EXPERIMENTAL STUDIES

DYNAMICS OF THE CONTENT OF REACTIVE OXYGEN SPECIES AND THE STATE OF THE GLUTATHIONE SYSTEM IN THE ORAL CAVITY DURING SUBCHRONIC INTOXICATION WITH THE FUNGICIDE THIRAM AND ITS ANTIOXIDANT CORRECTION

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Thiram is a dithiocarbamate derivative, which is used as a fungicide for seed dressing and spraying during the vegetation period of plants, and also as an active vulcanization accelerator in the production of rubber-based rubber products. In this study the content of reactive oxygen species (ROS) and the state of the glutathione system have been investigated in the oral fluid and gum tissues of adult male Wistar rats treated with thiram for 28 days during its administration with food at a dose of 1/50 LD_{50}. Thiram induced formation of ROS in the oral cavity; this was accompanied by an imbalance in the ratio of reduced and oxidized forms of glutathione due to a decrease in glutathione and an increase in its oxidized form as compared to the control. Thiram administration caused an increase in the activity of glutathione-dependent enzymes (glutathione peroxidase, glutathione transferase, and glutathione reductase). However, the time-course of enzyme activation in the gum tissues and oral fluid varied in dependence on the time of exposure to thiram. In the oral fluid of thiram-treated rats changes in the antioxidant glutathione system appeared earlier. The standard diet did not allow the glutathione pool to be fully restored to physiological levels after cessation of thiram intake. The use of exogenous antioxidants resveratrol and an *Echinacea purpurea* extract led to the restoration of redox homeostasis in the oral cavity.

Key words: fungicide thiram; dithiocarbamates; oral cavity; oxidative stress, glutathione and glutathione-dependent enzymes; antioxidants

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INTRODUCTION

Pesticides are the most effective means of controlling crop pests. The circulation of residual quantities of pesticides and their metabolites in environmental components and migration along food chains lead to constant entry into the human body in small doses, chronic intoxication, and the potential risk of development of various diseases [1].

Thiram (dimethylcarbamothioylsulfanyl *N,N*-dimethylcarbamodithioate) is a multifunctional compound from the group of dimethyldithiocarbamates; it is used primarily as a fungicide with insecticidal and bactericidal effects in agriculture. In soil, thiram undergoes degradation with the formation of toxic dimethylcarbamosulfonic and dimethylaminothioxomethanesulfonic acids [2, 3]. The presence of sulfur in the thiram molecule determines its use as a vulcanization accelerator in the production of rubber-based products; furthermore, the additives are found in urban dust and can have a negative effect on humans [4, 5]. Thiram can enter the human body through the skin, respiratory tract, and orally. The population can be exposed to thiram and its degradation products indirectly through air, water, and food products [1, 6]. After oral administration thiram is actively absorbed and distributed throughout the body within two hours. In animals, thiram is metabolized in the liver and spleen with formation of dimethyldithiocarbamate and tetramethylthiourea; carbon disulfide is also formed in the lungs. Metabolites are excreted in feces, urine, and through the lungs [4].

In animals, pesticides, depending on their physicochemical properties, can accumulate in different tissues and organs, causing physiological and biochemical effects at various levels of organization of living organisms. The general effect of pesticides in animals is associated with the induction of oxidative stress, accompanied by damage to biomolecules and development of dysfunction of cells and tissues [6, 7].

The glutathione system plays an important role in antioxidant protection; changes in the functioning of this system serve as a valuable biochemical marker of oxidative stress developed during exposure to pesticides. Reduced glutathione (GSH) is an important component for maintenance of cellular redox homeostasis; its thiol group is involved in scavenging hydroxyl radicals and singlet oxygen. GSH acts as a cosubstrate for glutathione peroxidase and glutathione-S-transferase, which replenish active forms of the antioxidant vitamins C and E; it also regulates expression of genes of the endogenous antioxidant system. Glutathione peroxidases (GPx; EC 1.11.1.9) and glutathione-S-transferases (GST; EC 2.5.1.18) catalyze the process of detoxification of lipid hydroperoxides, hydrogen peroxide and electrophiles; their catalytic effectiveness depends on the level of glutathione as a hydrogen donor. Glutathione

reductase (GR; EC 1.8.1.7) is a key enzyme in the regeneration of GSH from GSSG by using NAD(P)H as a reducing agent, thus reducing the need for *de novo* synthesis of GSH [8, 9].

Mechanisms of oxidative stress development and antioxidant protection are quite variable and depend on the active substance and dose of the pesticide, the route of its administration and duration of exposure, as well as involvement of the biological system and target cells [10–13].

The toxic effect of dithiocarbamates is based on their high affinity for the thiol groups of glutathione and glutathione enzymes. Dithiocarbamates induce the production of ROS and inhibit the glutathione system due to oxidation of the thiol groups of the disulfide bridge of the pesticide or direct binding [3, 14]. Studies have examined the effects of thiram on the cells of various organs. The dose dependence and specificity of molecular cellular damage and biochemical defects of glutathione-dependent enzymes have been demonstrated in various experimental models and human cells [2, 15, 16].

The potential protective effects of exogenous antioxidants are actively investigated in the context of correction of elevated levels of oxidative stress and prevent depletion of the endogenous antioxidant system [17]. Convincing evidence exists that resveratrol (trans-3,4,5-trihydroxystilbene), a polyphenol of plant origin, exhibits antioxidant effects [18]. The antioxidant activity of resveratrol is determined by the presence in its structure of two phenolic rings with hydroxyl groups, providing the possibility of electron delocalization and metal chelation. Inhibition of ROS production by resveratrol is also due to increased expression of various antioxidant enzymes. Resveratrol promotes an increase in GSH by activating Nrf2 and upregulating γ-glutamylcysteine synthetase [19]. Resveratrol and its metabolites accumulate in tissues and some metabolites can be reversibly converted into resveratrol [20].

The *Echinacea purpurea* (L.) extract is a complex of substances, some of which have proven antioxidant properties [21]. The antioxidant potential of *E. purpurea* is due to the scavenging of free radicals, mainly by derivatives of caffeic acid [22], as well as the mobilization of antioxidant reserves due to the more intensive use of vitamins A and E by the body [23].

During oral intake of pesticides, the oral cavity becomes the first target and barrier. In the oral cavity, pesticides bind to lipoproteins or albumins, which facilitate their distribution throughout the body. Carbamate pesticides easily form complexes with biomolecules, which complicate pesticide metabolism and prevent their elimination from the body. Exposure to sulfur-containing pesticides, including thiram, can lead to changes in the keratin structure of epithelial cells due to the formation of disulfide bridges. It has been found that pesticides disrupt the connection of the epithelial layer of the oral mucosa with the underlying lamina densa [24]. In turn, the dense lamina is located on a layer of adipose tissue, which acts as a target for the accumulation of fat-soluble pesticides. Pesticides lead to changes in epithelial cells and the microbiome of the oral cavity; this increases the risk of developing caries, periodontal diseases, and odontogenic infections [24]. Research has shown that oxidative stress of organs and biological fluids of the oral cavity underlies the toxic effects of xenobiotics and pathological processes [25–28]. There is insufficient information about the effect of thiram on the antioxidant system of oral fluid and gum tissue during subchronic oral administration in small doses and the possibilities of correction with exogenous antioxidants.

The aim of this study was to investigate the dynamics of ROS content and the functioning of the glutathione antioxidant system in the oral cavity during oral subchronic intoxication with thiram and correction with plant antioxidants.

MATERIALS AND METHODS

The study was performed using male albino Wistar rats at the Experimental Biological Clinic of the Kursk State Medical University in the autumn-winter period. At the beginning of the experiment, the animals were 8 weeks old and had body weight of 200–220 g. The animals were kept in individual cages under standard conditions with free access to food and clean drinking water.

Rats were randomly divided into groups of 10 animals each. The control groups (C_7, C_{14}, C_{21}, C_{28}, C_{56}) consisted of healthy intact rats that received standard food. Rats of the "Thiram" groups (T_7, T_{14}, T_{21}, T_{28}, T_{st}, T_e, T_r), received thiram (tetramethylthiuram disulfide 97%, CAS No. 137-26-8, Sigma-Aldrich, USA) at a dose of 1/50 LD_{50} (1.6 mg) [29] once a day for 28 days in the morning as a part of feed pellets, then during the day the animals had unlimited access to clean food. This method of the pesticide administration corresponds to the intake of the toxicant under natural conditions and eliminates the physiological stress of the animal.

To study the ability of animals to restore redox homeostasis by means of endogenous and exogenous antioxidants after cessation of thiram intake, in the next 28 days rats received the following treatments: (i) rats of the "Standard" subgroup (T_{st}) (n=10) received the usual standard food; (ii) rats of the "Echinacea" (T_e) subgroup (n=10) received the *E. purpurea* extract (Echinacea, 0.2 g tablets, Vifitech, Russia) with food at a dose of 3.43 mg/day, (iii) rats of the "Resveratrol" (T_r) subgroup (n=10) received resveratrol at a dose of 1.71 mg/day (Resveratrol in capsules, 100 mg, SOLGAR, USA) [25]. To study the dynamics of the state of the glutathione system, samples of oral fluid and gum tissue were obtained on days 7, 14, 21, and 28

of intoxication in groups C_7, C_{14}, C_{21}, C_{28}, T_7, T_{14}, T_{21}, T_{28}, as well as on day 56 from the start of the experiment in groups C_{56}, T_{st}, T_e, T_r. Unstimulated oral fluid was collected in a volume of 50 μl from living rats using a JoanLab pipette dispenser (DPAOP-1-10-100 JoanLab, Lenpipet Thermo Scientific, Russia) and centrifuged for 20 min at 1500 rpm (rotor F-45-12-11) (MiniSpin, Eppendorf, Germany). To obtain gum tissue, experimental animals were subjected to decapitation under ether anesthesia. The tissue washed with cold saline, was weighed, crushed and homogenized (Potter homogenizer, Sartorius, Germany) in cold 0.1 M potassium phosphate buffer (pH 7.4, 0°C) in a tissue-buffer ratio 1:6 (based on the weight of the wet tissue). The resulting homogenate and oral fluid were used for determination of the amount of ROS, the content of GSH and GSSG, and the activity of the glutathione enzymes GPx, GST, and GR.

The total amount of ROS evaluated with H_2O_2 as a standard was determined using the OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence) (STA-347, 96 tests, Cell Biolabs, USA) at an excitation wavelength of 480 nm and an emission wavelength of 530 nm. The concentration of reduced and oxidized forms of glutathione was assessed using the Enzyme-linked Immunosorbent Assay Kit For Glutathione (GSH) (CEA294Ge, 96 tests, Cloud-Clone Corp., USA) and the Enzyme-linked Immunosorbent Assay Kit For Oxidized Glutathione (GSSG) (CEK518Ge, Cloud-Clone Corp.). GPx activity was determined using the Glutathione Peroxidase Activity Colorimetric Assay Kit (K762, BioVision, USA). GST activity was assayed using Enzyme-linked Immunosorbent Assay Kit For Glutathione S Transferase Alpha1 (GSTa1) (SEA609Ra96, Cloud-Clone Corp.). GR activity was assayed with the OxiSelect™ Glutathione Reductase Assay Kit (STA-812, Cell Biolabs) was used. All measurements were carried out on a Varioscan Flash microplate reader (Thermo Fisher Scientific, USA), following the recommendations of the kit manufacturers.

Statistical data processing was carried out using the STATISTICA 13.0 program. The hypothesis of normal distribution of quantitative indicators was tested using the Shapiro-Wilk test. Since most of the data were not normally distributed, quantitative parameters were presented as median and lower and upper quartile values (Me [Q1–Q3]). To compare two independent datasets, the nonparametric Mann-Whitney U test was used. Differences between two groups were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Thiram intake of at a dose of 1/50 LD_{50} (as a part of natural food) for 28 days led to a statistically significant increase in the ROS production in the gum tissue (Table 1) and in the oral fluid (Table 2) as compared with the control group of rats; ROS production reached the maximum value on day 28. The induction of ROS production could be caused both by the direct action of the pesticide in the oral cavity, and indirectly due to transport of thiram and its metabolites by the blood into the gum tissue and salivary glands.

In the gum tissues and oral fluid (OF) in the control groups (C_7–C_{28}), the ROS content varied slightly (the interquartile range IQR_{gum}=45.85–49.22, the interquartile range IQR_{of}=13.79–14.39). This suggests the maintenance of oxidative homeostasis at an optimal level by antioxidant systems. The intake of thiram led to an increase in the interquartile range of the ROS amount in the gum tissue (the interquartile range IQR_{gum}=42.87–89.05) (Table 1) and oral fluid (the interquartile range IQR_{of}=25.43–37.21) (Table 2). The highest variation was observed on day 28 of thiram treatment.

The presence of a disulfide group in the structure of thiram identifies the glutathione system as a key target of action. Starting from day 14 of intoxication the thiram intake resulted in a statistically significant decrease in the gum tissue GSH content as compared with control groups of rats (Table 1). In the oral fluid of rats (groups T_7–T_{28}) a decrease in the amount of GSH compared to intact rats was already noted on day 7 of thiram intake (Table 2). The reduction in the GSH content in the oral fluid and gum tissue may be attributed to the ability of thiram to oxidize the glutathione SH group or bind directly to it thus forming conjugates; this prevents damage of important SH groups of proteins and cofactors [9, 10]. Oxidation of glutathione by thiram led to a statistically significant increase in the content of the oxidized form of GSSG in the gum tissue and oral fluid of rats already on day 7 of intoxication as compared to intact animals (Tables 1, 2).

The thiol-disulfide ratio, which under physiological conditions is 100:1, is an important indicator of cellular redox homeostasis [3]. In the gum tissue and oral fluid of rats non-exposed to thiram, this indicator varied within the range of 117.82–195.66 and 144.80–159.78, respectively (Tables 1, 2). Dietary intake of thiram led to a statistically significant decrease in the GSH/GSSG ratio compared to control throughout the entire period of experimental exposure; the decrease in this indicator below the physiological level reached a minimum on day 28 of the experimental intoxication: 31.76 in the gum tissue and 34.56 in the oral fluid, respectively (Tables 1, 2).

In the gum tissues of rats exposed to thiram intoxication, a statistically significant increase in the activity of the GR enzyme compared to the control was noted on days 21 and 28 of intoxication (Table 1). In the oral fluid a 1.92-fold increase in activity was detected already on day 14 of thiram intake (Table 2). Increased GR activity can be considered as a compensatory mechanism in maintaining cell redox

Table 1. Parameters of oxidative stress in the gum tissues of rats in the control group (C) and rats exposed to thiram intoxication (T) for 28 days

Day of experiment							
Day 7		Day 14		Day 21		Day 28	
C_7	T_7	C_{14}	T_{14}	C_{21}	T_{21}	C_{28}	T_{28}
ROS content μmol/l							
103.50 [83.02–132.24]	151.32 [110.82–176.76]	101.48 [85.04–130.89]	151.14 [130.45–173.32]	105.16 [84.12–133.21]	148.05 [129.55–182.10]	105.30 [84.38–132.64]	161.65 [111.92–200.97]
p=0.04326		p=0.02881		p=0.02323		p=0.03546	
GSH content, μg/ml							
566.63 [374.35–632.65]	349.52 [289.49–386.19]	520.27 [368.11–557.38]	291.72 [251.19–401.68]	522.97 [310.25–564.88]	272.61 [176.38–312.83]	517.11 [365.71–573.28]	196.21 [128.27–296.19]
p=0.07526		p=0.03546		p=0.00684		p=0.00048	
GSSG content, μg/ml							
3.08 [2.14–4.13]	5.92 [4.34–6.73]	2.95 [2.16–3.91]	8.83 [6.45–9.68]	3.15 [2.42–3.98]	8.06 [5.55–9.39]	2.98 [2.44–4.13]	7.54 [5.61–10.48]
p=0.00684		p=0.00049		p=0.00033		p=0.00001	
GSH/GSSG							
195.66 [94.68–234.34]	59.84 [45.40–74.14]	117.82 [123.24–275.95]	39.63 [28.76–45.91]	161.85 [109.26–230.70]	36.09 [17.33–52.78]	176.77 [99.42–228.74]	31.76 [13.33–39.78]
p=0.00893		p=0.00013		p=0.00021		p=0.00004	
GPx activity, mU/ml							
35.08 [29.09–45.49]	55.31 [38.29–78.38]	32.30 [27.95–43.28]	63.00 [36.48–78.34]	34.16 [31.24–46.12]	50.55 [36.31–58.33]	36.61 [32.41–46.73]	49.76 [37.73–66.19]
p=0.01854		p=0.01150		p=0.14314		p=0.05243	
GST activity, mU/ml							
0.16 [0.12–0.17]	0.29 [0.19–0.32]	0.16 [0.14–0.19]	0.35 [0.23–0.42]	0.17 [0.13–0.19]	0.37 [0.28–0.41]	0.17 [0.15–0.19]	0.41 [0.29–0.48]
p=0.00151		p=0.00008		p=0.0004		p=0.00105	
GR activity, mU/ml							
6.76 [6.48–8.89]	8.08 [6.63–10.17]	6.88 [5.79–7.71]	9.18 [7.06–11.22]	7.24 [6.95–7.81]	9.84 [8.13–12.64]	7.31 [6.15–7.54]	10.20 [7.74–14.52]
p=0.43587		p=0.24745		p=0.03546		p=0.00893	

C_7-C_{28} – control group, T_7-T_{28} – thiram intoxication. Here and in subsequent tables p – the level of statistical significance between groups of control and thiram treated animals (Mann-Whitney test).

homeostasis. In most tissues, the rate of GSSG reduction is higher compared to the synthesis of reduced glutathione. A decrease in the amount of glutathione due to its direct oxidation by thiram and participation in the mechanisms of ROS detoxification excessively induced by thiram under maintenance of GR activity at the level of the control rats, leads to a predominance of the rate of GSH oxidation over the rate of its regeneration and, as a consequence, a significant decrease in the GSH/GSSG ratio. The inability to synthesize GSH *de novo* and a decrease in export from the cytoplasm of cells with increased needs in the intracellular pool of GSH for detoxification of thiram may cause a decrease in the GSH content and an increase in GR activity in the oral fluid at an earlier time as compared to the gum tissue.

During subchronic intake of thiram as a part of the feed, a statistically significant increase in the activity of GPx and GST in the gum tissue and GPx in the oral fluid compared to control groups of rats was noted from day 7 of intoxication. A significant increase in GST activity in the oral fluid occurred only on day 28 of the thiram treatment (Tables 1, 2). Using GSH as a substrate GPx and GST reduce the effects of oxidative stress by scavenging free radicals, thus reducing GSH reserves and impairing the thiol/disulfide balance. Another mechanism of GST participation in ROS detoxification occurs through glutathione binding to the substrate or nucleophilic substitution with the formation of glutathione GSR conjugates; in this case glutathione regeneration becomes impossible [4]. An increase

Table 2. Parameters of oxidative stress in the oral fluid of rats of rats in the control group (C) and rats exposed to thiram intoxication (T) for 28 days

Day of experiment							
Day 7		Day 14		Day 21		Day 28	
C_7	T_7	C_{14}	T_{14}	C_{21}	T_{21}	C_{28}	T_{28}
ROS content, μmol/l							
26.65 [20.17–34.56]	45.10 [34.69–60.12]	29.17 [20.89–35.17]	53.02 [37.73–63.87]	29.55 [21.42–35.21]	54.40 [36.95–69.46]	29.96 [21.36–35.42]	56.36 [34.07–71.28]
p=0.00520		p=0.00288		p=0.00105		p=0.00389	
GSH content, μg/ml							
249.26 [199.87–310.76]	165.80 [107.33–185.12]	244.87 [204.87–315.10]	132.35 [126.25–165.70]	246.04 [208.10–310.84]	139.94 [87.39–154.57]	247.50 [205.95–311.95]	99.81 [87.32–133.21]
p=0.00520		p=0.00288		p=0.00033		p=0.00008	
GSSG content, μg/ml							
1.53 [1.04–1.81]	2.92 [1.97–3.13]	1.50 [1.27–1.83]	2.67 [2.35–3.45]	1.58 [1.24–1.82]	3.55 [2.70–4.86]	1.63 [1.27–1.97]	4.02 [2.74–4.31]
p=0.00033		p=0.00001		p=0.00001		p=0.00013	
GSH/GSSG							
150.11 [141.13–266.54]	59.00 [47.71–82.61]	159.78 [116.72–224.08]	44.29 [39.71–58.76]	152.35 [133.65–247.85]	37.89 [24.31–48.68]	144.80 [109.33–235.37]	34.56 [16.55–37.64]
p=0.00001		p=0.00002		p=0.00001		p=0.00001	
GPx activity, mU/ml							
21.56 [17.15–31.34]	41.58 [25.38–46.82]	21.13 [18.53–32.10]	49.18 [34.62–38.59]	24.80 [21.42–34.62]	54.99 [27.97–58.69]	24.28 [22.74–33.65]	46.28 [36.15–70.84]
p=0.00389		p=0.00151		p=0.00520		p=0.00073	
GST activity, mU/ml							
0.29 [0.21–0.36]	0.38 [0.32–0.39]	0.31 [0.24–0.33]	0.37 [0.29–0.46]	0.32 [0.28–0.37]	0.39 [0.28–0.47]	0.30 [0.26–0.38]	0.56 [0.45–0.69]
p=0.07526		p=0.05243		p=0.19032		p=0.00001	
GR activity, mU/ml							
3.27 [2.45–3.96]	4.08 [3.92–5.06]	3.40 [2.39–4.02]	6.53 [4.19–7.41]	3.42 [2.51–3.87]	6.23 [4.56–8.10]	3.46 [2.40–3.85]	6.01 [5.41–9.28]
p=0.05243		p=0.00389		p=0.00021		p=0.00001	

C_7-C_{28} – control group, T_7-T_{28} – thiram intoxication.

in GST activity in the oral fluid of rats only on day 28 of intoxication compared to gum tissue can be considered as a specific way of protection against excessive depletion of glutathione in biological fluids. A decrease in the amount of GSH and an increase in GST activity may also be associated with their participation in S-glutathionylation of proteins to protect cysteine residues from irreversible oxidation by thiram [3].

We have investigated the glutathione status of the cells of the gum tissue and oral fluid of rats after cessation of the thiram intake followed by the use of standard food during the next 28 days (T_{st} group) (Table 3). In the gum tissues and oral fluid of rats in the T_{st} group, the content of ROS was 1.35 and 1.79 times higher, respectively, as compared to intact rats (C_{56}). No statistically significant differences in ROS were found as compared to the group of rats treated with thiram on day 28 of the experiment (T_{28}). The amount of GSH and GSSG in the gum tissue and oral fluid after cessation of the thiram intake also did not reach the values of the control group, and the content of reduced and oxidized forms of glutathione remained comparable to their amount on day 28 of intoxication.

The antioxidant system of the gum tissue was also unable to restore the thiol-disulfide ratio in cells after cessation of thiram intake (it was 5.69 times lower compared to the control). There was a statistically significant (1.48 fold) increase in the GSH/GSSG ration

Table 3. Parameters of oxidative stress in the gum tissues and oral fluid of rats in the control group (C) and rats exposed to thiram intoxication (T) during their maintenance on the standard diet for 28 days after cessation of thiram administration

Gum tissues			Oral fluid		
T_{28}	C_{56}	T_{st}	T_{28}	C_{56}	T_{st}
ROS content, μmol/l					
161.65 [111.92–200.97]	105.80 [85.26–133.82]	142.95 [119.27–178.43]	56.36 [34.07–71.28]	27.43 [20.35–34.52]	48.97 [37.54–68.32]
—	—	p=0.68421#	—	—	p=0.85342#
		p=0.04328*			p=0.00105*
GSH content, μg/ml					
196.21 [128.27–296.19]	521.58 [370.82–570.73]	291.13 [152.45–307.76]	99.81 [87.32–133.21]	249.88 [190.87–308.98]	135.15 [92.29–152.55]
—	—	p=0.19032#	—	—	p=0.48125#
		p=0.00389*			p=0.00073*
GSSG content, μg/ml					
7.54 [5.61–10.48]	3.11 [2.37–4.15]	7.32 [5.08–8.63]	4.02 [2.74–4.31]	1.59 [1.21–1.95]	2.64 [2.37–2.99]
—	—	p=0.48125#	—	—	p=0.07526#
		p=0.00008*			p=0.00209*
GSH/GSSG					
31.76 [13.33–39.78]	184.91 [99.16–25.63]	32.49 [2.59–51.06]	34.56 [16.55–37.64]	148.52 [107.63–19.95]	51.20 [38.90–60.72]
—	—	p=0.39305#	—	—	p=0.01854#
		p=0.00021*			p=0.00130*
GPx activity, mU/ml					
49.76 [37.73–66.19]	36.55 [31.69–44.27]	38.92 [35.28–53.26]	46.28 [36.15–70.84]	24.63 [21.69–35.13]	40.19 [31.54–47.64]
—	—	p=0.35268#	—	—	p=0.31500#
		p=0.27986*			p=0.00684*
GST activity, mU/ml					
0.41 [0.29–0.49]	0.17 [0.15–0.19]	0.26 [0.20–0.27]	0.56 [0.45–0.69]	0.33 [0.29–0.37]	0.39 [0.34–0.44]
—	—	p=0.01854#	—	—	p=0.00520#
		p=0.00288*			p=0.07526*
GR activity, mU/ml					
10.20 [7.74–14.52]	7.11 [6.58–8.76]	9.91 [8.25–12.39]	6.01 [5.41–9.28]	3.40 [2.56–4.08]	6.49 [5.01–9.14]
—	—	p=0.57874#	—	—	p=0.585343#
		p=0.02881*			p=0.00008*

C – control group, T – thiram intoxication. p: # – as compared with the parameters on day 28 of intoxication (T_{28}; $p<0.05$); * – as compared to control C_{56} ($p<0.05$).

in the oral fluid compared to day 28 of intoxication, but this parameter did not reach the level of control group rats. GR activity in rat gum tissue cells and oral fluid after cessation of thiram intake for 28 days remained at the increased level compared to animals of the control group (C_{56}); however, the level of GR activity did not differ from that in the group of rats on day 28 of intoxication (T_{28}). GPx activity in the gum tissue after cessation of thiram intake in the T_{st} group corresponded to the enzyme activity in the group of intact rats. In the oral fluid of rats in the T_{st} group, it was statistically significantly higher (1.63 times) compared to animals of the control group and remained at the level observed on day 28

Table 4. Parameters of oxidative stress in the gum tissues and oral fluid of the control group rats (C) and rats exposed to thiram intoxication (T) during their maintenance on the diet supplemented with exogenous antioxidants for 28 days after cessation of thiram administration

Gum tissue				Oral fluid			
T_{28}	C_{56}	T_e	T_r	T_{28}	C_{56}	T_e	T_r
ROS content, μmol/l							
161.65 [111.92–200.97]	105.80 [85.26–133.82]	106.69 [87.34–125.87]	85.51 [64.58–118.56]	56.36 [34.07–71.28]	27.43 [20.35–34.52]	30.60 [26.97–43.24]	26.51 [15.27–29.81]
—	—	p=0.03546#	p=0.00684#	—	—	p=0.00209#	p=0.00001#
		p=0.97051*	p=0.31500*			p=0.24745*	p=0.35268*
GSH content, μg/ml							
196.21 [128.27–296.19]	521.58 [370.82–570.73]	324.79 [268.54–395.43]	355.42 [310.32–382.34]	99.81 [87.32–133.21]	249.88 [190.87–308.98]	209.99 [171.28–312.55]	240.66 [201.94–332.26]
—	—	p=0.00520#	p=0.00049#	—	—	p=0.00001#	p=0.00033#
		p=0.07526*	p=0.07526*			p=0.73936*	p=0.63053*
GSSG content, μg/ml							
7.54 [5.61–0.48]	3.11 [2.37–4.15]	4.63 [2.99–6.03]	3.81 [3.01–4.21]	4.02 [2.74–4.31]	1.59 [1.21–1.95]	1.55 [1.30–2.25]	1.41 [1.26–1.56]
—	—	p=0.00389#	p=0.00002#	—	—	p=0.00004#	p=0.00004#
		p=0.04328*	p=0.39305*			p=0.91180*	p=0.39305*
GSH/GSSG							
31.76 [13.33–39.78]	184.91 [99.16–225.63]	62.32 [54.33–89.81]	81.54 [73.04–127.02]	34.56 [16.55–37.64]	148.52 [107.63–219.95]	163.05 [120.10–193.69]	140.39 [121.17–245.66]
—	—	p=0.00021#	p=0.00002#	—	—	p=0.00001#	p=0.00001#
		p=0.02323*	p=0.07526*			p=0.91180*	p=0.97051*
GPx activity, mU/ml							
49.76 [37.73–66.19]	36.55 [31.69–44.27]	36.37 [29.57–47.96]	36.15 [21.98–43.29]	46.28 [36.15–70.84]	24.63 [21.69–35.13]	23.51 [21.26–26.01]	24.57 [21.13–27.14]
—	—	p=0.16549#	p=0.05243#	—	—	p=0.00001#	p=0.00001#
		p=0.97051*	p=0.52885*			p=0.24745*	p=0.31500*
GST activity, mU/ml							
0.41 [0.29–0.49]	0.17 [0.15–0.19]	0.19 [0.16–0.21]	0.16 [0.14–0.22]	0.56 [0.45–0.69]	0.33 [0.29–0.37]	0.34 [0.31–0.38]	0.29 [0.20–0.34]
—	—	p=0.00209#	p=0.00151#	—	—	p=0.00001#	p=0.00001#
		p=0.48125*	p=0.68421*			p=0.63053*	p=0.27986*
GR activity, mU/ml							
10.20 [7.74–4.52]	7.11 [6.58–8.76]	8.75 [5.95–0.43]	5.83 [4.65–8.39]	6.01 [5.41–9.28]	3.40 [2.56–4.08]	4.15 [3.38–5.17]	3.16 [2.52–4.37]
—	—	p=0.123001#	p=0.00288#	—	—	p=0.00288#	p=0.00008#
		p=0.58874*	p=0.10512*			p=0.10512*	p=0.91180*

C – control group, T – thiram intoxication. p: # – as compared with the parameters on day 28 of intoxication (T_{28}; $p<0.05$); * – as compared to control C_{56} ($p<0.05$).

of intoxication. GST activity in the gum tissues of rats kept for postintoxication 28 days on the standard diet decreases by 1.58 times compared to day 28 of thiram intoxication (group T_{28}), but did not reach the level of enzyme activity seen in intact rats (C_{56}). In the oral fluid of rats, after cessation of thiram intake, a more pronounced decrease in GST activity occurred as compared with day 28 of intoxication (T_{28}), reaching the enzyme activity values of the control group of animals (C_{56}).

To restore the state of the glutathione system after the thiram action, the corrective effect of exogenous antioxidants, resveratrol and a preparation of the *E. purpurea* extract, was studied (Table 4).

The addition of resveratrol to standard food for 28 days after exposure to thiram led to a statistically significant decrease in the amount of ROS and GSSG in gum tissue cells (1.89 and 1.98 times, respectively), an increase in both the GSH content (1.81 times) and the GSH/GSSG ratio (2.57 times). The activity of GST and GR decreased by 2.56 and 1.75 times, respectively, reaching the corresponding values in the control group rats (C_{56}) (Table 4). The *E. purpurea* extract reduced the amount of ROS (1.52 times) and GST activity (2.16 times) in the gum tissues of rats. At the same time, an increase of 1.66 times in the intracellular pool of GSH was noted compared to day 28 of intoxication; on day 56 of the experiment, this parameter reached the values of intact rats. There were no significant effects on the amount of GSSG and the GSH/GSSG balance, or GR activity in the gum tissues of rats treated with echinacea; the values of these parameters remained at the level seen on day 28 of thiram intoxication. Administration of resveratrol and echinacea into the diet of rats with food did not have a pronounced effect on the activity of GPx in the gum tissues compared to day 28 of thiram intoxication (group T_{28}) (Table 4).

The most pronounced effect on the ROS content and the glutathione system in the oral fluid of rats during recovery after thiram intoxication was observed when resveratrol and echinacea were added into food (Table 4). All the studied parameters (the amount of ROS, the content of GSH, GSSG, their ratio, the activity of GPx, GST, GR) reached the values of intact rats on day 56 of the experiment (group C_{56}).

CONCLUSIONS

Our study has shown, the thiram intake at a dose of 1/50 LD_{50} for 28 days induced oxidative stress in the gum tissue and oral fluid of rats, characterized by increased production of ROS followed by the imbalance in the glutathione system, and a compensatory increase in the activity of glutathione-dependent enzymes. In the oral fluid, an imbalance of glutathione and an increase in the activity of GPx and GST appeared earlier than in the gum tissue. After cessation of the thiram intake, the use of standard food could not normalize the studied parameters of the glutathione antioxidant system. Addition of exogenous antioxidants resveratrol and the *E. purpurea* extract to the diet led to normalization of the glutathione system and glutathione-dependent enzymes, reaching control values. Resveratrol has the most pronounced antioxidant effect, which leads to a state of physiological balance of the thiol-disulfide ratio GSH/GSSG due to an increase in the amount of glutathione, a decrease in the ROS content, oxidized glutathione and the activity of glutathione-dependent enzymes.

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COMPLIANCE WITH ETHICAL STANDARDS

The study was approved by the Regional Ethics Committee of Kursk State Medical University (protocol No. 7 of November 30, 2018), carried out in accordance with the ethical principles of working with animals for scientific purposes governed by "Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes" and national standards. This article does not contain results from studies involving human subjects.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ДИНАМИКА СОДЕРЖАНИЯ АКТИВНЫХ ФОРМ КИСЛОРОДА И СОСТОЯНИЯ СИСТЕМЫ ГЛУТАТИОНА В РОТОВОЙ ПОЛОСТИ ПРИ СУБХРОНИЧЕСКОЙ ИНТОКСИКАЦИИ ФУНГИЦИДОМ ТИРАМ И АНТИОКСИДАНТНОЙ КОРРЕКЦИИ

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Тирам — производное дитиокарбамата, используется как фунгицид для протравливания семян и опрыскивания в период вегетации растений, а также в качестве активного ускорителя вулканизации при производстве резинотехнических изделий на основе каучука. В работе определено содержание активных форм кислорода (АФК) и состояние системы глутатиона в ротовой жидкости и тканях десны взрослых самцов крыс линии Wistar при поступлении тирама в дозе 1/50 LD_{50} в течение 28 дней в составе корма. Тирам индуцирует образование АФК в полости рта; при этом отмечается нарушение баланса в соотношении восстановленной и окисленной форм глутатиона за счёт снижения глутатиона и увеличения его окисленной формы по сравнению с контролем. Установлено повышение активности глутатионзависимых ферментов (глутатионпероксидазы, глутатионтрансферазы, глутатионредуктазы) при поступлении тирама; при этом отмечена вариабельность в сроках активации ферментов в тканях десны и ротовой жидкости в зависимости от времени экспозиции пестицидом. В ротовой жидкости крыс при пероральном поступлении тирама нарушения антиоксидантной глутатионовой системы проявляются в более ранний период времени. Стандартный корм не позволяет в полной мере восстановить глутатионовый пул до физиологических показателей после прекращения поступления тирама. Использование экзогенных антиоксидантов ресвератрола и экстракта эхинацеи пурпурной приводит к восстановлению редокс-гомеостаза в полости рта.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: фунгицид тирам; дитиокарбаматы; ротовая полость; окислительный стресс, глутатион и глутатионзависимые ферменты; антиоксиданты

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