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Antioxidant Effect of the Complex Action of Vitamin E and Ethylthiosulfanylate in the Liver and Kidneys of Rats under Conditions of Chrome(VI)-Induced Oxidative Stress

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Abstract: The aim of our study was to investigate the efficacy and benefits of the complex effect of vitamin E and ethylthiosulfanylate (ETS) on the state of the pro/antioxidant system in the liver and kidneys of rats under the condition of Cr(VI)-induced oxidative stress. Rats were divided into 8 groups. Groups received: I (control) - physiological solution (150 µl) for 7 days; II – oil solution (1 ml) for 14 days; III, IV, VII, VIII - $K_2Cr_2O_7$ (2.5 mg Cr(VI)/kg body weight (b.w)) for 7 (III, IV) and for 14 (VII, VIII); V - vitamin E (20 mg/kg b.w) for 14 days; VI, VII, VIII - vitamin E in complex with ETS (100 mg/kg b.w) for 14 days. Results report that $K_2Cr_2O_7$ caused Cr(VI)-induced oxidative stress due to activation of lipid peroxidation (LP) processes. Cr(VI) action for 7 days caused compensatory activation of the antioxidant defense system (AOS) in both tissues. However, the longer action of Cr(VI) was accompanied by depletion of AOS enzyme activity and GSH content. The complex effect of vitamin E and ETS reduced the intensity of Cr(VI)-induced oxidative stress in both rat tissues. Our results indicate about positive antioxidant properties of vitamin E and ETS under the condition of Cr(VI) toxicity.

Keywords: rats; antioxidant system; oxidative stress; vitamin E; ethylthiosulfanylate; potassium bichromate; peroxidation.

1. Introduction

The active use of chromium for industrial and agricultural purposes led to a significant accumulation of Cr-containing compounds in the environment [1-3]. Chromium compounds are one of the most common pollutants in aquatic and terrestrial ecosystems [4-6]. The main industries that require active use of Cr-containing compounds are leather tanning, wood preservatives, industrial welding of metals, chrome plating, chromate, and ferrochromate production [1]. Chromium is a natural metal, found mostly in two different forms: trivalent chromium (Cr(III)) and hexavalent chromium (Cr(VI)). Cr(III) is the last stage of chromium oxidation. The trivalent form of chromium is common in all biological systems, thermodynamically stable, and exhibits strong properties for forming coordination compounds. The hexavalent form (Cr(VI)) exhibits strong toxic and carcinogenic properties and is usually presented as oxygen-containing compounds of chromates (CrO_4^{2-}) and dichromates ($Cr_2O_7^{2-}$) [1,7]. The high toxicity of Cr(VI) compounds is associated with the ability to be easily absorbed

and rapidly transported into the cells through the sulfate channels [8,9]. Then Cr(VI) is reduced to Cr(III) in several stages after penetration into the cell. A large number of reactive oxygen species (ROS) is generated during the process of Cr(VI) reduction [10,11]. Activation of ROS formation leads, in turn to the development of oxidative stress and tissue damage. Cr(VI) reduction processes are also accompanied by cytotoxicity, genotoxicity, carcinogenicity, and apoptosis through the p53 regulatory gene modulation [12-15]. The AOS system is the main barrier in the Cr(VI)-induced oxidative stress counteraction [9]. Enzymatic and non-enzymatic components of the AOS system, such as GSH and NADPH-dependent flavoenzymes, stimulate and accelerate the reduction of highly toxic Cr(VI) to much less toxic Cr(III). In turn, enzymes such as SOD, CAT, GP, and non-enzymatic GSH tripeptide neutralize many ROS, which are formed during the reduction of Cr(VI) [16,17]. However, prolonged Cr(VI)-induced oxidative stress leads to depletion of AOS system resources and provokes cell, tissue, and oxidative organ damage due to increased pro-oxidant processes [9,18]. Cr(VI)-containing compounds, such as potassium dichromate ($K_2Cr_2O_7$) at a dose of 8 mg/kg body weight causes acute hepatotoxicity in rats due to increasing necrotic and inflammatory processes and depletion of AOS system resources in liver tissue of animals [19]. Single subcutaneous injection of $K_2Cr_2O_7$ at a dose of 10 mg/kg also results in Cr(VI)-induced oxidative stress and damage to rat liver tissue, followed by degenerative changes in histoarchitecture and dilatation of hepatic sinusoids. A similar dose of Cr(VI) causes degenerative changes in tubular epithelial cells, cystic dilatation of tubules, congestion of blood vessels, hyaline casts, and dilatation of bowmans space in kidneys of rats [14]. Hepato- and nephrotoxicity caused by $K_2Cr_2O_7$ is accompanied by acute Cr(VI) -induced oxidative stress. Cr(VI)-induced toxicity stimulates, in particular, the formation of ROS, hyperactivation of peroxidation processes, inhibition of antioxidant enzymes activity, decreasing of cellular GSH, as well as depletion of non-protein sulfhydryl groups and accumulation of Cr(VI) liver and kidneys of rats and mice [14,20,21]. It is believed that the maintenance of antioxidant status is an important factor for reducing and preventing the negative effects of Cr(VI)-induced oxidative stress [14,22,23]. Neutralization of Cr(VI) is carried out by enzymatic reduction to Cr(V) and subsequent transformation into Cr-containing salts with the participation of GR and NADPH. Non-enzymatic antioxidants such as vitamin E, ascorbic acid, N-acetylcysteine, garlic powder, and GSH have the ability to reduce oxidative damage caused by $K_2Cr_2O_7$ [24,25]. Vitamin E is considered the most effective fat-soluble non-enzymatic antioxidant, which protects the cell membrane from radical-induced peroxidation, stimulates the activation of antioxidant enzymes, and reduces the intensity of oxidative stress caused by heavy metal-induced toxicity. The action of vitamin E at a dose of 100 and 125 mg/kg body weight for 2 and 6 weeks, respectively, reduces the level of $K_2Cr_2O_7$-induced peroxidation processes and restores GSH content and SOD activity in the liver and kidneys of rats [14,19,22]. Vitamin E at a dose of 125 mg/kg body weight also exhibits antioxidant and anti-inflammatory properties and reduces the intensity of Cr(VI)-induced toxicity in the liver and kidneys of rats [22].

Ethylthiosulfanylate belongs to a class of thiosulfonate compounds. Thiosulfonates are synthetic analogs of natural biologically active organosulfur compounds obtained from garlic, onion, broccoli, and cauliflower. Thiosulfonates are more stable than their natural analogs, exhibit a wide range of biological properties, and are characterized by low toxicity. Many researchers studied the anticancer, anti-inflammatory, antifungal, antimicrobial, and immunomodulatory properties of thiosulfonates in recent years. However, there are not sufficient studies about the antioxidant properties of thiosulfonates. It is known that

thiosulfonates are able to modulate transcription factors, which are involved in the activation of AOS system genes [26,27]. The antioxidant effect of these compounds is manifested in the ability to reduce the intensity of GSH pool depletion and thiobarbituric acid reactive substances (TBARS) formation in rat liver under the condition of oxidative stress [28]. There is very little known about the antioxidant properties of thiosulfonates under the toxic action of heavy metals. However, natural organosulfur compounds showed a positive antioxidant effect against the Cr(VI)-induced oxidative stress [23,29-31]. Our previous studies also indicate that ethylthiosulfanylate exhibits antioxidant properties and partially eliminates the negative effects of $K_2Cr_2O_7$-induced oxidative stress in rat liver tissue [32].

Therefore, given the positive antioxidant properties of vitamin E, as well as thiosulfonates and their natural analogs, our study aimed to investigate the efficacy and benefits of the complex effect of vitamin E and ethylthiosulfanylate on the state of the pro/antioxidant system in the liver and kidneys of rats under the condition of Cr(VI)-induced oxidative stress.

2. Materials and Methods

The section «Materials and Methods» was prepared by analogy with our previous publication [32].

2.1. Experimental design.

In our work, we used 40 Wistar male rats weighing 130-140g. We formed 8 groups of animals (5 rats per group): 1 control group and 7 experimental groups. All rats were housed in standard conditions and received standard feed and drinking water ad libitum.

Intact control rats in group I received one intraperitoneally injection of physiological saline (150 µl) once a day for 7 days. The animals of experimental groups III and IV were treated intraperitoneally with $K_2Cr_2O_7$ dissolved in 150 µl of physiological saline (Cr(VI) concentration 2.5 mg/kg body weight) for 7 and 14 days, respectively.

Group II received intragastrically 1000 µl of sunflower oil solution (trademark «Oleina»; DSTU 4492: ISO 14024) once a day for 14 days and immediately after that, physiological saline (150 µl) were given intraperitoneally once a day for 7 days.

Group V: were injected daily intragastrically with an oil solution of vitamin E at a dose of 20 mg/kg body weight for 14 days and then immediately after that, physiological saline (150 µl) were administered intraperitoneally once a day for 7 days.

Group VI: were injected daily intragastrically with a complex of oil solution of vitamin E [20 mg/kg body weight] and ethylthiosulfanylate (ETS) [100 mg/kg body weight] for 14 days and then immediately after that injected daily intraperitoneally with 150 µl of physiological saline solution for 7 days.

Group VII/Group VIII: received intragastrically complex oil solution of vitamin E [20 mg/kg body weight] and ETS [100 mg/kg body weight] for 14 days and then immediately after that received $K_2Cr_2O_7$ intraperitoneally daily at a dose 2.5 mg Cr(VI)/kg body weight per day for 7 days/14 days.

All manipulations with animals in our work were consistent with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and “Common Ethical Principles for Animal Experiments” (Ukraine, 2001). Permission to conduct research was obtained from the Committee on Bioethics of the Institute of Animal Biology NAAS of Lviv (Protocol № 80).

The work studied the effects of the newly synthesized ETS (ethyl 4-aminobenzenethiosulfonate) compound in complex with vitamin E on the rat body. ETS was synthesized at the department of technology of biologically active compounds, pharmacy, and biotechnology of National University "Lviv Polytechnic" according to the protocol described in detail in the paper [33, 34].

After decapitation of the animals, which occurred under thiopental anesthesia, the kidneys were collected. All procedures on kidneys were performed at 4 °C. The research material was the kidney homogenates of rats, which were prepared on 0.05 M Tris-HCl buffer with pH 7.4 in the ratio 1 g of tissue and 9 ml of buffer (1:9, weight/volume) and then centrifuged for 15 minutes at 1000 g. After centrifugation in obtained supernatants, the content of GSH, peroxidation products level, and antioxidant enzyme activity were determined.

2.1.1. Groups of animals.

Groups of animals were compared according to the following scheme (Figure 1). Group I is an intact control in relation to experimental groups III and IV, which did not receive an oil solution. Group II controls in relation to experimental groups V, VI, VII, and VIII, which received an oil solution. We recorded the percentage (%) change in indicators for III and IV experimental groups relative to a group I (intact control). We also recorded the % change in indicators for V, VI, VII, and VIII experimental groups relative to group II (oil control). At the final stage, we analyzed % change in indicators of III/IV experimental groups relative to a group I (intact control) and compared it with the % change in indicators of VII/VIII experimental groups relative to group II (oil control).

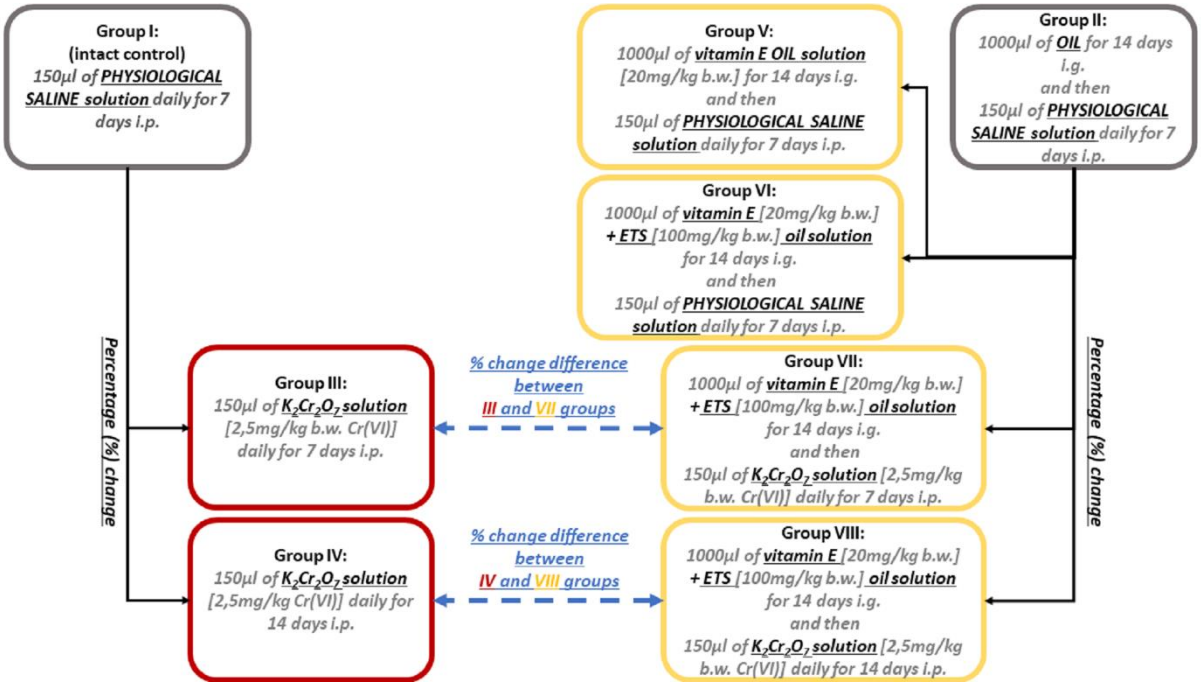

Figure 1. Groups of animals: Note: ETS (ethylthiosulfanylate), i.p. (intraperitoneally), i.g. (intragastrically), b.w. (body weight).

2.2. Processing.

2.1.1. Concentration of LHP.

The measurement of LHP (lipid hydroperoxides) level was performed in consistent with the methods of trichloroacetic acid-induced proteins precipitation and ethanol-induced lipid

extraction [35]. Ammonium thiocyanate interacts with lipid ethanol extracts and initiates the colored reaction. Absorbance recording of the colored product was performed spectrophotometrically (λ 480 nm). LHP level (SU/g tissue) was calculated as the difference between the control and experimental samples.

2.2.2 Concentration of TBARS.

Evaluating the concentration of TBARS (thiobarbituric acid reactive substances) is based on the principle of malondialdehyde and thiobarbituric acid interaction under the conditions of acidity and a high temperature [35]. The result of malondialdehyde and thiobarbituric acid interaction is the color reaction. The colored product absorbance recording was performed spectrophotometrically (λ 535 nm, and λ 580 nm) and TBARS level was calculated as nmol MDA/g tissue.

2.2.3. Activity of GP.

The measurement of GP (glutathione peroxidase) enzymatic activity is performed in the presence of GSH before and after adding of tertiary butyl hydroperoxide [35]. Evaluating the GP activity is based on the principle of GSH oxidation rate. SH-groups of GSH molecule is oxidized in the presence of 2-Nitrobenzoic acid. Dinitrophenyl anion is formed as a result of GSH oxidation. Absorbance recording of the colored product was performed spectrophotometrically (λ 412 nm) and GP activity was calculated in nmol GSH/min.×mg protein.

2.2.4. Activity of GR.

The measurement of GR (glutathione reductase) enzymatic activity was performed in the presence of oxidized glutathione and NADPH [35]. The evaluating of GR activity is based on the principle of oxidized glutathione reduction rate. The absorbance recording was performed spectrophotometrically (λ 340 nm) for 1 minute at 37 °C. The rate of extinction lowering is an indicator of the reaction intensity. GR activity was calculated in μmol NADPH/min.×mg protein.

2.2.5. Concentration of GSH.

The evaluating of the GSH (reduced glutathione) level is based on the principle of thionitrophenyl anion formation (colored product) after binding of 2-Nitrobenzoic acid to the SH-group of GSH molecule [36]. The value of GSH concentration depends on the intensity of the color reaction. The colored product absorbance recording was performed spectrophotometrically (λ 412 nm) and GSH content was calculated as mmol GSH/g tissue.

2.2.6. Activity of SOD.

The measurement of SOD (superoxide dismutase) enzymatic activity was performed in the presence of NADH and phenazine methosulfate [36]. The evaluating of the SOD activity is based on the principle of nitroblue tetrazolium reduction. The intensity of inhibition of the nitroblue tetrazolium reduction process indicates the intensity of enzyme activity. The absorbance recording was performed spectrophotometrically (λ 540 nm) and SOD activity was calculated in standard units per 1 mg of protein.

2.2.7. Activity of CAT.

The measurement of CAT (catalase) enzymatic activity was performed in the presence of molybdenum salts, which interact with hydrogen peroxide [36]. The colored product was formed as a result of the reaction. The colored product absorbance recording was performed spectrophotometrically (λ 410 nm) and CAT activity was calculated in mmol/minute × 1 mg of protein.

2.2.8. Protein concentration.

The concentration of total protein in the tissue homogenates was measured by the Lowry Method [37] by using the kits "Simko LTD" (Ukraine, Lviv). The measuring of all absorbance values was performed on a spectrophotometer, “Unico” 1205 (USA).

2.3. Statistical analysis.

All experimental data were statistically analyzed by Microsoft Excel software using one-way analysis of variance (ANOVA) and Tukey-Kramer test. All experimental values were calculated as mean values (M) ± standard error (S.E.M.) and were considered as statistically significant at $P < 0.05$.

3. Results and Discussion

The section «Results and Discussions» was prepared by analogy with our previous publication [32].

3.1. Oxidative stress markers.

We found that intraperitoneal administration of potassium dichromate for 7 and 14 days leads to an increase in the content of oxidative stress markers in the liver and kidney tissue of male rats. Exposure to $K_2Cr_2O_7$ at a dose of 2.5 mg Cr(VI)/kg body weight for 7 (III group) and 14 days (IV group) caused a significant increase of TBARS content in liver of animals in comparison with group I (control) by 69 and 75%, respectively (Table 1). The TBARS level was also significantly elevated in kidney tissue of rats of III and IV experimental groups relative to the control by 41 and 46%, respectively. A similar dose of Cr(VI) led to a significant increase of LHP concentration in rat liver of III and IV experimental groups compared to group I by 112 and 127%, respectively. The LHP level was also significantly increased in rat kidney tissue after 7 (III group) and 14 days (IV group) of Cr(VI) action in comparison with the control group by 39 and 56%, respectively.

The reason for the increase in peroxidation process intensity in the liver and kidneys of rats is Cr(VI) -induced hyperactivation of hydroxyl and superoxide radicals generation. Cr(VI)-induced ROS formation causes damage to the structure of lipid components of the cell membrane and, as a result, provokes an increase of TBARS content [19,20,38]. Intermediates of Cr(VI) reduction enter into Fenton-like reactions, interact with hydrogen peroxide, and initiate hydroxyl radicals [19]. According to the literature, Cr(VI) enhances the intensity of superoxide radicals generation by activating NADPH-oxidase [39] and xanthine-xanthine oxidase enzymatic complexes [40].

Table 1. The content of indicators of oxidative stress in the liver and kidneys of rats (M±S.E.M., n=5).

Groups of animals	I – Control	II – Oil	III – Cr 7 days	IV – Cr 7 days	V – vit. E	VI – vit. E+ETS	VII – vit E+ETS + Cr 7 days	VIII – vit E+ETS + Cr 14 days
	Liver							
LHP. SU/g tissue	0.220±0.011	0.269±0.02 ***	0.466±0.061 ***	0.5±0.091 ***	0.238±0.008 *** #	0.228±0.035 *** #	0.316±0.033 *** #	0.41±0.06 *** #
TBARS. nmol/g tissue	2.96±0.06	2.60±0.13 ***	4.99±0.65 ***	5.18±0.34 ***	2.27±0.23 *** #	2.19±0.20 *** #	2.59±0.23 *** #	3.19±0.09 *** #
	Kidneys							
LHP. SU/g tissue	0.330±0.031	0.345±0.023 **	0.459±0.046 **	0.516±0.032 **	0.309±0.040 ** #	0.319±0.017 ** #	0.400±0.031 ** #	0.452±0.030 ** #
TBARS. nmol/g tissue	4.91±0.39	4.96±0.25 ***	6.92±0.43 ***	7.17±0.25 ***	4.72±0.36 *** #	4.79±0.24 *** #	6.01±0.65 *** #	6.48±0.36 *** #

Note: the statistically significant difference II, III, IV, V, VI, VII, VIII groups compared to the group I (control) is: **–*** (P<0.01 – P <0.001); the statistically significant difference V, VI, VII, VIII groups compared to the group II is: # (P<0.05).

Intragastric administration of vitamin E (group V) and vitamin E in complex with ETS (group VI) during 14 days led to a significant decrease in the content of TBARS in the liver of animals relative to group II by 13 and 16%, respectively (Table 1). A slight decrease in the level of TBARS was also recorded in the kidney tissue of rats of V and VI experimental groups compared with group II by 5 and 3%, respectively. The LHP content was significantly decreased in V and VI experimental groups in rat liver compared to group II by 12 and 16%, respectively. There was also a significant decrease of LHP level in the rat kidney tissue of similar experimental groups relative to group II by 10 and 8%, respectively.

The TBARS content in rat liver was remained at the level of indicators of group II after 14 days of complex pretreatment with vitamin E and ETS by the next actin of Cr(VI) for 7 days (group VII). The previous complex impact of vitamin E and ETS by the next action of Cr(VI) for 14 days led to increased TBARS level in liver tissue of group VIII compared to group II by 23%. However, the increase in TBARS content in the liver tissue of animals of group VIII (23%) compared to group II was 52% lower than the percentage increase of TBARS content in rat liver of group IV (75%) compared to the group I.

The concentration of TBARS increased in rat kidney tissue after 14 days of complex pretreatment with vitamin E and ETS by the next action of Cr(VI) for 7 (VII group) and 14 days (VIII group) compared to the group II by 21 and 31%, respectively. However, the intensity of increase in the content of TBARS in rat kidneys of groups VII (21%) and VIII (31%) relative to group II was by 25 and 21% lower than the percentage increase of TBARS level in kidney homogenates of groups III (46%) and IV (52%) compared to the group I.

Complex pretreatment with vitamin E and ETS by the next actin of Cr(VI) for 7 days (group VII) and 14 days (group VIII) caused an increase of LHP content in liver tissue of rats compared to group II by 17 and 52%, respectively. However, the increase of LHP level in rat liver of groups VII (17%) and VIII (52%) relative to group II was by 97 and 75% lower than the percentage increase of LHP content in liver homogenates of groups III (114%) and IV (127%) compared to the group I.

The concentration of LHP elevated in kidneys of animals of groups VII and VIII relative to group II by 16 and 31%, respectively.

However, the increase of LHP content in rat kidneys of groups VII (16%) and VIII (31%) compared to group II was by 23 and 25% lower than the percentage increase of LHP level in kidney tissue of groups III (39%) and IV (56%) relative to the group I.

The literature data report that vitamin E is characterized by effective antioxidant properties, inhibits lipid peroxidation processes, and reduces ROS level *in vitro* and *in vivo* [22]. Oral administration of vitamin E attenuates Cr(VI)-induced hepatotoxicity and reduces the content of TBARS in the liver of rats [19]. The reason for the decrease in the intensity of peroxidation processes in rat liver tissue may also be the antioxidant property of the sulfoether group, which is a structural component of the ETS molecule [32,42,43]. This functional group has the properties to reduce LHP content [41]. S-alkylthiosulfonates, which are the synthetic structural analogs of ETS, inhibit the xanthine-xanthine oxidase system activity and ROS generation [27]. Vitamin E is an important component of the cytoplasm and cell membrane. The antioxidant effect of vitamin E prevents the activation of chain reactions of lipid autoxidation due to the neutralization of peroxyl and alkoxide radicals. Peroxyl radicals also react more rapidly with vitamin E than with cell membrane lipids [14].

Thus, the toxic effect of Cr(VI) leads to an elevation of TBARS and LHP content in the liver and kidney tissue of animals. However, the previous impact of vitamin E with ETS reduces the intensity of peroxidation processes in the liver and kidneys of rats under the action Cr(VI)-induced oxidative stress.

3.2. Glutathione antioxidant system.

There was an increase in the activity of GP in the liver tissue of rats under the action of Cr(VI) for 7 (III group) and 14 days (IV group) relative to a group I by 55 and 15%, respectively (Table 2).

Table 2. Indicators of glutathione antioxidant system in liver and kidneys of rats (M±S.E.M., n=5).

Groups of animals	I – Control	II – Oil	III – Cr 7 days	IV – Cr 7 days	V – vit. E	VI – vit. E+ETS	VII – vit E+ETS + Cr 7 days	VIII – vit E+ETS + Cr 14 days
	Liver							
GP. nmol/min.×mg prot.	22.75±1.99	25.70±1.32 **	35.17±2.54 **	26.10±1.90 **	27.68±2.80 **	26.93±0.90 **	23.40±1.15 **	24.04±0.94 **
GR. µmol/min.×mg prot.	1.83±0.16	1.89±0.24 **	1.77±0.07 **	1.51±0.15 **	2.50±0.15 **	2.38±0.24 **	2.27±0.10 **	2.30±0.13 **
GSH. mmol/g tissue	0.179±0.007	0.193±0.009 ***	0.201±0,012 ***	0.118±0.013 ***	0.219±0.006 *** #	0.230±0.009 *** #	0.229±0.008 *** #	0.238±0.013 *** #
	Kidneys							
GP. nmol/min.×mg prot.	96.52±5.06	104.38±12.56 ***	178.21±14.17 ***	70.77±4.88 ***	124.34±14.53 *** #	127.69±11.94 *** #	143.69±11.91 *** #	87.76±10.97 *** #
GR. µmol/min.×mg prot.	2.41±0.37	2.34±0.34 **	1.33±0.09 **	1,37±0.15 **	2.41±0.22 ** #	2.52±0,17 ** #	1.93±0.17 ** #	1.49±0.13 ** #
GSH. mmol/g tissue	0.149±0.011	0.159±0.009 ***	0.154±0.009 ***	0.097±0.003 ***	0.208±0.014 ***	0.229±0.028 ***	0.200±0.018 ***	0.210±0.026 ***

Note: the statistically significant difference II, III, IV, V, VI, VII, VIII groups compared to the group I (control) is: **–*** (P<0.01 – P <0.001); the statistically significant difference V, VI, VII, VIII groups compared to the group II is: # (P<0.05).

There was also activation of GP by 85% in the kidneys of group III animals, but 14 days of Cr(VI) action was accompanied by decreasing GP activity by 27% relative to group I.

Intraperitoneal administration of potassium dichromate for 7 days (group III) caused an increase in the content of cellular GSH in rat liver tissue by 12% compared with the control (group I). GSH level did not differ from the control values in the kidneys of animals of group III ($K_2Cr_2O_7$ 14 days). However, there was a decrease in GSH pool in the liver and kidneys of rats after 14 days of Cr(VI) action (group IV) relative to a group I by 34% and 36%, respectively. In turn, the high sensitivity of kidney tissue to Cr(VI)-induced toxicity may be the reason for the GP's inactivation in the kidneys of group IV animals [1,16].

Authors also suggest that the mechanism of GP inactivation under the condition of Cr(VI) toxicity is carried out by attaching Cr(VI) to the active site of the enzyme and direct displacement of cofactors-metals from the active site [25].

The activity of GR did not change in the liver of animals of group III relative to the control. But 14 days of Cr(VI) exposure led to a decrease in GR activity in rat liver tissue of animals by 17% in comparison to group I. GR activity inhibition was also observed in the kidney tissue of animals after 7 (III group) and 14 (IV group) days of $K_2Cr_2O_7$ injection compared to the group I by 45 and 43%, respectively.

We assume that a Cr(VI)-an induced decrease of GSH content may be the reason for GR suppression in both tissues of rats [44-46]. GSH molecules provide direct neutralization of free radicals, play a key role in the mechanisms of antioxidant protection of cells [47], and are involved in the processes of Cr(VI) reduction [19]. Therefore, the decrease in GSH content in the liver of rats affected by Cr(VI) might be a consequence of intensive use of GSH molecules in the processes of ROS and free radicals neutralization under the conditions of $K_2Cr_2O_7$-induced oxidative stress.

The literature data also describes the direct mechanism of GR activity inhibition due to the specific binding of heavy metal to thiol/thiolate redox pair and histidine residue in the catalytic center of the reduced form of the enzyme. Then the bound metal ion causes changes in the bending of the isoalloxazine ring of FAD and the hydrophobicity of its microenvironment. As a result, the enzymatic activity of GR is inhibited [48].

Administration of vitamin E (group V) and vitamin E in complex with ETS (group VI) caused an increase in the GSH level in the liver of rats relative to group II by 15 and 21%, respectively. The previous complex impact of vitamin E and ETS by the next action of Cr(VI) for 7 (group VII) and 14 days (group VIII) led to an elevation of GSH content in liver tissue compared to group II by 21 and 23%, respectively.

We did not find a statistically significant difference in changes of GSH content in animal tissues after the administration of vitamin E and vitamin E in complex with ETS. We observed only a tendency to increase GSH levels in the kidneys of animals after treatment for 14 days with vitamin E and vitamin E in complex with ETS.

According to the literature, vitamin E exhibits hepato- and nephroprotective properties against Cr(VI)-induced toxicity, restores GSH content and supports the activity of AOS enzymes [14,19]. The authors also report that thiosulfonates are responsible for the Nrf2-dependent activation of antioxidant responsive elements (ARE), which induce the activation of antioxidant defense system enzymes and free radicals scavenging. Thiosulfonate-mediated ARE activation stimulates the activity of genes encoding γ-GCS. Perhaps similar mechanisms are involved in increasing GSH content under the action of ETS [26]. Literature data also indicate that ARE stimulation induces GR and GS gene expression. These enzymes play a key role in the synthesis and reduction of GSH molecules [49].

Thiosulfonate molecules also have the ability to transform into mono-, di- and trisulfides. It is possible that sulfur-containing transformation products of thiosulfonates may be involved in the biosynthesis of new GSH molecules [29].

Complex pretreatment with vitamin E and ETS by the next actin of Cr(VI) for 7 days (group VII) and 14 days (group VIII) showed only a tendency to the restoration of GP and GR activity in rat liver. However, we did not find a statistically significant difference in this case.

The action of vitamin E in particular (group V) and in combination with ETS (group VI) for 14 days led to an activation of GP in the kidney tissue of animals relative to group II by 19 and 22%, respectively. The previous complex effect of vitamin E and ETS by the next action of Cr(VI) for 7 days caused an increase in the activity of GP by 38% in rat kidneys of group VII compared to group II (Table 2). However, the increase of GP activity in rat kidneys of group VII (38%) compared to group II was 47% lower than the percentage hyperactivation of GP in rat kidney tissue of group III (85%) relative to a group I.

In turn, GP activity was suppressed by 16% in kidney tissue of rats after the previous impact of vitamin E in complex with ETS for 14 days by the next action of Cr(VI) for 14 days (group VIII) compared to the group II. However, the decrease of GP activity in kidney tissue of animals of groups VIII (16%) compared to group II was 11% lower than the percentage GP inactivation in rat kidneys of group III (27%) relative to group I. A slight increase in GR activity (8%) was observed after 14 days of complex exposure to vitamin E and ETS in the kidney tissue of animals of group VI relative to group II.

The previous complex impact of vitamin E and ETS by the next action of Cr(VI) for 7 (group VII) and 14 days (group VIII) caused a decrease of GR activity in rat kidneys relative to group II by 17 and 36%, respectively.

However, the intensity of decrease of GR activity in rat kidney tissue of groups VII (17%) and VIII (36%) relative to group II was by 28 and 7% lower than the percentage inactivation of GR activity in kidney homogenates of groups III (45%) and IV (43%) compared to the group I.

We hypothesize that the partial stabilization of GP activity and decrease in the intensity of GR suppression in rat kidneys under the action of Cr(VI) were mediated by the antioxidant effect of the vitamin E and ETS complex. The results of the studies described above indicated that previous exposure to vitamin E and ETS attenuated the intensity of Cr(VI)-induced LP processes in the kidneys of rats. According to the literature, a sharp increase of TBARS, LHP, and protein peroxidation product content is accompanied by disruption of the activity of GSH-related AOS enzymes [50]. It is possible that the complex antioxidant effect of vitamin E and ETS stabilizes the enzymatic activity of GP and GR by reducing LHP and TBARS content in kidney tissue of animals.

Thus, intraperitoneal injection of $K_2Cr_2O_7$ for 7 days leads to a slight compensatory activation of GP and an increase of GSH content in the liver of rats. Slightly GP stimulation is also observed after 14 days of Cr(VI) action. However, 14 days of $K_2Cr_2O_7$ exposure causes depletion of the hepatic GSH pool and inhibition of GR activity. Cr(VI)-induced depletion of GSH content eliminates in liver tissue by complex intragastric pretreatment of vitamin E and ETS. Cr(VI) action for 7 days leads to compensatory activation of GP and suppression of GR in the kidneys of rats. In turn, 14 days of $K_2Cr_2O_7$ exposure causes inactivation of GP, GR and depletion of renal GSH content. The previous complex impact of vitamin E and ETS attenuates the intensity of GR inactivation and stabilizes the activity of GP in rat kidney tissue under conditions of $K_2Cr_2O_7$-induced oxidative stress.

3.3. Antioxidant enzymes.

Intraperitoneal injection of potassium dichromate for 7 and 14 days led to a decrease in the activity of SOD in liver tissue of animals of groups III and IV relative to a group I by 17 and 33%, respectively (Table 3). SOD activation was observed after 7 days of Cr(VI) treatment in rat kidney tissue of group III (21%), but 14 days of Cr(VI) action caused suppression of SOD enzymatic activity in the kidneys of animals of group IV (18%) compared to the group I.

Table 3. Indicators of antioxidant enzyme activity in liver and kidneys of rats (M±S.E.M., n=5).

Groups of animals	**I – Control**	**II – Oil**	**III – Cr 7 days**	**IV – Cr 7 days**	**V – vit. E**	**VI – vit. E+ETS**	**VII – vit E+ETS + Cr 7 days**	**VIII – vit E+ETS + Cr 14 days**
	Liver							
SOD, U/mg prot.	23,49±0,56	21,67±2,08 **	19,45±0,71 **	15,79±1,95 **	29,13±2,19 **	26,13±3,63 **	35,18±5,78 **	25,38±1,17 **
CAT, mmol/min ×mg prot.	16,27±0,49	14,47±0,84 *	18,05±1,91 *	14,17±0,38 *	16,57±1,29 * #	19,20±1,05 * #	16,98±1,39 * #	15,70±0,78 * #
	Kidneys							
SOD, U/mg prot.	67,56±6,08	59,39±4,52 *	82,04±3,19 *	55,33±6,64 *	52,20±5,09 *	53,11±7,28 *	53,86±5,77 *	55,45±5,31 *
CAT, mmol/min ×mg prot.	18,89±1,21	18,03±0,87 **	21,73±0,85 **	16,66±1,01 **	22,22±1,10 ** #	23,00±1,25 ** #	20,45±0,86 ** #	18,35±0,48 ** #

Note: the statistically significant difference II, III, IV, V, VI, VII, VIII groups compared to the group I (control) is: *–*** (P<0.05 – P <0.01); the statistically significant difference V, VI, VII, VIII groups compared to the group II is: # (P<0.05).

The activity of CAT increased by 11% after 7 days of Cr(VI) exposure, but 14 days of $K_2Cr_2O_7$ administration was accompanied by a decrease of CAT activity by 13% in rat liver tissue of group IV relative to group I. Cr(VI) toxicity for 7 days led to activation of CAT (by 15%), but $K_2Cr_2O_7$ toxicity for 14 days caused a decrease in CAT enzymatic activity in kidney tissue of animals of group IV compared to the group I.

The authors report that the reason for the activation of SOD (liver and kidney) and CAT (liver) in rat tissues of group III may be antioxidant gene overexpression or compensatory mechanisms of the AOS system against Cr(VI)-induced oxidative stress [51,52].

Analysis of the literature data also indicates that Cr(VI) is a strong inhibitor of SOD enzymatic activity. Perhaps, this data may explain the inhibition of SOD activity after longer toxic actin of Cr(VI) in rat tissues of group IV (Cr(VI) for 14 days). Toxic action of Cr(VI) leads to inhibition of SOD enzymatic activity and impairments of SOD molecular structure due to intensification of peroxidation processes [53]. Heavy metals, including Cr(VI), have the ability to inactivate the AOS enzymes after direct binding to the active site of corresponding enzymes [54]. After SOD inactivation is suppressed, dismutation processes O_{2-} to H_2O_2. As a consequence, Cr(VI)-induced stimulation of O_2^- formation and inhibition of O_2^- utilization processes may be the cause of AOS enzyme inactivation, including CAT [55].

Vitamin E in particular (group V) and in combination with ETS (group VI) stimulated CAT activity in the liver tissue of animals relative to group I by 15 and 33%, respectively. There was also a probable activation of CAT in rat kidneys of V and VI experimental groups compared to group II by 23 and 28%, respectively (Table 3).

Complex pretreatment with vitamin E and ETS by the next actin of Cr(VI) for 7 days (group VII) and 14 days (group VIII) caused an increase of CAT activity in rat liver tissue

relative to group II by 17 and 9%, respectively. CAT activation was also observed in rat kidneys of group VII (13%) relative to group II. However, CAT enzymatic activity in kidney tissue of animals of group VIII remained at the level of indicators of group II.

Literature data report that vitamin E prevents depletion of enzymatic activity of SOD, CAT, and other AOS enzymes in tissues of mice and rats under the condition of oxidative stress [56,57]. Vitamin E also attenuates Cr(VI)-induced oxidative stress in rat testes due to restoring SOD and CAT activity and reducing LP processes intensity [58].

Thiosulfonates are involved in the Nrf2-dependent activation of AOS gene stimulation [26]. Stimulation of Nrf2 leads, in turn to increased expression of genes encoding CAT [59]. Allicin, one of the natural analogs of thiosulfonates, is involved in activating genes responsible for the expression of SOD, CAT, and Nrf2 [60]. It is possible that the above antioxidant properties of vitamin E, thiosulfonates, and their natural analogs may be the reason for restoring CAT enzymatic activity under action of Cr(VI)-induced oxidative stress.

4. Conclusions

It is little known about the antioxidant properties of thiosulfonates. There is also enough information describing the protective properties of thiosulfonates against heavy metal-induced toxicity in the tissues of the animal organism. Our previous studies indicate that ETS pretreatment may be effective in correcting Cr(VI)-induced liver toxicity in rat organisms. We assume that further studies of the antioxidant properties of ETS in combination with antioxidant compounds and cellular reductants are important for better understanding of the role of thiosulfonates in the mechanisms of heavy metals-induced oxidative stress prevention.

Generalization of obtained results indicates that $K_2Cr_2O_7$ action leads to Cr(VI)-induced hepato- and nephrotoxicity due to the intensification of LP processes and elevation of LHP and TBARS formation in both animals tissues. The AOS system involves compensatory mechanisms to counteract Cr(VI)-induced oxidative stress. These mechanisms are accompanied by activation of SOD, CAT, and GP in kidney tissue, as well as by stimulation of CAT, GP, and accumulation of GSH in the liver of rats after 7 days of Cr(VI) exposure. However, a longer action of Cr(VI) for 14 days leads to depletion of AOS system resources due to inactivation of antioxidant enzymes (SOD, CAT, GR, GP) and depletion of GSH pool in liver and kidney tissues of animals. The complex of vitamin E and ETS exhibits antioxidant effect against Cr(VI)-induced toxicity. Intragastric pretreatment of this complex for 14 days is manifested with a decrease of Cr(VI)-induced LP processes in the liver and kidneys of rats. The previous impact of vitamin E and ETS also prevents depletion of CAT and GSH in the liver, as well as eliminates intensity of CAT inactivation, stabilizes GP and GR activity in kidney tissue of animal under the condition of $K_2Cr_2O_7$-induced oxidative stress. Vitamin E in particular and in complex with ETS suppresses LHP elevation and stimulates CAT in both rat tissues. The antioxidant effect of these compounds also leads to hepatic GSH accumulation and renal GP activation.

Obtained results indicate that vitamin E and ETS pretreatment partially stabilized Cr(VI)-induced disturbance in the mechanisms of antioxidant defense system action in rat kidneys. Moreover, the results of our study may become a part of the background for creating effective methods of prevention and correction of the antioxidant and pro-oxidant states in kidneys affected by the action of Cr(VI)-induced oxidative stress.

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Conflicts of Interest

The authors declare no conflict of interest.

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