

EFFECT OF PEPTIDES ON ANTIOXIDANT SYSTEM ENZYMES IN RATS WITH HEAVY METALS, PHOSPHOORGANIC PESTICIDES INTOXICATION

The aim of this work was to examine the effect of heavy metal ions and phosphororganic pesticides which contain glyphosate on activity antioxidative enzymes in the serum and liver of rats. The effect of peptide tsysteil-histidil-tyrosil-histidil-isoleucine on the state of antioxidant protection (superoxide dismutase, catalase) and lipid peroxidation was reached. The peptide exhibits antioxidant activity, the correction of the peptide increases antioxidant enzymes activity and concentration of glutathione.

KEY WORDS: **antioxidant system, chronic effect, rats, correction, the peptide.**

INTRODUCTION. Metallic elements are intrinsic components of the environment. Their presence is considered unique in the sense that it is difficult to remove them completely from the environment once they enter in it. Metal constitute an important class of toxic substance which are encountered in numerous occupational and environmental circumstances. The impact of these toxic agents on human health is currently an area of intense interest due to the ubiquity of exposure. With the increasing use of a wide verity of metals in industry and in our daily life, problems arising from toxic metal pollution of the environment have assumed serious dimensions [8, 9].

Some heavy metals have bio-importance as trace elements but the biotoxic effects of many of them in human biochemistry are of great concern. Hence, there is a need for proper understanding of mechanism involved, such as the concentrations and oxidation states, which make them harmful.

It is also important to know their sources, leaching processes, chemical conversions and their modes of deposition in polluting the environment, which essentially supports life. Literature sources point to the fact that these metals are released into the environment by both natural and anthropogenic means, especially mining and industrial activities, and automobile exhausts. They leach into the underground waters, moving along water pathways and eventually depositing in the aquifer,

or are washed away by run-off into surface waters thereby resulting in water and subsequently soil pollution. Poisoning and toxicity in ecosystem occur frequently through exchange and co-ordination mechanisms. When ingested, they form stable biotoxic compounds, thereby mutilating their structures and hindering bioreactions of their functions [11, 13].

Copper is a naturally-occurring metallic element. It is present in all animals and plants and is an essential nutrient for humans and animals in small amounts. The major sources of environmental copper releases include the mining, smelting and refining of copper, industries producing products from copper such as wire, pipes and sheet metal, and fossil fuel combustion. Water pipes are made of copper and bath fixtures may be made from brass and bronze alloys that contain copper. The principal source of copper in drinking water results from the leaching of copper from pipes and bath fixtures due to acidic water. Blue-green stains left in bath fixtures are a sign of the presence of copper in water. Other releases of copper to the environment include agricultural use against plant diseases and treatments applied to water bodies to eliminate algae [12, 14].

Copper is a component of several enzymes necessary for normal metabolic functions in humans. Effects of copper deficiency can include anemia, low numbers of white blood cells, osteoporosis in infants and children, and defects in con-

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nective tissue leading to skeletal problems. Effects of acute poisoning from ingestion of excessive copper can cause temporary gastrointestinal distress with symptoms such as nausea, vomiting, and abdominal pain. Liver toxicity was seen in doses high enough that resulted in death. High levels of exposure to copper can cause destruction of red blood cells, possibly resulting in anemia.

Mammals have efficient mechanisms to regulate copper stores in the body such that they are generally protected from excess dietary copper levels. However, at high enough levels, chronic overexposure to copper can damage the liver and kidneys.

For centuries, lead has been mined and used in industry and in household products. Modern industrialization, with the introduction of lead in mass-produced plumbing, solder used in food cans, paint, ceramic ware, and countless other products resulted in a marked rise in population exposures in the 20th century.

The dominant source of worldwide dispersion of lead into the environment for the past 50 years has clearly been the use of lead organic compounds as antiknock motor vehicle fuel additives. Since leaded gasoline was introduced in 1923, its combustion and resulting contamination of the atmosphere has increased background levels everywhere [9, 13].

Although a worldwide phase-out of leaded gasoline is in progress, it is still being used all over the world. The current annual worldwide production of lead is approximately 5.4 million tons and continues to rise. Sixty percent of lead is used for the manufacturing of batteries (automobile batteries, in particular), while the remainder is used in the production of pigments, glazes, solder, plastics, cable sheathing, ammunition, weights, gasoline additive, and a variety of other products.

Such industries continue to pose a significant risk to workers, as well as surrounding communities. In response to these risks, many developed countries over the last 25 years have implemented regulatory action that has effectively decreased actual exposures to the general population. However, exposures remain high or are increasing in many developing countries through a rapid increase in vehicles combusting leaded gasoline and polluting industries. Moreover, some segments of the population in developed countries remain at high risk of exposure because of the persistence of lead paint, lead plumbing, and lead-contaminated soil and dust, particularly in areas of old urban housing. Individuals will absorb more lead in their food if their diets are deficient in calcium, iron, or zinc. Other more unusual sources of lead exposure also continue to be sporadically found, such as improperly

glazed ceramics, lead crystal, imported candies, certain herbal folk remedies, and vinyl plastic toys [11, 14].

The general body of literature on lead toxicity indicates that, depending on the dose, lead exposure in children and adults can cause a wide spectrum of health problems, ranging from convulsions, coma, renal failure, and death at the high end to subtle effects on metabolism and intelligence at the low end of exposures.

Stimulation of generation of reactive oxygen species (ROS) plays a very important role in the mechanisms of the toxic effect of Pb^{2+} . ROS stimulate the activation of lipid peroxidation of the biomolecules [8].

The main aim of the present study was to investigate the effect of Pb^{2+} and Cu^{2+} upon antioxidant enzymes activity of rats after adding a lead acetate.

METHODS OF RESEARCH. We used lab nonlinear white rats – males of three age periods: puberty (youth, 70–90 g body weight and 2 to 3 months age); mature (average, weighing 170–210 g and 5–8 months age.) and aged rats processes in which catabolic processes prevail over anabolic (body weight 250–300 g and 20–24 months age) animals to study the combined affected of lead acetate, copper sulfate, glyphosate and correction for peptide. Age of rats was determined by the scheme V. I. Makhinko and V. N. Nikitin [6].

Subchronic lesions in rats was modelled by intragastric administration of water solution of Lead Acetate at a dose of 11 mg/kg (1/20 LD50), Copper Sulfate at a dose of 13 mg/kg (1/20 LD50), Glyphosate (in herbicide Roundup) at a dose of 250 mg/kg (1/20 LD50). Toxicants were administered in combination. Dechlorinated drinking tap water to intact animals was added. The correction effect was research by the during 10 days intragastric administration of water solution of peptide tsysteil-histidil-tyrosil-histidil-isoleucine in a dose of 9 mg/kg body weight (concentration of amino acids in the blood) from 20th day of the experiment after 6 hours after toxins. The peptides were synthesized at the Department of supramolecular chemistry and biochemistry Institute of High Technology of Taras Shevchenko Kyiv National University.

After acclimatization to the laboratory conditions, the animals were randomly divided into groups (ten rats each) placed in individual cages and classified as follow:

Group I (control normal group): Rats received no xenobiotics served as control nontreated for all experimental groups.

Group II (Combination exposed group): Rats received Lead-acetate (1/20 of LD50 (11 mg/kg

body weight), Copper-sulfate (1/20 LD50) (13 mg/kg body weight), Glyphosate (in herbicide Roundup) (1/20 LD50) (250 mg/kg body weight) orally and once per day over a period of 30 days.

Group III (Combination exposed with peptide correlation group): Rats received water solution of peptide tsysteil-histidil-tyrosil-histidil-isoleucine (concentration of amino acids in the blood) (9 mg/kg body weight) after 6 hours toxins exposed at 20th day of the experiment orally and once per day over a period of 10 days.

Euthanasia of rats was performed by bloodletting under the conditions of sodium thiopental anaesthesia on the 31st day after heavy metals and glyphosate exposed, and peptide correction.

We determined the activity of superoxidodismutase (SOD. 1.15.1.1) by method [2]; catalase (CAT, CE 1.11.1.9) at [4], glutathione (GSH) [1], glutathione peroxidase (EC 1.11.1.9, GSH-Px) [7] and glutathione reductase (GSH-Rx) [10].

All rats stayed on vivarium with stable temperature and humidity condition while the whole experiment [3].

Statistical analysis was done using Statsoft STATISTICA by the Department of Virtual Educational Programs and Videos of I. Horbachevsky Ternopil State Medical University. The results were expressed as mean (M)±m and statistical significance was evaluated by one way ANOVA using SPSS (version 10.0) program followed by the post hoc test, least significant difference (LSD). Values were considered statistically significant when p<0.05 [5].

RESULTS AND DISCUSSION. Lipid peroxides in cells have been implicated as one of the principal

factors causing age-related damage to cells. Serum concentrations of glutation, as well as activities of the superoxide (COD), catalase, glutathione peroxidase and glutathione reductase in the homogenized liver and serum were measured.

Concentrations of glutation and activity of antioxidation enzymes decreased in the blood serum and liver, after the administration of Pb acetate, copper sulfate, glyphosate. Superoxide dismutases are metalloenzymes capable of scavenging the free oxygen radical superoxide. In the rats co-exposed to xenobiotics in the blood and the homogenates of the liver activity of SOD were lower compared to the control group rats.

Thus, SOD activity decrease significant in blood plasma and liver homogenates of all age groups animals under the influence of xenobiotics (Table 1). There were recorded the maximum change of this index in blood by the combined action of toxicants and it was been at 3-month rats – 39.9 %, 6-month – 62.2 % and 18-month – 49.1 %, compared with intact animals. Similar changes of SOD activity in the liver, and it was in the 3-month animal – 48.1 %, 6-month – 66.6 %, 18-month – 50.2 %, compared with the control. Such a decreasing SOD activity decreased can explained that lead ions, excessive concentration of copper ions and glyphosate activate LPO processes and produced hydrogen peroxide formation, which is inhibitor of the enzyme. Spontaneous dismutation of superoxide radical reaction leads to the formation of H₂O₂ at the low SOD activity, which cells CAT decomposed. However, enzyme activity of catalase decreased in the blood and liver in animals with chemical liver affection.

Table 1 – Activity of superoxide dismutase (S.U./g protein) and catalase (mkkat/g protein blood, mkat/g protein liver) in the blood and liver of animals infected by lead acetate, copper sulfate, glyphosate and administrated of the peptide as correction factor (M±m, n=10)

Index		Animal Groups		
		Control	Combined lesion	Lesion and peptide
Puberty				
SOD	blood, ×10 ³	0.138±0.004	0.055±0.003*	0.130±0.004**
	liver, ×10 ⁴	0.586±0.018	0.302±0.020*	0.536±0.019**
CAT	blood	4.09±0.10	2.38±0.10*	3.89±0.11**
	liver	0.318±0.089	0.189±0.007*	0.288±0.011**
Mature				
SOD	blood, ×10 ³	0.116±0.005	0.071±0.003*	0.110±0.003**
	liver, ×10 ⁴	0.518±0.019	0.345±0.011*	0.508±0.019**
CAT	Blood	3.23±0.07	2.57±0.08*	3.15±0.08**
	Liver	0.254±0.010	0.168±0.006*	0.238±0.0089**
Old				
SOD	blood, ×10 ³	0.110±0.004	0.054±0.003*	0.105±0.004**
	liver, ×10 ⁴	0.454±0.016	0.228±0.010*	0.435±0.016**
CAT	blood	2.93±0.06	1.57±0.06*	2.85±0.08**
	liver	0.221±0.004	0.111±0.004*	0.215±0.006**

Note. Here, in Table 2 and Table 3: * – significant results relatively intact animals (p<0.05); ** – Significant results regarding performance in rats combined lesions (p<0.05).

It was observed the maximum decrease activity of this enzyme in old animals by the combined action of copper sulfate, lead acetate and glyphosate. It was higher on 53.6 % and 50.2 % (compere with the controls) in blood plasma and liver homogenate respectively.

Concentration of glutathione also decreased in both these tissues. The levels of glutathione decreased by about 32.2 %, 31.5 % and 29.2 % in blood serum of three, six, twenty months old age rats respectively. In the rats co-exposed to xenobiotics in the blood activity of GSH-Px decreased on 54.4%, 61.5 % and 59.1 % in three, six, twenty months old months age rats respectively and the homogenates of the liver activity of this enzyme were lower on 50.3 %, 49.6 %, 53.1 % compared to the control group rats (Table 2, 3). Activity of GSH-Rx decreased also.

Decrease in glutation serum concentration and its liver content and an decrease activity of anti-oxidation systems enzyme in the serum and in the liver, were noted. The results of the study allow us

to hypothesize that heavy metals toxication with co-exposed of glyphosate are at enhanced risk of liver, kidney and other organs damage due to lipid peroxidation.

Comparison of the effect of the peptide on glutathione levels between the all groups' animals showed significantly increase concentration it on (Table 1–3). Concentration of glutathione and anti-oxidant enzymes activity increased in the blood serum and liver, after the administration of peptide to exposed rats.

The levels of glutathione increased by about 54 %, while GSH-Px, GSH-Rx, CAT and SOD activities by 102, 1 %, 97.3 %, 63.4 % and 136 % respectively in serum of 3 three months old rats, and in 2,1 time, 1,9 time, 1,8 time and 1,5 time in liver respectively.

So, the peptide tsysteil-histidil-tyrosil-histidil-isoleucine has antioxidation activity.

CONCLUSIONS. 1. 30-day intoxication by the copper sulfate, the lead acetate and the glyphosate

Table 2 – Contain of glutathione (GSH) (mmole/L), activity of glutathione peroxidase (GSH-Px) mkmole/(min·g protein)) and glutathione reductase (GSH-Rx) mkmole/(min·g protein)) in the blood of animals infected by lead acetate, copper sulfate, glyphosate and administrated of the peptide as correction factor (M±m, n=10)

Index	Animal Groups		
	Control	Combined lesion	Lesion and peptide
Puberty			
GSH, mmole/L	6.10±0.08	3.95±0.05*	6.09±0.07**
GSH-Rx, mkmole/(min·g protein)	17.8±0.7	9.5±0.4*	17.6±0.5**
GSH-Px, mkmole/(min·g protein)	46.9±0.8	25.5±1.2*	46.8±0.9**
Mature			
GSH, mmole/L	5.87±0.07	4.02±0.06*	5.85±0.08
GSH-Rx, mkmole/(min·g protein)	15.3±0.6	9.4±0.5*	14.8±0.4**
GSH-Px, mkmole/(min·g protein)	42.9±0.7	26.4±0.9*	42.6±0.8**
Old			
GSH, mmole/L	5.65±0.08	4.00±0.07*	5.58±0.07**
GSH-Rx, mkmole/(min·g protein)	12.9±0.5	6.5±0.5*	12.2±0.5**
GSH-Px, mkmole/(min·g protein)	35.7±0.7	21.1±0.9*	35.6±0.7**

Table 3 – Contain of glutathione (GSH) (mmole/L), activity of glutathione peroxidase (GSH-Px) mkmole/(min·g protein)) and glutathione reductase (GSH-Rx) mkmole/(min·g protein)) in the liver of animals infected by lead acetate, copper sulfate, glyphosate and administrated of the peptide as correction factor (M±m, n=10)

Index	Animal Groups		
	Control	Combined lesion	Lesion and peptide
Puberty			
GSH, mmole/L	5.64±0.07	3.63±0.07*	5.45±0.07**
GSH-Rx, mkmole/(min·g protein)	10.4±0.4	4.8±0.3*	10.1±0.5**
GSH-Px, mkmole/(min·g protein)	15.3±0.6	7.6±0.5*	15.0±0.5**
Mature			
GSH, mmole/L	5.22±0.09	4.23±0.08*	5.07±0.04**
GSH-Rx, mkmole/(min·g protein)	9.2±0.5	5.4±0.3*	9.0±0.4**
GSH-Px, mkmole/(min·g protein)	12.9±0.5	6.5±0.4*	12.7±0.5**
Old			
GSH, mmole/L	4.91±0.08	3.73±0.08*	4.84±0.08**
GSH-Rx, mkmole/(min·g protein)	7.8±0.4	4.2±0.2*	7.6±0.4**
GSH-Px, mkmole/(min·g protein)	9.8±0.6	4.6±0.3*	9.4±0.6**

form of Roundup in threshold doses (1/20 DL50) are inactivate antioxidant system. Concentrations of glutathione and activity of antioxidation enzymes decreased in the blood serum and liver, after the

administration of Pb acetate, copper sulfate, glyphosate.

2. The effect of peptide tsysteil-histidil-tyrosil-histidil-isoleucine on the state of antioxidant protection

REFERENCES

1. Горячковский А. М. Клиническая биохимия в лабораторной диагностике / А. М. Горячковский. – Одесса : Экология, 2005. – 607 с.
2. Камышников В. С. Справочник по клинико-биохимической лабораторной диагностике / В. С. Камышников. – Минск : Беларусь, 2002. – 1. – С. 546–447.
3. Кожем'якіна Ю. М. Науково-практичні рекомендації з утримання лабораторних тварин та роботи з ними / Ю. М. Кожем'якіна, О. С. Хромова, М. А. Філоненко. – К. : Авіцена, 2002. – 156 с.
4. Метод определения активности каталазы / М. А. Королюк, Л. И. Иванова, Н. Г. Майорова, В. Е. Токарев // Лаб. дело. – 1988. – № 1. – С. 16–19.
5. Лакин Г. Ф. Биометрия / под ред. Г. Ф. Лакина. – М. : Высш. школа, 1990. – 352 с.
6. Махинько В. И. Константы роста и функциональные периоды развития в постнатальной жизни белых крыс / В. И. Махинько, В. Н. Никитин // Молекулярные и физиологические механизмы возрастного развития. – К., 1975. – С. 308–326.
7. Прохорова М. И. Методы биохимических исследований (липидный и энергетический обмен) : учеб. пособ. / под ред. М. И. Прохоровой. – Л. : Изд-во Ленинград. ун-та, 1982. – 272 с.
8. Droge W. Free radicals in the physiological control of cell function / W. Droge // *Physiol. Rev.* – 2002 – **82**, № 47. – P. 201–208.
9. Ernst E. Heavy metals in traditional Indian remedies / E. Ernst // *Eur. J. Clin. Pharmacol.* – 2002. – **57**. – P. 891–906.
10. Howard S. A. The relative effectiveness of human plasma glutathione peroxidase as a catalyst for the reduction of hydroperoxides by glutathione / S. A. Howard, W. C. Hawkes // *Biol. Trace Element Res.* – 1998. – **61**. – P. 127–136.
11. Frei B. Content of antioxidants, preformed lipid hydroperoxides and cholesterol as predictors of the susceptibility of human LDL to metal ion-dependent and independent oxidation / B. Frei, J. M. Gaziano // *J. Lipid Res.* – 1993. – **34**. – P. 2135–2145.
12. Peroxiredoxin II is essential for sustaining life span of erythrocytes in mice / T. H. Lee, S. U. Kim, S. L. Yu [et al.] // *Blood.* – 2003. – **101**. – P. 5033–5038.
13. Morais S. Heavy metals and human health / S. Morais, F. G. Costa, M. L. Pereira // In: Oosthuizen J, editor. *Environmental health – emerging issues and practice.* – 2012. – P. 227–246.
14. Martin S. Human health effects of heavy metals / S. Martin, W. Griswold // *Environmental Science and Technology Briefs for Citizens.* – 2009. – **15**. – P. 1–6.

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ТЕРНОПІЛЬСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО

ВПЛИВ ПЕПТИДІВ НА АКТИВНІСТЬ ФЕРМЕНТІВ АНТИОКСИДАНТНОЇ СИСТЕМИ В ЩУРІВ З ІНТОКСИКАЦІЄЮ ВАЖКИМИ МЕТАЛАМИ, ФОСФОРОРГАНІЧНИМИ ПЕСТИЦИДАМИ

Резюме

У даній роботі вивчено вплив іонів важких металів та фосфорорганічних пестицидів, які містять гліфосат, на активність ферментів антиоксидантної системи в сироватці крові й печінці щурів. Досліджено вплив пептиду цистеїл-гістидил-тирозил-гістидил-ізолейцину на стан системи антиоксидантного за-

хисту. Виявлено, що пептид проявляє антиоксидантну активність, про що свідчить відновлення активності ферментів антиоксидантної системи та вмісту глутатіону.

КЛЮЧОВІ СЛОВА: антиоксидантна система, хронічний ефект, щури, корекція, пептид.

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ТЕРНОПОЛЬСКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ ИМЕНИ И. Я. ГОРБАЧЕВСКОГО

ВЛИЯНИЕ ПЕПТИДОВ НА АКТИВНОСТЬ ФЕРМЕНТОВ АНТИОКСИДАНТНОЙ СИСТЕМЫ У КРЫС С ИНТОКСИКАЦИЕЙ ТЯЖЕЛЫМИ МЕТАЛЛАМИ, ФОСФОРОРГАНИЧЕСКИМИ ПЕСТИЦИДАМИ

Резюме

В данной работе изучено влияние ионов тяжелых металлов и фосфорорганических пестицидов, содержащих глифосат, на активность ферментов антиоксидантной системы в сыворотке крови и печени крыс. Исследовано влияние пептида цистеил-гистидил-тирозил-гистидил-изолейцина на состояние системы антиоксидантной защиты. Выявлено, что пептид проявляет антиоксидантную активность, о чем свидетельствует восстановление активности ферментов антиоксидантной системы и содержания глутатиона.

КЛЮЧЕВЫЕ СЛОВА: антиоксидантная система, хронический эффект, крысы, коррекция, пептид.

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