ has impact on neurotransmitters involved in the regulation of eating behaviour, mood and food cravings [57].

However, the role of Cr(III) in the regulation of appetite and body composition remains a matter of controversy, because the mechanism responsible for that is still unknown [53, 54, 58–60].

Several studies conducted on humans showed that supplementation with Cr(III), also combined with exercise, did not cause significant changes in body composition among students [59] and women with moderate obesity [53].

In this study, dietary supplementation with Cr3 at doses of 100 to1000 mg Cr(III)·kg^{-1} for 4 weeks did not influence the food intake, body mass gain, feeding efficiency ratio and internal organ masses in healthy female rats. Thus, the results of this study are consistent with the results of previous experiments, where rats were treated with Cr3 [26, 27, 33, 58, 61], as well as the results from the other Cr(III) compounds [5, 17, 20, 28, 33, 62].

There were no differences observed in the body mass after daily gavage administration of $Cr(pic)_3$ (1 mg Cr·kg^{-1} body mass), $CrCl_3$ (1 mg·kg^{-1} body mass), and Cr3 (33 μg and 1 mg Cr·kg^{-1} body mass) in Zucker lean, Zucker obese or ZDF rats [63]. Also, Stout et al. [64] conducted an experiment on male and female F344/N rats and B6C3F1 mice, which for 2 years, had been exposed to $Cr(pic)_3$ at up to 5 % of their diet in feed. The experiment revealed that $Cr(pic)_3$ had no influence on the animals' body mass. Similarly, there were no effects on body mass noted in another study after 13-week supplementation with niacin-bound chromium(III) complex at doses of 5 to 125 mg·kg^{-1} diet in male and female rats [21]. However, 52-week supplementation with this compound at a dose of

Table 5 The effect high dietary doses of Cr3 blood hematological in female rat (mean±SEM)

Index	Experimental groups					ANOVA
	Control (1 mg·kg^{-1})	A (100 mg·kg^{-1})	B (200 mg·kg^{-1})	C (500 mg·kg^{-1})	D (1000 mg·kg^{-1})	
Hb (mmol·dm^{-3})	8.85±0.16	9.03±0.31	9.03±0.07	9.19±0.10	8.90±0.14	NS
HCT (%)	18.37±0.44	19.78±0.95	19.23±0.23	19.24±0.40	18.54±0.74	NS
MCV (fl)	50.65±0.48	50.65±0.92	50.78±0.76	50.82±0.39	50.10±0.29	NS
MCH (pg)	19.22±0.12	18.72±0.42	19.07±0.18	19.58±0.29	19.44±0.27	NS
MCHC (g·dl^{-1})	76.70±0.56	74.44±1.36	75.43±0.84	76.60±0.91	78.32±1.22	NS
RDW (%)	14.40±0.16	14.53±0.29	14.12±0.30	14.53±0.26	15.17±0.36	NS

25 mg Cr(III)·kg^{-1} diet decreased the body mass gain, without causing significant changes in organ masses of male and female Sprague-Dawley rats at a similar level of dietary intake [22].

Yoshida et al. [18] observed no change in the body mass. However, dietary supplementation with $Cr(pic)_3$ and $CrCl_3$ at 100 μg·g^{-1} for 28 days decreased the liver mass. In contrast, Zha et al. [65] found that dietary supplementation with CrNano at doses of 75 to 450 μg·kg^{-1} diet increased the body mass gain and feeding efficiency in male Sprague-Dawley rats.

In this study, we observed no effect of Cr3 on serum glucose, total cholesterol, LDL cholesterol and HDL cholesterol concentration except serum triglycerides concentration. The dietary supplementation with Cr3 at doses of 500 and 1000 mg Cr·kg^{-1} diet decreased serum triglycerides concentration, as compared with the rats receiving 200 mg Cr·kg^{-1} diet.

Herring et al. [6] demonstrated that long-term 15-month exposure to Cr3 did not have significant effect on glucose levels in male Wistar rats on traditional and cafeteria-style diets. However, Sun et al. [26, 61] reported that plasma insulin, total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides, but not glucose, were lowered after 12 and 24 weeks of intravenous treatment with 20 μg Cr·kg^{-1} body mass as Cr3 in healthy and type 2 diabetic Sprague-Dawley rats.

The oral administration of Cr3 at levels of 250, 500 or 1000 μg·kg^{-1} body mass lowered fasting plasma insulin, triglycerides, total cholesterol and LDL-cholesterol levels in healthy Sprague-Dawley rats, but it had no effect on plasma glucose and HDL cholesterol [27].

In another study, Bennett et al. [58] found that Cr3 at doses of 1, 5 and 10 mg Cr·kg^{-1} lowered plasma insulin, leptin and triglycerides concentrations, but had no effect on plasma HDL, LDL and total cholesterol after 10 weeks of treatment in male Sprague-Dawley rats.

Previous studies by Sun et al. [61] indicated that Cr3 lowered total cholesterol, LDL and HDL cholesterol and triglycerides levels, but did not affect the levels of insulin and glucose in the blood after 12 weeks of intravenous administration of Cr3 at a dose of 20 mg·kg^{-1} b.w. in normal male rats.

Clodfelder et al. [27] and Sun et al. [26] showed that 24-week supplementation with Cr3 at doses of 250 to 1000 mg·kg^{-1} and a dose of 5 to 20 mg·kg^{-1} b.w., respectively, significantly reduced fasting insulin levels, total cholesterol, LDL, HDL and triglycerides in the blood serum, and after a 2-h glucose tolerance test, it reduced insulin and glucose levels in healthy and diabetic type 1 and 2 rats. This was in agreement with the results obtained by Sahin et al. [66], who demonstrated that CrPic reduced the blood glucose and total cholesterol levels as well as free fatty acid concentration and it increased the serum insulin level and the composite insulin sensitivity index in rats.

Significantly lower blood glucose, total cholesterol and HDL cholesterol in the blood were also reported by Yang et al. [25]. In contrast to previous reports, they indicated increased concentration of triglycerides in obese mice, which had been supplemented with complex Cr(III) with phenylalanine $Cr(_{D}\text{-Phe})_3$ at an amount of 150 mg Cr·kg^{-1} dose·day^{-1} for 6 weeks. These authors also demonstrated the inhibitory effect of $Cr(_{D}\text{-Phe})_3$ on lipid peroxidation in a dose-dependent manner.

In this study, there were no significant changes in the concentration of total protein, urea, creatinine and ALT and AST in the serum, suggesting the absence of abnormalities in the liver and kidney function in female rats supplemented with Cr3 at doses of 100 to 1000 mg Cr(III)·kg^{-1} of diet. Anderson et al. [67] obtained similar results and showed that supplementation with $CrCl_3$ and $Cr(pic)_3$ up to 100 mg·kg^{-1} diet (9 mg Cr·kg^{-1}·day^{-1}) for 20 weeks did not cause changes in the concentration of creatinine, total protein and ALT and AST in male Harlan Sprague-Dawley rats.

According to reports, Cr(III) compounds are of low toxicity to animals [68]. This lack of toxicity was also confirmed in many previous studies with different Cr(III) compounds following oral administration [21, 22, 28, 33, 34]. The daily oral administration of the chromium rutin complex (CrRC), chromium folate complex (CrFC) and chromium stachyose complex at a dose of 3.0 mg Cr·kg^{-1} for 2 weeks did not affect the AST, ALT and ALP activities in normal mice, but decreased the activities of serum AST, ALT and ALP in diabetic mice [20]. However, Yoshida et al. [18] observed that there were increased activities of serum AST and ALT in male Wistar rats supplemented with CrPic at a dose of 100 μg Cr·g^{-1}. On the other hand, Michaliński et al. [69] showed a decrease in the ALT, AST and creatine kinase (CK) activity after 3 weeks of supplementation with CRC454 and CrPic at a dose of 42 μg Cr(III)·kg^{-1} b.w. in rats with type 1 diabetes. Wang et al. [70] reported an increase in urea, immunoglobulin M and G concentrations and a decrease in total protein in the serum of pigs on a diet supplemented with 200 μg Cr·kg^{-1} diet, given as CrNano.

Latest studies on animals, cell cultures and humans (patients) seem to demonstrate the same kind of beneficial effects or clear cellular effects of Cr supplements [11, 17, 20, 24, 71–74].

Conclusions

The results of this experiment suggest that even high doses of Cr3 (100–1000 mg Cr·kg^{-1} diet) do not significantly affect overall nutritional indices and most biochemical, morphological and haematological indices in the blood of healthy female Wistar rats.

Conflict of Interest The authors declare that they have no conflicts of interests.

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