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-
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)/m³. 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-
Vitamins 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$_2$Cr$_2$O$_7$ 50 mg/kg bw (intratracheal, single dose); G1 (N = 6) – Na$_2$Cr$_2$O$_7$ 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$_2$CrO$_4$ different doses: 3 μmol, 6 μmol, 9 μmol (for 24 h); G1 – vitamin E 20 μmol (pretreatment for 24 h) and after that Na$_2$CrO$_4$ different doses: 3 μmol, 6 μmol, 9 μmol (for 24 h) | G1 vs. CG - in all doses, clonogenic survival↔ - in group with Na$_2$CrO$_4$, 6 μm apoptosis↔ |
| 17        | rats, Wistar, adult, female | CG (N = 6) – K$_2$Cr$_2$O$_7$ 10 mg/kg bw (s.c. injection, single dose); G1 (N = 6) – K$_2$Cr$_2$O$_7$ 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$_2$Cr$_2$O$_7$, 0.4 mg/kg bw (i.p. injection for 26 days); G1 (N = 8) – K$_2$Cr$_2$O$_7$, 0.4 mg/kg bw (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↑, Δ5β-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$_2$CrO$_4$ different doses: 2 μmol, 4 μmol (for 24 h); G1 – vitamin E 25 μmol (pretreatment for 24 h) and after that Na$_2$CrO$_4$ different doses 2 μmol, 4 μmol (for 24 h) | G1 vs. CG - Cr-DNA adduct↔ |
|           |                             | CG – Na$_2$CrO$_4$ 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$_2$CrO$_4$ 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) – K₂Cr₂O₇ 10 mg/kg bw (s.c. injection, single dose); G1 (N = 6) – K₂Cr₂O₇ 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) – K₂Cr₂O₇ 8 mg/kg bw/day (orally for 6 weeks); G1 (N = 6) – K₂Cr₂O₇ 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 K₂Cr₂O₇ 500 μmol (for 8 h); G1 – vitamin E 100 μmol (pretreatment for 20 h) and after that K₂Cr₂O₇ 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) – K₂Cr₂O₇ 12 mg/kg bw (i.p. injection, 6 times over 2 weeks); G1 (N = 6) – K₂Cr₂O₇ 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)↑ |
| 25 | guinea pigs, male | CG – vitamin C deficient diet (for 2 weeks) and K₂Cr₂O₇ 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 K₂Cr₂O₇ 4 mg/kg bw (i.p., injected 24 h before death) | G1 vs. CG – PCEs with MN↔, PCE (%)/(PCE+NCE)↑ |
| 26 | murine macrophages J774 | CG – K₂Cr₂O₇ 1 μg/ml (for 24 h); G1 – vitamin E 50 μg/ml (pretreatment for 30 min) and after that K₂Cr₂O₇ 1 μg/ml (for 24 h) | G1 vs. CG – NR uptake↔, LDH↔, caspase activity↔, DCFH-DA↔, NO↓, superoxide radical↓, GSH↑, GP↑, SOD↑, rhodamine↑, PI↔, phagocytosis↑, cell proliferation↑ |
| 27 | human leukemic T-lymphocyte MOLT-4 cells | CG – K₂Cr₂O₇ 400 μmol (for 8 h); G1 – α-tocopherol 25 μmol (pretreatment for 16 h) and after that K₂Cr₂O₇ 400 μmol (for 8 h) | G1 vs. CG – MDA↓ |
| 28 | | CG – K₂Cr₂O₇ 200 μmol (for 2 h); G2 – α-tocopherol 25 μmol (pretreatment for 16 h) and after that K₂Cr₂O₇ 200 μmol (for 2 h) | G2 vs. CG – DPCs↓, protein carbonyls↓ |
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].

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 normochromic erythrocytes to polychromatophilic erythrocytes) but it showed no antianogenic effect (decreased amount of micronuclei in polychromatophilic erythrocytes being observed). In a murine macrophage study, pretreatment with vitamin E in human leukemic lymphocytes exposed to Cr(VI) was studied. Rezanka et al. [25] concluded that vitamin E reduced the toxicity of Cr(VI) by reducing nitric oxide production and enhancing phagocyte activity and macrophage proliferation [23]. Matzgersdorff et al. [26] studied the influence of pretreatment with vitamin E in human leukemic lymphocytes exposed to Cr(VI). In the study using rats and guinea pigs, vitamin E did not inhibit the production of free radicals and enhanced phagocyte activity and macrophage proliferation [23].

In the study of Balakrishnan et al. [21], it was shown that vitamin E reduced the concentration of protein and lipid peroxidation products in the kidneys, as well as reducing the concentration of antioxidants in the liver and increasing the activity of antioxidant enzymes. Vitamin E reduced the concentration of lipid peroxidation products in the liver and increasing the activity of antioxidant enzymes. Vitamin E reduced the concentration of protein and lipid peroxidation products in the kidneys, as well as reducing the concentration of antioxidants in the liver and increasing the activity of antioxidant enzymes.

**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; LD₅₀ – 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 – polychromatophilic 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; Δ³β-HSD – Δ³β-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.
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) – K₂Cr₂O₇ 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 K₂Cr₂O₇ 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 K₂Cr₂O₇ 60 μmol/kg bw (i.p. injection, single dose) and sacrificed after 48 h; G4 (N = 8) – combined premix dose of ascorbic acid and K₂Cr₂O₇ (i.p. injection, single dose, 2:1 ratio) and sacrificed after 48 h | G1,2,3 vs. CG  
- serum: TSH↑, FT₄↓, FT₃↓  
- follicular density↑  
G4 vs. CG  
- serum: FT₄↔, FT₃↔, 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) – K₂Cr₂O₇ 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 K₂Cr₂O₇ 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↑ |
| 31        | rats, Wistar, adult, male | CG – K₂Cr₂O₇, 16 μmol (for 24 h); G1 – vitamin C 200 μmol (pretreatment for 2 h) and after that K₂Cr₂O₇, 16 μmol (for 24 h) | G1 vs. CG  
- AST↑, ALT↑, IL-1β↑, TNF-α↑, LT14, GSH↑, SOD↑, Trx↑ |
| 32        | HK-2 cells (kidney cells) | CG – K₂Cr₂O₇; G1 – vitamin C 100 μmol (pretreatment for 2 h) and after that K₂Cr₂O₇ | G1 vs. CG  
- serum: creatinine↑, BUN↓  
- homogenates and BBM: BBM enzymes (AP, GGTase, LAP, MIT)↑  
- Pi uptake by BBMV↑  
- kidney: MDA↑, SH↑, CAT↑ |
| 19        | mice, Swiss albino, adult, male | CG (N = 24) – CrO₃ 10 mg/kg bw (i.p. injection, single dose) and sacrificed after 8 weeks; G1 (N = 24) – CrO₃ 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↓ |
| Reference | Research model                                                                 | Study description                                                                 | Main results                                                                 |
|-----------|-------------------------------------------------------------------------------|----------------------------------------------------------------------------------|------------------------------------------------------------------------------|
| 33        | rats, Wistar, female pups                                                      | CG (N = 18) – K₂Cr₂O₇, 200 mg/l (via mother’s milk during 1–21 PND) and after that regular diet and water; G1 (N = 18) – K₂Cr₂O₇, 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)                                                           |
| 34        | SIGC rat granulosa cell line                                                   | CG – K₂Cr₂O₇, 12.5 μmol (for 24 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that K₂Cr₂O₇, 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↑                                                  |
| 35        | granulosa cells from ovaries from rats, Sprague Dawley, female                 | CG – K₂Cr₂O₇, 10 μmol (for 48 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that K₂Cr₂O₇, 10 μmol (for 48 h) | G1 vs. CG                                                                    |
|           |                                                                                |                                                                                  | – cell proliferation↑                                                          |
|           |                                                                                |                                                                                  |                                                                                  |
|           |                                                                                | CG – K₂Cr₂O₇, 10 μmol (for 24 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that K₂Cr₂O₇, 10 μmol (for 24 h) | G1 vs. CG                                                                    |
|           |                                                                                |                                                                                  | – CDKs↑                                                                      |
|           |                                                                                |                                                                                  | – cyklin: D₂↑, E₂↑, B₁↑, PCNA↑                                               |
|           |                                                                                |                                                                                  | – CDKs: p15↑, p16↑, p27↓                                                     |
|           |                                                                                |                                                                                  | – mRNA levels FSH-R↑, ER-β↑                                                  |
| 35        | granulosa cells from ovaries from rat, Sprague Dawley, female 22–25-day-old    | CG – 10 μmol K₂Cr₂O₇, 10 μmol (for 12 h); G1 – 1 mmol vitamin C (pretreatment for 24 h) and after that K₂Cr₂O₇, 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↑,                       |
|           |                                                                                |                                                                                  | phosphorylation 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 – Na₂CrO₄ different doses: 2 μmol, 4 μmol (for 24 h);
G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na₂CrO₄ different doses 2 μmol, 4 μmol (for 24 h)

CG – Na₂CrO₄ different doses: 8 μmol, 9 μmol, 10 μmol (for 24 h);
G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na₂CrO₄ different doses 8 μmol, 9 μmol, 10 μmol (for 24 h)

G1 vs. CG
– Cr-DNA adduct↔, clastogenic activity (% metaphase chromosomes damaged, total damage in 100 metaphase)↓

15 rats, Sprague Dawley, adult, male and female

CG (N = 6) – Na₂CrO₄, 50 mg/kg bw (intratracheal, single dose);
G1 (N = 6) – Na₂CrO₄, 50 mg/kg bw (intratracheal, single dose) and vitamin C 75 mg/kg bw/day (orally, for 3 weeks);
G2 (N = 6) – Na₂CrO₄, 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 – Na₂CrO₄, 15 μmol (for 2 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na₂CrO₄, 15 μmol (for 2 h)

CG – Na₂CrO₄, 200 μmol (for 2 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na₂CrO₄, 200 μmol (for 2 h)

G1 vs. CG
– alkali-labile sites↓, DPCs↑, GR↑, cellular levels of chromium↑

CG – Na₂CrO₄, 5 μmol (single dose); G1 – ascorbate 0.25–1.5 mmol (pretreatment for 6 h) and after that 10 μmol Na₂CrO₄ (single dose)

CG – BSO 10 μmol (pretreatment 24 h prior to dye treatment) and after that Na₂CrO₄, 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 Na₂CrO₄, 80 μmol (30 min after dye loading)

G1 vs. CG
– % survival in the clonogetic assay↑

37 human adenocarcinoma cell line-A549

CG – Na₂CrO₄, in PBS 80 μmol (for 4 h); G1 – vitamin C 1 mmol (pretreatment for 6 h) and after that Na₂CrO₄, in PBS 80 μmol (for 4 h)

CG – Na₂CrO₄, 5 μmol (single dose); G1 – ascorbate 0.25–1.5 mmol (pretreatment for 6 h) and after that 10 μmol Na₂CrO₄ (single dose)

CG – BSO 10 μmol (pretreatment 24 h prior to dye treatment) and after that Na₂CrO₄, 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 Na₂CrO₄, 80 μmol (30 min after dye loading)

G1 vs. CG
– intracellular chromium accumulation↓

G1 vs. CG
– DCF fluorescence in A549 cell↑
HLF- cells (LL-24 cell line), from 5-year-old male

CG – Na$_2$CrO$_4$ different doses: 3 µmol, 6 µmol, 9 µmol (for 24 h);
G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na$_2$CrO$_4$ different doses: 3 µmol, 6 µmol, 9 µmol (for 24 h)

CG – Na$_2$CrO$_4$, 6 µmol (for 24 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na$_2$CrO$_4$, 6 µmol (for 24 h)

CG – Na$_2$CrO$_4$, 75 µmol (for 2 h); G1 – vitamin C 1 mmol (pretreatment for 24 h) and after that Na$_2$CrO$_4$, 75 µmol (for 2 h)

CG – Na$_2$CrO$_4$, 9 µmol (for 24 h); G1 – vitamin C 1 mmol (pretreatment) and after that Na$_2$CrO$_4$, 9 µmol (for 24 h)

CG (N = 6) – Na$_2$CrO$_4$, 20 mg/kg bw (i.p. injection, single dose)
G1 (N = 6) – ascorbic acid 40 mg/kg bw (i.p. injection, single dose, pretreatment for 30 min) and after that Na$_2$CrO$_4$, 20 mg/kg bw (i.p. injection, single dose) and sacrificed after 24 h

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

rats (Rattus Norvegicus var. Alba), Wistar, male
CG (N = 6) – K$_2$CrO$_4$, 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$_2$CrO$_4$, 12 mg/kg bw (i.p. injection, 6 times over 2 weeks)

peripheral blood lymphocytes
CG – K$_2$CrO$_4$, 100 µmol (for 6 h); G1 – vitamin C 200 µmol (pretreatment for 2h) and after that K$_2$CrO$_4$, 100 µmol (for 6 h)
G2 – vitamin C 200 µmol and K$_2$CrO$_4$, 100 µmol (for 6 h)

human leukemic T-lymphocyte MOLT-4 cells
GC – K$_2$CrO$_4$, 400 µmol (for 8 h); G1 – ascorbate 1 mmol (pretreatment for 16 h) and after that K$_2$CrO$_4$, 400 µmol (for 8 h)

SeSF cell lines, Indo-Pacific dolphin
CG – K$_2$CrO$_4$, 12.5 µmol (for 24 h); G1 – vitamin C 50 µmol and K$_2$CrO$_4$, 12.5 µmol (for 24 h)

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.
murine macrophages J774  
CG – K₂CrO₄, 1 μg/ml (for 24 h); G1 – vitamin C 25 μg/ml (pretreatment for 30 min) and after that K₂CrO₄, 1 μg/ml (for 24 h)  
G1 vs. CG  
- NR uptake↓, LDH↓, caspase activity↓, DCFH-DA↓, NO↓, superoxide radical↓, GSH↑, GPx↑, SOD↑, rhodamine↑, PI↓, phagocytosis↑, cell proliferation↑

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↑

yeast *Saccharomyces cerevisiae* ZIM 1825, ZIM 1836, ZIM 327  
CG – K₂CrO₄, 0.8 mmol; G1 – 1 mmol ascorbic acid (pretreatment) and after that K₂CrO₄, 0.8 mmol  
G1 vs. CG  
- relative cell viability↑, mitotic gene conversions and reverse mutation of *S. cerevisiae*↓

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 kinase; CDKIs – cyclin-dependent kinase inhibitors; DCF – dichlorofluorescein; E2 – estradiol; ER-α – estradiol receptor α; ER-β – estradiol receptor β; ERK – extracellular-signal-regulated kinase; FSH-R – FSH receptor; FT₃ – free triiodothyronine; FT₄ – free thyroxine; GC – granulosa cells; GGT – γ-glutamyl transferase; H₂O₂ – 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; MIT – 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; TR – 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.
Protective 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 estriadiol 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 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 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].

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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).
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$_2$Cr$_2$O$_7$ 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$_2$Cr$_2$O$_7$ 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$_2$Cr$_2$O$_7$ 60 μg/kg bw/day (i.p. injection, for 5 days); G1 (N = 40) – K$_2$Cr$_2$O$_7$ 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$_2$Cr$_2$O$_7$ 6% LD$_{50}$/day (orally, for 42 days); G1 (N = 15) – K$_2$Cr$_2$O$_7$ 6% LD$_{50}$/day (orally, for 42 days) and Na$_2$SeO$_3$ 0.63 mg/kg bw/day (orally, for 42 days); G2 (N = 15) – K$_2$Cr$_2$O$_7$ 6% LD$_{50}$/day (orally, for 42 days) and Na$_2$SeO$_3$ 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$_2$Cr$_2$O$_7$ 67 mg/kg bw/day (orally, for 21 days); G1 (N = 6) – K$_2$Cr$_2$O$_7$ 67 mg/kg bw/day (orally, for 21 days) and Na$_2$SeO$_3$ 0.5 mg/kg of diet/day (orally, for 21 days) | G1 vs. CG – cerebrum and cerebellum: MDA↓, GSH↑, NPSH↑, vitamin C↑, CAT↓, GPx↑, SOD↑, AChE↑, LDH↑ – plasma: LDH↓ – severe brain damage↓ |
| 52        | rats, Wistar, adult, female | CG (N = 6) – K$_2$Cr$_2$O$_7$ 67 mg/kg bw/day (orally, for 21 days); G1 (N = 6) – K$_2$Cr$_2$O$_7$ 67 mg/kg bw/day (orally, for 21 days) and Na$_2$SeO$_3$ 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) – K₂Cr₂O₇, 6% LD₅₀/day (orally, for 42 days); G1 (N = 15) – K₂Cr₂O₇, 6% LD₅₀/day (orally, for 42 days) and Na₂SeO₃ 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↑, Ca²⁺-ATPase activity↑, MMP↑  
- G1 vs. CG  
- G1 vs. CG  
- G1 vs. CG  
- G1 vs. CG  
- G1 vs. CG  |
| 54        | rats, Wistar, adult, female | CG (N = 6) – K₂Cr₂O₇, 67 mg/kg bw/day (orally, for 21 days); G1 (N = 6) – K₂Cr₂O₇, 67 mg/kg bw/day (orally, for 21 days) and Na₂SeO₃ 0.5 mg/kg of diet/day (orally, for 21 days) | G1 vs. CG  
- liver: MDA↓, CAT↓, SOD↓, GP↑, 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) – K₂Cr₂O₇, 7.83 mg/kg bw/day (orally, for 42 days); G1(N = 15) – K₂Cr₂O₇, 7.83 mg/kg bw/day (orally, for 42 days) and Na₂SeO₃ 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↑, Ca²⁺-ATPase activity↑, MMP↑  
- G1 vs. CG  
- G1 vs. CG  
- G1 vs. CG  
- G1 vs. CG  |
| 56        | chickens, Arbor Acres (1-day-old) | CG (N = 10) – K₂Cr₂O₇, 8% LD₅₀/day (orally, for 5 weeks); G1 (N = 10) – K₂Cr₂O₇, 8% LD₅₀/day (orally, for 5 weeks) and nano-Se 0.5 mg/kg bw/day (orally, for 5 weeks); G2 (N = 10) – K₂Cr₂O₇, 8% LD₅₀/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 K₂Cr₂O₇, 8% LD₅₀/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) – K₂Cr₂O₇, 8% LD₅₀/day (orally, for 5 weeks); G1 (N = 20) – K₂Cr₂O₇, 8% LD₅₀/day (orally, for 5 weeks) and nano-Se 0.5 mg/kg bw/day (orally, for 5 weeks); G2 (N = 20) – K₂Cr₂O₇, 8% LD₅₀/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 K₂Cr₂O₇, 8% LD₅₀ (orally, for 3 weeks) | G1, G2 vs. CG  
- mRNA levels of FASN in the liver↓  
- mRNA levels of ACOX1 in the liver↑  
- the protein levels of FASN in the liver↓  
- the protein levels of ACOX1 in the liver↑  
- antibody expression levels of FASN in the liver↓  
- antibody expression levels of ACOX1 in the liver↑  |
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↓

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↑, GPx↑, CAT↑, AChE↑

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↔

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↑
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 Ca²⁺-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
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, B₆ 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  | CG (N = 9) – K$_2$Cr$_2$O$_7$ 0.630 mg/kg bw (i.t., 1/week for 1 month); G1 (N = 8) – K$_2$Cr$_2$O$_7$ 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 |
|           | Dawley, male   |                     | – 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↓ |
| 63        | rats, Wistar   | CG (N = 6) – physiological saline; C1 (N = 6) – K$_2$Cr$_2$O$_7$ 0.5 mg/kg bw (i.p. injection, single dose) and sacrificed 3, 5, 7 days after the injection; G2 (N = 6) – K$_2$Cr$_2$O$_7$ 5 mg/kg bw (i.p. injection, single dose) and sacrificed 3, 5, 7 days after the injection | G1 vs. CG |
|           | female         |                     | – 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 (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)↑ |
|           |                |                     | – histopathological changes in the kidney↑ |
| 64        | rats, Sprague  | CG (N = 9) – K$_2$Cr$_2$O$_7$ 0.630 mg/kg bw (i.t., 1/week for 1 month); G1 (N = 8) – K$_2$Cr$_2$O$_7$ 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 |
|           | Dawley, male   |                     | – CCl6 concentration in the serum↔, urinary 8-OHdG↓, average density of CCl6 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(C₂H₃O₂)₂ 1.5 mmol/kg (for 2 h or 4 h); G1 – Na₂Cr₂O₇ 100 μmol/kg (for 2 h or 4 h) and after that Zn(C₂H₃O₂)₂ 1.5 mmol/kg (for 4 h) | G1 vs. CG – expression of MT mRNA in the liver↓ |
|           |                 | CG – Zn(C₂H₃O₂)₂ 1.5 mmol/kg (for 4 h); G1 – Na₂Cr₂O₇ 100 μmol/kg (for 8 h) and after that Zn(C₂H₃O₂)₂ 1.5 mmol/kg (for 4 h) | G1 vs. CG – expression of MT mRNA in the liver↔ |
|           |                 | CG – Zn(C₂H₃O₂)₂ 1.5 mmol/kg (for 2 h); G1 – Na₂Cr₂O₇ 100 μmol/kg (for 2 h) and after that Zn(C₂H₃O₂)₂ 1.5 mmol/kg (for 4 h) | G1 vs. CG – levels of MT protein↓ |
|           |                 | CG – Zn(C₂H₃O₂)₂ 1.5 mmol/kg (for 4 h or 8 h); G1 – Na₂Cr₂O₇ 100 μmol/kg (for 4 h) and after that Zn(C₂H₃O₂)₂ 1.5 mmol/kg (for 4 h) | G1 vs. CG – levels of MT protein↔ |
| 66        | Arthrobacter sp. 61B | CG – K₂CrO₄ 200 mg/l; G1 – ZnSO₄ 50 mg/l and K₂CrO₄ 200 mg/l | G1 vs. CG – accumulation of Cr↑; growth of bacteria↑ |
|           | A. globiformis sp. 151B | CG – K₂CrO₄ 100 mg/l; G1 – ZnSO₄ 50 mg/l and K₂CrO₄ 100 mg/l | G1 vs. CG – accumulation of Zn – higher in Arthrobacter sp. 61B than in A. globiformis sp. 151B |
| 67        | human diploid dermal fibroblasts | CG – K₂CrO₄ 10 μmol (for 48 h); G1 – ZnSO₄ 100 μmol (pretreatment for 24 h) and after that K₂CrO₄ 10 μmol (for 48 h) | G1 vs. CG – ROS↓, DNA damage↓, GPₓ↑, SOD↑, expression of MT IIA↑, activated p53 protein↓, fragmented PARP↓, active caspase-3↓ |
human tumor cell line Hep-2

CG – K2CrO4 10 μmol/l (for 24 h); G2 – ZnSO4 50 μmol/l (for 24 h) and K2CrO4 10 μmol/l (for 24 h)

G1 vs. CG – proliferation↑

CG – K2CrO4 50 μmol/l (for 24 h); G2 – ZnSO4 100 μmol/l (for 24 h) and K2CrO4 50 μmol/l (for 24 h)

G1 vs. CG – proliferation↓

CG – K2CrO4 10 μmol/l (for 12 h); G1 – ZnSO4 100 μmol/l (for 12 h) and K2CrO4 10 μmol/l (for 12 h)

G2 – K2CrO4 150 μmol/l (for 12 h); G1 – ZnSO4 100 μmol/l (for 12 h) and K2CrO4 150 μmol/l (for 12 h)

G1 vs. CG – production: H2O2↓, superoxide anion↓

CG – K2CrO4 10 μmol/l (for 12 h); G1 – ZnSO4 10 μmol/l (for 12 h) and K2CrO4 150 μmol/l (for 12 h)

G1 vs. CG – mitochondrial membrane depolarization↑

CG – K2CrO4 150 μmol/l (for 12 h); G1 – ZnSO4 100 μmol/l (for 12 h) and K2CrO4 150 μmol/l (for 12 h)

G1 vs. CG – caspase-3 activation↓

CG – K2CrO4 10 μmol/l (for 12 h) and 10 μmol/l K2CrO4 (for 12 h)

G1 vs. CG – caspase-3 activation↑

CG – K2CrO4 50 μmol/l (for 12 h); G2 – ZnSO4 100 μmol/l (for 12 h) and K2CrO4 50 μmol/l (for 12 h)

G1 vs. CG – cleavage of PARP↓

CG – K2CrO4 10 μmol/l (for 24 h); G1 – ZnSO4 100 μmol/l (for 24 h) and K2CrO4 10 μmol/l (for 24 h)

G1 vs. CG – cleavage of PARP↑

CG – K2CrO4 50 μmol/l (for 24 h); G2 – ZnSO4 100 μmol/l (for 24 h) and K2CrO4 50 μmol/l (for 24 h)

G1 vs. CG – MT expression in cells↑

CG – Zn deficient medium; G1 – Zn medium deficient and Zn 50 μmol (for 2 days)

G1 vs. CG – number of transformed foci↓

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

CC16 – club (Clara) cell secretory protein; i.g. – intragastric administration; i.t. – intatracheal instillation; MT IIA – metallothionein 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.
REFERENCES

1. Vincent JB. Chromium: celebrating 50 years as an essential element? Dalton Trans. 2010;39(16):3787–94, https://doi.org/10.1039/b920480f.

2. European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific opinion on dietary reference values for chromium. EFSA J. 2014;12(10):3845, https://doi.org/10.2903/j.efsa.2014.3845.

3. International Agency for Research on Cancer (IARC) [Internet]. Lyon: The Agency; 1990 [cited 2020 Jul 18]. Monograph on the evaluation of carcinogenic risks to humans. Chromium, nickel and welding. Available from: https://publications.iarc.fr/67.

4. National Institute for Occupational Safety and Health [Internet]. Washington: The Institute; 2013 [cited 2020 Jul 20]. Criteria for a recommended standard: Occupational exposure to hexavalent chromium. Available from: https://www.cdc.gov/niosh/docs/2013128/pdfs/2013_128.pdf?id=10.26616/NIOSHPUB2013128.

5. Stanislawksa M, Janasik B, Kuras R, Malachowska B, Halatek T, Wasowicz W. Assessment of occupational exposure to stainless steel welding fumes – A human biomonitoring study. Toxic Lett. 2020;329:47–55, https://doi.org/10.1016/j.toxlet.2020.04.019.

6. International Programme on Chemical Safety [Internet]. Geneva: WHO; 2013 [cited 2020 Jul 18] Concise International Chemical Assessment Document 78. Inorganic chromium (VI) compounds. Available from: https://apps.who.int/iris/handle/10665/90560.

7. Yatera K, Morimoto Y, Ueno S, Noguchi S, Kawaguchi T, Tanaka T, et al. Cancer risks of hexavalent chromium in the respiratory tract. J UOEH 2018;40(2):157–72, https://doi.org/10.7888/juoh.40.157.

8. Li H, Chen Q, Li S, Yao W, Li L, Shi X, et al. Effect of Cr(VI) exposure on sperm quality: human and animal studies. Ann Occup Hyg. 2001;45(7):505–11, https://doi.org/10.1016/S0003-4878(01)00004-7.

9. Wang T, Jia G, Zhang J, Ma Y, Feng W, Liu L, et al. Renal impairment caused by chronic occupational chromate exposure.
1. Alexander J, Aaseth J. Uptake of chromate in human red blood cells and isolated rat liver cells: the role of the anion carrier. Analyst. 1995;120(3):931–3, https://doi.org/10.1039/AN9952000931.

11. Ray RR. Adverse hematological effects of hexavalent chromium: an overview. Interdiscip Toxicol. 2016;9(2):55–65, https://doi.org/10.1515/intox-2016-0007.

12. Ding M, Shi X. Molecular mechanisms of Cr(VI)-induced carcinogenesis. Mol Cell Biochem. 2002;234:293–300, https://doi.org/10.1023/A:1015975218920.

13. Santonen T, Alimonti A, Bocca B, Duca RC, Galea KS, Godderis L, et al. Setting up a collaborative European human biological monitoring study on occupational exposure to hexavalent chromium. Environ Res. 2019;177:108583, https://doi.org/10.1016/j.envres.2019.108583.

14. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097, https://doi.org/10.1371/journal.pmed.1000097.

15. Hemmati AA, Nazari Z, Ranjbari N, Torfi A. Comparison of the preventive effect of vitamin C and E on hexavalent chromium induced pulmonary fibrosis in rat. Inflammopharmacol. 2008;16(4):195–7, https://doi.org/10.1007/s10787-008-7004-4.

16. Carlisle DL, Pritchard DE, Sinhg J, Owens BM, Blankenship LJ, Orenstein JM, et al. Apoptosis and P53 induction in human lung fibroblasts exposed to chromium (VI): Effect of ascorbate and tocopherol. Toxicol Sci. 2000;55(1):60–8, https://doi.org/10.1093/toxsci/55.1.60.

17. Balakrishnan R, Satish Kumar CS, Rani MU, Kavita K, Boobalan G, Reddy AG. Evaluation of protective action of α-tocopherol in chromium-induced oxidative stress in female reproductive system of rats. J Nat Sc Biol Med. 2013;4(1):87–93, https://doi.org/10.4103/0976-9668.107266.

18. Chandra AK, Chatterjee A, Ghosh R, Sarkar M. Vitamin E-supplementation protect chromium (VI)-induced spermatogenic and steroidogenic disorders in testicular tissues of rats. Food Chem Toxicol. 2010;48(3):972–9, https://doi.org/10.1016/j.fct.2010.01.008.

19. Acharya UR, Mishra M, Mishra I, Tripathy RR. Potential role of vitamins in chromium induced spermatogenesis in Swiss mice. Environ Toxicol Pharmacol. 2004;15(2–3):53–9, https://doi.org/10.1016/j.etap.2003.08.010.

20. Blankenship LJ, Carlisle DL, Wise JP, Orenstein JM, Dye LE, Patierno SR. Induction of apoptotic cell death by particulate lead chromate: differential effects of vitamins C and E on genotoxicity and survival. Toxicol Appl Pharmacol. 1997;146(2):270–80, https://doi.org/10.1006/taap.1997.8237.

21. Shati AA. Ameliorative effect of vitamin E on potassium dichromate-induced hepatotoxicity in rats. J King Saud University Sci. 2014;26(3):181–9, https://doi.org/10.1016/j.jksus.2013.12.001.

22. Santonen T, Alimonti A, Bocca B, Duca RC, Galea KS, Godderis L, et al. Setting up a collaborative European human biological monitoring study on occupational exposure to hexavalent chromium. Environ Res. 2019;177:108583, https://doi.org/10.1016/j.envres.2019.108583.

23. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097, https://doi.org/10.1371/journal.pmed.1000097.

24. Chorvatovičová D, Ginter E, Košinová A, Zloch Z. Effect of vitamins C and E on toxicity and mutagenicity of hexavalent chromium in rat and guinea pig. Mutat Res Lett. 1991;262(1):41–6, https://doi.org/10.1016/0165-7992(91)90104-C.

25. V andana S, Ram S, Ilavazhagan M, Kumar GD, Banerjee PK. Comparative cytoprotective activity of vitamin C, E and beta-carotene against chromium induced oxidative stress in murine macrophages. Biomed Pharmacother. 2006;60(2):71–6, https://doi.org/10.1016/j.biopha.2005.04.005.

26. Mattagajasingh SN, Misra BR, Misra HP. Carcinogenic chromium(VI)-induced protein oxidation and lipid peroxidation: implications in DNA–protein crosslinking.
J Appl Toxicol. 2008;28(8):987–97, https://doi.org/10.1002/jat.1364.

27. Kumari RR, Kumar P, Mondal TK. Effect of Vitamin E and Selenium on haematological parameters in sub-acute toxicity of hexavalent chromium in Broiler Chick. Natl J Physiol Pharm Pharmacol. 2013;3(2):158–61, https://doi.org/10.5455/njppp.2013.3.150-153.

28. Fernandes MAS, Geraldes CFGC, Oliveira CR, Alporim MC. Chromate–induced human erythrocytes haemoglobin oxidation and peroxidation: influence of vitamin E, vitamin C, salicylate, deferoxamine, and N-ethylmaleimide. Toxicol Lett. 2000;114(1–3):237–43, https://doi.org/10.1016/S0378-4274(00)00167-3.

29. Qureshi IZ, Mahmood T. Prospective role of ascorbic acid (vitamin C) in attenuating hexavalent chromium-induced functional and cellular damage in rat thyroid. Toxicol Ind Health. 2010;26(6):349–59, https://doi.org/10.1177/0748233 710371109.

30. Zhong X, Zeng M, Bian H, Zhong C, Xiao F. An evaluation of the protective role of vitamin C in reactive oxygen species-induced hepatotoxicity due to hexavalent chromium in vitro and in vivo. J Occup Med Toxicol. 2017;12:15, https://doi.org/10.1186/s12995-017-0161-x.

31. Fatima S, Mahmood R. Vitamin C attenuates potassium dichromate–induced nephrotoxicity and alterations in renal brush border membrane enzymes and phosphate transport in rats. Clinica Chimica Acta. 2007;386(1–2):94–9, https://doi.org/10.1016/j.cca.2007.08.006.

32. Li WJ, Yang CL, Chow KC, Kuo TW. Hexavalent chromium induces expression of mesenchymal and stem cell markers in renal epithelial cells. Mol Carcinog. 2016;55(2):182–92, https://doi.org/10.1002/mc.22268.

33. Banu SK, Samuel JB, Arosh JA, Burghardt RC, Aruldhas MM. Lactational exposure to hexavalent chromium delays puberty by impairing ovarian development, steroidogenesis and pituitary hormone synthesis in developing Wistar rats. Toxicol Appl Pharmacol. 2008;232(2):180–9, https://doi.org/10.1016/j.taap.2008.06.002.

34. Stanley JA, Lee J, Nithy TK, Arosh JA, Burghardt RC, Banu SK. Chromium-VI arrests cell cycle and decreases granulosa cell proliferation by down-regulating cyclin-dependent kinases (CDK) and cyclins and up-regulating CDK-inhibitors. Reprod Toxicol. 2011;32(1):112–23, https://doi.org/10.1016/j.reprotox.2011.05.007.

35. Banu SK, Stanley JA, Lee J, Stephen SD, Arosh JA, Hoy er PB, et al. Hexavalent chromium–induced apoptosis of granulosa cells involves selective sub-cellular translocation of Bcl-2 members, ERK1/2 and p53. Toxicol Appl Pharmacol. 2011;251(3):253–66, https://doi.org/10.1016/j.taap.2011 .01.011.

36. Sugiyama M, Tsuzuki K, Ogura R. Effect of ascorbic acid on DNA damage, cytotoxicity, glutathione reductase, and formation of paramagnetic chromium in Chinese hamster V-79 cells treated with sodium chromate(VI). J Biol Chem. 1991;266(6):3383–6.

37. Martin BD, Schoenhard JA, Hwang JM, Sugden KD. Ascorbate is a pro-oxidant in chromium-treated human lung cells. Mutat Res Fund Mol Mech Mutagen. 2006;610(1–2):74–84, https://doi.org/10.1016/j.mrgentox.2006.06.014.

38. Afolaranmi GA, Grant MH. The effect of ascorbic acid on the distribution of soluble Cr and Co ions in the blood and organs of rats. J Appl Toxicol. 2013;33(3):220–6, https://doi. org/10.1002/jat.1744.

39. Xiao F, Chen D, Luo L, Zhong X, Xie Y, Zou L, et al. Time-order effects of vitamin C on hexavalent chromium-induced mitochondrial damage and DNA-protein crosslinks in cultured rat peripheral blood lymphocytes. Mol Med Rep. 2013;8(1):53–60, https://doi.org/10.3892/mmr.2013.1462.

40. Yu X, Yu RQ, Gui D, Zhang X, Zhan F, Sun X, et al. Hexavalent chromium induces oxidative stress and mitochondria-mediated apoptosis in isolated skin fibroblasts of Indo-Pacific humpback dolphin. Aquat Toxicol. 2018;203:179–86, https://doi.org/10.1016/j.aquatox.2018.08.012.

41. Poljšak B, Gazdag Z, Jenko-Brinovec Š, Fusi Š, Pesti M, Belagj J, et al. Pro-oxidative vs. antioxidative properties of ascorbic acid in chromium(VI)-induced damage: an in vivo
and in vitro approach. J Appl Toxicol. 2005;25(6):535–48, https://doi.org/10.1002/jat.1093.
42. Yousef MI, El-Demerdash FM, Kamil KI, Elaswad FAM. Ameliorating effect of folic acid on chromium(VI)-induced changes in reproductive performance and seminal plasma biochemistry in male rabbits. Reprod Toxicol. 2006;21(3):322–8, https://doi.org/10.1016/j.reprotox.2005.09.005.
43. El-Demerdash F, Yousef MI, Elaswad FAM. Biochemical study on the protective role of folic acid in rabbits treated with chromium (VI). J Environ Sci Health B. 2006;41(5):731–46, https://doi.org/10.1080/03601230600704282.
44. Alimba CG, Dhillon V, Bakare AA, Fenech M. Genotoxicity and cytotoxicity of chromium, copper, manganese and lead, and their mixture in WIL2-NS human B lymphoblastoid cells is enhanced by folate depletion. Mutat Res Genet Toxicol Environ Mutagen. 2016;(798–799):35–47, https://doi.org/10.1016/j.mrgentox.2016.02.002.
45. Wang TC, Jia G, Zhang J, Ma YH, Liu LZ, Zhang N, et al. Vitamin B12 and folate deficiency and elevated plasma total homocysteine in workers with chronic exposure to chromate. Occup Environ Med. 2011;68:870–5, https://doi.org/10.1016/j.ijhmed.2010.063305.
46. Wang TC, Song YS, Yu SF, Zhang J, Wang H, Gu YE, et al. Association of folate deficiency and selected tumor marker concentrations in long-term hexavalent chromium exposed population. Int J Hyg Environ Health. 2014;217(1):88–94, https://doi.org/10.1016/j.ijhheh.2013.03.013.
47. Anand SS. Protective effect of vitamin B6 in chromium-induced oxidative stress in liver. J Appl Toxicol. 2005;25(5):440–3, https://doi.org/10.1002/jat.1077.
48. Hassanin KMA, Abd El-Kawi SH, Hashem KS. The prospective protective effect of selenium nanoparticles against chromium-induced oxidative and cellular damage in rat thyroid. Int J Nanomedicine. 2013;8:1713–20, https://doi.org/10.2147/IJN.S42736.
49. Mohamed HZE, Ragab IK, Ghafeer HH. A histological study on the possible protective effect of selenium against chromium-induced thyrotoxicity in adult male albino rats. Egyptian J Histol. 2016;39(1):1–11, https://doi.org/10.1097/01.EHX.0000481747.20806.2d.
50. Hao P, Zhu Y, Wang S, Wan H, Chen P, Wang Y, et al. Selenium administration alleviates toxicity of chromium(VI) in the chicken brain. Biol Trace Elem Res. 2017;178(1):127–35, https://doi.org/10.1007/s12011-016-0915-9.
51. Soudani N, Troudi A, Ben Amara I, Bouazziz H, Boudawara T, Zeghal N. Ameliorating effect of selenium on chromium (VI)-induced oxidative damage in the brain of adult rats. J Physiol Biochem. 2012;68(3):397–409, https://doi.org/10.1007/s13105-012-0152-4.
52. Soudani N, Sefi M, Ben Amara I, Boudawara T, Zeghal N. Protective effects of Selenium (Se) on Chromium (VI) induced nephrotoxicity in adult rats. Ecotoxicol Environ Safet. 2010;73(4):671–8, https://doi.org/10.1016/j.ecoenv.2009.10.002.
53. Wan H, Zhu Y, Chen P, Wang Y, Hao P, Cheng Z, et al. Effect of various selenium doses on chromium(IV)-induced nephrotoxicity in a male chicken model. Chemosphere. 2017;174:306–14, https://doi.org/10.1016/j.chemosphere.2017.01.143.
54. Soudani N, Sefi M, Ben Amara I, Boudawara T, Zeghal N. Effects of selenium on chromium (VI)-induced hepatotoxicity in adult rats. Exp Toxicologic Pathol. 2011;63(6):541–8, https://doi.org/10.1016/j.etp.2010.04.005.
55. Wang Y, Liu Y, Wan H, Zhu Y, Chen P, Hao P, et al. Moderate selenium dosing inhibited chromium (VI) toxicity in chicken liver. J Biochem Mol Toxicol. 2017;31(8):e21916, https://doi.org/10.1002/jbt.21916.
56. Xueting L, Rehman MU, Mehmood K, Huang S, Tian X, Xiaoxing W, et al. Ameliorative effects of nano-elemental selenium against hexavalent chromium-induced apoptosis in broiler liver. Environ Sci Pollut Res. 2018;25(16):15609–15, https://doi.org/10.1007/s11356-018-1758-z.
57. Luo M, Huang S, Zhang J, Zhang L, Mehmood K, Jiang J, et al. Effect of selenium nanoparticles against abnormal fatty acid metabolism induced by hexavalent chromium in chicken’s liver. Environ Sci Pollut Res. 2019;26(21):21828–34, https://doi.org/10.1007/s11356-019-05397-3.
58. Soudani N, Troudi A, Bouaziz H, Ben Amara I, Boudawara T, Zeghal N. Cardioprotective effects of selenium on chromium (VI)-induced toxicity in female rats. Ecotox Environ Safe. 2011;74(3):513–20; https://doi.org/10.1016/j.ecoenv.2010.06.009.

59. Soudani N, Ben Amara I, Troudi A, Hakim A, Bouaziz H, Aydi Makni F, et al. Oxidative damage induced by chromium (VI) in rat erythrocytes: protective effect of selenium. J Physiol Biochem. 2011;67(4):577–88; https://doi.org/10.1007/s13105-011-0104-4.

60. Chen P, Zhu Y, Wan H, Wang Y, Hao P, Cheng Z, et al. Effects of the oral administration of K2Cr2O7 and Na2SeO3 on Ca, Mg, Mn, Fe, Cu, and Zn contents in the heart, liver, spleen, and kidney of chickens. Biol Trace Elem Res. 2017;180(2):285–96; https://doi.org/10.1007/s12011-017-0999-x.

61. Cemeli E, Carder J, Anderson D, Guillamet E, Morillas MJ, Creus A, et al. Antigenotoxic properties of selenium compounds on potassium dichromate and hydrogen peroxide. Teratog Carcinog Mutagen. 2003;23(S2):53–67; https://doi.org/10.1002/tcm.10080.

62. Song Y, Wang T, Pu J, Guo J, Chen Z, Wang Y, et al. Multielement distribution profile in Sprague-Dawley rats: Effects of intratracheal instillation of Cr(VI) and Zn intervention. Toxicol Lett. 2014;226(2):198–205; https://doi.org/10.1016/j.toxlet.2014.02.008.

63. Chmielnicka J, Świetlicka E, Nasiadek M. Essential elements as early indicators of hexavalent chromium nephrotoxicity. Ecotox Environ Safe. 2002;53(1):20–6; https://doi.org/10.1006/cesa.2001.2152.

64. Zhao L, Song Y, Pu J, Guo J, Wang Y, Chen Z, et al. Effects of repeated Cr(VI) intratracheal instillation on club (Clara) cells and activation of nuclear factor-kappa B pathway via oxidative stress. Toxicol Lett. 2014;231(1):72–81; https://doi.org/10.1016/j.toxlet.2014.09.011.

65. Alcedo JA, Misra M, Hamilton JW, Wetterhahn KE. The genotoxic carcinogen chromium(VI) alters the metal-inducible expression but not the basal expression of the metallothionein gene in vivo. Carcinogenesis. 1994;15(5):1089–92; https://doi.org/10.1093/carcin/15.5.1089.

66. Tsibakhashvili NY, Kalabegishvili TL, Rcheulishvili AN, Gin portray EN, Lomidze LG, Gvarjaladze DN, et al. Effect of Zn(II) on the reduction and accumulation of Cr(VI) by Arthrobacter species. J Ind Microbiol Biotechnol. 2011;38(11):1803–8; https://doi.org/10.1007/s10295-011-0967-y.

67. Rudolf E, Červinka M. The role of intracellular zinc in chromium(VI)-induced oxidative stress, DNA damage and apoptosis. Chem Biol Interact. 2006;162(3):212–27; https://doi.org/10.1016/j.cbi.2006.06.005.

68. Rudolf E, Červinka M, Cerman J. Zinc has ambiguous effects on chromium (VI)-induced oxidative stress and apoptosis. J Trace Elem Med Bio. 2005;18(3):251–60; https://doi.org/10.1016/j.jtemb.2004.09.004.

69. Kimura T, Onoder A, Okumura F, Nakanishi T, Itoh N. Chromium (VI)-induced transformation is enhanced by Zn deficiency in BALB/c 3T3 cells. J Toxicol Sci. 2015;40(3):383–7; https://doi.org/10.2131/jts.40.383.