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CAN THE EFFECTS OF CHROMIUM COMPOUNDS EXPOSURE BE MODULATED BY VITAMINS AND MICROELEMENTS?

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Abstract
Chromium (Cr) is a very common element. It occurs in 2 oxidation states, Cr(III) and Cr(VI). Although Cr(III) is not considered an element essential for mammals, it raises lots of controversy due to its role in the body. While Cr(III) action should be considered an effect of pharmacological action, Cr(VI) is included in the first group of carcinogens for humans. Moreover, it induces numerous pathological changes in the respiratory, urinary, reproductive and digestive systems. In addition, Cr(VI) is used in many industry branches, causing millions of workers all over the world to be exposed to Cr(VI) compounds. A considerable number of the occupationally exposed individuals are in favor of a deep analysis of the mechanisms of Cr(VI) action and a search for a way to reduce its negative impact on the human body. Numerous reactive oxygen species inducing oxidative stress and causing various damage are produced during Cr(VI) reduction in the cells. A good balance between antioxidants and pro-oxidants can reduce Cr(VI)-induced damage. The influence of vitamins and microelements on the adverse Cr(VI) effects has no systematic research results summary. Therefore, this work focuses on the role of dietary antioxidants such as vitamins and microelements in the prevention of Cr(VI) adverse health effects. Numerous studies have revealed a protective influence of vitamins (mainly vitamins E and C) as well as microelements (especially selenium) on the reduction of Cr(VI)-induced adverse changes. A potential protective effect of these ingredients may be useful in occupational groups that are particularly exposed to Cr(VI). However, more research in this area is required. Int J Occup Med Environ Health. 2021;34(4):461–90

Key words:
zinc, folic acid, selenium, hexavalent chromium, vitamin E, vitamin C

INTRODUCTION

Chromium (Cr) is an element that occurs in various degrees of valency. The Cr compounds that occur naturally contain the element in the third degree of valency (Cr(III)), whereas Cr derivatives in the sixth degree of valency (Cr(VI)) are the result of operation of many industries. The first publication on the biological function of Cr(III) dates back to the 1950s. Since then, Cr(III) has been listed as an essential element. Such a theory was supported by, *inter alia*, studies carried out in patients on long-term parenteral nourishment, in whom the symptoms of Cr deficiency were manifested by glucose intol-

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erance, which disappeared after the addition of an extra amount of this element [1].

Despite the fact that Cr was considered an essential element for mammals for as long as 60 years, in-depth research on biochemistry and nutrients, carried out according to modern standards, has not confirmed this statement. In 2014, the European Food Safety Authority (EFSA) [2] clearly stated: "no evidence of beneficial effects associated with Cr intake in healthy subjects" as well as that "the setting of an Adequate Intake for Cr is also not appropriate."

Clinical studies involving the use of proportionally lower doses of Cr have not shown any beneficial effects of supplementation, but in rodent studies, such supranutritional doses may have a beneficial effect. In fact, Cr is an element that nowadays raises a lot of controversy due to its functions in the body. Currently, it is believed that the Cr(III) effect should be rather considered an effect of pharmacological action. A completely different action is attributed to this element in the sixth degree of oxidation. The International Agency for Research on Cancer (IARC) has classified Cr(VI) compounds as carcinogenic to humans, belonging to group 1 [3]. The National Institute of Occupational Safety and Health has also classified all Cr(VI) compounds to the group of carcinogenic agents [4].

As Cr(VI) compounds are used in various industries, many occupational groups are exposed to Cr(VI). This exposure concerns, *inter alia*, welders, painters, tanners, and workers in electroplating, chrome-plating, chromate production, chromate pigment production and construction industries [4]. It is estimated that exposure to Cr(VI) concerns several millions of workers all over the world. Welders constitute an occupational group that is particularly exposed to Cr effects. The chemical composition of welding fumes is diverse, and it depends on the welding technique as well as the applied material [5]. Welding fumes have been classified as the first group of carcinogens by the IARC and they contain, *inter alia*, large amounts of Cr(VI) or nickel compounds [3]. It is estimated that all over the world there are 110 million people who are exposed to welding fumes. In the USA, there are >550 000 workers exposed to Cr(VI), with stainless steel welders constituting 127 000 of that number [4].

Painters are another occupational group working in exposure to Cr(VI) compounds. They use paints containing Cr(VI) compounds. Spray painters in the aviation industry are exposed to an adverse effect of Cr(VI) during painting, but also as a result of sanding and abrasive blasting [4]. Electroplating workers, and chrome-plating workers in particular, constitute another big occupational group exposed to Cr(VI) effects. Their exposure results from the fact that, in the process of Cr plating, an electrolytic solution of chromic acid is applied, which contains Cr(VI) compounds. Additionally, during chrome-plating, chromic acid mist is produced.

Moreover, Cr(VI) compounds are also used during leather tanning – where during the process of tanning sole Cr(VI) is applied. Chromate production workers are exposed to Cr(VI) during Cr compounds production from chromite ore (e.g., sodium and potassium dichromate, sodium and potassium chromate, and ammonium dichromate). Also individuals who during their work use Cr(VI) compounds to produce other materials, such as stainless steel, dyes, paints, coatings, plastic colorants, anticorrosive agents, wood preservatives, fireproof materials, printer ink, and fungicides, are exposed to Cr(VI). Construction industry workers are exposed to Cr(VI) during works in which grout is used. In the USA, the number of workers in this sector is estimated to be 1 045 500 [3,4].

Chromium compounds are used in many industries because they affect, *inter alia*, such properties as hardenability, creep, strength, resistance to corrosion, and oxidation [3]. These wide possibilities of using Cr(VI) compounds in the economy have resulted in workers in many industries being exposed to the harmful effects of these compounds.

Generally, Cr(VI) may induce a number of adverse health effects [3,4]. Inhalation is the most common way of exposure to Cr(VI), while dermal and oral exposure occurs to a lesser extent. The systems that are especially exposed to Cr(VI) effects are respiratory, gastrointestinal, reproductive and urinary systems. Moreover, it has been observed that Cr(VI) may have a negative influence on immunological and hematological systems as well as on development [4]. The main system exposed to the adverse effects of Cr(VI) is the respiratory system. Exposure to Cr(VI) in the work environment may lead to non-cancer as well as cancer-related respiratory diseases. It has been shown that exposure to Cr(VI) may contribute to the induction of nasal irritation, perforation and ulceration as well as to asthma development [4,6,7]. Lung cancer is the most common type of neoplasm in workers exposed to Cr(VI). The first case of lung cancer in workers exposed to Cr(VI) was observed already in 1930. The estimated risk of death due to lung cancer is 6 in 1000 workers in the case of exposure to 1 μg Cr(VI)/m^3. A correlation between exposure and total Cr, genotype, and lung cancer risk has also been observed.

Moreover, cases of workers suffering from nose and nasal sinus cancer after exposure to Cr(VI) compounds have been described [7]. A few cases of gastrointestinal cancer in people exposed to Cr(VI) have also been analyzed. In addition, some studies have found increased mortality due to stomach cancer as well as an association between exposure to this ingredient and cancer of the whole gastrointestinal tract [6]. Adverse health effects caused by Cr(VI) also concern the genitourinary system. It has been observed that in men occupationally exposed to Cr(VI) there are adverse changes in reproductive parameters with respect to the quality of semen, *inter alia*, decreased sperm count, sperm motility, semen volume and abnormal sperm morphology [8]. In individuals chronically exposed to Cr, tubular and glomerular impairment has also been observed [9]. Finally, exposure to Cr(VI) may contribute to the occurrence of dermal changes such as rashes, ulcers, sores and sensitization, which may be a reason of dermatitis [6].

Compared to other Cr forms, Cr(VI) compounds are considerably more toxic and carcinogenic. This stems from the fact that Cr(VI) can penetrate the cell membrane, which is not the property of Cr(III). A similar structure of chromate anion to sulfate anion and hydrogen phosphate anion means that it can be transported through the cell membrane thanks to the anion transport system [10]. After penetrating the cell membrane, Cr(VI) is reduced to Cr(V), and then to Cr(III). The Cr reduction process may take place in the presence of various molecules, e.g., ascorbic acid, glutathione (GSH) and glutathione reductase (GR). During the reduction process, reactive oxygen species (ROS) are produced, which may lead to oxidative damage, e.g., of proteins, fat and deoxyribonucleic acid (DNA). The DNA damage caused by the action of Cr(VI) is associated with an increased formation of DNA adducts, DNA-protein cross-links (DPCs), and DNA strand break. Moreover, Cr(VI) may have a negative impact on gene expression, signaling pathways and cell apoptosis [11,12].

Bearing in the mind these results, it is generally believed that ROS play an important role in Cr(VI)-induced carcinogenesis. As presented above, human biomonitoring provides important data on the combined exposure via all routes of exposure. It is believed that a biomonitoring study on occupational exposure to Cr(VI) has a unique set-up including multiple countries collecting biomonitoring and industrial hygiene information on exposure to Cr(VI) using harmonized protocols [13].

Moreover, it is also known that biological antioxidants can protect cells from various kinds of oxidative damage. Therefore, enzymatic and non-enzymatic antioxidants, acting as natural defense mechanisms against oxidative stress, may also decrease the negative effects of Cr(VI) [12]. It seems that an analysis of data on the relationship between Cr(VI) concentrations and selected vita-

mins and microelements may be important when assessing the effects of exposure to this carcinogen. Therefore, this work focuses on the role of dietary antioxidants, such as vitamins and microelements, in the prevention and treatment of Cr(VI) adverse health effects.

METHODS

In this review, electronic databases such as PubMed and Scopus were used. In order to find relevant articles, a compilation of the following key words was applied: hexavalent chromium, chromium VI, Cr(VI), diet, nutrient, vitamin C, vitamin E, selenium (Se), antioxidants, zinc (Zn), and folic acid. Antioxidants (such as folic acid and Zn) were included because they may quench the ROS which are formed in the Cr reduction process. The review was elaborated based on "The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration" [14].

As a result of the search performed by the authors, 1003 articles were collected. Of this number, articles irrelevant to the subject, duplicated in the databases, and those in which a whole range of bioactive diet ingredients were examined were excluded. The authors did not include studies with the whole range of bioactive dietary ingredients, as there is a limit of literature references in the publishing house. The analysis eventually included 55 original, reviewed articles in English (53 *in vitro* and *in vivo* studies, and 2 human studies), in which a potentially modulating effect of vitamins and microelements on Cr(VI)-induced toxicity has been studied. Articles published in 1991–2020 were used, 90.9% of which were those published after 2000.

RESULTS

Vitamins as a modulator of Cr(VI)-induced toxicity

Vitamin E

The influence of vitamin E on the decrease in the effects of Cr(VI)-induced toxicity has been examined using animal models (8 studies on rats, mice, guinea pigs and chickens) as well as cell lines (6 studies). Different doses of vitamin E and various times of exposure to Cr(VI) have been applied. A summary of the results of those studies is presented in Table 1 [15–28].

In the study using rats exposed to Cr(VI), in a group of animals receiving vitamin E compared to the group not receiving it, the following beneficial changes were observed: a lower lung mass and a lower intensity of pulmonary fibrosis, which were associated with a decrease in the concentration of hydroxyproline in the lungs [15]. However, in the study using human lung fibroblast cells exposed to Cr(VI) with pretreatment with vitamin E, no significant effects on clonogenic survival, apoptosis, and Cr-DNA adduct levels, compared to the cells without pretreatment with vitamin E, were shown [16].

Three studies using animal models concerned the influence of Cr(VI) and vitamin E on the reproductive system [17–19]. In the study using female rats exposed to Cr(VI), a protective effect of vitamin E on the reproductive system was indicated, including a normalization of the frequency of the estrous cycle, a reduction of the concentration of lipid and protein peroxidation products in the ovaries, and an increase in the activity of antioxidant enzymes [17]. Chandra et al. [18], in the study using male rats, also observed a protective influence of vitamin E in the case of exposure to Cr(VI), including an increase in the antioxidant barrier activity and a decrease in the concentration of lipid peroxidation products in the testes, in the group receiving vitamin E, an increase in the number of sperm and semen, the activity of enzymes involved in steroidogenesis and serum testosterone levels.

In the studies using male mice, the supplementation of vitamin E resulted in a decrease in the concentration of lipid peroxidation products in the testes, an increase in the number of sperm, and a decrease in sperm abnormalities, thereby showing a protective effect against the harmful effects of Cr(VI) in the testes [19]. At the same time, in the *in vitro* study in Chinese hamster ovary cells, vitamin E did not show

Table 1. Results of *in vivo* and *in vitro* studies with Cr(IV) exposure and vitamin E treatment in articles published over the years 1991–2020, collected on PubMed and Scopus in July 2020*

Reference	Research model	Study description	Main results
15	rats, Sprague Dawley, adult male and female	CG (N = 6) – $Na_2Cr_2O_7$ 50 mg/kg bw (intratracheal, single dose); G1 (N = 6) – $Na_2Cr_2O_7$ 50 mg/kg bw (intratracheal, single dose) and vitamin E 20 mg/kg bw/day (orally, for 3 weeks)	G1 vs. CG – lung: weight↓, hydroxyproline concentration↓
16	HLF-cells (LL-24 cell line), from 5-year-old male human	CG – Na_2CrO_4 different doses: 3 μmol, 6 μmol, 9 μmol (for 24 h); G1 – vitamin E 20 μm (pretreatment for 24 h) and after that Na_2CrO_4 different doses: 3 μmol, 6 μmol, 9 μmol (for 24 h)	G1 vs. CG – in all doses, clonogenic survival↔ – in group with Na_2CrO_4 6 μm apoptosis↔
		CG – Na_2CrO_4 75 μmol (for 2 h); G1 – vitamin E 20 μmol (pretreatment for 24 h) and after that Na_2CrO_4 75 μmol (for 2 h)	G1 vs. GC – Cr-DNA adducts↔
17	rats, Wistar, adult, female	CG (N = 6) – $K_2Cr_2O_7$ 10 mg/kg bw (s.c. injection, single dose); G1 (N = 6) – $K_2Cr_2O_7$ 10 mg/kg bw (s.c. injection, single dose) and α-tocopherol 125 mg/kg bw/day (by oral gavage for 14 days)	G1 vs. CG – ovaries: SOD↑, GSH↑, TBARS↓, protein carbonyls↓ – liver: TP↑, ALT↓ – kidney: BUN↓, creatinine↓ – ovaries: cholesterol↓ – in G1 normal frequency of estrous cycle – in CG abnormal frequency of estrous cycle
18	rats, Sprague Dawley, male albino	CG (N = 8) – $K_2Cr_2O_7$ 0.4 mg/kg bw/day (i.p. injection for 26 days); G1 (N = 8) – $K_2Cr_2O_7$ 0.4 mg/kg bw/day (i.p. injection for 26 days) and vitamin E 50 mg/kg bw/day (orally for 26 days)	G1 vs. CG – epididymal histoarchitecture – near normal – number of stage VII spermatids↑, epididymal sperm number↑ – testis: TBARS↓, SOD↑, CAT↑, 17β-HSD↑, $\Delta^5$3β-HSD↑ – serum: T↑, FSH↓, LH↑
19	mice, Swiss albino, adult, male	CG (N = 24) – CrO_3 10 mg/kg bw (i.p. injection, single dose) and sacrificed after 8 weeks; G1 (N = 24) – CrO_3 10 mg/kg bw (i.p. injection, single dose) and vitamin E 100 mg/kg bw (i.p. injection, single dose) and sacrificed after 8 weeks	G1 vs. CG – testes: TBARS↓ – sperm: count↑, abnormality↓
20	CHO-AA8 cells Chinese hamster	CG – Na_2CrO_4 different doses: 2 μmol, 4 μmol (for 24 h); G1 – vitamin E 25 μmol (pretreatment for 24 h) and after that Na_2CrO_4 different doses 2 μmol, 4 μmol (for 24 h)	G1 vs. CG – Cr-DNA adduct↔
		CG – Na_2CrO_4 different doses 6 μmol, 7 μmol, 8 μmol, 9 μmol (for 24 h); G1 – vitamin E 25 μmol (pretreatment for 24 h) and after that Na_2CrO_4 different doses 6 μmol, 7 μmol, 8 μmol, 9 μmol (for 24 h)	G1 vs. CG – survival↔

Table 1. Results of *in vivo* and *in vitro* studies with Cr(IV) exposure and vitamin E treatment in articles published over the years 1991–2020, collected on PubMed and Scopus in July 2020* – cont.

Reference	Research model	Study description	Main results
21	rats, Wistar, adult, female	CG (N = 6) – $K_2Cr_2O_7$ 10 mg/kg bw (s.c. injection, single dose); G1 (N = 6) – $K_2Cr_2O_7$ 10 mg/kg bw (s.c. injection, single dose) and α-tocopherol 125 mg/kg bw/day (oral gavage for 14 days)	G1 vs. CG – TEC↑, Hb↑, PCV↑, MCV↑, MCH↑, TLC↑ – liver and kidney: SOD↑, GSH↑, TBARS↓, protein carbonyls↓ – liver: TP↑, ALT↓ – kidney: BUN↓, creatinine↓
22	rats, *Rattus Norvegicus*, adult, male	CG (N = 6) – $K_2Cr_2O_7$ 8 mg/kg bw/day (orally for 6 weeks); G1 (N = 6) – $K_2Cr_2O_7$ 8 mg/kg bw/day and vitamin E 100 mg/kg bw/day (orally for 6 weeks)	G1 vs. CG – liver: TBARS↓, GSH↑, SOD↑, Cr levels↔, glycogen content↑, protein content↑, DNA content↑ – serum: Cr levels↔ – hepatic histological (normal)
23	cell culture hepatocytes, Wistar rats, male	GC – DMSO 0.5% (pretreatment for 20 h) and after that $K_2Cr_2O_7$ 500 μmol (for 8 h); G1 – vitamin E 100 μmol (pretreatment for 20 h) and after that $K_2Cr_2O_7$ 500 μmol (for 8 h)	G1 vs. CG – Cr in homogenates and subcellular fractions↔ – GSH↑, vitamin C↑, vitamin E↑,GR↔, SOD↔, CAT↔, LDH leakage↓ MDA↓
24	rats, *Rattus norvegicus* var. *alba*, Wistar, male	CG (N = 6) – $K_2Cr_2O_7$ 12 mg/kg bw (i.p. injection, 6 times over 2 weeks); G1 (N = 6) – $K_2Cr_2O_7$ 12 mg/kg bw (i.p. injection, 6 times over 2 weeks) and α-tocopherol 20 mg/kg bw (orally, 5 times/week for 2 weeks)	G1 vs. CG – kidney: vitamin C↔ – lungs: vitamin C↑ – liver: vitamin E↑, MDA↔ – PCEs with MN↔, PCE (%)/(PCE+NCE)↑
	guinea pigs, male	CG (N = 6) – vitamin C deficient diet (for 2 weeks) and $K_2Cr_2O_7$ 4 mg/kg bw (injected i.p., 24 h before death); G1 (N = 6) – pretreatment for 2 weeks: vitamin C deficient diet (2 weeks) and α-tocopherol 10 mg/animal/day, and after that $K_2Cr_2O_7$ 4 mg/kg bw (i.p., injected 24 h before death)	G1 vs. CG – PCEs with MN↔, PCE (%)/(PCE+NCE)↑
25	murine macrophages J774	CG – $K_2Cr_2O_7$ 1 μg/ml (for 24 h); G1 – vitamin E 50 μg/ml (pretreatment for 30 min) and after that $K_2Cr_2O_7$ 1 μg/ml (for 24 h)	G1 vs. CG – NR uptake↔, LDH↔, caspase activity↔, DCFH-DA↔, NO↓, superoxide radical↓, GSH↔, GP_x↑, SOD↑, rhodamine↑, PI↔, phagocytosis↑, cell proliferation↑
26	human leukemic T-lymphocyte MOLT-4 cells	CG – K_2CrO_4 400 μmol (for 8 h); G1 – α-tocopherol 25 μmol (pretreatment for 16 h) and after that K_2CrO_4 400 μmol (for 8 h)	G1 vs. CG – MDA↓
		CG – K_2CrO_4 200 μmol (for 2 h); G2 – α-tocopherol 25 μmol (pretreatment for 16 h) and after that K_2CrO_4 200 μmol (for 2 h)	G2 vs. CG – DPCs↓, protein carbonyls↓

27	broiler chicks	CG (N = 6) – K_2CrO_4 one-third of LD_{50}/day (syringe into the ventriculus, for 30 days); G1 (N = 6) – pretreatment for 1 week with vitamin E 300 mg/day and Se 1.8 mg/day orally, and after that for 30 days K_2CrO_4 one-third of LD_{50}/day and vitamin E 300 mg/day and Se 1.8 mg/day orally (syringe into the ventriculus)	G1 vs. CG (after 30 days of treatment) – Hb↑, PCV↑, clotting time↓, TEC↑, TLC↑
28	human erythrocytes	CG – 4 mmol dichromate (for 1 h); G1 – vitamin E 20 µmol (pretreatment for 2 h) and after that 4 mmol dichromate (for 1 h)	G1 vs. CG – hemoglobin oxidation↑

ALT – alanine transaminase; bw – body weight; BUN – blood urea nitrogen; CAT – catalase; CG – control group; DCFH-DA – 2',7'-dichlorofluorescein diacetate; DMSO – dimethyl sulfoxide; DPCs – DNA-protein cross-link coefficient; FSH – follicle-stimulating hormone; GPx – glutathione peroxidase; GR – glutathione reductase; GSH – glutathione; Hb – hemoglobin; i.p. injection – intraperitoneal injection; LD_{50} – median lethal dose; LDH – lactate dehydrogenase; LH – luteinizing hormone; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MDA – malondialdehyde; MN – micronuclei; NCE – normochromatic erythrocytes; NO – nitric oxide; NR – neutral red; PCE – polychromatic erythrocytes; PCV – packed cell volume; PI – propidum iodide; s.c injection – subcutaneous injection; SOD – superoxide dismutase; TBARS – thiobarbituric acid-reactive substances; TEC – total erythrocyte count; T – testosterone; TLC – total leukocyte count; TP – total protein; $\Delta^5$3β-HSD – $\Delta^5$3β-hydroxysteroid dehydrogenase; 17β-HSD – 17β-hydroxysteroid dehydrogenase.
G1 – group 1; G2 – group 2.
* The results are presented according to the effects on the respiratory, reproductive, digestive, urinary and circulatory systems.
↑ – significant increase; ↓ – significant decrease; ↔ – no significant changes.

a protective effect on Cr(VI)-induced apoptosis and did not reduce the number of adduct products [20].

In 2 studies carried out using rats exposed to Cr(VI), it was observed that the administration of vitamin E reduced the severity of hepatotoxicity by reducing the concentration of lipid peroxidation products in the liver and increasing the activity of antioxidant barrier enzymes [21,22].

In the study by Balakrishnan et al. [21], it was shown that vitamin E reduced nephrotoxicity, *inter alia*, by reducing the concentration of protein and lipid peroxidation products in the kidneys, as well as reducing the concentration of creatinine and blood urea nitrogen (BUN), compared to the group exposed to Cr(VI) but without vitamin E treatment. However, pretreatment with vitamin E increased the concentration of vitamins C and E, and GSH in the hepatocytes of rats, but it had no influence on the activity of antioxidant enzymes. These results suggest that the protective effect of vitamin E on the harmful effects of Cr(VI) may result from the increasing non-enzymatic antioxidants concentration [23].

Chorvatovičová et al. [24], in the study using rats and guinea pigs, demonstrated that, in the case of exposure to Cr(VI), vitamin E had a cytoprotective effect (an increase in vitamin C concentration in the lungs, and vitamin E in the liver, as well as an increase in the ratio of normochromatic erythrocytes to polychromatic erythrocytes), but it showed no antimutagenic effect (no decreased amount of micronuclei in polychromatic erythrocytes being observed). In a murine macrophage study, pretreatment with vitamin E showed no inhibitory effect on Cr(VI)-induced cytotoxicity. Vitamin E, to a relatively low extent, inhibited the production of nitric oxide and enhanced phagocyte activity and macrophage proliferation [25]. Mattagajasingh et al. [26] studied the influence of pretreatment with vitamin E in human leukemic lymphocytes exposed to Cr(VI), in which they observed some beneficial effects of vitamin E associated with the reduction of malondialdehyde (MDA) and protein carbonyls.

In turn, Kumari et al. [27] observed that the administration of vitamin E together with Se mitigated the effect of Cr(VI) and improved hematological parameters in chickens. On the contrary, in the study on human erythrocytes exposed to Cr(VI), pretreatment with vitamin E increased the oxidation of hemoglobin [28].

Vitamin E has been shown in both *in vitro* and *in vivo* studies to reduce the adverse changes induced by Cr(VI) in the lungs, testes and ovaries, the liver and kidneys, as well as to exhibit cytoprotective activity and improve hematological parameters. Only a few *in vitro* studies have shown no evidence of a protective effect of vitamin E (in studies on human lung fibroblast cells, Chinese hamster ovary cells and human erythrocytes).

Vitamin C

Table 2 presents the results of studies performed using animal models (9 studies) and cell lines (15 studies) with exposure to Cr(VI), in which a protective effect of vitamin C has been analyzed [15,16,19,20,24–26,28–41].

In a rat model, Quereshi and Mahmood [29] analyzed the influence of pretreatment, simultaneous treatment or a premix with vitamin C on the Cr(VI)-induced toxicity. A beneficial effect of vitamin C was observed after the application of a premix consisting of vitamin C and Cr(VI). The administration of the premix normalized hormones concentration (the serum thyroid stimulating hormone, free thyroxine, free triiodothyronine) as well as resulted in the lack of significant changes in follicular density and epithelial cell height. Only the nuclear cytoplasmic ratio and the epithelial follicular index were slightly altered. After the administration of the premix, a similar structure to the normal structure of the thyroid gland was also observed. However, such effects were not observed in the case of pretreatment and simultaneous treatment with vitamin C and Cr(VI).

Zhong et al. [30] carried out a study using rats, and an *in vitro* study using human hepatocytes. In both experiments, a protective effect of vitamin C on the toxic effect of Cr(VI) was shown. In the case of the *in vivo* study, pretreatment with vitamin C decreased the activity of liver enzymes in the serum and the concentration of lipid peroxidation products, as well as increased the activity of antioxidant enzymes in the liver and free radical scavenging capacity. Moreover, the increased content of Cr(VI) was observed in stool, and the decreased content in the blood and the liver. On the other hand, in the case of the *in vitro* study, the hepatoprotective effect was associated with a decrease in the activity of liver enzymes, the level of proinflammatory cytokines and an increase in the activity of antioxidant enzymes, compared to the cells treated with Cr(VI) but without vitamin C.

In the study by Fatima and Mahmood [31] performed using rats exposed to Cr(VI), a renoprotective effect of vitamin C was demonstrated. Pretreatment with vitamin C lowered creatinine concentration in the serum as well as BUN. Additionally, it increased the activity of brush border membrane enzymes in homogenates and in brush border membrane vesicles, the activity of antioxidant enzymes and the transport of phosphate, but it decreased the lipid peroxidation products concentration in rats' kidneys, compared to the group without pretreatment with vitamin C. In turn, in the case of the *in vitro* study performed using renal epithelial cells, it was observed that Cr(VI) might increase the eukaryotic elongation factor-2 concentration, which increases the expression of mesenchymal cell markers (including paxillin). That, in turn, might induce morphological changes in the kidney cells, whereas pretreatment with vitamin C decreased paxillin expression [32].

In several studies, a protective effect of vitamin C on the reproductive system was analyzed [19,20,33–35]. Vitamin C alleviated the adverse effect of Cr(VI) in the testes of mice by reducing the lipid peroxidation products concentration in the testes and by increasing sperm count, and reducing sperm abnormality [19]. Banu et al. [33] analyzed the pro-

Table 2. Results of *in vivo* and *in vitro* studies with Cr(IV) exposure and vitamin C treatment in articles published over the years 1991–2020, collected in PubMed and Scopus in July 2020*

Reference	Research model	Study description	Main results
29	rats, Sprague Dawley, adult, male	CG (N = 8) – physiological saline 0.9% and sacrificed after 48 h; G1 (N = 8) – $K_2Cr_2O_7$ 60 μmol/kg bw(i.p. injection, single dose) and sacrificed after 48 h; G2 (N = 8) – ascorbic acid 120 mg/kg bw (i.p. injection, single dose, pretreatment for 1h) and after that $K_2Cr_2O_7$ 60 μmol/kg bw (i.p. injection, single dose) and sacrificed after 48 h; G3 (N = 8) – simultaneously, but separate injections: ascorbic acid 120 mg/kg bw (i.p injection, single dose) and $K_2Cr_2O_7$ 60 μmol/kg bw (i.p. injection, single dose) and sacrificed after 48 h; G4 (N = 8) – combined premix dose of ascorbic acid and $K_2Cr_2O_7$ (i.p. injection, single dose, 2:1 ratio) and sacrificed after 48 h	G1,2,3 vs. CG – serum: TSH↑, FT_4↓, FT_3↓ – follicular density↑ G4 vs. CG – serum: FT_4↔, FT_3↔, TSH↔ – follicular density↔, epithelial cell height↔, epithelial follicular index↑, nuclear cytoplasmic ratio↓, – thyroid gland structure – near normal
30	rats, Sprague Dawley, adult, male and female	CG (N = 8) – $K_2Cr_2O_7$ 17.68 mg/kg bw/day (by gavage, for 7 days); G1 (N = 8) – vitamin C 500 mg/kg bw/day (by gavage, pretreatment 0.5 h, for 7 days) and after that $K_2Cr_2O_7$ 17.68 mg/kg bw/day (by gavage, for 7 days)	G1 vs. CG – stool: Cr content↑ – blood: Cr content↓ – liver: Cr content↓, MDA↓, SOD↑, GSH↑ – serum: AST↓, ALT↓ – free radical scavenging capacity↑
	human L-02 hepatocyte line	CG – $K_2Cr_2O_7$ 16 μmol (for 24 h); G1 – vitamin C 200 μmol (pretreatment for 2 h) and after that $K_2Cr_2O_7$ 16 μmol (for 24 h)	G1 vs. CG – AST↓, ALT↓, IL-1β↓, TNF-α↓, LTB4↓, GSH↑, SOD↑, Trx↑
31	rats, Wistar, adult, male	CG – $K_2Cr_2O_7$ 15 mg/kg bw (i.p. injection, single dose) and sacrificed after 48 h; G1 – vitamin C 250 mg/kg bw (i.p. injection, single dose, pretreatment for 6 h) and after that $K_2Cr_2O_7$ 15 mg/kg bw (i.p. injection, single dose) and sacrificed after 48 h	G1 vs. CG – serum: creatinine↓, BUN↓ – homogenates and BBM: BBM enzymes (AP, GGTase, LAP, MIT)↑ – Pi uptake by BBMV↑ – kidney: MDA↓, SH↑, CAT↑
32	HK-2 cells (kidney cells)	CG – $K_2Cr_2O_7$; G1 – vitamin C 100 μmol (pretreatment for 2 h) and after that $K_2Cr_2O_7$	G1 vs. CG – expression of paxillin↓
19	mice, Swiss albino, adult, male	CG (N = 24) – CrO_3 10 mg/kg bw (i.p. injection, single dose) and sacrificed after 8 weeks; G1 (N = 24) – CrO_3 10 mg/kg bw (i.p. injection, single dose) and vitamin C 10 mg/kg bw (i.p. injection, single dose) and sacrificed after 8 weeks	G1 vs. CG – testes: TBARS↓ – sperm: count↑, abnormality↓

Table 2. Results of *in vivo* and *in vitro* studies with Cr(IV) exposure and vitamin C treatment in articles published over the years 1991–2020, collected in PubMed and Scopus in July 2020* – cont.

Reference	Research model	Study description	Main results
33	rats, Wistar, female pups	CG (N = 18) – $K_2Cr_2O_7$ 200 mg/l (via mother's milk during 1–21 PND) and after that regular diet and water; G1 (N = 18) – $K_2Cr_2O_7$ 200 mg/l and vitamin C 500 mg/l (via mother's milk during 1–21 PND) and after that 21 PND regular diet and water	G1 vs. CG (results after 65 PND) – plasma: Cr levels↓ – ovary: Cr levels↓ – onset of puberty – normal – follicle number↑ – plasma: E2↑, T↑, P4↑, – LH↔, FSH↔ (normal)
	SIGC rat granulosa cell line	CG – $K_2Cr_2O_7$ 12.5 μmol (for 24 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that $K_2Cr_2O_7$ 12.5 μmol (for 24 h)	G1 vs. CG – expression of: StAR↑, SF-1↑, 17β-HSD1↑, 17β-HSD2↑, FSH-R↑, LH-R↑,Er-α↑, ER-β↑
34	granulosa cells from ovaries from rats, Sprague Dawley, female	CG – $K_2Cr_2O_7$ 10 μmol (for 48 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that $K_2Cr_2O_7$ 10 μmol (for 48 h)	G1 vs. CG – cell proliferation↑
		CG – $K_2Cr_2O_7$ 10 μmol (for 24 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that $K_2Cr_2O_7$ 10 μmol (for 24 h)	G1 vs. CG – CDKs↑ – cyklin: D2↑, E2↑, B1↑, PCNA↑ – CDKls: p15↓, p16↓, p27↓ – mRNA levels FSH-R↑, ER-β↑
35	granulosa cells from ovaries rat, Sprague Dawley, female 22–25-day-old	CG – 10 μmol $K_2Cr_2O_7$ 10 μmol (for 12 h); G1 – 1 mmol vitamin C (pretreatment for 24 h) and after that $K_2Cr_2O_7$ 10 μmol (for 12 h)	G1 vs. CG – apoptosis of granulosa cells↓, cleavage of caspase-3 in GC↓, cleavage PARP in GC↓, expression Bcl-2, Bcl-XL in GC↑, levels of BAX, BAD↓, levels of HSP70, HSP90 proteins↑, phosporylation of Bad protein at-ser-112, ser-136↑, translocation Bax, Bad to mitochondria↓, phosphorylation p-BAD-112, pBAD-136 in cytosol↑, HSP90 (mitochondrial expression)↑, phosphorylation of ERK1/2↓, phosphorylation of JNK↓, phosphorylation of AKT↑, expression and phosphorylation of p53↓, expression of MDM-2 protein↑, translocation of p53 to the mitochondria↓

20	CHO-AA8 cells, Chinese hamster	CG – Na_2CrO_4 different doses: 2 μmol, 4 μmol (for 24 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na_2CrO_4 different doses 2 μmol, 4 μmol (for 24 h)	G1 vs. CG – Cr-DNA adduct↔, clastogenic activity (% metaphase chromosomes damaged, total damage in 100 metaphase)↓
		CG – Na_2CrO_4 different doses: 8 μmol, 9 μmol, 10 μmol (for 24 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na_2CrO_4 different doses 8 μmol, 9 μmol, 10 μmol (for 24 h)	G1 vs. CG – survival↑
15	rats, Sprague Dawley, adult, male and female	CG (N = 6) – $Na_2Cr_2O_7$ 50 mg/kg bw (intratracheal, single dose); G1 (N = 6) – $Na_2Cr_2O_7$ 50 mg/kg bw (intratracheal, single dose) and vitamin C 75 mg/kg bw/day (orally, for 3 weeks); G2 (N = 6) – $Na_2Cr_2O_7$ 50 mg/kg bw (intratracheal, single dose) and vitamin C 75 mg/kg bw/day + vitamin E 20 mg/kg bw/day (orally, for 3 weeks)	G1 vs. CG – lung weight↓, hydroxyproline concentration↔ G2 vs. CG – lung weight↓, hydroxyproline concentration↓
36	V-79 cells, Chinese hamster	CG – Na_2CrO_4 15 μmol (for 2 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na_2CrO_4 15 μmol (for 2 h)	G1 vs. CG – alkali-labile sites↓, DPCs↑, GR↑, cellular levels of chromium↑
		CG – Na_2CrO_4 200 μmol (for 2 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na_2CrO_4 200 μmol (for 2 h)	G1 vs. CG – levels of Cr(V)↓, levels of Cr(III) complex↑, cellular reduction of Cr(VI) to Cr(III) ↑
37	human adenocarcinoma cell line-A549	CG – $Na_2Cr_2O_7$ in PBS 80 μmol (for 4 h); G1 – vitamin C 1 mmol (pretreatment for 6 h) and after that $Na_2Cr_2O_7$ in PBS 80 μmol (for 4 h)	G1 vs. CG – % survival in the clonogetic assay↑
		CG – $Na_2Cr_2O_7$ 5 μmol (single dose); G1 – ascorbate 0.25–1.5 mmol (pretreatment for 6 h) and after that 10 μmol $Na_2Cr_2O_7$ (single dose)	G1 vs. CG – intracellular chromium accumulation↓
		CG – BSO 10 μmol (pretreatment 24 h prior to dye treatment) and after that $Na_2Cr_2O_7$ 80 μmol (30 min after dye loading); G1 – BSO (pretreatment for 24 h prior to dye treatment) and after that 1 mmol ascorbate (pretreatment 6 h prior to dye treatment) and $Na_2Cr_2O_7$ 80 μmol (30 min after dye loading)	G1 vs. CG – DCF fluorescence in A549 cell↑

Table 2. Results of *in vivo* and *in vitro* studies with Cr(IV) exposure and vitamin C treatment in articles published over the years 1991–2020, collected in PubMed and Scopus in July 2020* – cont.

Reference	Research model	Study description	Main results
16	HLF- cells (LL-24 cell line), from 5-year-old male	CG – Na_2CrO_4 different doses: 3 μmol, 6 μmol, 9 μmol (for 24 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na_2CrO_4 different doses: 3 μmol, 6 μmol, 9 μmol (for 24 h)	G1 vs. CG – clonogenic survival↔
		CG – Na_2CrO_4 6 μmol (for 24 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na_2CrO_4 6 μmol (for 24 h)	G1 vs. CG – apoptosis↔
		CG – Na_2CrO_4 75 μmol (for 2 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na_2CrO_4 75 μmol (for 2 h)	G1 vs. GC – Cr-DNA adducts↔
		CG – Na_2CrO_4 9 μmol (for 24 h); G1 – vitamin C 1 mmol (pretreatment) and after that Na_2CrO_4 9 μmol (for 24 h)	G1 vs. CG – p53 induction↔
38	rats, Sprague Dawley, adult, male	CG (N = 6) – $Na_2Cr_2O_7$ 20 mg/kg bw (i.p. injection, single dose) and sacrificed after 24 h; G1 (N = 6) – ascorbic acid 40 mg/kg bw (i.p. injection, single dose, pretreatment for 30 min) and after that $Na_2Cr_2O_7$ 20 mg/kg bw (i.p. injection, single dose) and sacrificed after 24 h	G1 vs. CG – organs and blood: levels Cr↔ – urine and feces: total Cr excreted↔
	human plasma	CG – Cr(VI) 5000 μg/l; G1 – ascorbic acid 1 g/l (pretreatment for 30 min) and after that Cr(VI) 5000 μg/l	G1 vs. CG – reduction of Cr(VI)↑
24	rats (*Rattus Norvegicus var. Alba*), Wistar, male	CG (N = 6) – $K_2Cr_2O_7$ 12 mg/kg bw (i.p. injection, 6 times over 2 weeks); G1 (N = 6) – vitamin C 100 mg/kg bw (i.p. injection, 6 times over 2 weeks) and $K_2Cr_2O_7$ 12 mg/kg bw (i.p. injection, 6 times over 2 weeks)	G1 vs. CG – lungs: vitamin C↑ – kidney: vitamin C↔ – liver: vitamin E↑, vitamin C↑, MDA↔ – PCEs with MN↓, PCE (%)/(PCE+NCE)↑
39	peripheral blood lymphocytes rats, Sprague Dawley, male	CG – $K_2Cr_2O_7$ 100 μmol (for 6 h); G1 – vitamin C 200 μmol (pretreatment for 2h) and after that $K_2Cr_2O_7$ 100 μmol (for 6 h) G2 – vitamin C 200 μmol and $K_2Cr_2O_7$ 100 μmol (for 6 h)	G1 vs. CG – PBL viability↑, DPCs↔, MDA content↓, ROS levels↓ G2 vs. CG – PBL viability↑, DPCs↓, MDA content↓, ROS levels↓
26	human leukemic T-lymphocyte MOLT-4 cells	GC – K_2CrO_4 400 μmol (for 8 h); G1 – ascorbate 1 mmol (pretreatment for 16 h) and after that K_2CrO_4 400 μmol (for 8 h)	G1 vs. CG – MDA↓
40	ScSF cell lines, Indo-Pacific dolphin	CG – K_2CrO_4 12.5 μmol (for 24 h); G1 – vitamin C 50 μmol and K_2CrO_4 12.5 μmol (for 24 h)	G1 vs. CG – cell viability↑, expression of p53↓

25	murine macrophages J774	CG – $K_2Cr_2O_7$ 1 μg/ml (for 24 h); G1 – vitamin C 25 μg/ml (pretreatment for 30 min) and after that $K_2Cr_2O_7$ 1 μg/ml (for 24 h)	G1 vs. CG – NR uptake↓, LDH↓, caspase activity↓, DCFH-DA↓, NO↓, superoxide radical↓, GSH↑, GPx↑, SOD↑, rhodamine↑, PI↓, phagotycytosis↑, cell proliferation↑
28	human erythrocytes	CG – dichromate 4 mmol (for 1 h); G1 – vitamin C 1 mmol (pretreatment for 2 h) and after that dichromate 4 mmol (for 1 h)	G1 vs. CG – hemoglobin oxidation↑
41	yeast *Sacharomyces cerevisiae* ZIM 1825, ZIM 1836, ZIM 327	CG – $K_2Cr_2O_7$ 0.8 mmol; G1 – 1 mmol ascorbic acid (pretreatment) and after that $K_2Cr_2O_7$ 0.8 mm	G1 vs. CG – relative cell viability↑, mitotic gene conversions and reverse mutation of *S. cerevisiae*↓
		CG –$K_2Cr_2O_7$ 0.5 mmol (for 8.5 h); G1 – 1 mmol ascorbic acid (pretreatment) and after that $K_2Cr_2O_7$ 0.5 mmol (for 8.5 h)	G1 vs. CG – total accumulated Cr in yeast↑
		CG – 0.1 mmol Cr(VI) (for 50 min); G1 – 1 mmol ascorbic acid (pretreatment for 12 h) and after that $K_2Cr_2O_7$ 0.1 mmol (for 50 min)	G1 vs. CG – formation of H_2O_2 in yeast↓
		CG – Cr(VI) 0.1 mmol; G1 – ascorbic acid 1 mmol (pretreatment for 9 h or 1 h) and after that $K_2Cr_2O_7$ 0.1 mmol	G1 vs. CG – levels of 8-OHdG in yeast DNA↑, superoxide radical↓

AP – alkaline phosphatase; AST – aspartate aminotransferase; BAD – Bcl-xL/Bcl-2-associated death promoter; BAX – BCL-2–associated X protein; BBM – brush border membrane; BBMV – brush border membrane vesicles; Bcl-2 – B-cell lymphoma 2; Bcl-XL – B-cell lymphoma-extra large; BSO – buthionine sulfoximine; CDK – cyclin-dependent kinases; CDKls – cyclin-dependent kinase inhibitors; DCF – dichlorofluorescein ; E2 – estriadol; ER-α – estriadol receptor α; ER-β – estriadol receptor β; ERK – extracellular-signal-regulated kinase; FSH-R – FSH receptor; FT_3 – free triiodothyronine; FT_4 – free thyroxine; GC – granulosa cells; GGTase – γ-glutamyl transferase; H_2O_2 – hydrogen peroxide; HSP70 – heat shock proteins-70; HSP90 – heat shock proteins-90; IL-1β – interleukin-1β; JNK – c-Jun N-terminal kinase; LAP – leucine aminopeptidase; LH-R – LH receptor; LTB4 – leukotriene B4; MDM2 – murine double minute 2; MlT – maltase; P4 – progesterone; PARP – poly (ADP–ribose) polymerase; PBL – peripheral blood lymphocytes; PBS – phosphate buffered saline; PCNA – proliferating cell nuclear antigen; Pi – inorganic phosphate; PND – postnatal days; ROS – reactive oxygen species; SF-1 – steroidogenic factor 1; SH – sulfhydryl; StAR – steroidogenic acute regulatory protein; TNFα – tumor necrosis factor-α; Trx – thioredoxin; TSH – thyroid stimulating hormone; 17β-HSD1 – 17β-hydroxysteroid dehydrogenases type 1; 17β-HSD2 – 17β-hydroxysteroid dehydrogenases type 2; 8-OHdG – 8-hydroxy-2'deoxyguanosine.
G3 – group 3; G4 – group 4.
Other abbreviations as in Table 1.
* The results are presented according to the effects on the endocrine, digestive, urinary, reproductive, respiratory and circulatory systems.

tective effect of vitamin C on the reproductive system in both *in vitro* and *in vivo* studies. The beneficial effect in the case of the *in vivo* study was associated with a reduced level of Cr in the plasma and ovaries, a normal pubertal onset, and an increased number of follicles. In addition, increased levels of steroid hormones (estradiol, testosterone, progesterone) as well as normal pituitary hormonal profiles (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]) were observed. In the case of the *in vitro* study in granular cells, pretreatment with vitamin C increased expression of enzymes, regulators of protein and steroidogenic factors participating in steroidogenesis, and LH, FSH, and estradiol receptors.

The protective effect of vitamin C was also analyzed in 2 other *in vitro* studies in ovarian granulosa cells [34,35]. Pretreatment with vitamin C reduced the negative effect of Cr(VI) on the cell cycle; it increased cell proliferation, decreased cyclin-dependent kinase inhibitors, and increased the mRNA levels of FSH and estriadol receptors β [34]. In the study by Banu et al. [35], vitamin C partially mitigated the adverse effect of Cr(VI) in several signaling pathways that lead to granular cell apoptosis. The beneficial effect of vitamin C was observed, to a more extent, after a shorter time of cells exposure (12-hour exposure) to Cr(VI) than after 24 h. Another *in vitro* study performed using Chinese hamster ovary cells also indicated a protective influence of pretreatment with vitamin C that included a decrease of cells apoptosis (higher survival) and a decrease in clastogenic activity [20].

The influence of vitamin C on the changes induced by Cr(VI) in the lungs has also been studied. In the study on rats, no reduced pulmonary fibrosis was observed after the administration of vitamin C. On the other hand, a protective effect against the effect of Cr(VI) in the lungs was shown in the case of the simultaneous administration of vitamin C and vitamin E (a decrease in lung weight and hydroxyproline concentration) [15]. Pretreatment of Chinese hamster lung fibroblasts (V-79 cells) with vitamin C at lower doses of Cr(VI) reduced the alkali-labile sites, increased the level of GR and increased the reduction of Cr(VI) to Cr(III), while pretreatment with vitamin C at higher doses of Cr(VI) increased DPCs [36]. In human adenocarcinoma cell line-A549 subjected to the effect of Cr(VI), pretreatment with vitamin C increased the percent survival in the clonogetic assay and decreased intracellular Cr accumulation. Pretreatment with vitamin C revealed a mitigating effect regardless of the GSH level in the cell (increased DCF fluorescence) [37]. On the other hand, a study on HLF-cells exposed to Cr(VI) did not demonstrate a beneficial effect of pretreatment with vitamin C in such parameters as the clonogenic survival, apoptosis, and Cr-DNA adducts [16].

The distribution of Cr in the organs and blood, and the effect of vitamin C on its elimination, have been analyzed in both *in vivo* and *in vitro* studies. In the *in vitro* study in human plasma, it was shown that pretreatment with vitamin C increased the reduction of Cr(VI). In an animal model, pretreatment with vitamin C did not decrease the level of Cr in the organs (such as the liver, kidneys, the heart, the brain, lungs, the spleen or testes) and blood, as well the amount of Cr excreted with urine and feces [38].

Chorvatovičová et al. [24], in the study using rats exposed to Cr(VI), observed that the intraperitoneal injection of vitamin C showed an anti-mutagenic effect against Cr(VI) due to a reduction in the number of micronuclei in polychromatic erythrocytes in the bone marrow cells of rats. After the administration of vitamin C, a normalization of vitamin C levels in the lungs and the liver was also observed, with no significant changes in the concentration of MDA in the rats' liver.

Xiao et al. [39], in the study in which they used rats peripheral blood lymphocytes, demonstrated that vitamin C mitigated the harmful effects of Cr(VI). Pretreatment with vitamin C as well as treatment simultaneously with Cr(VI) increased the ability of lymphocytes to survive, decreased

mitochondrial damage (by reducing the level of MDA) and decreased the level of ROS. Exposure to Cr(VI), simultaneous with vitamin C treatment, reduced DNA damage (DPCs decreased) but this effect was not observed with vitamin C pretreatment. In turn, in the case of the *in vitro* study on human leukemic T-lymphocyte (MOLT-4) cells exposed to Cr(VI), pretreatment with vitamin C reduced lipid peroxidation by reducing MDA concentration [26]. Vitamin C in ScSF cell lines exposed to Cr(VI) increased cell viability and decreased p53 protein expression, which suggests a potential effect on reducing cell apoptosis [40].

On the other hand, pretreatment of murine macrophages J774 exposed to Cr(VI) with vitamin C reduced cytotoxicity (a decreased level of neutral red dye uptake and lactate dehydrogenase), apoptosis (a decreased level of caspase activity), and oxidative stress (decreased levels of 2′,7′-dichlorofluoresceindiacetate, nitric oxide, superoxide radical, and an increased activity of antioxidant enzymes), while it increased cell proliferation and phagocytosis [25]. Increased hemoglobin oxidation was also observed in human erythrocytes pretreated with vitamin C and after that exposed to Cr(VI) [28].

Poljšak et al. [41], in their study carried out on the yeast *Sacharomyces cerevisiae*, showed that pretreatment with vitamin C reduced the cytotoxicity and genotoxicity caused by Cr(VI) (through, *inter alia*, an increase in viability and a decrease in conversion and reverse mutation frequency of *Sacharomyces cerevisiae*, as well as a decrease in peroxide formation and superoxide radical).

Vitamin C has been found to alleviate the adverse effects of Cr(VI) in many organs: the thyroid gland, the liver, kidneys, testes, ovaries and lungs. Moreover, it showed antimutagenic and cytoprotective activity in an *in vitro* study. The protective effect of vitamin C was not demonstrated in one of the studies in an animal model and *in vitro* studies (on human lung fibroblast cells and human erythrocytes).

Other vitamins

A modulating effect of folic acid on Cr(VI)-induced toxicity has been studied on animal models (2 studies), *in vitro* (1 study), and in workers exposed to Cr(VI) (2 studies).

In the study by Yousefa et al. [42], the influence of folic acid on the adverse effects of Cr in seminal plasma of rabbits was analyzed. For 10 weeks, the first group of rabbits was orally administered sodium chromate at a dose of 5 mg/kg bw/day, while the second group was administered folic acid at a dose of 8.3 μg/kg bw/day, together with the same dose of sodium chromate as in the case of the first group. In the group that received folic acid together with Cr(VI), an increase in the relative testes and epididymis weight, increased plasma testosterone, and higher levels of ejaculate volume, sperm concentration, total sperm output, sperm motility, percent of normal sperm and total functional sperm fraction, compared to the group not receiving folic acid, were observed. The administration of folic acid to the rabbits also decreased the level of thiobarbituric acid-reactive substance and increased the level of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and acid phosphatase in the seminal plasma. Moreover, the following changes in biochemical parameters were observed: a decrease in total lipid, triglycerides, glucose and urea, and an increase in total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and albumin.

In the study by El-Demerdash et al. [43], also performed using rabbits, folic acid (administered orally at a dose of 8.3 μg/kg bw/day together with 5 mg/kg bw/day of Cr(VI) for 10 weeks) decreased Cr(VI)-induced toxicity. This effect was associated, *inter alia*, with a decreased concentration of thiobarbituric acid-reactive substances in the liver, testes, the brain, and kidneys, as well as with maintaining a correct level of sulfhydryl groups and the activity of glutathione S-transferase (GST). Moreover, folic acid normalized enzymes activity (*inter alia*, AST, ALT, alkaline phosphatase, acid phosphatase and lactate dehydrogenase), as well as alleviated changes in biochemi-

cal parameters (total protein, globulin, glucose, urea, creatinine, bilirubin, total lipids and cholesterol). Human B-lymphoblastoid cell line (WIL2-NS) studies revealed that folic acid deficiency with Cr(VI) exposure increased chromosomal DNA damage, the frequencies of micronucleated, nucleoplasmic bridge and nuclear bud binucleated cells, as well as lowered the nuclear division index and the percentage of necrosis [44].

In a study conducted in workers chronically exposed to Cr(VI), adverse metabolic changes have been observed, including increased blood homocysteine concentration in the individuals with lower levels of vitamin B_{12} and folic acid [45]. In another study in workers with long-term exposure to Cr(VI) compounds, a lower serum level of folic acid, a higher level of homocysteine and a concentration of tumor markers (such as carcinoembryonic antigen, neuron-specific enolase, squamous cell carcinoma antigen, cytokeratin fragment antigen 21-1, cancer antigen 72-4 and α-fetoprotein) in the plasma have been observed. Moreover, a negative correlation between serum folic acid concentration and plasma tumor markers (carcinoembryonic antigen, neuron-specific enolase and squamous cell carcinoma antigen) was observed [46].

On the other hand, Anand [47] investigated the effect of vitamin B_6 on the intensification of oxidative stress induced by Cr(VI) in the liver of rats. Vitamin B_6 pretreatment and simultaneous treatment with Cr(VI) reduced lipid peroxidation, while increasing non-enzymatic antioxidants concentration (such as vitamins C and E, and GSH) and the activity of enzymatic antioxidants (such as glutathione peroxidase [GPx], catalase, superoxide dismutase [SOD], GST, GR) in comparison with the group not receiving this vitamin.

Microelements as a modulator of Cr(VI)-induced toxicity

Selenium

In 13 studies using animal models and in 1 *in vitro* study, the influence of Se with respect to the reduction of the adverse effects of Cr(VI) was analyzed (Table 3) [48–61].

In 2 of the studies, the influence of Se on the reduction of Cr(VI)-induced thyrotoxicity was analyzed. Hassanin et al. [48], in rats exposed to Cr(VI), observed that nano-Se reduced the intensity of oxidative stress, which was associated with an increase in the activity of antioxidant enzymes and a decrease in the concentration of lipid peroxidation products. Moreover, in the group receiving nano-Se, thyroid hormones concentration was close to normal values, and no adverse changes in the structure of the thyroid gland were observed. In the study carried out by other authors using adult male rats, nano-Se also reduced adverse histological changes in the thyroid induced by Cr(VI) action [49].

The protective effect of Se in the case of exposure to Cr(VI) has also been analyzed with respect to the reduction of brain damage. In the study carried out on chickens, it was shown that lower doses of Se reduced the harmful effects of Cr(VI) in the brains of chickens by reducing the intensity of oxidative stress (increasing the activity of antioxidant enzymes and reducing the concentration of lipid peroxidation products in the brain). Also, a reduced brain-body ratio and an increased level of membrane mitochondrial potential (MMP) and activity of Ca^{2+}-ATPase in the brain were observed. On the other hand, higher doses of Se did not show the above-mentioned beneficial changes and, what is more, they increased oxidative stress intensity [50]. In another study, in the case of exposure to Cr(VI), a neuroprotective effect of Se on the brains of rats was also observed, which was associated with a reduction in the intensity of oxidative stress and histopathological changes in the brain [51].

Animal models have also been used to analyze a renoprotective effect of Se in the case of exposure to Cr(VI). The administration of Se to rats compensated for such kidney marker levels as creatinine, urea, uric acid, urinary volume, creatinine clearance and a decreased concentration of lipid peroxidation products, as well as increased antioxidant enzymes activity. Selenium also reduced histo-

Table 3. Results of *in vivo* and *in vitro* studies with Cr(IV) exposure and selenium treatment in articles published over the years 1991–2020, collected in PubMed and Scopus in July 2020*

Reference	Research model	Study description	Main results
48	rats, albino Wistar, adult, male	CG (N = 5) – $K_2Cr_2O_7$ 60 μmol/kg bw (i.p. injection, single dose on the third day of the study); G1(N = 5) – nano-selenium 0.5 mg/kg bw/day (i.p. injection, for 5 days) and $K_2Cr_2O_7$ 60 μmol/kg bw (i.p. injection, single dose on the third day of the study)	G1 vs. CG – serum: FT_3↑, FT_4↑, GSH↑, MDA↓, CAT↓, SOD↓ – morphometric parameters: follicular epithelial cell height↓, area percentage of trichrome-stained collagen↓, area percentage of ki67 – positive nuclei↓, histopathological changes in thyroid tissues – not observed in the G1
49	rats, Sprague Dawley, adult, male	CG (N = 40) – $K_2Cr_2O_7$ 60 μg/kg bw/day (i.p. injection, for 5 days); G1 (N = 40) – $K_2Cr_2O_7$ 60 μg/kg bw/day (i.p. injection, for 5 days) and nano-Se 0.5 mg/kg bw/day (i.p. injection, for 5 days)	G1 vs. CG – thyroid gland: follicular structure (near normal) – thyroid gland: parafollicular structure (near normal) – preponderance of larger follicles over smaller – the structure follicular cells (near normal) – the structure parafollicular cells (near normal) – in the cytoplasm of follicular epithelial cells: expression of iNOS↓ – mean follicular epithelial cell height↓
50	chickens, Hyland brown (1-day-old), male	CG (N = 15) – $K_2Cr_2O_7$ 6% LD_{50}/day (orally, for 42 days); G1 (N = 15) – $K_2Cr_2O_7$ 6% LD_{50}/day (orally, for 42 days) and Na_2SeO_3 0.63 mg/kg bw/day (orally, for 42 days); G2 (N = 15) – $K_2Cr_2O_7$ 6% LD_{50}/day (orally, for 42 days) and Na_2SeO_3 5.0 mg/kg bw/day (orally, for 42 days)	G1 vs. CG – brain-body ratio↓ – brain: GSH↑, SOD↔, MDA↓, activity of Ca^{2+}-ATPase↑, MMP↑ G2 vs. CG – brain-body ratio↑ – brain: GSH↓, SOD↑, MDA↔, activity of Ca^{2+}-ATPase↓, MMP↓
51	rats, Wistar, adult, female	CG (N = 6) – $K_2Cr_2O_7$ 67 mg/kg bw/day (orally, for 21 days); G1 (N = 6) – $K_2Cr_2O_7$ 67 mg/kg bw/day (orally, for 21 days) and Na_2SeO_3 0.5 mg/kg of diet/day (orally, for 21 days)	G1 vs. CG – cerebrum and cerebellum: MDA↓, GSH↑, NPSH↑, vitamin C↑, CAT↓, GP_x↓, SOD↓, AChE↑, LDH↑ – plasma: LDH↓ – severe brain damage↓
52	rats, Wistar, adult, female	CG (N = 6) – $K_2Cr_2O_7$ 67 mg/kg bw/day (orally, for 21 days); G1 (N = 6) – $K_2Cr_2O_7$ 67 mg/kg bw/day (orally, for 21 days) and Na_2SeO_3 0.5 mg/kg of diet/day (orally, for 21 days)	G1 vs. CG – plasma: creatinine↓, urea↔, uric acid↑ – urine: creatinine↔ urea↑, uric acid↓ – urinary volume↓ – creatinine clearance↑ – kidney: MDA↓, GSH↔, NPSH↑, MT↔, CAT↑, GPx↑, SOD↓ – pathological lesions in kidney sections↓

Table 3. Results of *in vivo* and *in vitro* studies with Cr(IV) exposure and selenium treatment in articles published over the years 1991–2020, collected in PubMed and Scopus in July 2020* – cont.

Reference	Research model	Study description	Main results
53	chickens, Hyland (1-day-old) male	CG (N = 15) – $K_2Cr_2O_7$ 6% LD_{50}/day (orally, for 42 days); G1 (N = 15) – $K_2Cr_2O_7$ 6% LD_{50}/day (orally, for 42 days) and Na_2SeO_3 0.63 mg/kg bw/day (orally, for 42 days)	G1 vs. CG – organ coefficient in the kidney↓, renal damage↓ – kidney: MDA↓, GSH↑, T-SOD activity↑, Ca^{2+}-ATPase activity↑, MMP↑
54	rats, Wistar, adult, female	CG (N = 6) – $K_2Cr_2O_7$ 67 mg/kg bw/day (orally, for 21 days); G1 (N = 6) – $K_2Cr_2O_7$ 67 mg/kg bw/day (orally, for 21 days) and Na_2SeO_3 0.5 mg/kg of diet/day (orally, for 21 days)	G1 vs. CG – liver: MDA↓, CAT↓, SOD↓, GP_x↑, GSH↑, LDH↑ – plasma: ALT↓, AST↓, bilirubin↓, LDH↓, TC↓, TG↓, LDL-C↓, HDL-C↑, AI↓ – severe liver damage↓
55	chickens, Hyland (1-day-old), male	CG (N = 15) – $K_2Cr_2O_7$ 7.83 mg/kg bw/day (orally, for 42 days); G1(N = 15) – $K_2Cr_2O_7$ 7.83 mg/kg bw/day (orally, for 42 days) and Na_2SeO_3 0.57 mg/kg bw/day (orally, for 42 days)	G1 vs. CG – ratio of the liver/body weight↓ – liver: MDA↓, GSH↑, T-SOD activity↑, Ca^{2+}-ATPase activity↑, MMP↑
56	chickens, Arbor Acres (1-day-old)	CG (N = 10) – $K_2Cr_2O_7$ 8% LD_{50}/day (orally, for 5 weeks); G1 (N = 10) – $K_2Cr_2O_7$ 8% LD_{50}/day (orally, for 5 weeks) and nano-Se 0.5 mg/kg bw/day (orally, for 5 weeks); G2 (N = 10) – $K_2Cr_2O_7$ 8% LD_{50}/day (orally, for 2 weeks) and after that nano-Se 0.5 mg/kg bw/day (orally, for 3 weeks); G3 (N = 10) – nano-Se 0.5 mg/kg bw/day (orally, pretreatment for 2 weeks) and after that $K_2Cr_2O_7$ 8% LD_{50}/day (orally, for 3 weeks)	G1, G2, G3 vs. CG – liver gene expression: Bcl-2↑, caspase-3↓, Bax↓ – protein expression levels: Bcl-2↑, caspase 3↓, Bax↔
57	chickens, Arbor Acres (1-day-old)	CG (N = 20) – $K_2Cr_2O_7$ 8% LD_{50}/day (orally, for 5 weeks); G1 (N = 20) – $K_2Cr_2O_7$ 8% LD_{50}/day (orally, for 5 weeks) and nano-Se 0.5 mg/kg bw/day (orally, for 5 weeks); G2 (N = 20) – $K_2Cr_2O_7$ 8% LD_{50}/day (orally, for 2 weeks) and after that nano-Se 0.5 mg/kg bw/day (orally, for 3 weeks); G3 (N = 20) – nano-Se 0.5 mg/kg bw/day (orally, pretreatment for 2 weeks) and after that $K_2Cr_2O_7$ 8% LD_{50} (orally, for 3 weeks)	G1, G2 vs. CG – mRNA levels of FASN in the liver↓ G1, G2, G3 vs. CG – mRNA levels of ACOX1 in the liver↑ G1 vs. CG – the protein levels of FASN in the liver↓ G1, G2 vs. CG – the protein levels of ACOX1 in the liver↑ G1, G2, G3 vs. CG – antibody expression levels of FASN in the liver↓ – antibody expression levels of ACOX1 in the liver↑

58	rats, Wistar, adult, female	CG (N = 6) – $K_2Cr_2O_7$ 67 mg/kg bw/day (orally, for 21 days); G1 (N = 6) – $K_2Cr_2O_7$ 67 mg/kg bw/day (orally, for 21 days) and Na_2SeO_3 0.5 mg/kg of diet/day (orally, for 21 days)	G1 vs. CG – heart: MDA↓, GSH↑, NPSH↑, vitamin C↑, CAT↓, SOD↓, GPx↓, LDH↑ – plasma: ALT↓, AST↓, bilirubin↓, TC↓, TG↓, LDL-C↓, HDL-C↑, AI↓, LDH↓ – heart damage↓
59	rats, Wistar, adult, female	CG (N = 6) – $K_2Cr_2O_7$ 67 mg/kg bw/day (orally, for 21 days); G1 (N = 6) – $K_2Cr_2O_7$ 67 mg/kg bw/day (orally, for 21 days) and Na_2SeO_3 0.5 mg/kg of diet/day (orally, for 21 days)	G1 vs. CG – RBC↑, Hb↑, WBC↓, MEF↓ – erythrocytes: MDA↓, GSH↑, NPSH↑, vitamin C↑, carbonyl formation↓, sulfhydryl oxidation↑, SOD↑, GP_x↑, CAT↑, AChE↑
60	chicken, Hyland (1-day-old), male	CG (N = 15) – $K_2Cr_2O_7$ 22.4 mg/kg bw/day (orally, for 42 days); G1(N = 15) – $K_2Cr_2O_7$ 22.4 mg/kg bw/day (orally, for 42 days) and Na_2SeO_3 0.63 g/kg bw/day (orally, for 42 days)	G1 vs. CG – Cr contents: in the heart↓, in the liver↔, in the spleen↔, in the kidney↔ – Ca contents: in the heart↓, in the liver↓, in the spleen↑, in the kidney↓ – Mn contents: in the heart↔, in the liver↑, in the spleen↑, in the kidney↓ – Cu contents: in the heart↓, in the liver↓, in the spleen↓, in the kidney↓ – Zn contents: in the heart↑, in the liver↑, in the spleen↓, in the kidney↔ – Fe contents: in the heart↔, in the liver↓, in the spleen↔, in the kidney↔ – Mg contents: in the heart↔, in the liver↔, in the spleen↔, in the kidney↔
61	strain TA102 (*Salmonella typhymurium*)	CG – $K_2Cr_2O_7$ 20 μmol; G1 – $K_2Cr_2O_7$ 20 μmol and sodium selenate 100 μmol	CG vs. G1 – genotoxic effect↓
	human lymphocytes	G1 – $K_2Cr_2O_7$ 300 mm and sodium selenite in doses: 100 mmol, 300 mmol, 500 mmol, 1 mmol; G2 – $K_2Cr_2O_7$ 300 mmol and sodium selenite in doses: 100 mmol, 300 mmol, 500 mmol, 1 mmol; G3 – $K_2Cr_2O_7$ 300 mmol and selenous acid in doses: 5 mmol, 10 mmol, 15 mmol	– G1 in higher concentration DNA damage↓ – G2, G3 in higher concentration DNA damage↑

Table 3. Results of *in vivo* and *in vitro* studies with Cr(IV) exposure and selenium treatment in articles published over the years 1991–2020, collected in PubMed and Scopus in July 2020* – cont.

Reference	Research model	Study description	Main results
61 – cont.	TK-6 cell line, human lymphoblastoid	CG – $K_2Cr_2O_7$ 5000 µmol (for 3 h); G1 – sodium selenite 10 µmol (pretreatment for 30 min) and after that $K_2Cr_2O_7$ 5000 µmol (for 3 h)	G1 vs. CG – genotoxicity (tail moment)↓
		CG – $K_2Cr_2O_7$ 5000 µmol (for 3 h); G1 – $K_2Cr_2O_7$ 5000 µmol (for 3 h) and sodium selenite 10 µmol	G1 vs. CG – genotoxicity (tail moment)↑

AChE – acetylcholinesterase; ACOX1 – acyl-coenzyme A oxidase 1; AI – atherogenic index; ; FASN – fatty acids synthase; HDL-C – high-density lipoprotein cholesterol; iNOS – anti-inducible nitric oxide synthase; LDL-C – low-density lipoprotein cholesterol; MEF – mean erythrocyte fragility; MMP – mitochondrial membrane potential; MT – metallothionein; NPSH – non-protein thiols; RBC – red blood cells; TC – total cholesterol; TG – triacylglycerol; WBC – white blood cells.
Other abbreviations as in Table 1 and 2.
* The results are presented according to the effects on the endocrine, nervous, urinary, digestive and circulatory systems.

pathological changes in the kidneys caused by Cr(VI) [52]. In the study by Wan et al. [53] carried out on chickens, different doses of Se were used, and the most favorable effect was observed when low doses of this ingredient were applied (e.g., 0.63 mg/kg bw/day). In the case of exposure to Cr(VI), low doses of Se showed a renoprotective effect, including a reduction of the organ coefficient of the kidney, renal damage, a decreased concentration of lipid peroxidation products, and an increased activity of antioxidant enzymes.

In subsequent studies, the effect of Se in terms of hepatoprotection has been analyzed. In the study using rats exposed to Cr(VI), in which Se was administered, a reduction in the lipid peroxidation products concentration was observed, along with a normalization of the non-enzymatic antioxidant concentration (GSH) and the activity of antioxidant enzymes, compared to the group that did not receive Se. The administration of Se also reduced liver damage, which was observed in histopathological examinations, and it normalized both lipid parameters and lactate dehydrogenase concentration in the liver. It also decreased the serum levels of liver enzymes and bilirubin [54].

In the study using chickens, an effect of different doses of Se on the reduction of hepatotoxicity during exposure to Cr(VI) was also analyzed. It was shown that low doses of Se could alleviate the adverse changes induced by Cr(VI) in the liver of chickens. The administration of 0.57 mg/kg bw/day of Se reduced hepatotoxicity by lowering the concentration of lipid peroxidation products and increasing antioxidant enzymes activity. Additionally, a reduction in the ratio of the liver/body weight, an increase in Ca^{2+}-ATPase in the liver and changes suggesting a reduction in mitochondrial damage in the liver were indicated [55]. In another study, chickens were also used as an animal model in order to study apoptosis in the liver. The administration of Se at various points of time (pretreatment, simultaneous treatment, posttreatment) reduced the apoptosis

in the chickens' liver by reducing pro-apoptotic gene expression (Bax and caspase-3) and increasing anti-apoptotic gene expression (B-cell lymphoma 2) [56].

Luo et al. [57] investigated how the duration of Se administration influenced the fatty acids metabolism in the liver. Regardless of whether Se was administered simultaneously, before or after exposure to Cr(VI), similar results were obtained. Namely, Se reduced the abnormal metabolism of fatty acids in the liver (*inter alia*, by reducing the mRNA levels and protein levels of fatty acid synthase, and increasing the mRNA levels and protein levels of acyl-coenzyme A oxidase 1).

A study on animal models also showed that, in the case of exposure to Cr(VI), Se might have a beneficial effect on the circulatory system. Selenium displayed a cardioprotective effect by reducing lipid peroxidation products concentration in the heart, increasing non-enzymatic antioxidants activity (GSH, non-protein thiols and vitamin C) and normalizing antioxidant enzymes activity (catalase, SOD and GPx). Moreover, in the group receiving Se, only mild changes in the histopathological tests of the heart were observed. Additionally, favorable changes in the lipid profile and normalization of the liver enzymes levels were found in that study [58]. It has been shown that in rats' erythrocytes Se attenuates changes induced by Cr(VI). An increase in the number of erythrocytes and hemoglobin, as well as a reduction in oxidative stress and adverse hematological changes in erythrocytes, were observed [59].

A beneficial effect of Se in the case of exposure to Cr(VI) has also been analyzed with respect to the contents of various elements in individual organs. Chen et al. [60], in the study performed using chickens, observed that exposure to Cr(VI) induced changes in the contents of trace elements in various organs, and that low doses of Se alleviated the intensity of some of them. More specifically, Se increased the contents of manganese and Zn, and decreased the contents of copper, calcium and iron, in the heart, the liver, the spleen and kidneys.

Cemeli et al. [61], in their *in vitro* study using *Salmonella typhimurium*, human lymphocytes and human lymphoblastoid cell line, analyzed the influence of Se on Cr(VI)-induced genotoxicity. In the study on TA102 strain, they observed that sodium selenate decreased the genotoxic effect, whereas other Se compounds, such as sodium selenite and selenous acid, did not demonstrate such properties. A reduction of DNA damage was also observed in human lymphocytes exposed to Cr(VI) with sodium selenite treatment. However, treatment with sodium selenite and selenous acid did not reduce DNA damage. In turn, in the TK6 lymphoblastoid cell line exposed to Cr(VI), pretreatment with sodium selenite slightly decreased genotoxicity. Such an effect was not achieved with the use of simultaneous exposure to Cr(VI) and treatment with sodium selenite. The study results indicate that sodium selenate shows a better effect with respect to Cr(VI)-induced genotoxicity reduction.

The studies performed on animal models exposed to Cr(VI) have shown the alleviating effect of Se in many organs: the thyroid, the brain, kidneys, the liver, the heart and erythrocytes. In an *in vivo* study, Se also influenced the distribution of elements in the organs and to reduce genotoxicity.

Zinc

The influence of Zn on the reduction of adverse Cr(VI)-induced changes has been studied on animal models (4 studies) and cell lines (4 studies). The results are presented in Table 4 [62–69].

In the studies on rats, a protective effect of Zn against Cr(VI)-induced toxicity has been analyzed. In groups of rats that were exposed to Cr(VI) and that were administered Zn, the restoration of the normal concentration of some elements (in particular, calcium, magnesium, iron and Se) in the whole blood, red blood cells and in the lungs was observed. Moreover, the administration of Zn decreased 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentration in the urine [62]. Chmielnicka et al. [63], in

a rat model exposed to Cr(VI), observed a higher urinary excretion of endogenous Zn; its excretion increased already on day 1 after the injection of a high dose of Cr(VI), and on day 3 after the injection of a lower dose of Cr(VI). What is more, in the case of a higher dose of Cr(VI), disturbances in the metabolism and the release of Zn have been observed and, additionally, some histopathological changes have been found to appear in the kidney. In the study by Zhao et al. [64], Zn showed no attenuating influence on Cr(VI)-induced changes in the lungs of rats as it did not reduce damage in club cells in rats. More specifically, no significant changes in the concentration of club (Clara) cell secretory protein (CC16) in the serum or in the average density of CC16 in the lung tissue were observed, while the urinary excretion of 8-OHdG was lower.

In the liver of chick embryos, the ability of Cr(VI) to affect the basal and Zn-inductible expression of hepatic metallothionein was examined. It was shown that Cr(VI), depending on the duration of treatment (2 or 4 h), decreased Zn-induced mRNA expression. Such an effect was not observed after 8 h of exposure to Cr(VI). Additionally, 2-hour Cr(VI) pretreatment reduced the levels of metallothionein, but pretreatment for 4 h or 8 h did not affect this parameter to a significant extent [65].

In the study on bacterial strains (*Arthrobacter sp. 61B* and *A. globiformis sp. 151B*), the influence of Zn on the accumulation and reduction of Cr(VI) was analyzed. The study demonstrated that in the presence of Zn, the bacteria had an increased ability to accumulate Cr(VI), while maintaining their ability to grow. It was further demonstrated that the affinity of Zn accumulation was by an order of magnitude higher in *Arthrobacter sp. 61B* than in *A. globiformis sp. 151B*. A low dose of Zn (50 mg/l) did not reduce the formation of Cr(V) and Cr(III), but a high dose (for example 200 mg/l) showed such an effect. The study in question showed that reduction and accumulation of Cr(VI) depended on the type of bacterial strain and the applied doses of Zn and Cr(VI) [66].

In an *in vitro* study on the human diploid dermal fibroblasts exposed to Cr(VI), pretreatment with Zn reduced oxidative stress, DNA damage and cell apoptosis [67]. In turn, the study on human tumor cell line Hep-2 revealed that the protective effect of Zn depended on the applied dose of Cr(VI). Zinc was found to reduce oxidative stress and cell apoptosis in the case of a lower exposure to Cr(VI) – 10 μmol/l, while at higher doses (50 or 100 μmol/l) it enhanced the adverse effects of Cr(VI) [68]. In the study by Kimura et al. [69], it was shown that Zn deficiency in BALB/3T3 A31-1-1 cells reduced the expression of metallothionein, as a result of which the adverse effect of Cr(VI) might be more pronounced. Zinc deficient cells were supplemented with this component, and a decrease in susceptibility to transformation (through a decreased number of transformed foci) was demonstrated.

In animal model studies, the protective effect of Zn was also demonstrated by normalizing the concentrations of certain elements in the blood and lungs. In one study, Zn was not found to protect the lungs of rats. In the study on chicken embryos, depending on the treatment time, Cr(VI) influenced the Zn-induced mRNA expression of metallothionein. Zinc also influences the accumulation and reduction of Cr(VI) in bacteria. Under *in vitro* conditions, an alleviating effect of Zn in human diploid dermal fibroblast, human tumor cell line Hep-2 and BALB/3T3 A31-1-1 cells has been observed.

CONCLUSIONS

The results of both *in vitro* and experimental animal studies indicate a potentially beneficial effect of vitamins C, E, B_6 and folic acid, as well as microelements such as Se and Zn, on reducing the negative health effects caused by exposure to Cr(VI). However, the results of the experiments are not conclusive. Several studies have not shown any protective effect of vitamins E and C or folic acid. Differences in the study results may depend on the dose of the vitamins, microelements and Cr(VI) used, as well as

Table 4. Results of *in vivo* and *in vitro* studies with Cr(IV) exposure and zinc treatment, collected in PubMed and Scopus in July 2020*

Reference	Research model	Experimental design	Main results
62	rats, Sprague Dawley, male	CG (N = 9) – $K_2Cr_2O_7$ 0.630 mg/kg bw (i.t., 1/week for 1 month); G1 (N = 8) – $K_2Cr_2O_7$ 0.630 mg/kg bw (i.t., 1/week for 1 month) and $ZnSo_4$ 10 mg/kg bw/day (i.g., for 1 month)	G1 vs. CG – Ca, Mg, Fe, Se concentrations in whole blood↑ – Co, Mg, Fe concentrations in the RBC↓ – Se concentrations in the RBC↑ – Ca, Mg, Fe concentrations in the lungs↑ – urinary 8-OHdG↓
		CG (N = 8) – $K_2Cr_2O_7$ 0.063 mg/kg bw (i.t., 1/week for 1 month); G1 (N = 10) – $K_2Cr_2O_7$ 0.063 mg/kg bw (i.t., 1/week for 1 month) and $ZnSO_4$ 10 mg/kg bw/day (ig., for 1 month)	G1 vs. CG – Ca, Se concentrations in whole blood↑ – Ca, Cd, Mg concentrations in the RBC↓ – Se concentrations in the RBC↑ – Ca, Mg concentrations in the lungs↑ – urinary 8-OHdG↓
63	rats, Wistar, female	CG (N = 6) – physiological saline; C1 (N = 6) – K_2CrO_4 0.5 mg/kg bw (i.p. injection, single dose) and sacrificed 3, 5, 7 days after the injection; G2 (N = 6) – K_2CrO_4 5 mg/kg bw (i.p. injection, single dose) and sacrificed 3, 5, 7 days after the injection	G1 vs. CG – urinary excretion of endogenous Zn (3 and 7 days after the injection)↑ – levels of endogenous Zn in the kidney (1, 3, 7 days after the injection)↑ – levels of endogenous Zn in the liver (1, 3, 7 days after the injection)↔ G2 vs. CG – urinary excretion of endogenous Zn (1, 3, 7 days after the injection)↑ – levels of endogenous Zn in the kidney (1, 3, 7 days after the injection)↑ – levels of endogenous Zn in the liver (1, 3, 7 days after the injection)↑ – histopathological changes in the kidney↑
64	rats, Sprague Dawley, male	CG (N = 9) – $K_2Cr_2O_7$ 0.630 mg/kg bw (i.t., 1/week for 1 month); G1 (N = 8) – $K_2Cr_2O_7$ 0.630 mg/kg bw (i.t., 1/week for 1 month) and $ZnSO_4$ 10 mg/kg bw/day (ig., for 1 month)	G1 vs. CG – CC16 concentration in the serum↔, urinary 8-OHdG↓, average density of CC16 in the lung tissue↔

Table 4. Results of *in vivo* and *in vitro* studies with Cr(IV) exposure and zinc treatment, collected in PubMed and Scopus in July 2020* – cont.

Reference	Research model	Experimental design	Main results:
65	14-day-old chick embryos	CG – $Zn(C_2H_3O_2)_2$ 1.5 mmol/kg (for 2 h or 4 h); G1 – $Na_2Cr_2O_7$ 100 µmol/kg (for 2 h or 4 h) and after that $Zn(C_2H_3O_2)_2$ 1.5 mmol/kg (for 4 h)	G1 vs. CG – expression of MT mRNA in the liver↓
		CG – $Zn(C_2H_3O_2)_2$ 1.5 mmol/kg (for 4 h); G1 – $Na_2Cr_2O_7$ 100 µmol/kg (for 8 h) and after that $Zn(C_2H_3O_2)_2$ 1.5 mmol/kg (for 4 h)	G1 vs. CG – expression of MT mRNA in the liver↔
		CG – $Zn(C_2H_3O_2)_2$ 1.5 mmol/kg (for 2 h); G1 – $Na_2Cr_2O_7$ 100 µmol/kg (for 2 h) and after that $Zn(C_2H_3O_2)_2$ 1.5 mmol/kg (for 4 h)	G1 vs. CG – levels of MT protein↓
		CG – $Zn(C_2H_3O_2)_2$ 1.5 mmol/kg (for 4 h or 8 h); G1 – $Na_2Cr_2O_7$ 100 µmol/kg (for 4 h or 8 h) and after that $Zn(C_2H_3O_2)_2$ 1.5 mmol/kg (for 4 h)	G1 vs. CG – levels of MT protein↔
66	*Arthrobacter sp. 61B* *A. globiformis sp. 151B*	CG – K_2CrO_4 200 mg/l; G1 – $ZnSO_4$ 50 mg/l and K_2CrO_4 200 mg/l	G1 vs. CG – accumulation of Cr↑, growth of bacteria↑
		CG – K_2CrO_4 100 mg/l; G1 – $ZnSO_4$ 50 mg/l and K_2CrO_4 100 mg/l	G1 vs. CG – accumulation of Zn – higher in *Arthrobacter sp. 61B* than in *A. globiformis sp. 151B*
	A. globiformis sp. 151B	CG – $ZnSO_4$ 50 mg/l and K_2CrO_4 50 mg/l; G1 – $ZnSO_4$ 50 mg/l and K_2CrO_4 200 mg/l	G1 vs. CG – formation of Cr(V) and Cr(III) complexes↑
		CG – $ZnSO_4$ 50 mg/l and K_2CrO_4 100 mg/l; G1 – $ZnSO_4$ 200 mg/l and K_2CrO_4 100 mg/l	G1 vs. CG – formation of Cr(V) and Cr(III) complexes↓
67	human diploid dermal fibroblasts	CG – K_2CrO_4 10 µmol (for 48 h); G1 – $ZnSO_4$ 100 µmol (pretreatment for 24 h) and after that K_2CrO_4 10 µmol (for 48 h)	G1 vs. CG – ROS↓, DNA damage↓, GP_x↑, SOD↑, expression of MT IIA↑, activated p53 protein↓, fragmented PARP↓, active caspase-3↓

68	human tumor cell line Hep-2	CG – K_2CrO_4 10 μmol/l (for 24 h); G2 – $ZnSO_4$ 50 μmol/l (for 24 h) and K_2CrO_4 10 μmol/l (for 24 h)	G1 vs. CG – proliferation↔
		CG – K_2CrO_4 50 μmol (for 24 h); G2 – $ZnSO_4$ 100 μmol/l (for 24 h) and K_2CrO_4 50 μmol/l (for 24 h)	G1 vs. CG – proliferation↓
		CG – K_2CrO4 10 μmol/l (for 12 h); G1 – $ZnSO_4$ 100 μmol/l (for 12 h) and K_2CrO_4 10 μmol/l (for 12 h)	G1 vs. CG – production: H_2O_2↓, superoxide anion↓
		G2 – K_2CrO_4 150 μmol/l (for 12 h); G1 – $ZnSO_4$ 100 μmol/l (for 12 h) and K_2CrO_4 150 μmol/l (for 12 h)	G1 vs. CG – production: H_2O_2↑, superoxide anion↑
		CG – K_2CrO_4 10 μmol/l (for 12 h); G1 – $ZnSO_4$ 10 μmol/l (for 12 h) and K_2CrO_4 150 μmol/l (for 12 h)	G1 vs. CG – mitochondrial membrane depolarization↔
		CG – K_2CrO_4 150 μmol/l (for 12 h); G1 – $ZnSO_4$ 100 μmol/l (for 12 h) and K_2CrO_4 150 μmol/l (for 12 h)	G1 vs. CG – mitochondrial membrane depolarization↑
		CG – K_2CrO_4 10 μmol/l (for 12 h); G1 – $ZnSO_4$ 100 μmol/l (for 12 h) and 10 μmol/l K_2CrO_4 (for 12 h)	G1 vs. CG – caspase-3 activation↓
		CG – K_2CrO_4 50 μmol/l (for 12 h); G2 – $ZnSO_4$ 100 μmol/l (for 12 h) and K_2CrO_4 50 μmol/l (for 12 h)	G1 vs. CG – caspase-3 activation↑
		CG – K_2CrO_4 10 μmol/l (for 24 h); G1 – $ZnSO_4$ 100 μmol/l (for 24 h) and 10 μmol/l K_2CrO_4 (for 24 h)	G1 vs. CG – cleavage of PARP↓
		CG – K_2CrO_4 50 μmol/l (for 24 h); G2 – $ZnSO_4$ 100 μmol/l (for 24 h) and K_2CrO_4 50 μmol/l (for 24 h)	G1 vs. CG – cleavage of PARP↑
69	BALB/3T3 A31-1-1 cells	CG – Zn deficient medium; G1 – Zn medium deficient and Zn 50 μmol (for 2 days)	G1 vs. CG – MT expression in cells↑
		CG – 100 μmol Cr(VI) (for 3 h); G1 – Zn medium deficient and Zn 50 μmol (for 2 days) and after that 100 μmol Cr(VI) (for 3 h)	G1 vs. CG – number of transformed foci↓

CC16 – club (Clara) cell secretory protein; i.g. – intragastric administration; i.t. – intatracheal instillation; MT IIA – metalothionein IIA.
Other abbreviations as in Tables 1, 2 and 3.
* The results are presented according to the effects on the circulatory, respiratory, urinary, respiratory and digestive systems.

on the exposure time. Initial concentrations of vitamins and microelements in the tissues and organs may also affect the results. It may seem that the studies carried out on cells or using experimental animals indicate a protective effect of selected vitamins and some microelements against the toxic effect of Cr(VI). However, one should remember that *in vitro* and *in vivo* studies are not equivalent to the widely performed human research, both in general and exposed populations. So far such studies have not been conducted. Taking into consideration the promising results of cell line and animal studies, such studies in humans should definitely be conducted.

It is important that the study populations consume these vitamins and microelements with diet in accordance with the values specified in the nutrition standards for the population. There are no studies showing the effectiveness of using higher doses of antioxidants in reducing the adverse effects of Cr(VI).The potential protective effect of these vitamins and microelements makes it possible to use them in the groups that are particularly exposed to Cr(VI), such as welders, painters, tanners and workers in electroplating, chrome-plating, chromate production, chromate pigment production and construction industries. The individuals who are occupationally exposed to compounds containing Cr(VI) should consume food products rich, in particular, in vitamins C, E and B_6, and folic acid, as well as in Se and Zn. Many foods are rich in vitamin C (rosehips, blackcurrants, paprika, kiwi, strawberries, broccoli, Brussels sprouts, kohlrabi), vitamin E (vegetable oils, pumpkin, sunflower and sesame seeds, almonds, avocados, sprouts and legumes) and vitamin B6 (red meat, fish and legumes), as well as folic acid (green leafy vegetables) Se and Zn (cereal products, meat and nuts). Preventive measures should be taken, including nutritional education with respect to the recommended food products. Additionally, the diet of people who are occupationally exposed to Cr(VI) should be monitored.

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