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INFLUENCE OF HEAVY METALS ON ANTIOXIDANT SYSTEM AND BIOCHEMICAL INDEXES IN RATS

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Abstract. *The study was undertaken to examine the effect of heavy metals on the antioxidant system and biochemical indexes in the organism of rats. The influence of heavy metals on indexes The influence of heavy metals on the indicators of the antioxidant system (the antioxidant enzyme activities – glutathione peroxidase, glutathione reductase, catalase, and superoxide dismutase) and the processes of lipid peroxidation (the content of hydroperoxides and products of thiobarbituric acid) was determined. It is established, that the antioxidant system functions more intensively in blood and liver of rats under the action of heavy metals. The study of enzyme activity showed the activation of the latter under conditions of heavy metal intoxication 1.5–2.0 times (depending on the heavy metal) compared with the control. We found that blood levels of total and direct bilirubin, creatinine, and urea increased in intoxicated rats from all experimental groups compared with intact animals. However, a decrease in the content of albumin, total protein, cholesterol, and triglycerides was also found in all experimental groups, in comparison with intact rats. Under the action of heavy metals, the activity of total α-amylase, lactate dehydrogenase, and glucose concentration increases in blood of rats. According to the results of studies in intoxicated animals compared with the intact group, there was a change in the cation-anion pool, in particular, a tendency to decrease the content of sodium and inorganic phosphorus and increase chlorides, magnesium, calcium, and potassium.*

Keywords: *rats, blood, liver, copper, zinc, cadmium, lead, antioxidant system*

Introduction

Environmental contamination by heavy metals raises concerns about human health. It has become evident that increasing human activities have modified the global cycle of heavy metals, including the toxic non-essential elements. Heavy metals are among the most toxic and it is reported that their increased concentration in agricultural soils is known to come from the application of phosphate fertilizers, sewage sludge, wastewater, and pesticides. The accumulation of toxicants in the air, soil, plants, water, and sewage sludge has overwhelmed the natural capacity in many ecosystems, resulting in the potential for humans to be exposed to heavy metals (Djuric et al., 2015; Saghazadeh & Rezaei, 2017).

As necessary microelements for physiological activities, copper and zinc play a significant part in normal development and organisms' homeostasis maintenance. However, high-level exposure to these elements may also induce adverse health effects (Maret et al., 2006; Cdos & Fernandes, 2008). The toxic effect of heavy metals is due to their influence on metabolic enzyme systems and induced oxidative stress in the animal body. The toxic molecular mechanisms of different heavy metals vary, although they also have some similarities (El Yamani et al., 2017; Husak et al., 2018).

Oxidative stress is the imbalance that occurs when there is an increased production of free radicals that exceeds the body's ability to neutralize it. Alteration of chemical reactions at the cellular level leads to the appearance of free radicals and peroxides that affect the intracellular structures – proteins, lipids, and DNA, with the disruption of intrinsic mechanisms at this level. Free radicals are normally produced in the body due to the influence of external factors, such as pollution, cigarette smoke, or internal, due to intracellular metabolism when antioxidant mechanisms are exceeded (Fig. 1).

Oxidative stress under the action of heavy metals defined as a persistent imbalance between the production of highly reactive molecular species (chiefly oxygen and nitrogen) and antioxidant defense, is implicated in a broad variety of chronic and acute diseases, including such diseases as diabetes (Wang et al., 2014; Kumar & Sharma, 2019).

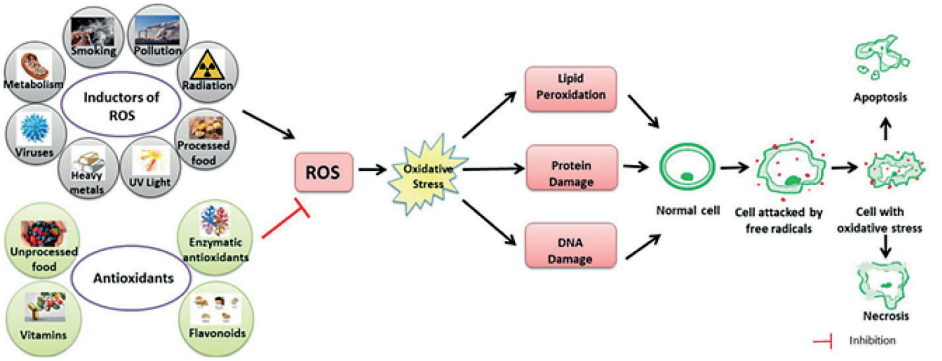

Fig. 1. Schematic figure of the link between reactive oxygen species (ROS), oxidative stress and their effects on the body

Source: Sharifi-Rad et al. (2020).

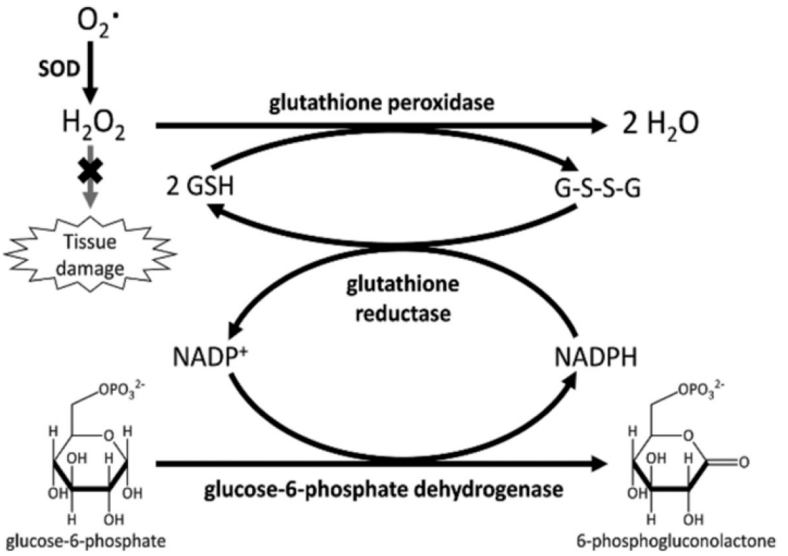

Fig. 2. Primary enzymes (SOD or peroxidases) act directly in scavenging ROS. Secondary enzymes, such as glutathione reductase and glucose-6-phosphate dehydrogenase, support the action of primary enzymes regenerating NAPDH and reduced glutathione.

Source: Sharifi-Rad et al. (2020).

Negative environmental factors, including heavy metals, lead to a breakdown of antioxidant protection due to any external influence and cause increased free radical oxidation. This is accompanied by a change in the conformation of lipids, which leads to a violation of the structural and functional properties of biomembranes, increasing their lability and permeability, imbalance of enzyme systems of membranes, disruption of electron transport chains in mitochondria. In addition, the products of free radical oxidation damage proteins, thiol compounds, and nucleotide phosphates, change the degree of glycolysis, damage nuclear DNA with the formation of its single-strand breaks (Lodovici & Bigagli, 2011).

The activity of the thiol-disulfide system may indicate the state of antioxidant reserves of the body, it responds to its effects and the external nature of the change of redox potential, which characterizes the ratio of the concentration of renewed sulfhydryl (SH) and oxidized disulfide (SS) groups (thiol-disulfide coefficient). It is known that the TDC can be an in-integrative indicator of adaptive capabilities of the organism (Fig. 2) (Sharifi-Rad et al., 2020).

The aim of this study is to assess the influence of copper, zinc, lead, and cadmium on the functioning of the antioxidant system and biochemical indexes in rat tissues.

Materials and methods of research

The study was conducted on white male rats of the same age, weighing 180–

200 g, kept under standard conditions of a vivarium, with free access to food and water. Five groups of animals were formed: the first – intact (control), the second – rats orally administered a solution of copper sulfate at a dose of 3 mg/kg, the third – rats orally administered a solution of zinc sulfate at a dose of 2 mg/kg, the fourth – rats orally administered a solution of cadmium sulfate at a dose of 1.5 mg/kg, the fifth – rats orally administered a solution of lead nitrate at a dose of 1.7 mg/kg. Intoxication was performed within 14 days, then the rats were decapitated under ether anesthesia for extraction of blood and liver for further research. The work was carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (Strasbourg, France, 1986).

Blood was obtained by well-known methods and preparations of a homogeneous fraction of liver cells were conducted by differential centrifugation (Severyn & Soloveva, 1989). The content of thiobarbituric acid reactive substances (TBARS) was determined by Stalnaya & Haryshvyly (1977), diene conjugates – by Gavrilov et al. (1988). In the course of the research, we used the following methods to determine the activity of enzymes: superoxide dismutase (EC 1.15.1.1) by Orehovych (1977), catalases (EC 1.11.1.9) by Koroliuk (1988), glutathione peroxidase (GP) (EC 1.11.1.9) and glutathione transferase (GT) (EC 2.5.1.18) by Mannervik (1985), Vlasova et al. (1990). The content of reduced form of glutathione (GSH) was established by the Ellman method (1959).

The content of heavy metals in blood and liver was determined by spectrometric method (Havezov & Tsalev, 1983), using the absorption mode in acetylene-air flame on the atomic absorption spectrometer (SpectrAA-55B, VARIAN, USA). Standard solutions of these metals were used as controls.

Blood biochemical analysis (activity of alkaline phosphatase (EC 3.1.3.1), alanine aminotransferase (EC 2.6.1.2), aspartate aminotransferase (EC 2.6.1.1), gamma-glutamyl transpeptidase (EC 2.3.2.2), lactate dehydrogenase (EC 1.1.1.27), cholinesterase (EC 3.1.1.8), total α-amylase (EC 3.2.1.1), bilirubin (total and direct), creatinine, urea, glucose, albumin, total protein, cholesterol, triglycerides, chlorides, magnesium, inorganic phosphorus, calcium, sodium, and potassium) was performed using a semi-automatic biochemical analyzer (Micro Lab 300, Netherlands).

The probability of the results was determined using Student's t-test. Statistical calculations were performed using the Microsoft Excel 2007 program (Kucherenko et al., 2001).

Analysis of recent researches and publications

It was found that in rats intoxicated with copper, zinc, cadmium, and lead ions, activation of lipid peroxidation was revealed in blood and liver, which was assessed by the accumulation of TBARS (Table 1).

Copper sulfate intoxication leads to an increase in TBARS by 40% in blood and 31% in the liver, zinc sulfate – by 42% in blood and 31% in the liver, cadmium sulfate – by 66% in blood and 38% in the liver, lead nitrate – by 61% in blood and 36% in the liver, in comparison with the control group of animals.

The content of diene conjugates in rat tissues (Table 2) was determined as the ratio of optical density at 233 and 218 nm.

1. The content of TBARS in rat tissues under conditions of heavy metal intoxication (M ± m, n = 8)

Rats	TBARS	
	Blood, mmol/L	Liver, μmol/mg protein
Intact	1.34 ± 0.05	0.74 ± 0.03
Intoxicated with $CuSO_4$	1.87 ± 0.09*	0.97 ± 0.04*
Intoxicated with $ZnSO_4$	1.91 ± 0.04*	0.95 ± 0.05*
Intoxicated with $CdSO_4$	2.23 ± 0.08*	1.02 ± 0.07*
Intoxicated with $Pb(NO_3)_2$	2.16 ± 0.05*	1.01 ± 0.05*

Note: * P < 0.05 compared with intact rats.

After intoxication of rats with heavy metal ions, the content of diene conjugates in blood and liver tissues increases. Thus, the content of diene conjugates in blood increased by 16% after copper sulfate intoxication, 18% – zinc sulfate, 24% – cadmium sulfate, 26% – lead nitrate, compared with the control group.

The body's antioxidant defense system controls and inhibits all stages of free radical reactions, from their initiation to the formation of hydroperoxides and malondialdehyde.

Studies of the activity of superoxide dismutase and catalase are shown in Table 3.

Therefore, intoxication with heavy metal ions leads to a decrease in the activity of SOD and CAT in the studied tissues of rats, especially in the case of intoxication with cadmium and lead ions.

The study of the activity of glutathione-dependent enzymes in rat tissues is shown in Table 4.

In blood of rats under conditions of copper sulfate intoxication, the

2. The content of diene conjugates in rat tissues under conditions of heavy metal intoxication (M ± m, n = 8)

Rats	Diene conjugate content (E233/E218)	
	Blood	Liver
Intact	0.84 ± 0.04	0.97 ± 0.05
Intoxicated with $CuSO_4$	0.98 ± 0.07	1.02 ± 0.09
Intoxicated with $ZnSO_4$	0.99 ± 0.05	1.04 ± 0.07
Intoxicated with $CdSO_4$	1.04 ± 0.06*	1.10 ± 0.08*
Intoxicated with $Pb(NO_3)_2$	1.06 ± 0.09*	1.08 ± 0.04*

Note: * P < 0.05 compared with intact rats.

3. The activity of superoxide dismutase (SOD) and catalase (CAT) in rat tissues under conditions of heavy metal intoxication (M ± m, n = 8)

Rats	Blood		Liver	
	SOD (IU/mg of protein)	CAT (μmol/L min)	SOD (IU/mg of protein)	CAT (μmol/L min)
Intact	0.83 ± 0.05	11.2 ± 1.10	2.83 ± 0.32	0.18 ± 0.03
Intoxicated with $CuSO_4$	0.68 ± 0.02	10.1 ± 0.90	2.68 ± 0.17	0.12 ± 0.02*
Intoxicated with $ZnSO_4$	0.70 ± 0.04	10.7 ± 0.70	2.71 ± 0.15	0.14 ± 0.03*
Intoxicated with $CdSO_4$	0.60 ± 0.03*	8.5 ± 0.90*	1.37 ± 0.14*	0.09 ± 0.01*
Intoxicated with $Pb(NO_3)_2$	0.62 ± 0.05*	8.9 ± 0.80*	1.72 ± 0.19*	0.11 ± 0.01*

Note: * P < 0.05 compared with intact rats.

4. The activity of glutathione peroxidase (GP) and glutathione transferase (GT) and the content of reduced form of glutathione (GSH) in rat tissues under conditions of heavy metal intoxication (M ± m, n = 8)

Rats	Blood			Liver		
	GP (mmol/ min•L)	GT (mmol/ min•L)	GSH (mmol/L)	GP (μmol/ min•mg protein)	GT (μmol/ min • mg protein)	GSH (μmol/mg • protein)
Intact	0.273 ± 0.12	68.0 ± 4.71	0.379 ± 0.04	0.37 ± 0.02	0.48 ± 0.05	0.80 ± 0.04
Intoxicated with $CuSO_4$	0.214 ± 0.11*	35.7 ± 3.68*	0.294 ± 0.03*	0.34 ± 0.03	0.46 ± 0.07	0.67 ± 0.05
Intoxicated with $ZnSO_4$	0.211 ± 0.14*	34.1 ± 3.52*	0.276 ± 0.07*	0.36 ± 0.05	0.44 ± 0.03	0.62 ± 0.07
Intoxicated with $CdSO_4$	0.170 ± 0.09*	27.4 ± 2.90*	0.252 ± 0.02*	0.28 ± 0.04*	0.39 ± 0.02*	0.31 ± 0.03*
Intoxicated with $Pb(NO_3)_2$	0.181 ± 0.10*	29.7 ± 3.10*	0.263 ± 0.05*	0.30 ± 0.03*	0.41 ± 0.04*	0.39 ± 0.05*

Note: * P < 0.05 compared with intact rats.

following decreases: GP activity by 22%, GT activity by 47% and the content of reduced glutathione by 23%; zinc sulfate – GP activity by 23%, GT activity by 50% and the content of reduced glutathione by 27%; cadmium sulfate – GP activity by 38%, GT activity by 60% and the content of reduced glutathione by 34%; lead nitrate – GP activity by 34%, GT activity by 57% and the content of reduced glutathione by 31%, respectively, compared with the control group of animals.

Under conditions of intoxication with copper sulfate and zinc sulfate, the activity of GP and GT in the liver of rats changes slightly. The activity of GP and GT in the liver under exposure to cadmium ions is reduced by 25% and 19%, respectively, compared with the control. Under exposure to lead ions, the activity of GP and GT in the liver

5. Biochemical indexes of rat blood serum under conditions of heavy metal intoxication (M ± m, n = 8)

Indexes	Intact	Intoxicated rats			
		Cu	Zn	Cd	Pb
Alkaline phosphatase, U/L	291.2 ± 27.40	535.4 ± 85.21*	537.3 ± 86.11*	589.5 ± 87.16*	554.7 ± 86.94*
Alanine aminotransferase, U/L	78.4 ± 6.34	132.8 ± 10.73*	135.3 ± 11.12*	176.2 ± 12.31*	163.6 ± 12.11*
Aspartate aminotransferase, U/L	162.3 ± 14.87	253.6 ± 21.32*	254.9 ± 22.41*	338.7 ± 31.24*	285.4 ± 23.10*
γ-Glutamyl transpeptidase, U/L	24.7 ± 2.21	39.2 ± 3.91*	40.5 ± 4.17*	43.4 ± 4.85*	41.2 ± 4.38*
Lactate dehydrogenase, U/L	323.5 ± 32.12	650.3 ± 54.74*	653.4 ± 55.23*	724.6 ± 61.14*	692.1 ± 55.73*
Cholinesterase, U/L	34.3 ± 4.91	41.4 ± 5.13	40.1 ± 5.03	43.2 ± 5.94*	42.3 ± 5.18*
Total α-amylase, U/L	517.8 ± 89.84	746.3 ± 115.12*	749.2 ± 123.14*	809.3 ± 141.25*	792.1 ± 134.21*
Bilirubin:					
total, μmol/L	3.6 ± 0.21	3.8 ± 0.20	3.9 ± 0.23	4.9 ± 0.27*	4.7 ± 0.29*
direct, μmol/L	0.9 ± 0.01	1.0 ± 0.02	1.0 ± 0.03	1.2 ± 0.03*	1.2 ± 0.03*
Creatinine, μmol/L	0.9 ± 0.01	1.0 ± 0.02	1.0 ± 0.03	1.2 ± 0.03*	1.2 ± 0.03*
Urea, mmol/L	69.3 ± 6.12	102.4 ± 8.93*	104.1 ± 9.23*	118.5 ± 11.54*	112.6 ± 10.15*
Glucose, mmol/L	6.2 ± 0.90	11.4 ± 1.25*	11.1 ± 1.15*	12.9 ± 2.32*	12.3 ± 2.21*
Albumin, g/L	5.1 ± 0.70	6.7 ± 1.10*	7.1 ± 1.32*	8.3 ± 1.41*	8.1 ± 1.38*
Total protein, g/L	42.6 ± 3.31	33.8 ± 2.52	34.9 ± 2.73	31.4 ± 2.21*	32.1 ± 2.32*
Cholesterol mmol/L	74.7 ± 3.70	61.3 ± 2.43	62.1 ± 2.63	56.2 ± 2.12*	58.4 ± 2.21*
Triglycerides, mmol/L	1.2 ± 0.07	0.9 ± 0.02*	0.9 ± 0.03*	0.8 ± 0.01*	0.8 ± 0.02*
Chlorides, mmol/L	1.0 ± 0.02	0.5 ± 0.02*	0.5 ± 0.03*	0.4 ± 0.01*	0.4 ± 0.02*
Magnesium, mmol/L	86.3 ± 7.43	106.1 ± 9.72	105.7 ± 9.50	112.4 ± 10.92*	111.3 ± 10.23*
Inorganic phosphorus, mmol/L	1.7 ± 0.12	2.3 ± 0.21*	2.4 ± 0.24*	2.6 ± 0.28*	2.5 ± 0.25*
Calcium, mmol/L	2.5 ± 0.23	1.4 ± 0.10*	1.5 ± 0.12*	1.2 ± 0.09*	1.3 ± 0.10*
Sodium, mmol/L	1.9 ± 0.11	2.9 ± 0.20*	2.8 ± 0.25*	3.5 ± 0.37*	3.3 ± 0.45*
Potassium, mmol/L	144.2 ± 12.34	127.3 ± 11.81*	128.1 ± 11.90*	122.3 ± 11.15*	123.2 ± 11.75*
Alkaline phosphatase, U/L	5.3 ± 0.30	7.4 ± 0.60*	7.6 ± 0.71*	8.4 ± 0.90*	8.1 ± 0.84*

Note: * $P < 0.05$ compared with intact rats.

of rats decreased by 19% and 15%, respectively, compared with control animals.

It should be noted that the content of reduced form of glutathione in the liver of intoxicated rats decreased more intensively: $CuSO_4$ – by 17%, $ZnSO_4$ – by 23%, $CdSO_4$ – by 61%, $Pb(NO_3)_2$ – by 51%, compared with the control group of animals. This change, in our opinion, can be explained by the fact that glutathione is involved in the protective reactions of cellular organelles.

Studies have shown that the action of heavy metals reduces the activity of antioxidant system enzymes and the

concentration of reduced glutathione in blood of animals.

Biochemical analysis of rat blood under the influence of xenobiotics on metabolic processes in the body is shown in Table 5. The study of enzyme activity showed the activation of the latter under conditions of heavy metal intoxication in 1.5–2.0 times (depending on a xenobiotic), compared with control, which is an important sign of confirmation of pathological processes, taking into account the organ-specificity of enzymes.

A decrease in the de Ritis ratio was found in all groups of intoxicated rats, compared with the group of intact rats, which indicates inflammatory processes in the liver.

The increase in the activity of the studied enzymes is probably caused by the destruction of hepatocytes and the development of intrahepatic cholestasis, which causes their intensive entry into blood. In addition, the change in the activity of gamma-glutamyl transpeptidase in the serum is of important diagnostic value in the simultaneous defeat of the liver parenchyma and hepatobiliary tract and its activity is a sign of hepatotoxicity. The increase in the level of gamma-glutamyl transpeptidase also indicates the stimulation of the activity of microsomal enzymes.

Hypoproteinemia and a decrease in albumin content in the serum of rats under conditions of intoxication may indicate a decrease in the intensity of protein synthesis in hepatocytes.

Thus, the increase in serum activity of the studied enzymes may indicate damage to liver cells, and may also be a consequence of the mobilization of protective and compensatory mechanisms in the entry of xenobiotics into the body.

Any functional manifestation of a living organism is provided by the action of the corresponding enzyme systems, so the change in the activity of the corresponding enzymes correlates with other studied blood biochemical parameters. We found that in intoxicated rats of all experimental groups, levels of total and direct bilirubin, creatinine, urea, and glucose were increased compared with intact animals. However, a decrease in the content of albumin, total protein, cholesterol, and triglycerides was also found in all experimental groups, compared with intact rats.

According to the results of studies in intoxicated animals, compared with the intact group, there was a change in the cation-anion pool, in particular, a tendency to decrease in the content of sodium and inorganic phosphorus, and increase in chlorides, magnesium, calcium, and potassium. This change is probably due to a decrease in total protein and albumin levels, as well as an increase in organic acids.

The entry of heavy metals into a living organism leads to occurrence of oxidative stress, which triggers a set of interdependent pathological reactions that cause the activation of the pool and accumulation of TBARS, as shown in our previous works. Such data are consistent with the indicators of the thiol-disulfide balance system (Table 6) in the protein fraction of blood and liver in rats.

In blood of all experimental groups, the thiol-disulfide ratio decreased: in the second – by 1.5 times, in the third – by 1.7 times, in the fourth and fifth – almost by 3 times, compared with a group of intact rats. A decrease in thiol-disulfide ratio was also found in the liver of all experimental groups: in the second – by 1.8 times, in the third – by 2 times, in the fourth – almost by 3 times, and the fifth – by 2.3 times, compared

6. Thiol-disulfide balance of blood and liver of rats under conditions of heavy metal intoxication (M ± m, n = 8)

Indexes	Intact	Intoxicated rats			
		Cu	Zn	Cd	Pb
Blood, μmol/mL					
-SH-groups	14.36 ± 1.52	12.84 ± 1.42	12.61 ± 1.23	11.08 ± 1.0*	11.76 ± 1.21*
-SS-groups	4.53 ± 0.63	6.05 ± 0.82*	6.73 ± 0.87*	10.41 ± 0.7*	10.29 ± 0.67*
Thiol-disulfide ratio	3.17 ± 0.58	2.12 ± 0.32*	1.87 ± 0.28*	1.06 ± 0.17*	1.14 ± 0.19*
Liver, μmol/g					
-SH-groups	19.72 ± 1.94	15.24 ± 1.6*	14.76 ± 1.48*	12.87 ± 1.29*	13.73 ± 1.32*
-SS-groups	5.34 ± 0.78	7.45 ± 0.72*	7.94 ± 0.72*	9.68 ± 0.95*	8.79 ± 0.64*
Thiol-disulfide ratio	3.69 ± 0.62	2.04 ± 0.31*	1.86 ± 0.24*	1.33 ± 0.19*	1.56 ± 0.21*

Note: * $P < 0.05$ compared with intact rats.

with control. A decrease in the thiol-disulfide ratio indicates an increase in the concentration of free radicals and depletion of antioxidants' sedentary reserves of the body, which is a reflection of the dynamics of positive process under the negative action of xenobiotics.

Changes in the biochemical function of the liver are accompanied by signs of toxic hepatitis with hepatocellular insufficiency, as evidenced by increased activity of aminotransferases, lactate dehydrogenase, decreased cholesterol and triglycerides, increased glucose, and impaired excretory function of the liver – increase in the presence of gamma-glutamyl transpeptidase, alkaline phosphatase. Increased activity of cholinesterase, total α-amylase, and creatinine is a sign of renal failure, pathology of filtration in the glomeruli of the kidneys, and the occurrence of nephritis.

The content of total protein and albumin in the serum during intoxication with the studied heavy metals is reduced, which indicates damage to liver and kidney cells. An increase in urea content in the serum is a sign of increased protein catabolism, acute renal failure, as well as a shift in the relationship between the processes of urea formation and its excretion in the urine. It was found that in all groups of intoxicated animals, the content of inorganic sodium and phosphorus is reduced and chlorides, magnesium, calcium, and potassium is increased, which is a sign of nephritis and previously mentioned disorders of acid-base status (compensated acidosis).

Conclusions and future perspectives

This article summarizes the results obtained, indicating a violation of the prooxidant-antioxidant balance. It should be noted that the glutathione peroxidase system is universal in the decomposition of peroxides and prevents the initiation of secondary reactions of lipid oxidation and participates in the inactivation of products of oxidative metabolism of heavy metals in rats.

It was found that heavy metal intoxication of rats leads to the activation

of studied blood enzymes (alkaline phosphatase, alanine and aspartate aminotransferases, gamma-glutamyl transpeptidase, lactate dehydrogenase, cholinesterase, and total α-amylase) in comparison with the intact group.

In all experimental groups, the content of total and direct bilirubin, creatinine, urea, glucose, chloride, magnesium, calcium, and potassium was increased compared with intact animals.

A decrease in the content of albumin, total protein, cholesterol, triglycerides, sodium, and inorganic phosphorus was also found in all experimental groups, compared with intact rats.

Heavy metal intoxication in rats leads to a decrease in the content of -SH-groups and an increase in the content of -SS-groups in blood and liver of all experimental groups, as a consequence – a decrease in thiol-disulfide ratio indicating the strengthening of free radical processes oxidation, depletion of antioxidant reserves of the body, and confirmatory modifications of protein molecules.

In conclusion, oxidative stress is an important pathogenetic link for animals, and studies in this field may be important elements in the future to better understand and manage various diseases, including in humans.

References

Aggarwal, V., Tuli, H., Varol, A., Thakral, F., Yerer, M., Sak, K., … & Sethi, G. (2019). Role of reactive oxygen species in cancer progression: molecular mechanisms and recent advancements. Biomolecules, 9(11), 735. doi: 10.3390/biom9110735.

Antunes dos Santos, A., Ferrer, B., Marques Gonçalves, F., Tsatsakis, A. M., Renieri, E. A., Skalny, A. V., … & Aschner, M. (2018). Oxidative stress in methylmercury-induced cell toxicity. Toxics, 6(3), 47. doi: 10.3390/toxics6030047.

Engwa, G. A., Ferdinand, P. U., Nwalo, F. N., & Unachukwu, M. N. (2019). Mechanism and health effects of heavy metal toxicity in humans. In O. Karcioglu & B. Arslan (Eds.), Poisoning in the Modern World: New Tricks for an Old Dog? BoD–Books on Demand. doi: 10.5772/intechopen.82511.

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., & Sadeghi, M. (2021). Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. Frontiers in Pharmacology, 12, 643972. doi: 10.3389/fphar.2021.643972

Batáriová, A., Spěváčková, V., Beneš, B., Čejchanová, M., Šmíd, J., & Černá, M. (2006). Blood and urine levels of Pb, Cd and Hg in the general population of the Czech Republic and proposed reference values. International Journal of Hygieneand Environmental Health, 209(4), 359-366. doi: 10.1016/j.ijheh.2006.02.005.

Cao, Z. R., Cui, S. M., Lu, X. X., Chen, X. M., Yang, X., Cui, J. P., & Zhang, G. H. (2018). Effects of occupational cadmium exposure on workers' cardiovascular system. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi, 36(6), 474–477. doi: 10.3760/cma.j.issn.1001-9391.2018.06.025.

Carvalho Cdos, S., & Fernandes, M. N. (2008). Effect of copper on liver key enzymes of anaerobic glucose metabolism from freshwater tropical fish Prochilodus lineatus. Comparative Biochemistry & Physiology, 151, 437-442.

Djordjevic, V. R., Wallace, D. R., Schweitzer, A., Boricic, N., Knezevic, D., Matic, S., … & Buha, A. (2019). Environmental cadmium exposure and pancreatic cancer: evidence from case control, animal and in vitro studies. Environment International, 128, 353-361. doi: 10.1016/j.envint.2019.04.048.

Dongre, N. N., Suryakar, A. N., Patil, A. J., Ambekar, J. G., & Rathi, D. B. (2011). Biochemical effects of lead exposure on systolic &

diastolic blood pressure, heme biosynthesis and hematological parameters in automobile workers of north Karnataka (India). Indian Journal of Clinical Biochemistry, 26(4), 400-406. doi: 10.1007/s12291-011-0159-6.

Djuric, A., Begic, A., Gobeljic, B., Stanojevic, I., Ninkovic, M., Vojvodic, D., … & Djukic, M. (2015). Oxidative stress, bioelements and androgen status in testes of rats subacutely exposed to cadmium. Food and Chemical Toxicology, 86, 25-33.

Ellman, G. L. (1959). Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics, 82(1), 70-77.

El Yamani, N., Collins, A. R., Runden-Pran, E., Fjellsbo, L. M., Shaposhnikov, S., Zienolddiny, S., & Dusinska, M. (2017). In vitro genotoxicity testing of four reference metal nanomaterials, titanium dioxide, zinc oxide, cerium oxide and silver: Towards reliable hazard assessment. Mutagenesis, 32, 117-126.

Fay, M. J., Alt, L. A. C, Ryba, D., Salamah, R., Peach, R., Papaeliou, A., … & Prozialeck, W. C. (2018). Cadmium nephrotoxicity is associated with altered microRNA expression in the rat renal cortex. Toxics, 6(1), 16. doi: 10.3390/toxics6010016.

Gavrilov, V. B., Gavrilov, A. R., & Khmara, N. F. (1988). Measurement diene conjugates in plasma by UV absorption heptane and isopropanol extract. Laboratornoe case, 2, 60-63.

Havezov, I., & Tsalev, D. (1983). Atomic absorption analysis. Leningrad: Chemistry.

Husak, V. V., Mosiichuk, N. M., Kubrak, O. I., Matviishyn, T. M., Storey, J. M., Storey, K. B., & Lushchak, V. I. (2018). Acute exposure to copper induces variable intensity of oxidative stress in goldfish tissues. Fish Physiology and Biochemistry, 44, 841-852.

Kim, J.-J., Kim, Y.-S., & Kumar, V. (2019). Heavy metal toxicity: An update of chelating therapeutic strategies. Journal of Trace Elements in Medicine and Biology, 54, 226-231. doi: 10.1016/j.jtemb.2019.05.003.

Kim, T. H., Kim, J. H., Le Kim, M. D., Suh, W. D., Kim, J. E., Yeon, H. J., … & Jo, G. H. (2020). Exposure assessment and safe intake guidelines for heavy metals in consumed fishery products in the Republic of Korea. Environmental Science and Pollution Research, 27, 33042-33051. doi: 10.1007/s11356-020-09624-0.

Koroliuk, M. A. (1988). Method for determining activity of catalase in biological material, 2, 31-34.

Kucherenko, M. E., Babenyuk, Y. D., & Voytsitskyy, V. M. (2001). Modern methods of biochemical research. Kyiv: Fitosotsiotsentr.

Kulshrestha, A., Jarouliya, U., Prasad, G., Flora, S., & Bisen, P. S. (2014). Arsenic-induced abnormalities in glucose metabolism: Biochemical basis and potential therapeutic and nutritional interventions. World Journal of Translational Medicine, 3(2), 96-111. doi: 10.5528/wjtm.v3.i2.96.

Kumar, S, & Sharma, A. (2019). Cadmium toxicity: effects on human reproduction and fertility. Reviews on Environmental Health, 34, 327-338.

Lech, T., & Sadlik, J. K. (2017). Cadmium concentration in human autopsy tissues. Biological Trace Element Research, 179(2), 172-177. doi: 10.1007/s12011-017-0959-5.

Li, H., Fagerberg, B., Sallsten, G., Borné, Y., Hedblad, B., Engström, G., Barregard, L., & Andersson, E.M. (2019). Smoking-induced risk of future cardiovascular disease is partly mediated by cadmium in tobacco: Malmö Diet and Cancer Cohort Study. Environmental Health, 18(1), 56. doi: 10.1186/s12940-019-0495-1.

Luo, L., Wang, B., Jiang, J., Huang, Q., Yu, Z., Li, H., … & Chen, S. (2021). Heavy metal contaminations in herbal medicines: determination. comprehensive risk assessments. Frontiers in Pharmacology, 11, 595335. doi: 10.3389/fphar.2020.595335.

Mannervik, B. (1985). Glutathione peroxidase. Methods in enzymology, 113, 490-495.

Ohta, H., & Ohba, K. (2020). Involvement of metal transporters in the intestinal uptake of cadmium. Journal of Toxicological Sciences, 45(9), 539-548. doi: 10.2131/jts.45.539.

Oluranti, O. I., Adeyemo, V. A., Achile, E. O., Fatokun, B. P., & Ojo, A. O. (2021). Rutin improves cardiac and erythrocyte membrane-bound ATPase activities in male rats exposed to cadmium chloride and lead acetate. Biological Trace Element Research, 1-10. doi: 10.1007/s12011-021-02711-4.

Orehovych, V. N. (1977). Modern methods in biochemistry. Moscow: Medicyna.

Patra, R. C., Swarup, D., & Dwivedi, S. K. (2001). Antioxidant effects of α tocopherol, ascorbic acid and l-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. Toxicology, 162 (2), 81-88. doi: 10.1016/s0300-483x(01)00345-6.

Proshad, R., Zhang, D., Uddin, M., & Wu, Y. (2020). Presence of cadmium and lead in tobacco and soil with ecological and human health risks in Sichuan province, China. Environmental Science and Pollution Research, 27, 18355-18370. doi: 10.1007/s11356-020-08160-1.

Rani, A., Kumar, A., Lal, A., & Pant, M. (2014). Cellular mechanisms of cadmium-induced toxicity: a review. International Journal of Environmental Health Research, 24(4), 378-399. doi: 10.1080/09603123.2013.835032.

Saghazadeh, A., & Rezaei, N. (2017). Systematic review and meta-analysis links autism and toxic metals and highlights the impact of country development status: Higher blood and erythrocyte levels for mercury and lead, and higher hair antimony, cadmium, lead, and mercury. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 79, 340-368.

Saha, P., & Paul, B. (2019). Assessment of heavy metal toxicity related with human health risk in the surface water of an industrialized area by a novel technique. Human and Ecological Risk Assessment Journal, 25, 966-987. doi: 10.1080/10807039.2018.1458595.

Severyn, S. E., & Soloveva, G. A. (1989). Praktikum on biochemistry. Moscow: MGU.

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., … & Sharifi-Rad, J. (2020). Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Frontiers in Pharmacology, 11, 694. doi: 10.3389/fphys.2020.00694.

Singh, Y. P., Patel, R. N., Singh, Y., Butcher, R. J., Vishakarma, P. K., & Singh, R. K. B. (2017). Structure and antioxidant superoxide dismutase activity of copper(II)hydrazone complexes. Polyhedron, 122, 1-15. doi: 10.1016/j.poly.2016.11.013.

Stalnaya, Y. D., & Haryshvyly, T. G. (1977). Modern methods in biochemistry. Moscow: Medicyna.

Vlasova, S. N., Shabunyna, E. I., & Pereslehyna, A. I. (1990). Glutathione dependent activity of enzymes in red blood cells chronic disease liver in children. Laboratornoe case, 8, 19-21.

Wang, Y., Mandal, A. K., Son, Y.-O., Pratheeshkumar, P., Wise, J. T. F., Wang, L., … & Chen, Z. (2018). Roles of ROS, Nrf2, and autophagy in cadmium-carcinogenesis and its prevention by sulforaphane. Toxicology and Applied Pharmacology, 353, 23-30. doi: 10.1016/j.taap.2018.06.003.

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Анотація. *Дослідження проводилося для вивчення впливу важких металів на антиоксидантну систему та біохімічні показники крові в організмі щурів. Встановлено вплив важких металів на показники антиоксидантної системи (активність ферментів антиоксидантної системи – глутатіонпероксидази, глутатіонредуктази, каталази та супероксиддисмутази) та процеси перекисного окиснення ліпідів (вміст гідропероксидів та продуктів тіобарбітурової кислоти) у щурів. Встановлено, що за дії важких металів у крові та печінці щурів інтенсивніше функціонує антиоксидантної системи. Дослідження активності ферментів показало активацію останніх в умовах інтоксикації важкими металами в 1,5–2,0 рази (залежно від важкого металу), порівнюючи з контролем. Встановлено, що в інтоксикованих щурів усіх дослідних груп підвищений рівень загального та прямого білірубіну, креатиніну, сечовини, порівнюючи з інтактними тваринами. Однак у всіх дослідних групах, порівнюючи з інтактними щурами, також виявлено зниження вмісту альбуміну, загального білка, холестеролу та тригліцеридів. За дії важких металів у крові щурів підвищується активність загальної α-амілази, лактатдегідрогенази та концентрації глюкози. За результатами досліджень у інтоксикованих тварин, порівнюючи з інтактною групою, спостерігалася зміна катіонно-аніонного пулу, зокрема, тенденція до зниження вмісту натрію та неорганічного фосфору, та збільшення вмісту хлоридів, магнію, кальцію та калію.*

Ключові слова: *щури, кров, печінка, мідь, цинк, кадмій, свинець, антиоксидантна система*