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EXPERIMENTAL PARTICIPATION OF PHARMACOLOGICAL SUBSTANCES IN MECHANISMS OF LEAD ACETATE TOXICITY

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The aim of the work is to study pharmacological substances that play a role of eNOS expression regulators in the modification of lead intoxication effects in the experiment.

Materials and methods. In the experiment, linear male rats of the same age were used: intact and with lead intoxication (120 heads). The study design was the following: group 1 – control; group 2 – intoxication with a lead acetate solution; group 3 – intact + L-nitroarginine methyl ester; group 4 – lead acetate + L-nitroarginine methyl ester; group 5 – intact + L-arginine; group 6 – lead acetate + L-arginine. The research carried out the study state of the redox reactions, the content of nitric oxide (NOx) stable metabolites, a lipid profile, the level of NO-synthase (eNOS) expression in the vascular endothelium, the main processes of urination and the activity of Na^+/K^+-ATPase in the renal tissue layers, as well as in the liver. The results were subjected to statistical processing.

Results. Saturnism caused the oxidative stress development, a decrease in the NO_x content in blood plasma, a violation of the L-arginine for eNOS bioavailability, and an endothelial dysfunction. Indicators of the impaired renal function were a decrease in the glomerular filtration rate (GFR), the tubular reabsorption of water, sodium, and the Na^+/K^+-ATPase activity. The damage to hepatocytes was evidenced by changes in the activity of organ-specific enzymes in the blood and Na^+/K^+-ATPase. L-arginine exhibited antioxidant properties, increased the NO_x content and the level of eNOS expression. The eNOS L-nitroarginine methyl ester inhibitor showed the effects opposite to L-arginine.

Conclusion. Biochemical markers of damage to kidney and liver cells during saturnism are indicators of the oxidative stress, NO_x deficiency and hemodynamic disturbances in them. These mechanisms involved the following pharmacological substances: an eNOS inhibitor, L-nitroarginine methyl ester, which caused a decrease in the expression level of the enzyme, and an eNOS inducer, L-arginine, which increased this indicator severity. The lead toxicity mechanisms have been implicated in the impaired cholesterol metabolism, contributing to the L-arginine reduced availability for eNOS and the NO_x production. Therefore, the use of L-arginine can be recommended as a regulator of the oxidative stress and an NO-producing endothelial function in other pathologies.

Keywords: lead acetate; lipid peroxidation; antioxidant system; total nitric oxide metabolites; endothelial dysfunction; L-arginine; L-NAME; kidney function; cholesterol; hepatocytes

Abbreviations: MPC – maximum permissible concentration; LPO – lipid peroxidation; AOS – antioxidant system; NO – nitric oxide; NO_x – total nitric oxide metabolites; eNOS – endothelial NO synthase; iNOS – inducible NO synthase; IP – inorganic phosphorus; Na^+/K^+-ATPase – sodium-potassium adenosine triphosphatase; L-NAME – L-nitroarginine methyl ester; TNF-α – tumor necrosis factor-α; iL-1β – interleukin 1β; iL-10 – interleukin 10; MDA – malonic dialdehyde; SOD – superoxide dismutase; CP – ceruloplasmin; TC – total cholesterol; LDL – low density lipoproteins; HDL – high density lipoproteins; GFR – glomerular filtration rate; ADMA – asymmetric dimethylarginine; ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGTP – gamma-glutamyl transpeptidase; AORs – active oxygen radicals; AOMs – active oxygen metabolites; FRO – free radical oxidation.

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УЧАСТИЕ ФАРМАКОЛОГИЧЕСКИХ ВЕЩЕСТВ В МЕХАНИЗМАХ ТОКСИЧНОСТИ АЦЕТАТА СВИНЦА В ЭКСПЕРИМЕНТЕ

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Цель. Изучение фармакологических веществ, играющих роль регуляторов экспрессии eNOS, в модификации эффектов свинцовой интоксикации в эксперименте.

Материал и методы. В эксперименте были использованы линейные крысы-самцы одного возраста: интактные и со свинцовой интоксикацией (120 голов). Дизайн исследования: группа 1– контроль; группа 2 – интоксикация раствором ацетата свинца; группа 3 – интактные + L-нитроаргинин метиловый эфир; группа 4 – ацетат свинца + L-нитроаргинин метиловый эфир; группа 5 – интактные + L-аргинин; группа 6 – ацетат свинца + L-аргинин. В исследовании проводилось изучение состояния окислительно-восстановительных реакций, содержания стабильных метаболитов оксида азота (NO_x), липидного профиля, уровня экспрессии NO-синтазы (eNOS) в эндотелии сосудов, основных процессов мочеобразования и активности Na^+/K^+-АТФ-азы слоёв почечной ткани, а также в печени. Результаты подвергались статистической обработке.

Результаты. Сатурнизм вызвал развитие окислительного стресса, снижение содержания NO_x в плазме крови, нарушение биодоступности L-аргинина для eNOS и дисфункцию эндотелия. Показателями нарушения функции почек были снижение скорости клубочковой фильтрации (СКФ), канальцевой реабсорбции воды, натрия и активности Na^+/K^+-АТФ-азы. О повреждении гепатоцитов свидетельствовало изменение активности органоспецифических ферментов в крови и Na^+/K^+-АТФ-азы. L-аргинин проявлял антиоксидантные свойства, повышал содержание NO_x и уровень экспрессии eNOS. Ингибитор eNOS – L-нитроаргинин метиловый эфир показал противоположные L-аргинину эффекты.

Заключение. Биохимическими маркерами повреждения клеток почек и печени при сатурнизме являются показатели окислительного стресса, дефицит NO_x и нарушение гемодинамики в них. В этих механизмах участвовали фармакологические вещества: ингибитор eNOS – L-нитроаргинин метиловый эфир, вызывавший снижение уровня экспрессии энзима, и индуктор eNOS – L-аргинин, повышавший степень выраженности этого показателя. В механизмах токсичности свинца участвовало нарушение обмена холестерина, способствующее сниженной доступности L-аргинина для eNOS и продукции NO_x. Следовательно, применение L-аргинина можно рекомендовать как регулятора окислительного стресса и NO-продуцирующей функции эндотелия при других патологиях.

Ключевые слова: ацетат свинца; перекисное окисление липидов; антиокислительная система; суммарные метаболиты оксида азота; дисфункция эндотелия; L-аргинин; L-NAME; функция почек; холестерин; гепатоциты

Список сокращений: ПДК – предельно допустимая концентрация; ПОЛ – перекисное окисление липидов; АОС – антиокислительная система; NO – оксид азота; NO_x – суммарные метаболиты оксида азота; eNOS – эндотелиальная NO-синтаза; iNOS – индуцибельная NO-синтаза; Рн – неорганический фосфор; Na^+/K^+-АТФ-аза – натрий-калиевая аденозинтрифосфатаза; L-NAME – L-нитроаргинин метиловый эфир; TNF-α – фактор некроза опухоли-α; iL-1β – интерлейкин 1β; iL-10 – интерлейкин 10; МДА – малоновый диальдегид; СОД – супероксиддисмутаза; ЦП – церулоплазмин; ОХС – общий холестерин; ЛПНП – липопротеины низкой плотности; ЛПВП – липопротеины высокой плотности; СКФ – скорость клубочковой фильтрации; АДМА – ассиметричный диметиларгинин; АЛТ – аланинаминотрансфераза; АСТ – аспартатаминотрансфераза; ГГТП – гамма-глутамилтранспептидаза; АРК – активные радикалы кислорода; АМК – активные метаболиты кислорода; СРО – свободно-радикальное окисление.

INTRODUCTION

Experimental and clinical studies conducted in recent years have shown the negative role of ecopathogenic factors and their participation in the development of vascular complications, an endothelial dysfunction and pathology of internal organs – kidneys and liver. In this aspect, heavy metals, which quite often exceed the maximum permissible concentration (MPC) in the environment, make their negative contribution. Scientists are also interested in the effect of lead on metabolism and the function of internal organs. When evaluating the toxicity of lead for the body, one should take into account its persistence and cumulative capacity for biological media in humans and animals [1–3]. Lead ions at low levels, which were previously considered safe, cause toxic effects [4–6]. Being a polytropic poison,

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lead is capable of disrupting the structure and function of the internal organs' cells. Lead nephropathy and hepatopathy are an integral part of the toxic effect; almost all elements of the nephron and hepatocyte are damaged [7, 8].

By changing the heme structure of blood hemoglobin, lead is the cause of anemia, hypoxia of the organ cells and the activation of lipid peroxidation (LPO) in erythrocytes and tissues [9-14]. Being a necessary component of body systems under physiological conditions, free radical oxidation (FRO) can be a factor in the development of a pathological process. However, it should be noted that there are very few published data that indicate the ability of lead to activate the lipid peroxidation (LPO) process in the blood and cells of the internal organs. Moreover, the increased activity of free radical reactions (FRRs) can cause not only negative phenomena, but also play the role of a pathogenetic link in a number of pathological processes in various nosologies. This process can be interpreted as a multifunctional stress response of the body to toxic effects. Developing an oxidative stress inhibits the production of nitric oxide (NO), which acts as an intracellular messenger and is involved in the implementation of response reactions from the cells of organs and tissues [15]. NO is formed by the enzymatic oxidation of L-arginine with NO synthase (eNOS). LPO reactions cause changes in lipoproteins in biological membranes, while increasing their hydrophilicity, permeability and disruption of lipid-protein interactions, including the participation of enzymes.

A few pieces of information in the literature are devoted to the activity analysis of Na^+/K^+-ATPase erythrocytes during the lead intoxication in the metallurgical industry workers [16]. Experimental studies on rats have shown that a systematic exposure to lead acetate is accompanied by its accumulation in the nephron structures and the impairment of the functional kidneys state. The contact duration with lead and the amount of cumulated substance can lead to chronic nephropathy, characterized by the development of inflammation and apoptosis of kidney cells [17].

Pro-inflammatory cytokines are involved in the mechanisms of nephropathy development: tumor necrosis factor-α (TNF-α), interleukin 1β (iL-1β), etc., with an insufficient level of anti-inflammatory cytokines – iL-10 in the nephron. At the same time, it should be noted that complex studies on changes in redox reactions and their role in the disturbance of NO metabolism, the activity and expression of NO synthase, a kidney and liver function in case of systematic poisoning of the body with lead acetate are insufficiently represented in the available literature. There are no literature data on the pharmacological drugs that play a role of the eNOS expression regulators in the lead intoxication; on the participation of L-arginine and its modified derivative L-NAME (L-nitroarginine methyl ester) in these processes. That was the basis for this experimental study.

THE AIM of the work is to study pharmacological substances that play a role of eNOS expression regulators in the modification of lead intoxication effects in the experiment.

MATERIALS AND METHODS

The studies were carried out on linear male rats of the same age group (10–14 months), weighing 200–280 grams: intact – control (n=20) and with a chronic lead intoxication caused by a daily administration of a lead acetate solution at the dose of 5 mg/kg of an animal body weight subcutaneously for 30 days (n=60). Against the background of the lead intoxication, the intact animals (n=40) were administered pharmacological substances: L-arginine (10 mg/kg) and L-NAME (25 mg/kg). During the experiments, the animals obtained from the vivarium of the Institute of Institute of Biomedical Research – branch of Vladikavkaz Scientific Center of the Russian Academy of Sciences were on a standard diet with a free access to water, and a natural light regime.

The experimental animals were divided into the following series: the 1st control group (n=20) – the studies on intact rats; the 2nd experimental group (n=20) with a systematic intoxication caused by a subcutaneous injection of a lead acetate solution (5 mg/kg of an animal body weight) for 30 days; the 3rd group (n=20) – intact rats + modified L-arginine (L-NAME); group 4 (n=20) – the rats intoxicated with lead acetate + L-NAME; group 5 (n=20) – the intact rats with the L-arginine administration; group 6 (n=20) – the rats intoxicated with lead acetate + L-arginine. All series of the experiments had a duration of 30 days. The experiments were carried out in accordance with the requirements for the work using animals in the experiment. The compliance with the international requirements for working with experimental animals, including humane treatment of them, is confirmed by the decision of the Institute of Biomedical Research – branch of Vladikavkaz Scientific Center of the RAS Ethics Committee (December 26, 2018, Protocol No. 6). The eNOS expression regulators, L-arginine (Dia-m LLC, Ajinomoto, Japan) and L-NAME (Etalon LLC, Cat. No. 5757 Sigma-Aldrich, USA) were used.

At the end of the experiments, the chest in intact and experimental rats was opened up under roush anesthesia, the blood was taken with sodium citrate anticoagulant from the left ventricle of the heart through a catheter, centrifuged in a laboratory centrifuge TsLMN-P10-01-ELEKON, ELEKON-M, Russia, for 10 min at 1500 rpm, the blood plasma was collected. The erythrocyte mass was washed twice with saline, then a certain volume was lysed. At the same time, the samples of kidney and liver tissues were taken, homogenized at +4°C and homogenates were obtained. The effectiveness of modeling was assessed by the content of lead in the blood and the development of intoxication syndrome by the intensity of the LPO-AOS system. The state of the antioxidant system (AOS) of the body was assessed by

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the activity of its enzymes – catalase in blood plasma spectrophotometrically according to the method of M.A. Korolyuk (1988) [19], and superoxide dismutase (SOD) – by the adrenaline oxidation method in erythrocyte hemolysate (Sirota TV, 1999) [20], the concentration of ceruloplasmin (CP) – by Ravin's method [21]. Cholesterol metabolism (CM) was determined according to the total cholesterol (TC), LDL-C, HDL-C and TAG in the blood plasma using kits (Vital Development Corporation, Russia). The degree of peroxidation was determined according to the change in the concentration of the end product of lipid peroxidation – malonic dialdehyde (MDA) in the hemolysate of erythrocytes, in the homogenates of the renal and hepatic tissues by the colorimetric method with thiobarbituric acid according to the method of Asacawa T. [18]. The state of the antioxidant system (AOS) of the body was assessed by the activity of its enzymes – catalases in blood plasma spectrophotometrically according to the method of M.A. Korolyuk (1988) [19] and superoxide dismutase (SOD) – by the method of adrenaline oxidation in erythrocyte hemolysate (Sirota TV, 1999) [20], the concentration of ceruloplasmin (CP) – by Ravin's method [21]. Cholesterol metabolism (CH) was determined according to the total cholesterol (TC), LDL-C, HDL-C and TAG in blood plasma using kits (Vital Development Corporation, Russia).

At the same time, the activity of enzymes – alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGTP) and alkaline phosphatase in blood plasma – was studied using the kits of the "Vital" company. For the analysis and determination of stable nitric oxide (NO_x) metabolites in the blood plasma, the method developed by V.A. Metelskaya was used. [22].

The study of the expression level of endothelial NO-synthase (eNOS) was carried out by Western blotting in the aorta endothelium in experimental animals, including the samples treatment in liquid nitrogen. The resulting content was placed in a centrifuge tube, washed with phosphate buffer 3 times, and the precipitate was centrifuged for 10 min at 1000 g. The precipitate obtained was subjected to the action of a lysis buffer in the volume of 100 µl. To determine the effect of the used pharmacological preparations, a comparative analysis of the eNOS expression level was used in comparison with control marker proteins. The data were expressed in arbitrary units, calculating the ratio of the studied band X in relation to the control data on each film. The determination of this indicator was carried out jointly with the biochemical laboratory of the National Medical Research Center for Therapy and Preventive Medicine. The toxicity of lead acetate in the experimental animals was determined according to the content of this heavy metal in the animals' blood. The metal was extracted with concentrated nitric acid, after which the lead content was determined spectrophotometrically on a Hewlett-Packard ICH-VSHP 4500 mass spectrophotometer (USA).

To study the functional nephron state, the conditions of spontaneous diuresis were used, the main processes of urine formation were evaluated: glomerular filtration rate (GFR) and tubular reabsorption of sodium (R_{Na}%) and water (R_{H2O}%), filtration charges of potassium and sodium. The value of glomerular ultrafiltration was determined by the clearance method by the ratio of urinary creatinine to its content in blood plasma, taking into account hourly diuresis. All kidney function indicators were calculated according to the formulas developed by Academician Natochin Yu.V. [23]. The activity of Na^+/K^+-ATPase in the homogenates of the cortical and medulla of the kidneys, as well as the liver, was determined by the method of Scow J.C. (1957). A specific activity was calculated per mg of protein per hour (µmolRn/mg protein/h). The protein in the samples was determined by the Lowry O.H. method. (1951) [24].

The results were statistically processed using the Microsoft Excel 2006 program and the Statistica 6.0 Package. The data obtained are characterized by a normal distribution in accordance with the Shapiro-Wilk test, and a parametric statistical method was used in their processing. The data were presented as mean (M) and error of mean (±m). The significance of differences was determined by Student's t-test, the statistical significance of differences between the groups was checked, and $p<0.05$ was considered the level of the statistical significance. Correlation coefficients were determined according to Pearson.

RESULTS AND DISCUSSION

Getting into the body of animals and humans, lead inhibits the synthesis of hemoglobin and causes the development of anemia, followed by tissue hypoxia. Violation of the oxygen transport blood function contributes to the formation of reactive oxygen species (ROSs) and activation of the LPO process. Being necessarily present in cell membranes FRO is an important factor in the normal functioning of cells, providing renewal of cell membrane phospholipids and the regulation of metabolism. However, an increased CPP activity can be the cause of negative manifestations and pathological processes. Nevertheless, it should be noted that the literature provides insufficient information on the ability of lead to activate FRO in the blood and internal organs as a pathogenetic link in vascular complications, its relationship with other metabolic processes, in particular with the regulation of the NO content, NO synthase activity, interaction with enzyme systems and participation in these processes of eNOS expression regulators – L-arginine and L-NAME. Systematizing and analyzing the results obtained on the nature of changes in redox reactions during the lead intoxication, it should be noted that there is an activation of lipid peroxidation according to a significant increase in erythrocytes in the MDA concentration, in the cells of the renal and hepatic tissues (Table 1). The MDA concentration in the hemolysate of erythrocytes increases by an average

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of 33.3% ($p<0.02$), in the cortical and medulla of the kidneys with a parenteral administration of the heavy metal – by 74.2% ($p<0.001$) and 25.4% ($p<0.001$), respectively, as well as in hepatocyte – by 94.1% ($p<0.001$).

In the interconnection with the FRO processes is the AOS of the organism, which limits lipid peroxidation in almost all of its links. The state of the AOS was judged by the activity of superoxide dismutase (SOD), catalase, and the concentration of ceruloplasmin (CP). The data showed an imbalance in the AOS – a decrease in the SOD activity and an increase in the level of catalase and CP (Table 1).

The analysis of the AOS activity data showed the SOD inhibition in erythrocytes by 62.2%, while the catalase activity and CP content in the blood serum increased by 69.5% and 27.6%, respectively. To understand the lack of unidirectionality in the change activity of AOS enzymes, the difference in their molecular structure is taken into account; while the catalase enzyme is more protected than SOD due to the presence of four heme molecules and 4 NADPH (nicotinamide adenine dinucleotide phosphate).

The intoxication syndrome development in the parenteral administration of lead acetate is characterized by a significant increase in the lead content in blood plasma, urine, kidney and liver tissues. A strong correlation was found out between the administered lead acetate dose and its concentration in the blood serum ($r=+0.95$; $p<0.001$). These results are consistent with the literature data [12]. Any xenobiotic, as a rule, is oxidized in the microsomal fraction of the hepatocyte, exerts its damaging effect and is excreted by the kidneys. In accordance with the data obtained, it was noted that during the lead intoxication, reactive oxygen radicals (RORs) induce the development of a systemic oxidative stress, which is accompanied by a decrease in the concentration of NO_x in the blood plasma (Table 1).

An analysis of the relationship between an increase in the MDA blood concentration in the NO_x level decrease showed a strong negative relationship between these indicators ($r=–0.69$; $p<0.001$). It should be notified that a disruption of the L-arginine-NO-synthase-NO signaling pathway plays a decisive role in the NO regulation of the vasodilator action. LPO products can disrupt the interaction between the oxygenase and reductase domains of NO synthase, as a result of which the enzyme begins to produce ROS and, accordingly, less NO is formed. In discussing these results, the causal relationship of reduced NO_x levels, the intensity of lipid peroxidation, the presence of the L-arginine substrate, its modified derivatives, the activity and the eNOS expression level should be notified. In this aspect, it should be assumed that in the development of L-arginine deficiency as an eNOS expression inducer, its use in the ornithine cycle might also play a role.

The literature data indicate an increased content of urea in the blood serum against the background of the lead intoxication, due to its production [25, 26]. Therefore, many researchers use the arginase enzyme inhibitors of the urea synthesis cycle to increase the concentration of the L-arginine substrate [27]. The reason for the L-arginine deficiency may be its conversion into asymmetric dimethylarginine (ADMA) and L-NAME, i.e., modified derivatives that play a competitive role with L-arginine and reduce its bioavailability for eNOS. In this regard, the effect of these amino acids on the content of LPO products, NOx, and eNOS expression was studied. A participation of the increased LPO activity, the MDA concentration in erythrocytes, the cells of the cortical and medulla of the kidneys and hepatocytes in the disruption of the NO_x production was shown in this study. Their relationship is confirmed by the presence of a negative correlation between these indicators. To establish the fact of the violation of the NO-producing endothelium function and the insufficient NO_x formation, the involvement of the expression level and the eNOS functional activity were studied. Another cause of the L-arginine deficiency is an increase in the modified L-arginine derivative level – ADMA and its analogue – L-NAME. The data obtained showed that the eNOS expression inhibitor caused the activation of the LPO process and, at the same time, an even more pronounced decrease in the NO_x level (Table 1). In contrast to this result, L-arginine showed its ability to increase the NO concentration during saturnism and at the same time provide a decrease in the LPO activity according to the MDA content (Table 2). The adaptive system under conditions of the reduced FRO activity under the influence of L-arginine showed an increase in the SOD activity and positive dynamics in relation to catalase and CP (Table 2).

In the regulation of the eNOS expression level and its activity, an important role is played by coenzymes, which lose their reduced state under an oxidative stress, as well as in the presence of an expression inhibitor – eNOS, an analogue of ADMA, L-NAME. To confirm this assumption, a study of the eNOS expression against the background of L-NAME during the lead acetate intoxication was undertaken. The data showed that in saturnism, L-NAME inhibited the eNOS expression by 23.9%, while L-arginine stimulated this expression by 29.05%. By normalizing redox reactions, L-arginine simultaneously increased the production and content of NO_x, herewith promoting vasodilation. The data obtained indicate the participation of L-NAME and L-arginine in the regulation of the expression level of the endothelial NO synthase. The results of the study confirm that the iNOS activation under the oxidative stress conditions is accompanied by a decrease in the eNOS expression level, which is responsible for the basic level of the NO_x production as the main vasodilator. The very fact of an increase in the level of the eNOS expression under the influence of the eNOS inducer L-arginine and a decrease in this indicator against the background of a modified amino acid derivative, L-NAME, gives priority to the results.

Table 1 – Influence of expression inhibitor eNOS – L-NAME on changes nature in indicators of oxidative stress and lipid metabolism during saturnism in the experiment

Indicators	Units of measurement	Control	Lead acetate	Intact + L-NAME	Lead acetate + L-NAME
MDA, erythrocytes	nmol/ml	4.74±0.16	6.32±0.015[a]	4.82±0.043[b]	6.59±0.03[b,c]
MDA, cortex	nmol/mg protein	3.18±0.22	5.54±0.02[a]	3.24±0.01[b]	5.67±0.02[b,c]
MDA, medulla	nmol/mg protein	4.25±0.059	5.33±0.009[a]	4.55±0.042[b]	5.48±0.015[b,c]
MDA, hepatocyte	nmol/mg protein	1.73±0.05	3.36±0.007[a]	1.77±0.07[b]	3.52±0.013[b,c]
SOD	c.u.	88.05±0.07	54.94±0.081[a]	82.1±1.67[b]	51.97±0.318[b,c]
Catalase	mkat/l	225.56±29.09	382.36±0.313[a]	285.6±4.63[b]	395.41±3.01[b,c]
CP	mg/l	339.14±6.59	432.29±1.14[a]	360.2±1.15[b]	448.6±3.18[b,c]
NO	µmol	50.95±0.65	29.38±0.029[a]	48.13±0.57[b]	28.33±0.32[b,cc]
TC	mmol/L	1.88±0.03	4.67±0.009[a]	2.08±0.01[b]	5.02±0.014[b,c]
LDL cholesterol	mmol/L	1.09±0.01	4.15±0.02[a]	1.11±0.01[b]	4.54±0.005[b,c]
HDL cholesterol	mmol/L	0.673±0.01	0.27±0.006[a]	0.65±0.03[b]	0.205±0.009[b,c]
TAG	mmol/L	0.246±0.011	0.55±0.009[a]	0.25±0.01[b]	0.61±0.007[b,c]

Note: MDA – malondialdehyde; SOD – superoxide dismutase; CP – ceruloplasmin; NO – nitric oxide; TC – total cholesterol; LDLs – low density lipoproteins; HDLs – high density lipoproteins; TAG – triacylglycerides; L-NAME – L-nitroarginine methyl ester; a – p<0.001 – significance of lead acetate relative to control; b – p<0.001, significance of lead acetate + L-NAME relative to intact + L-NAME; c – p<0.001 – significance of lead acetate + L-NAME relative to control; cc – p<0.01 – significance of lead acetate + L-NAME vs lead acetate.

Table 2 – Influence of eNOS expression inductor – L-arginine – on the nature of changes in oxidative stress and lipid metabolism in saturnism during the experiment

Indicators	Units of measurement	Control	Lead acetate	Intact + L-NAME	Lead acetate + L-NAME
MDA, erythrocytes	nmol/ml	4.74±0.16	6.32±0.015[a]	4.18±0.10[b]	6.05±0.1[b,cc]
MDA, cortex	nmol/mg protein	3.18±0.22	5.54±0.02[a]	2.8±0.05[b]	5.26±0.005[b,c]
MDA, medulla	nmol/mg protein	4.25±0.059	5.33±0.009[a]	3.9±0.06[b]	5.19±0.009[b,c]
MDA, hepatocyte	nmol/mg protein	1.73±0.05	3.36±0.007[a]	1.67±0.03[b]	3.22±0.013[b,c]
SOD	c.u.	88.05±0.07	54.94±0.081[a]	88.8±1.37[b]	57.18±0.38[b,c]
Catalase	mkat/l	225.56±29.09	382.36±0.313[a]	221.72±2.97[b]	370.17±3.12[b,c]
CP	mg/l	339.14±6.59	432.29±1.14[a]	336.4±6.39[b]	416.3±3.71[b,c]
NO	µmol	50.95±0.65	29.38±0.029[a]	53.25±0.08[b]	32.07±0.29[b,c]
TC	mmol/L	1.88±0.03	4.67±0.009[a]	1.84±0.02[b]	4.32±0.009[b,c]
LDL cholesterol	mmol/L	1.09±0.01	4.15±0.02[a]	1.03±0.03[b]	3.77±0.015[b,c]
HDL cholesterol	mmol/L	0.673±0.01	0.27±0.006[a]	0.69±0.03[b]	0.39±0.011[b,c]
TAG	mmol/L	0.246±0.011	0.55±0.009[a]	0.23±0.03[b]	0.49±0.007[b,c]

Note: MDA – malondialdehyde; SOD – superoxide dismutase; CP – ceruloplasmin; NO – nitric oxide; TC – total cholesterol; LDLs – low density lipoproteins; HDLs – high density lipoproteins; TAG – triacylglycerides; L-NAME – L-nitroarginine methyl ester; a – p<0.001 – significance of lead acetate relative to control; b – p <0.001, significance of lead acetate + L-NAME relative to intact + L-NAME; c – p<0.001 – significance of lead acetate + L-NAME relative to control; cc – p <0.01 – significance of lead acetate + L-NAME vs lead acetate.

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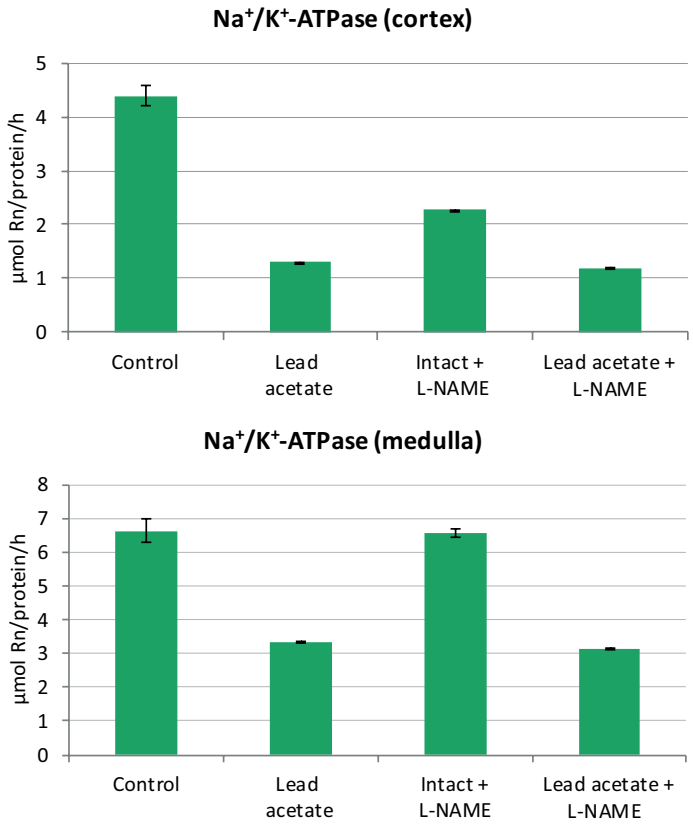

Figure 1 – Dynamics of changes in Na^+/K^+-ATPase activity during saturnism and inhibitor effect of eNOS expression – L-NAME

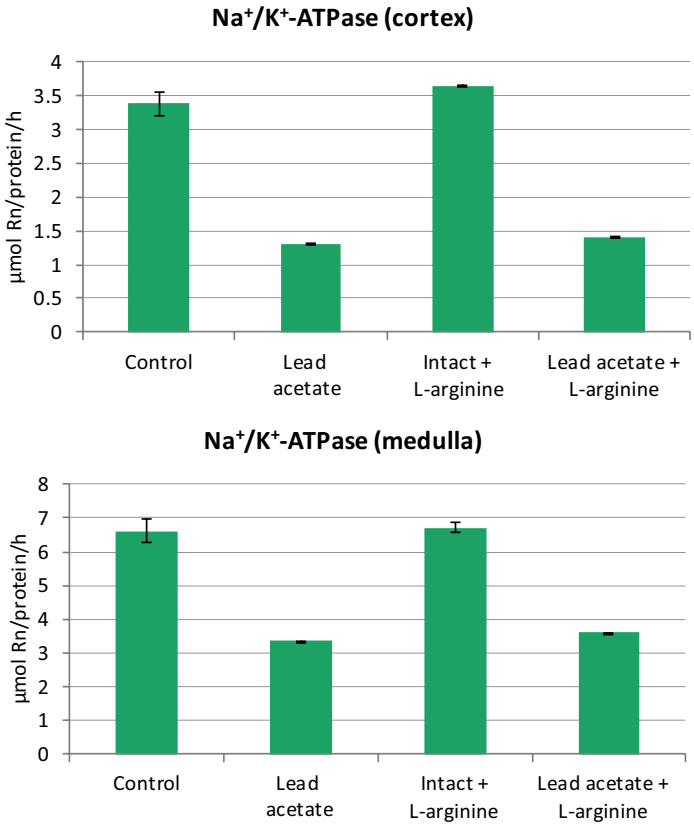

Figure 2 – Dynamics of changes in Na^+/K^+-ATPase activity during saturnism and influence of eNOS expression inducer – L-arginine

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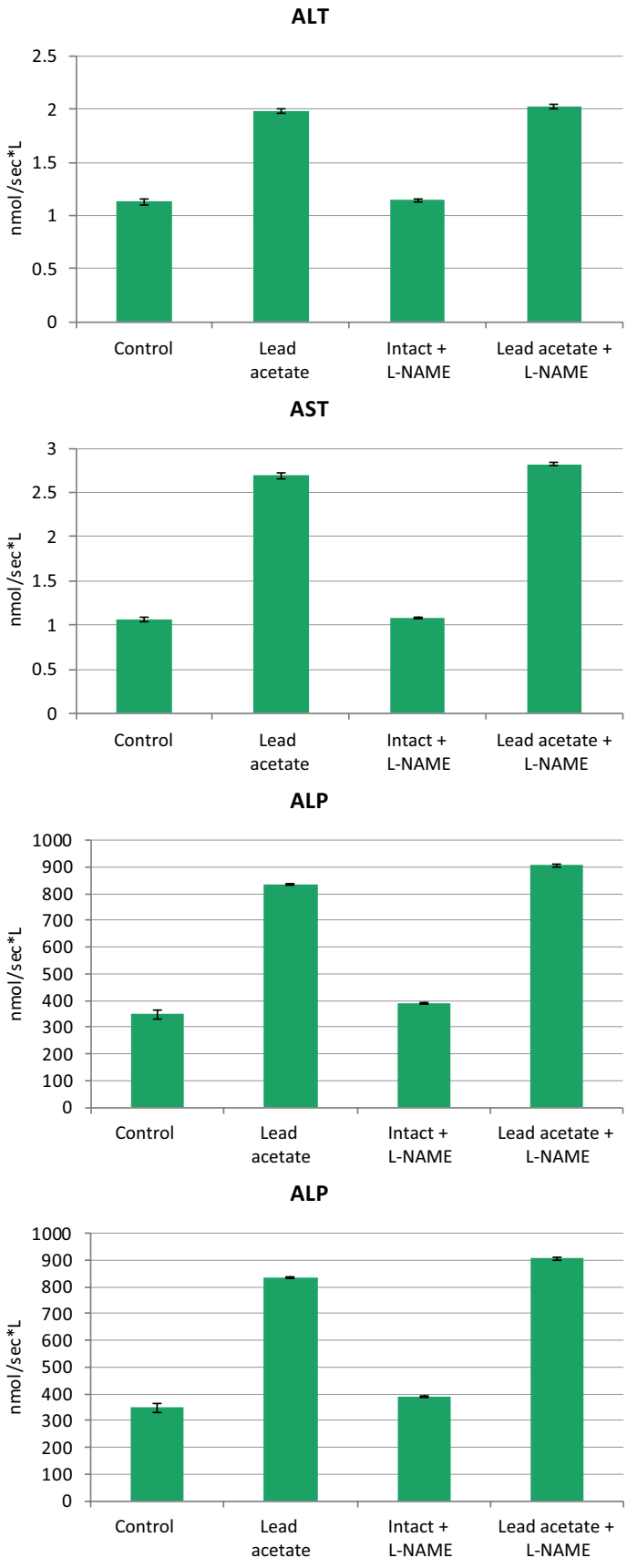

Figure 3 – Data on changes in the activity of organ-specific enzymes under the influence of an inhibitor of eNOS expression – L-NAME

Note: ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGTP – gamma-glutamyl transpeptidase; ALP – alkaline phosphatase.

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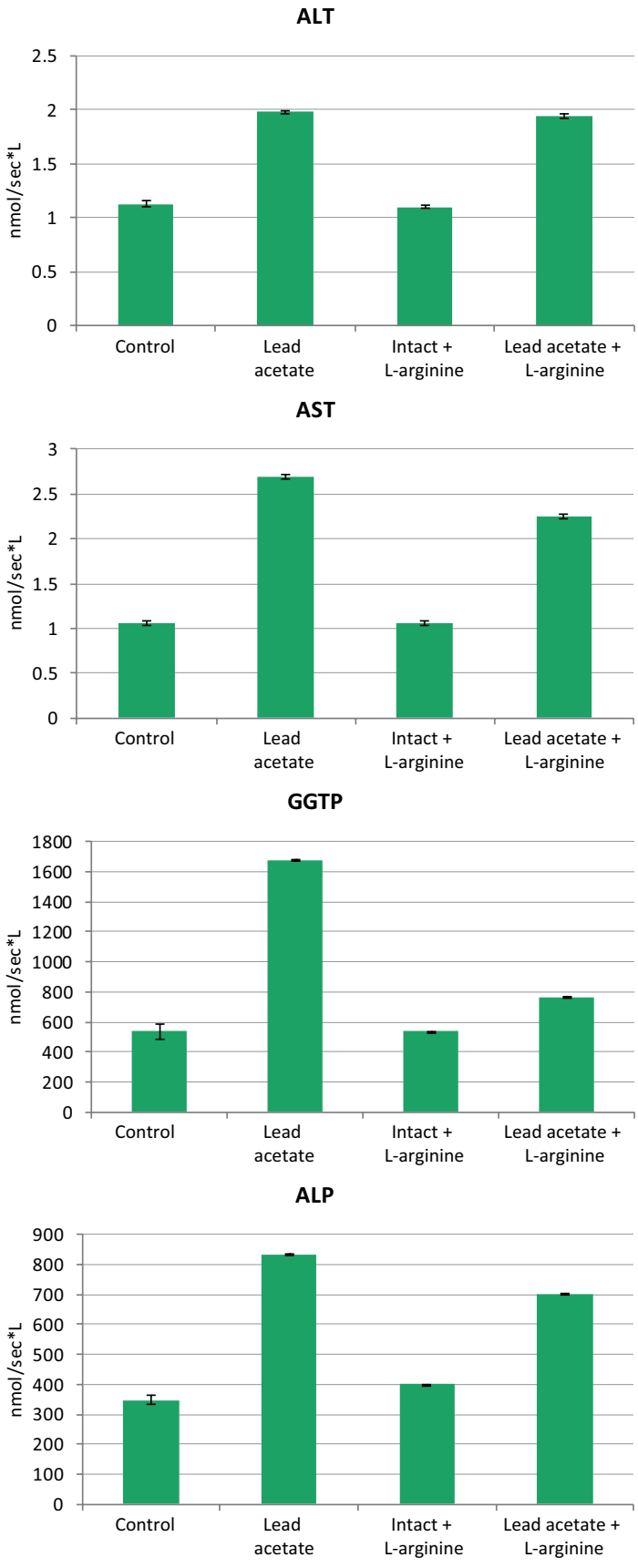

Figure 4 – Data on changes in the activity of organ-specific enzymes under the influence of the inducer of eNOS expression – L-arginine

Note: ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGTP – gamma-glutamyl transpeptidase; ALP – alkaline phosphatase.

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A concomitant role may be played by an impaired bioavailability of L-arginine to NO-synthase due to the inadequacy of the transport mechanism for the amino acid. In this regard, changes in cholesterol metabolism – hypercholesterolemia and hyper-β-lipoproteinemia – can contribute to the disruption availability of the L-arginine substrate. Our data on the lead intoxication showed an increase in the content of total cholesterol, LDL cholesterol in the blood serum and a decrease in HDL cholesterol (Tables 1, 2). Moreover, the results showed that under the oxidative stress conditions, the oxidative modification of LDL occurs, not only with the lipid component, but also with the β-apolipoprotein protein – apo B_{100}. The affinity of β-globulin for LDL receptors is impaired, their uptake by tissue cells is reduced, and the cholesterol content in the blood plasma is increased. Such changes in the metabolism of cholesterol lead to the interaction of lipoproteins with the receptors-"scavengers" of phagocytes, and their absorption. Lipids in the vascular endothelium accumulate; "foamy" cells – risk factors for atherogenesis – are formed. Under these conditions, the endothelium structure of the vascular wall changes, which leads to a disruption in the availability of L-arginine for NO synthase and a decrease in the production of NO_x as the main vasodilator. NOx deficiency is accompanied by hemodynamic changes, in particular in the nephron. A violation of the adequate interaction between the vascular tone of the afferent and efferent arterioles of the kidneys glomerulus causes a change in their functional state.

The analysis of the data obtained revealed a decrease in the GFR by 17.09% in the lead intoxication. At the same time, there was an increase in diuresis due to a significant decrease in the tubular reabsorption of water and sodium. Calculations of these indicators revealed that the levels of the tubular reabsorption of water and sodium are significantly reduced compared to the control by 0.26% and 3.01%, respectively.

To determine the cause-effect relations of sodium metabolism disorders in the nephron and a decrease in the level of the ion tubular reabsorption, the activity of the ATPase enzyme activated by Na and K was determined in the homogenates of the cortical and medulla of the renal tissue. The data showed the activity inhibition of Na^+/K^+-ATPase in both layers of the kidneys in the lead intoxication, as well as an even more significant change in the enzyme data when it was combined with L-NAME (Fig. 1). Conformational changes in the enzyme molecule as a result of the LPO process, which changes the molecular structure of phospholipids in renal tubular cells, were accompanied by a decrease in the ATPase activity (Fig. 1). The phospholipid structure violation of cell membranes is also confirmed by the literature data [16, 28].

Damage indicators of hepatocytes during the lead intoxication against the background of L-NAME were the data indicating an increase in the MDA content in them, a decrease in the function of Na^+/K^+-ATPase, as well as significant changes in the activity level of blood plasma enzymes: ALT, AST, GGTP and alkaline phosphatase (Fig. 1, 3). In contrast to these data, the administration of L-arginine to experimental animals caused the FRO suppression in the cells of the renal and hepatic tissues, the restoration of the phospholipid structure of the organs cell membranes, and an increase in the activity of Na^+/K^+-ATPase in them (Fig. 2). In blood plasma, under the influence of L-arginine, the data showed a decrease in the activity of organ-specific enzymes: ALT, AST, GGTP, and alkaline phosphatase (Fig. 4).

Therefore, biochemical markers of the hepatocyte membrane hydrophilicity and the increased permeability are the activity level of organ-specific enzymes in blood plasma, the modulation activity of Na^+/K^+-ATPase in liver cells.

Discussing these results in the lead intoxication, it was notified that lipid peroxidation contributes to the dysfunction of the endothelium and microcirculation, as well as nephropathy and hepatopathy. Comparing these biochemical data with the literature data, the activation of mitogen-activated protein kinase was notified, which triggers a certain sequence of reactions for the formation of pro-inflammatory proteins, an increase in the vascular tone and blood pressure [29].

An auxiliary role in the bioavailability disruption of L-arginine for eNOS is played by changes in cholesterol metabolism. The changes in cholesterol metabolism were accompanied by the development of pre-atherogenic changes in the vascular endothelium and were an obstacle to the availability of L-arginine to eNOS, which is also confirmed by the literature data [30].

Microcirculatory hemodynamic disorders in the nephron led to functional changes in the kidneys, as well as in the liver. Against the background of metabolic changes, functional disorders developed both in nephrons and in hepatocytes, including indicators of the main processes of urination, a decrease in the activity of the membrane enzyme Na^+/K^+-ATPase, a violation of cholesterol metabolism in the liver, and an increase in the activity of organ-specific enzymes in the blood. In these mechanisms, the regulatory role was played by pharmacological substances: the amino acid L-arginine and its modified derivative, L-NAME. Thus, the data obtained in the study with the lead intoxication on changes in the LPO-AOS system, the NO-producing endothelial function, including the level of the eNOS expression, as well as impaired renal and liver functions in one study, are priorities.

CONCLUSION

A direct correlation was found between the administered dose of lead acetate, the metal content in blood plasma, and the activity of lipid peroxidation in erythrocyte hemolysate. The POL activation is

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also notified in the homogenates of the cortical and medulla of the renal tissue, as well as in the liver, as evidenced by an increase in the concentration of the secondary product of the lipid peroxidation – MDA. A decrease in the AOS – SOD activity is responsible for the development of the oxidative stress. There is a direct positive correlation between the indicators characterizing the lead acetate dose, its content in the blood, organs and the intensity of the lipid peroxidation. The oxidative stress is accompanied by a decrease in the NOx content in blood plasma. Since eNOS is the main NO producer, the effect of the NO synthase expression level regulators was studied: the L-NAME inhibitor and L-arginine inducer. The data confirmed the participation of the NO-synthase inhibitor L-NAME, against the background of which LPO increases, the NO_x content and the level of eNOS expression decrease. In contrast, L-arginine promoted an increase in NO_x content, inhibition of FRO intensity, and an increase in the level of the eNOS expression. In the mechanism of the reduced NO_x level, a violation of the availability of L-arginine for the NO synthase also plays a role due to the changes in cholesterol metabolism – hypercholesterolemia and hyper-β-lipoproteinemia. In the vascular endothelium, pre-atherogenic structural changes develop; they are involved in reducing the availability of L-arginine for eNOS. The oxidative stress causes a decrease in NOx levels and a change in the functional state of the kidneys: polyuria, natriuresis, a decrease in the level of tubular reabsorption of water, sodium, and the Na^+/K^+-ATPase activity. At the same time, there is an activation of lipid peroxidation in hepatocytes, an increase in the permeability of the cell membrane, the activity level of the organ-specific enzymes in the blood serum. L-arginine caused an increase in the NO_x level, an increase in microcirculatory hemodynamics and an increase in the activity of Na^+/K^+-ATPase in the tissues of the kidneys and liver. It is important to note the unidirectional changes in the LPO process in erythrocytes, in the cells of the renal, hepatic tissues, which is the basis for recommendations for the use in clinical conditions of determining the FRO activity in erythrocytes to assess the dysfunction of internal organs in lead poisoning.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Sergey G. Dzugkoev – idea, planning and scientific guidance of the study, article writing; Fira S. Dzugkoeva, Olga I. Margieva – participation in the study design development, information search and analysis, analysis and interpretation of the data obtained, preparation of the final article version; Irina V. Mozhaeva, Olga I. Margieva, Anna E. Khubulova – pathology modeling, biochemical research, calculations.

REFERENCES

1. Boskabady M, Marefati N, Farkhondeh T, Shakeri F, Farshbaf A, Boskabady MH. The effect of environmental lead exposure on human health and the contribution of inflammatory mechanisms, a review. Environ Int. 2018 Nov;120:404–20. DOI:10.1016/j.envint.2018.08.013
2. Levin SM, Goldberg M. Clinical evaluation and management of lead-exposed construction workers. Am J Ind Med. 2000 Jan;37(1):23–43. DOI:10.1002/(sici)1097-0274(200001)37:1<23::aid-ajim4>3.0.co;2-u
3. de Souza ID, de Andrade AS, Dalmolin RJS. Lead-interacting proteins and their implication in lead poisoning. Crit Rev Toxicol. 2018 May;48(5):375–86. DOI:10.1080/10408444.2018.1429387
4. Ericson B, Gabelaia L, Keith J, Kashibadze T, Beraia N, Sturua L, Kazzi Z. Elevated Levels of Lead (Pb) Identified in Georgian Spices. Ann Glob Health. 2020 Sep 28;86(1):124. DOI:10.5334/aogh.3044
5. Mani MS, Kabekkodu SP, Joshi MB, Dsouza HS. Ecogenetics of lead toxicity and its influence on risk assessment. Hum Exp Toxicol. 2019 Sep;38(9):1031–59. DOI:10.1177/0960327119851253
6. Obeng-Gyasi E. Sources of lead exposure in various countries. Rev Environ Health. 2019 Mar 26;34(1):25–34. DOI:10.1515/reveh-2018-0037
7. Wrońska-Nofer T, Pisarska A, Trzcinka-Ochocka M, Hałatek T, Stetkiewicz J, Braziewicz J, Nofer JR, Wąsowicz W. Scintigraphic assessment of renal function in steel plant workers occupationally exposed to lead. J Occup Health. 2015;57(2):91–9. DOI:10.1539/joh.14-0115-OA
8. Alwaleedi SA. Haemato-biochemical changes induced by lead intoxication in male and female albino mice. Int J Recent Sci Res. 2015;6(Issue 5):3999–4004.
9. López-Vanegas NC, Hernández G, Maldonado-Vega M, Calderón-Salinas JV. Leukocyte apoptosis, TNF-α concentration and oxidative damage in lead-exposed workers. Toxicol Appl Pharmacol. 2020 Mar 15;391:114901. DOI:10.1016/j.taap.2020.114901
10. Omobowale TO, Oyagbemi AA, Akinrinde AS, Saba AB, Daramola OT, Ogunpolu BS, Olopade JO. Failure of recovery from lead induced hepatoxicity and disruption of erythrocyte antioxidant defence system in Wistar rats. Environ Toxicol Pharmacol. 2014 May;37(3):1202–11. DOI:10.1016/j.etap.2014.03.002
11. Nakhaee S, Amirabadizadeh A, Brent J, Mehrpour O. Impact of chronic lead exposure on liver and kidney function and haematologic parameters. Basic Clin Pharmacol Toxicol. 2019 May;124(5):621–8. DOI:10.1111/bcpt.13179

12. Wang H, Huang P, Zhang R, Feng X, Tang Q, Liu S, Wen F, Zeng L, Liu Y, Wang T, Ma L. Effect of lead exposure from electronic waste on haemoglobin synthesis in children. Int Arch Occup Environ Health. 2021 Jul;94(5):911–8. DOI:10.1007/s00420-020-01619-1
13. Sosedova LM, Vokina VA, Kapustina EA. Fetal programming in the formation of cognitive impairment in the modeling of lead intoxication in white rats. Bull Experim Biolog Med. 2018;166(11):559–64. Russian
14. Obeng-Gyasi E, Armijos RX, Weigel MM, Filippelli GM, Sayegh MA. Cardiovascular-Related Outcomes in U.S. Adults Exposed to Lead. Int J Environ Res Public Health. 2018 Apr 15;15(4):759. DOI:10.3390/ijerph15040759
15. Tejero J, Shiva S, Gladwin MT. Sources of Vascular Nitric Oxide and Reactive Oxygen Species and Their Regulation. Physiol Rev. 2019 Jan 1;99(1):311-379. DOI: 10.1152/physrev.00036.2017
16. Yücebilgiç G, Bilgin R, Tamer L, Tükel S. Effects of lead on Na(+)-K(+) ATPase and Ca(+2) ATPase activities and lipid peroxidation in blood of workers. Int J Toxicol. 2003 Mar-Apr;22(2):95–7. DOI:10.1080/10915810305096
17. Satarug S, C Gobe G, A Vesey D, Phelps KR. Cadmium and Lead Exposure, Nephrotoxicity, and Mortality. Toxics. 2020 Oct 13;8(4):86. DOI:10.3390/toxics8040086
18. Asakawa T, Matsushita S. Coloring conditions of thiobarbituric acid test, for detecting lipid hydroperoxides. Lipids. 2006;15:137–40. DOI:10.1007/BF02540959
19. Korolyuk MA, Ivanova LI, Mayorova I.G. Method for determining the activity of catalase. Laboratory Business. 1988;(1):16–9. Russian
20. Sirota TV. A new approach to the investigation of adrenaline autooxidation and its application for determination of superoxide dismutase activity. Voprosy Meditsinskoi Khimii, 1999;45(3):263–72. Russian
21. Afashagova MM, Marzhohova MYu, Akhokhova AV. The content of ceruloplasmin in the blood of patients with erysipelas. Fundamental Research. 2005;(5):103-4. Russian
22. Metelskaya V.A., Gumanova N.G. Screening-method for nitric oxide metabolites determination in human serum. Russian Clinical Laboratory Diagnostics. 2005;(6):15–8. Russian
23. Natochin YuV, Kutina AV. Novel approach to integrative renal functional characteristics in various types of diuresis. Nephrology (Saint-Petersburg). 2009;13(3):19–23. DOI:10.24884/1561-6274-2009-13-3-19-23. Russian
24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951 Nov;193(1):265–75.
25. Zhang G, Han S, Wang L, Yao Y, Chen K, Chen S. A Ternary Synergistic eNOS Gene Delivery System Based on Calcium Ion and L-Arginine for Accelerating Angiogenesis by Maximizing NO Production. Int J Nanomedicine. 2022 May 2;17:1987–2000. DOI:10.2147/IJN.S363168
26. Fan M, Gao X, Li L, Ren Z, Lui LMW, McIntyre RS, Teopiz KM, Deng P, Cao B. The Association Between Concentrations of Arginine, Ornithine, Citrulline and Major Depressive Disorder: A Meta-Analysis. Front Psychiatry. 2021 Nov 18;12:686973. DOI:10.3389/fpsyt.2021.686973
27. Koo BH, Lee J, Jin Y, Lim HK, Ryoo S. Arginase inhibition by rhaponticin increases L-arginine concentration that contributes to Ca^{2+}-dependent eNOS activation. BMB Rep. 2021 Oct;54(10):516–21. DOI:10.5483/BMBRep.2021.54.10.053
28. Dzugkoev S. G., Dzugkoeva F. S., Mozhaeva I. V., Margieva O. I. Analysis of changes in redox reactions in intoxication with nickel chloride and the NO-synthase inhibitor. Medical news of the North Caucasus. 2021;(4):422–4. DOI:10.14300/mnnc.2021.16102. Russian
29. Tarasova OS, Gaynullina DK. Rho-kinase as a key participant in the regulation of vascular tone in normal circulation and vascular disorders. “Arterial’naya Gipertenziya” (“Arterial Hypertension”). 2017;23(5):383–94. DOI:10.18705/1607-419X-2017-23-5-383-394
30. Jamwal S, Sharma S. Vascular endothelium dysfunction: a conservative target in metabolic disorders. Inflamm Res. 2018 May;67(5):391–405. DOI:10.1007/s00011-018-1129-8

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