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EFFECTS OF ETHYLTHIOSULFANYLATE AND CHROMIUM (VI) ON THE STATE OF PRO/ANTIOXIDANT SYSTEM IN RAT LIVER

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Ethylthiosulfanylate is alkyl ester of thiosulfoacid and belongs to the class of thiosulfonate compounds. Structurally, thiosulfonates are synthetic analogues of natural phytoncides. It is known that, natural organic sulfur-containing compounds are characterized by antioxidant and detoxification properties against heavy metals toxicity. Therefore, the purpose of the study was to investigate the influence of ethylthiosulfanylate, as a synthetic analogue of natural phytoncides, on the state of the pro/antioxidant system in the liver of laboratory rats exposed to Cr(VI). It was found that ethylthiosulfanylate exposure at a dose 100 mg/kg body weight daily for 14 days led to a decrease in the intensity of increasing of the lipid hydroperoxides (LHP) content in the rat liver caused by Cr(VI) action. In addition, ethylthiosulfanylate pretreatment prevented depletion of reduced glutathione (GSH) pool under the action of potassium dichromate oxidative stress and performed the accumulation of cellular GSH in rat liver.

K e y w o r d s: rats, liver, antioxidant system, oxidative stress, ethylthiosulfanylate, potassium dichromate, free radicals.

Chromium is a fairly common chemical element in the earth's crust and is capable of being in various oxidation states, among which Cr(VI) and Cr(III) forms are the most common. Hexavalent chromium compounds are used in various industry sectors including pigment production, manufacture of wood preservatives, leather tanning, anticorrosive processes in the production of kitchen utensils (electroplating) and chromite ore mining [1]. Cr(VI) is a toxic heavy metal. Violation of production standards may cause soil, air and water contamination by hexavalent chromium compounds. The high concentration of Cr(VI) in the soil leads to its accumulation in plant cells. People living near contaminated sites are at risk for health through the threat of consumption of products and water contaminated with hexavalent chromium compounds [1]. Toxicity of Cr(VI) associated with Hexavalent chromium can easily transport through the anionic channels in the plasma membranes. In the cytoplasm of the cells, Cr(VI) is reduced by cellular reductants through reactive intermediate forms Cr(V), Cr(VI) to Cr(III) form, which is much more stable. The redox couples Cr(VI)/(V), Cr(V)/(IV) are the cyclical electron donors in a Fenton-like reaction, which generates reactive oxygen species (ROS) leading to genomic DNA damage and oxidative deterioration of lipids and proteins [2].

Histopathological observations indicate that Cr(VI) exposure lead to cytological liver damages, which cause necrotic and apoptotic changes in rat hepatocytes [3]. Acute action of Cr(VI) by potassium dichromate administration at a dose 20 mg/kg body weight causes increase in malondialdehyde (MDA) content, depletion of pool of GSH, decrease in superoxide dismutase (SOD) and catalase (CAT)

activity in liver of male Swiss Albino mice [4]. Also, potassium dichromate action causes increase of urea concentration and creatinine level, decrease of total protein content in rat serum [5] and induces increase of alanine aminotransferase and total cholesterol in serum of Rabbit Doe [6]. Nowadays is carried out an active search for biologically active substances with antioxidants, detoxifying and cytoprotective properties, which are capable to attenuate the toxic effect of heavy metals, including Cr(VI) [3, 4, 7].

Ethylthiosulfanylate is alkyl ester of thiosulfoacid and belongs to the class of thiosulfonate compounds. Thiosulfonates are structurally analogs of natural sulfur-containing organic compounds with phytoncidal properties, which are obtained from garlic, onion, cauliflower and broccoli. It is known that synthetic thiosulfonates have a wider range of biological activity and are more stable than their natural analogs. Also thiosulfonates have a low level of toxicity for animal organism [8, 9]. The literature date report that natural organosulfur analogs of thiosulfonates exhibit a wide range of biological activity including effective antioxidant and detoxifying properties against heavy metals toxicity [10, 11]. In recent years is actively conducted investigations of the properties of thiosulfonates in different scientific directions [9]. Previous studies have shown that ethylthiosulfanylate is involved in regulation of the activity of the lipid metabolism enzymes and performs the redistribution of lipid classes and a decrease in mono-, di-, triglycerides and free fatty acids in the rat liver [8]. Administration of ethylthiosulfanylate induces the increase of total protein and albumin content in rat blood plasma [12]. Also, ethylthiosulfanylate is an effective agent against fungal infections and has antimicrobial effect [9].

The studies of thiosulfonates properties have shown that these compounds are also effective inhibitors of cancer cells proliferation and induce G2/M cell-cycle arrest and provoke apoptosis in WHCO1 oesophageal cancer cells by mechanism of S-thiolation [13].

Thiotaurine (2-aminoethane thiosulfonate) induces the immunomodulatory action by the activation of human neutrophils due to persulfidation of target proteins [14].

Propyl-Propane thiosulfonate exhibits an anti-inflammatory effect by suppression of pro-inflammatory mediators activity and improving the intestinal epithelial barrier integrity in mice with experimental colitis [15].

Thus, in recent years have been conducted many studies to understand the effects of thiosulfonates on the microorganisms, cancer cells proliferation and activity of immune system, but not enough is known about the effects of these compounds on the activity of antioxidant defense system enzymes in tissues of animal organism. Also, there are limited data describing the effect of thiosulfonates on the antioxidant and pro-oxidant states under the action of heavy metals.

Therefore, given that natural organosulfur analogs of thiosulfonates have the antioxidant properties, the purpose of our study was to investigate the influence of synthesized ethylthiosulfanylate on the state of the pro/antioxidant system in the liver of rats exposed to Cr(VI).

Materials and Methods

The research was conducted in the vivarium of the Institute of Animal Biology of NAAS on white male Wistar laboratory rats (130-140 g), which were randomly divided into 7 groups with 5 animals each group. Animals of all groups were fed with standard compound feed for laboratory rats with free access to drinking water and feed. The rats in the group I (intact control) were injected daily intraperitoneally with 150 µl of physiological saline solution for 7 days. Animals of III and IV research groups received potassium dichromate ($K_2C_{r2}O_7$) intraperitoneally at a dose 2.5 mg Cr(VI)/kg body weight per day for 7 days (group III) and for 14 days (group IV) [5]. Rats of group II were injected daily intragastrally with 1000 µl of oil for 14 days ("Oleina" oil, traditional: refined, deodorized, frozen; Producer of PJSC with II "DOEP"; certified according to State Standard of Ukraine 4492: 2017 and complies with ISO 14024) and then daily intraperitoneally injected with 150 µl of physiological saline solution for 7 days. Rats of group V were injected daily intragastrally with an oil solution of ethylthiosulfanylate at a dose of 100 mg/kg body weight for 14 days [8, 16] and then daily intraperitoneally injected with 150 µl of physiological saline solution for 7 days. Animals of VI and VII groups received intragastrally an oil solution of ethylthiosulfanylate at a dose 100 mg/kg body weight daily for 14 days and then daily received $K_2Cr_2O_7$ intraperitoneally at a dose 2.5 mg Cr(VI)/kg body weight per day for 7 days (group VI) and for 14 days (group VII). All procedures were made to minimize animal suffering and were followed the guidelines

of 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). At the work were studied the effects of the newly synthesized ethyl 4-aminobenzenethiosulphonate compound on the rat body 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 [17].

After decapitation of the animals, which occurred under thiopental anesthesia [18], the liver was collected. All procedures on liver was performed at 4°C. The research material was the liver 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 min at 1000 g. After centrifugation in obtained supernatants were determined content of GSH, peroxidation products level and antioxidant enzymes activity.

The content of LHP (lipid hydroperoxides) was determined according to the principle of precipitation of proteins with trichloroacetic acid solution and lipid extraction by ethanol action as described by [19]. This method is based on spectrophotometrically measurement the level of colored product, which formed by the interaction of the experimental extracts (ethanol extracts of lipids) with ammonium thiocyanate. The absorption was measured spectrophotometrically at λ 480 nm. The concentration of LHP was determined by the difference between values of control and experimental samples and expressed in standard units per 1 gram of tissue.

The content of TBARS (thiobarbituric acid reactive substances) in liver homogenates was determined by color reaction of MDA with thiobarbituric acid (TBA) as described by Korobeinikova [19]. The reaction was conducted at high temperature in in acidic environment. The level of colored product (colored complex of one MDA and two TBA molecules) was measured spectrophotometrically at λ 535 nm and λ 580 nm and the values were expressed as nmol of MDA per 1 g of tissue.

The content of CP (carbonyl group of proteins) was determined by the interaction of the carbonyl groups of amino acids with 2,4-dinitrophenylhydrazine (DNPH) with the formation of 2,4-dinitrophenylhydrazones as described by [19]. The absorption was measured spectrophotometrically at λ 370 nm and the values were expressed in nmol CP per 1 mg of protein.

SOD (superoxide dismutase, EC 1.15.1.1.) activity was determined by the level of inhibition in the rate of nitroblue tetrazolium reduction in the presence of NADH and phenazine methosulfate as described by [19]. The absorption was measured spectrophotometrically at λ 540 nm and the values were expressed in standard units per 1 mg of protein.

Method of CAT (catalase, EC 1.11.1.6) activity determining based on the principles of ability of hydrogen peroxide to form a stable color complex with molybdenum salts described by [19]. The level of colored product was measured spectrophotometrically at λ 410 nm against water and the values were expressed in mmol/min×mg of protein.

GP (glutathione peroxidase, EC 1.11.1.9) activity was established by the rate of oxidation of GSH before and after incubation with tertiary butyl hydroperoxide as described by [19]. The intensity of GSH oxidation was determined by the formation of colored product (dinitrophenyl anion) during the interaction of 5,5-dytiobis-2-nitrobenzoic acid (DTNBA) with SH-groups. The absorption was measured spectrophotometrically at λ 412 nm. The activity of GP was expressed in nmol GSH/min×mg of protein.

GR (glutathione reductase, EC 1.6.4.2) activity was determined in the reaction medium which consist of 2.5 ml of 0.15 M phosphate buffer (pH 7.4), 0.2 ml of oxidized glutathione (7.5 mM), 0.1 ml of tissue homogenate and 0.1 ml of NADPH (1.2 mM). The enzyme activity was determined spectrophotometrically at λ 340 nm for 1 min at 37°C. The GR activity was calculated by using molar absorption ratio for NADPH at a wavelength 340 nm and expressed in μmol of NADPH/min×mg of protein. The intensity of reaction depends on the tempo of extinction decrease. The principle of this method is based on determining the rate of glutathione reduction in the presence of NADPH as described by [19].

The content of GSH (reduced glutathione) in liver homogenates was performed according to the principle of measurement the level of formation of colored product – thionitrophenyl anion as described by [19]. Thionitrophenyl anion formation process is based on the interaction between DTNBA and SH-groups of GSH molecules. The absorption was measured spectrophotometrically at λ 412 nm. The content of GSH was expressed in mmol of GSH per gram of tissue.

The measuring of all absorbance values were performed on a spectrophotometer "Unico" 1205 (USA).

Statistical evaluation of the results was performed using arithmetic mean and standard error ($M \pm m$) and the variances between groups were tested for significance using one-way ANOVA, followed by Tukey-Kramer test.

The differences were statistically significant at $P < 0.05$. All calculations were performed using Microsoft Excel software.

Results and Discussion

According to the results of our studies, the level of oxidative stress markers was significantly increased in the liver tissue of rats after 7 and 14 days of potassium dichromate exposure. Under the action of Cr(VI), the content of LHP was significantly increased in the liver of animals of groups III and IV compared to the group I (control) by 50 and 158%, respectively (Table 1). The level of CP by Cr(VI) action was higher in rat liver homogenates of III experimental group compared to the group I by 6%. Concentration of CP was significantly increased by potassium dichromate exposure in rat liver tissue of group IV compared with group I by 49%. The injection of potassium dichromate did not lead to statistically significant changes in the content of TBARS, but we observe a low tendency to increase of TBARS level in experimental groups, which were administrated with Cr(VI). Literature data indicate that Cr(VI) can easily penetrate the cell membrane and reduce to Cr(III) [5]. The process of Cr(VI) reduction in biological systems is accompanied by the generation of large amounts of ROS, which cause a sharp activation of the processes of peroxidation of lipids, proteins and other components of biological systems [1, 5]. Also, the authors suggest that $K_2Cr_2O_7$ injection leads to increase of oxidative protein products and xanthine oxidase activity in serum of rats. Activation of xanthine-xanthine oxidase system causes ROS generation and provokes a directly oxidation of lipids, proteins and DNA in cells [7].

Therefore, intraperitoneal administration of potassium dichromate at a dose of 2.5 mg Cr(VI)/kg body weight per day for 7 and 14 days causes Cr(VI)-induced oxidative stress by increasing in the content of LHP and CP (group III and IV) in the liver of rats.

The effect of Cr(VI) by the previous influence of ethylthiosulfanylate was also accompanied by a significantly increase in the level of LHP by 74% in the liver tissue of rats of group VII compared to the group II (Table 1). However, the percentage increase in the content of LHP in the liver of animals of group VII in comparison with group II was by 84% lower than the percentage increase of the concentration of LHP in the liver homogenates of animals of group IV compared with group I. According to the literature, the sulfoether group, which is part of ethylthiosulfanylate, has antioxidant properties and is involved in the reduction processes of LHP [20] and this may be the reason of the decrease of the intensity of Cr(VI)-induced lipid peroxidation processes. It is known that compounds with sulfur atoms induce the process of lipid hydroperoxides decomposition into non-radical products [21]. Sulfur-containing compounds have the ability to inhibit the activity of the xanthine-xanthine oxidase system and attenuate ROS-induced lipids and proteins peroxidation [22]. Garlic organosulfur compounds also attenuate lipids peroxidation by inhibition of xanthine-xanthine oxidase-induced ROS generation [23]. In addition, during biotransformation processes, thiosulfonates are able to transform into other sulfur-containing compounds and thiols, which can be further used as a material for the synthesis of GSH molecules [24]. Hydrogen sulfide groups of GSH molecules are capable of direct non-enzymatic removal of hydrogen peroxide molecules [25]. Moreover, these properties of organosulfur compounds may be the reason for decrease of intensity of LHP formation by ethylthiosulfanylate pretreatment.

Therefore, the previous intragastric injection of ethylthiosulfanylate at a dose 100 mg/kg body weight daily for 14 days leads to a partially compensates of Cr(VI)-induced oxidative stress and attenuates the intensity of increasing in the concentration of LHP (group VII) in the liver of animals.

The results of the studies indicate that after 7 and 14 days of potassium dichromate exposure the activity of SOD was statistically lower in the rat liver of III and IV groups compared to the group I by 10 and 22%, respectively (Table 2). CAT activity did not change after 7 days of Cr(VI) action in liver tissue of animals of group III relative to the group I. However, after 14 days of potassium dichromate administration was observed decrease of CAT activity by 14% in the rat liver of group IV in comparison with group I. This may indicate about the inhibition of the activity of these enzymes by the prolonged action of Cr(VI). Our results are consistent with studies by

Table 1. The content of indicators of oxidative stress in liver of rats (M ± m, n = 7)

Groups of animals	LHP, SU/g tissue	TBARS, nmol/g tissue	CP, nmol/mg prot
I – Control	0.24 ± 0.01	4.88 ± 0.24	0.85 ± 0.07
II – Oil	0.23 ± 0.02***	4.91 ± 0.06	0.80 ± 0.02
III – Cr 7 days	0.36 ± 0.03***	5.70 ± 0.21	0.90 ± 0.08*
IV – Cr 14 days	0.63 ± 0.07***	5.57 ± 0.67	1.26 ± 0.16*
V – Ethylth.	0.30 ± 0.05***	5.04 ± 0.19	0.71 ± 0.13
VI – Ethylth.+ Cr 7 days	0.30 ± 0.02***	5.38 ± 0.07	0.81 ± 0.06
VII – Ethylth. + Cr 14 days	0.40 ± 0.03***#	5.31 ± 0.22	1.04 ± 0.08

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

other authors that the Cr(VI) is a potent inhibitor of SOD activity in vitro [4]. These authors suggest that the depletion of SOD activity by Cr(VI) exposure is caused by the damage of the structure of Cu-, Zn-dependent SOD, which can be caused by a sharp increase in the intensity of lipid peroxidation processes under Cr(VI) action [4]. Administration of Cr(VI) to Wistar rats also provokes the inhibition of SOD and CAT activity in the liver of these animals. The authors suggest that the high concentration of LHP, CP and TBARS under the condition of Cr(VI)-induced oxidative stress leads to the down-regulation of SOD and CAT enzymatic activity [6].

Therefore, 14 days of potassium dichromate exposure at a dose of 2.5 mg Cr(VI)/kg body weight leads to the decrease of SOD and CAT (group IV) activity in the rat liver tissue. And these changes may be a consequence of the inhibition of activity of these enzymes under the action of Cr(VI)-induced oxidative stress.

The effect of ethylthiosulfanylate exposure during the 14 days without Cr(VI) action did not cause the changes of CAT activity in liver tissue of rats of group V relative to the group II. The previous impact of ethylthiosulfanylate under the action of Cr(VI) by 7 days was accompanied by a decrease in CAT activity by 20% in the rat liver of animals of group VI compared with the group II (Table 2). This may indicate that ethylthiosulfanylate at a dose 100 mg/kg body weight is not able to stabilize the activity of CAT disrupted by Cr(VI)-induced oxidative stress. Potassium dichromate causes inactivation the most of antioxidant enzymes due to the direct binding of Cr(VI) to the active site of enzymes and after the displacement of metal co-factors from active sites [6]. However, after ethylthiosulfanylate pretreatment and the next action of Cr(VI) during 14 days was observed restoration of CAT activity in the rat liver of group VII to the values of activity such as by group II. We assume that the restoration of CAT activity in rat liver of group VII is not carried out with the direct participation of ethylthiosulfanylate, because we did not observe the restoration of CAT activity in rat liver tissue of group VI relative to the group II. Probably, restoration of CAT activity in this case may be caused by significantly higher increase of GSH content in liver of animals of group VII in comparison with all other experimental groups (Table 3). CAT performs enzymatic destruction of hydrogen peroxide to molecular oxygen and water. However, the large amounts of superoxide anion and hydrogen peroxide molecules, which are formed in a large quantity during the process of Cr(VI) reduction, may inhibit activity of this enzyme. Cr(VI) also attenuates the CAT activity in the process of heavy metal direct binding to the enzyme active site [6]. GSH is low molecular weight thiol with strong radical scavenging properties. It also plays an important role in the process of hydrogen peroxide decomposition [25]. Also, GSH molecule has the ability to form C(VI)-GSH complex during the process of Cr(VI) reduction [4]. And the ability of cellular GSH to decrease the content of Cr(VI) and level of hydrogen peroxide may be the reason for the restoration of CAT activity under the action of Cr(VI)-induced oxidative stress.

After potassium dichromate exposure content of GSH in the rat liver of III and IV experimental groups was significantly decreased by 41 and 33%, respectively (Table 3). Our results are consistent with studies by other authors that the action of Cr(VI) leads to depletion of GSH content in liver tissue [3].

Table 2. Activity of antioxidant enzymes in liver of rats (M ± m, n = 7)

Groups of animals	SOD, U/mg prot.	CAT, mmol/min×mg prot.
I – Control	11.52 ± 0.56	8.67 ± 0.41
II – Oil	9.89 ± 0.64*	8.82 ± 0.16**
III – Cr 7 days	10.43 ± 0.51*	9.00 ± 0.15**
IV – Cr 14 days	9.04 ± 0.71*	7.44 ± 0.31**
V – Ethylth.	11.76 ± 0.79*	8.48 ± 0.44** #
VI – Ethylth.+ Cr 7 days	8.91 ± 0.73*	7.08 ± 0.55** #
VII – Ethylth. + Cr 14 days	9.33 ± 0.82*	9.02 ± 0.42** #

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

It is known that the reduction process of Cr(VI) to Cr(III) in the cells of living organisms is accompanied with the formation of a large number of free radicals, ROS and LHP. These changes leads to increase of enzymatic processes of LHP reduction and non-enzymatic pathways of ROS neutralization by cellular GSH and that could be the reason of depletion of total GSH cellular pool in liver tissue [25]. GSH consumption also activates by the process of Cr(VI) binding to the molecule of GSH by the next reduction of Cr(VI) to Cr(III) [4]. Cr(VI) also induces the processes of NADPH content decreasing by activation of NADPH-oxidase and inhibition of glucose-6-phosphate dehydrogenase activity. As a consequence, Cr(VI)-induced decrease in the content of NADPH molecules may also cause a decrease in the efficiency of reduction process of GSSG to GSH [4].

In obtained results no statistically significant differences were observed between the values of GP activity in the rat liver tissue of all experimental group compared to the control.

GR activity was decreased after 7 (group III) and 14 days (group IV) of Cr(VI) action in the liver of animals compared to group I by 13 and 17%, respectively. GR catalyzes the enzymatic reduction of Cr(VI) to Cr(V) in the presence of NADPH. The reason for the inhibition of GR activity according to the literature may be the process of Cr(VI) reduction that causes decrease in NADPH content, inhibition of GR activity and as a result the decrease in cellular GSH content [4]. The authors suggest that all GSH-related enzymes are suppressed under the action of Cr(VI) [2]. Therefore, the administration of Cr(VI) at a dose of 2.5 mg Cr(VI)/kg body weight for 7 (group III) and 14 days (group IV) provokes the depletion of cellular pool of total GSH and causes inhibition of GR activity in the rat liver tissue.

Our results showed also the tendency to restoration of GR activity by previous impact of ethylthiosulfanylate under the toxic action of potassium dichromate. But statistically significant differences were not observed in this case between the values

Table 3. Indicators of glutathione AOS system in liver of rats (M ± m, n = 7)

Groups of animals	GP, nmol/min×mg prot.	GR, μmol/min×mg prot.	GSH, mmol/g tissue
I – Control	26.24 ± 0.35	1.71 ± 0.09	0.49 ± 0.04
II – Oil	26.64 ± 0.38	1.69 ± 0.03***	0.54 ± 0.07***
III – Cr 7 days	32.47 ± 3.65	1.49 ± 0.09***	0.29 ± 0.02***
IV – Cr 14 days	32.61 ± 0.42	1.42 ± 0.08***	0.33 ± 0.07***
V – Ethylth.	27.16 ± 2.39	2.07 ± 0.16***	0.99 ± 0.04*** ##
VI – Ethylth.+ Cr 7 days	27.27 ± 3.47	1.72 ± 0.12***	1.02 ± 0.12*** ##
VII – Ethylth. + Cr 14 days	28.69 ± 0.77	2.03 ± 0.12***	1.29 ± 0.15*** ##

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

of GR activity in rat liver of animals pretreated with ethylthiosulfanylate compared to the group II.

After 14 days of ethylthiosulfanylate exposure GSH content was significantly increased by 83% in the rat liver tissue of group V compared to the group II (Table 3). Ethylthiosulfanylate pretreatment under the Cr(VI) action for 7 and 14 days caused significantly increase of GSH content in the rat liver homogenates of VI and VII groups compared to the group II by 89 and 139%, respectively. And these changes may indicate about a decrease of the toxic effect of potassium dichromate by the previous impact of ethylthiosulfanylate.

According to the literature data, the structure and biochemical properties of thiosulfonates may be the reason for the increase of the content of GSH molecules in the liver of animals after 14 days of ethylthiosulfanylate exposure [24]. In addition, the increase in cellular GSH concentration by the action of ethylthiosulfanylate can be explained by the literature data according to which thiosulfonates are able to activate antioxidant-responsive elements (ARE), which are transcription factors of genes encoding antioxidant enzymes [26]. ARE stimulation also leads to the activation of the processes of synthesis and reduction of GSH and HADPH molecules, which are key elements necessary for the proper functioning of the enzymes of glutathione antioxidant system [26, 27]. The authors report that ARE-dependent stimulation is responsible for antioxidant defense genes expression that encode gamma-glutamylcysteine synthetase (γ-GCS) [27]. This enzyme (γ-GCS) catalyzes the first stage of GSH biosynthesis. Catalytic activity of γ -GCS induces the formation of gamma-glutamylcysteine molecule by condensation of cysteine and glutamate in the presence of ATP. Gamma-glutamylcysteine molecule is a key element necessary for the second stage of GSH biosynthesis with the participation of glutathione synthetase (GS). Activation of ARE leads also to the upregulation of GS and GR genes expression and provides positive effect on the processes of GSH synthesis and restoration [27].

Therefore, by intragastric administration of ethylthiosulfanylate at a dose of 100 mg/kg body weight increases the cellular GSH content (group V) in the rat liver tissue. The previous impact of ethylthiosulfanylate for 14 days at the same dose partially offset the negative impact of Cr(VI)-induced oxidative stress and as a result provokes restoration of GSH pool in rat liver (groups VI, VII).

In conclusion, our results report that potassium dichromate administration induced rat liver toxicity associated with oxidative stress. Such markers of peroxidation processes as LHP and CP increased under the action of Cr(VI). Also, SOD, CAT, GR activity and GSH content were decreased under the condition of potassium dichromate oxidative stress. However, we observed that ethylthiosulfanylate pretreatment showed potential antioxidant properties due to the attenuating the processes of Cr(VI)-induced lipid peroxidation. Also, previous ethylthiosulfanylate administration prevented depletion of GSH pool under the action of potassium dichromate oxidative stress and performed the accumulation of cellular GSH in rat liver. We assume that ethylthiosulfanylate-induced GSH accumulation may be also the reason for restoration of CAT activity impaired by Cr(VI) toxic effect. Pretreatment with ethylthiosulfanylate did not show statistically significant differences between the values of SOD, GR and CP. We observed only a tendency to restoration of activity of these enzymes and content of CP in rat liver after the ethylthiosulfanylate pretreatment. Analysis of the results of our studies indicates that ethylthiosulfanylate pretreatment has a positive antioxidant effect against the Cr(VI) toxicity in rat liver. In addition, the obtained results may be used by the implementation of effective methods of prevention and pharmacological correction of the antioxidant and pro-oxidant states in liver and other tissue under the conditions of heavy metals-induced oxidative stress.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВПЛИВ ЕТИЛТІОСУЛЬФАНІЛАТУ ТА ХРОМУ (VI) НА СТАН ПРО/ АНТИОКСИДАНТНОЇ СИСТЕМИ В ПЕЧІНЦІ ЩУРІВ

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Етилтіосульфанілат є алкільним етером тіосульфанілової кислоти та належить до класу сполук тіосульфонатів. У структурному плані тіосульфонати є синтетичними аналогами молекул природних фітонцидів. Відомо, що природні сульфурвмісні органічні сполуки характеризуються антиоксидантними та детоксикуючими властивостями по відношенню до токсичної дії важких металів. Метою досліджень було з'ясувати вплив етилтіосульфанілату як синтетичного аналога природних фітонцидів на стан про/антиоксидантної системи в печінці лабораторних щурів, що зазнавали впливу Cr(VI). Встановлено, що за дії етилтіосульфанілату в дозі 100 мг/кг маси тіла щурів протягом 14 діб спостерігалось зниження інтенсивності зростання вмісту гідропероксидів ліпідів (ГПЛ) у печінці, спричинене дією Cr(VI). Також попередній вплив етилтіосульфанілату запобігав вичерпанню запасів відновленого глутатіону (GSH) в умовах оксидативного стресу, спричиненого дією біхромату калію і сприяв накопиченню клітинного GSH у печінці щурів.

Ключові слова: щури, печінка, антиоксидантна система, оксидативний стрес, етилтіосульфанілат, біхромат калію, вільні радикали.

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