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INFLUENCE OF CHRONIC COMBINED INTOXICATION WITH ZINC, COPPER AND ARSENIC SALTS ON THE CHANGES IN HEMATOLOGIC BLOOD INDICATORS OF RATS

Abstract. This article indicated the investigation results of the chronic combined poisoning effect with zinc, copper and arsenic salts on hematologic blood indicators. Since one of the assessing criteria of the effects of toxic substances on the body is hematological indicators and one of the research objects are indicators of environmental contamination at the organism level, animal experiments have been performed to determine the cytological blood composition. As a result of the conducted investigations, it was revealed that in case of chronic combined intoxication with zinc and copper, copper and arsenic salts, the number of leucocytes increases, and with intoxication with zinc and arsenic salts only the amount of leucocytes decreases. In the first case, there is a development of so-called leucocytosis and leucopenia in the second case. There was also identified an increase in the concentration of erythrocytes, hemoglobin, and mean erythrocyte volume in all experimental groups. Possible causes of changes in morpho-functional blood parameters were revealed and substantiated as well. It was found out that certain groups of heavy metals possess various toxic effects.

Keywords: heavy metals, zinc, copper, arsenic, chronic combined intoxication, blood indicators.

Introduction. Environmental pollution caused by toxic elements emissions, such as heavy metals, is now attracting the interest of researchers all around the world, because toxic elements can accumulate in soil and crops, animal and human organisms [1]. As a result of the rapid development of industrial production, especially in developing countries, the use of heavy metals and synthetic chemicals has rapidly increased. Pollution of the environment with heavy metals creates a danger to human health in particular, as they can bio-accumulate at all levels of the food chain [2]. It is known from scientific studies that heavy metals such as copper, zinc, cadmium, chromium, manganese, lead, arsenic are considered to be toxic to both humans and animals [3].

As for Kazakhstan, pollution of the environment with toxic substances is a topical subject, especially in the areas of mining and processing industry. Akmola region is not an exception. There are mining deposits, where gold, uranium, titanium, iron, manganese, molybdenum are being extracted. One of the largest gold deposits is Vasilkovskoye deposit. The accompanying element in this gold deposit is arsenic, which is why during gold mining process, arsenic enters the environment. Also, soils in Kokshetau area contain such heavy metals as lead, copper, cadmium and zinc [4].

Zinc is considered to be an important element for all life forms, as it is a part of many body's enzymes, it promotes cell growth, takes part in the exchange of proteins, nucleic acids, and vitamin A. Therefore, zinc is of great importance for human health as a trace element [5]. The human body contains 2-3 g of zinc, and it also occurs in muscles, bones, liver, kidneys, lungs, brain, heart and pancreas [6]. 30-40% of zinc is concentrated in the cell nucleus, 50% in the cytosol, and the rest is a part of the cell membranes [5]. Zinc plays a special role in the maintenance of immune functions (cellular and humoral immunity). A lack of zinc can affect congenital and adaptive immunity [7]. Zinc deficiency is associated with acute and

chronic liver disease. Adding zinc to the diet helps protect against toxin-induced liver damage and is used in the treatment of hepatic encephalopathy [8].

However, zinc is considered to be relatively non-toxic to humans [9]. There are three main ways of getting zinc into the body: inhalation (by inhaling air polluted with heavy metals), alimentary (through the gastrointestinal tract during eating), through the skin [10]. Zinc is excreted from the body through the kidneys, skin and intestines [11]. An increase in the concentration of zinc in the body is often associated with copper deficiency. The absorption of copper decreases if there is an increase in the consumption of zinc [12].

Zinc intoxication can cause a decrease in the level of copper, immunity, lipoprotein and copper-containing enzymes. High doses of zinc negatively affect the physiology of urination [13]. They also can inhibit the absorption of copper, sometimes resulting in copper deficiency and, as a consequence, cause anemia. [14] Jerome Nriagu, professor of University of Michigan, has shown that zinc salts are irritating, can cause erosive pharyngitis, gastritis, gastrointestinal bleeding, pancreatitis and also oral, throat, and stomach injuries after swallowing. Prolonged zinc exposure leads to hypoplasia, anemia, leucopenia, and neutropenia, as well as impaired pancreatic function, resulting in increased release of amylase, lipase and alkaline phosphatase into the bloodstream [15].

Copper is an important element for cellular metabolism. It is a cofactor-oxidation-reduction reaction involving intracellular proteins and enzymes such as cytochrome oxidase and superoxide dismutase [16].

Also, copper is a composite component of all soils, an essential element for plants and animals, situated in small amounts in plants and animal organisms, although an increase in copper concentration leads to toxic effects on soil inhabitants. Large doses of copper can cause the destruction of red blood cells and, as a consequence, it can lead to the development of anemia [17]. The main organ, the target, which is struck by chronic copper intoxication firstly, is the liver. Studies conducted on animals have shown that taking large doses of copper can lead to liver and kidney disease. Also, due to the high concentration of copper, hemolytic anemia can be developed. It is caused by acute hepatic necrosis. In some cases, kidney functions may be impaired. As a result of the destruction of liver cells, a large amount of copper enters the bloodstream, damaging the red blood cells [18].

Hepatic and sometimes renal changes are the most common effects found in animals that receive high concentrations of copper. In the organisms of rats, mice, rabbits, chronic copper intoxication leads to mortality increase and retardation growth. In rats, which were subjected to copper sulfate intoxication, there were various cellular cell destruction and membrane disorders, increase in the amount of lysosomes, swelling of mitochondria and tubular microvillus [19].

Arsenic is one of the most toxic metalloids. It can be found in the environment, as a result of natural and anthropogenic influences. It appears in rocks, soil, air, water in small amounts. In nature, arsenic has organic and inorganic forms. Inorganic forms are mainly represented by the form of trivalent metaarsenite (As^{3+}) and pentavalent arsenate (As^{5+}) [20]. Studies of arsenic toxicity show that chronic effects can cause serious impairment of organ functions [21]. Arsenic is a serious carcinogen, intoxication which can lead to the development of malignant tumors, large tumors of the lungs, bladder, and prostate [22]. And also it seriously impacts the cardiovascular system, cause developmental anomalies, neurological and neurobiological disorders, diabetes, hearing loss, hematologic disorders - anemia, leucopenia and eosinophilia [23]. In areas with a high level of arsenic contamination, there was a high death rate from bladder, kidneys, skin and liver cancer [24].

Arsenic and its compounds are excreted from the body with urine and bile and breast milk in small quantities [25]. During the experiment on rats, using chronic arsenic primer, after 12 weeks, arsenic was found in the blood, liver and kidneys [26].

According to Khanturina G.R. date, with chronic zinc, copper and iron salts intoxication, a decrease in the level of leucocytes, erythrocytes, hemoglobin in the blood was revealed [27].

Despite the fact that the influence of heavy metals on the body is well known throughout the world, there is no evidence of the effect of chronic combined intoxication with salts of zinc, copper and arsenic. Therefore, the study of the combined chronic effects of zinc, copper and arsenic salts on the body is of a great interest.

Proceeding from the above mentioned, the purpose of our study was to study the influence of combined chronic intoxication with zinc, copper and arsenic salts on the change in hematological blood indicators of experimental animals.

Materials and methods. The experiments were carried out on 40 white uncontaminated rats. The laboratory rats were divided into four groups for these experiments. They were daily injected intragastrically with solutions of heavy metal salts for three months. The first group (n = 10) consisted of control animals, which were kept under standard conditions, including usual food and water diet. The animals of the second group (n = 10) were injected with solutions of zinc and copper salts, the dose of copper sulfate II was 13 mg/kg, zinc sulfate was 17.5 mg/kg. In the third group (n = 10), there were injected the solutions of copper and arsenic salts, the dose of copper sulfate II was 13 mg/kg, sodium arsenite was mg/kg. Animals of the fourth group (n = 10) were injected with solutions of zinc and arsenic salts, the dose of zinc sulfate was 17.5 mg/kg, sodium arsenite was 1 mg / kg.

Blood sampling is performed three months after the beginning of the experiment. During the experiment, all ethical norms and rules were maintained. Blood was taken from the carotid artery of experimental animals. Hematologic blood indicators were determined on a modern automatic hematological analyzer Nihon Kohden Celltac E (Japan). Blood indicators such as leucocytes, erythrocytes, hemoglobin, hematocrit (HCT), mean erythrocyte volume (MCV), mean hemoglobin in erythrocyte (MCH), mean hemoglobin concentration in erythrocyte (MCHC), ESR using various methods were determined. So, the number of leucocytes was determined with the help of the unified counting method in Goryaev's counting chamber, the erythrocyte concentration was determined by a unified method with 0.9% sodium chloride solution, the hemoglobin level was determined by the hemoglobin cyanide method [28]. The hematocrit was calculated by the formula:

$$\text{HCT}\,(\%) = \frac{RBC\,(109\,MCV\,(fl) * cells/l}{10}$$

The average volume of erythrocytes was calculated according to the formula:

$$\text{MCV} = \frac{Hematocrit\ in\ mcl^3}{\text{Number of erythrocytes in 1 mcl}}$$

The mean hemoglobin content in erythrocyte was calculated by the formula:

$$\text{MCH} = \frac{\text{Hemoglobin in g/l}}{First\ three\ numerals\ of\ erythrocytes\ concentration\ in\ 1l}\,(pg).$$

The mean concentration of hemoglobin in the erythrocyte was calculated by the formula:

$$\text{MCHC} = \frac{Hemoglobin\ in\ g/l}{Hematocrit\ in\ \%} * 100$$

ESR was determined with the help of PR-3 (ESR meter, Panchenkov's apparatus) [29]. The results were processed using *Microsoft Office Excel* software, *Statistica* for Windows. The arithmetical mean (M), the standard error of the arithmetic mean (m) were calculated. The significance of differences in the arithmetic mean was estimated using Student's t-test (t) and significance level (p).

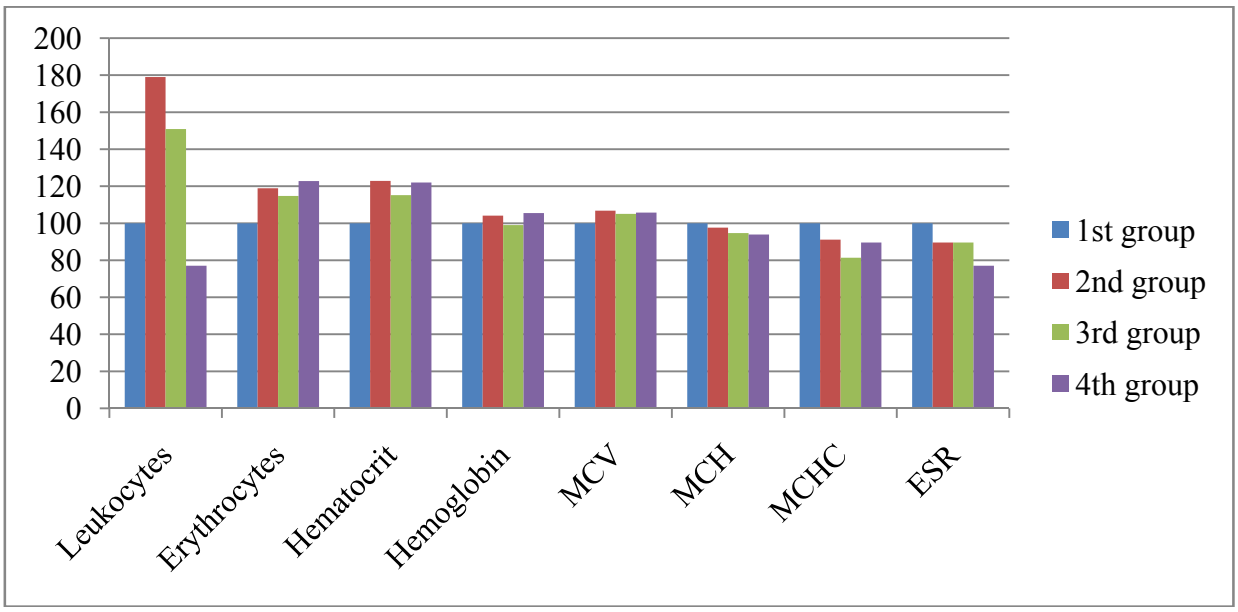
Research results and discussion. The results of our studies show that in case of chronic combined intoxication with zinc, copper and arsenic salts, the number of leucocytes in the blood in the second group increased by 78.89% ($p < 0.05$), in the third group by 50.88% ($p < 0, 05$), and in the fourth group it decreased by 30.94% ($p < 0.05$), in comparison with the control group. Leucocytosis is more marked in the second group (table, figure).

The development of leucocytosis is probably connected with the increased formation of leucocytes in the bone marrow and their release into the bloodstream. It is known from the literature that cytokines are formed in the leucocytes under the impact of toxins in leucocytes, which increase the proliferation and differentiation of leucocytes, as well as the release of the formed elements from the bone marrow. Leucopenia, which is in evidence among the animals of the fourth group, is probably connected with a decrease in the production of leucocytes and their release from the bone marrow into the blood, i.e. there is an oppression of leucopoiesis due to toxic effects on leucopoiesis tissue, as well as increased destruction of leucocytes in peripheral blood and bone marrow.

Hematological indicators of rats' blood with chronic combined intoxication salts of zinc, copper and arsenic salts

Blood indicators	Groups of experimental animals			
	1st group (control group)	2nd group (intoxication with zinc and copper salts)	3rd group (intoxication with copper and arsenic salts)	4th group (intoxication with zinc and arsenic salts)
Leucocytes , $*10^9$/L	6,82±0,11	12,20±0,33*	10,29±0,09*	4,71±0,32*
Erythrocytes,$*10^{12}$/L	7,8±0,16	9,27±0,03*	8,95±0,12*	9,58±0,07*
Hemoglobin, g/L	127±2,97	156,1±1,60*	146,3±2,2*	155±0,61*
Hematocrit, %	47,0±1,13	48,93±0,37	46,49±0,68	49,56±0,36
MCV, fL	49,4±0,64	52,78±0,27*	51,91±0,08**	52,24±0,59**
MCH, pg	17,25±0,04	16,84±0,15	16,33±0,06*	16,2±0,12*
MCHC, g/L	350±5,19	319,1±1,56*	284,8±21,08*	313,5±1,76*
ESR, mm/h	4,8±0,10	4,3±0,11**	4,3±0,11**	3,7±0,11*

*The differences are significant compared to the control group, with p <0.05; ** - the differences are significant in comparison with the control group, with p <0.01, n - the number of animals in the groups.



Dynamics of hematological indicators in chronic combined intoxication with salts of zinc, copper and arsenic

There were also changes in the leukocyte formula. The number of lymphocytes decreased in the second group by 14.42% (p <0.05), in the third group by 19.36% (p <0.05), in comparison with the control group. In the fourth group, the results were practically equal to the control group. The number of monocytes increased in the second group by 220% (p <0.05), in the third group by 155% (p <0.05), in the fourth group by 61.18% (p <0.05) in comparison with the control group of animals. The number of segmented neutrophils increased in the second group by 26.5% (p <0.05), in the third group by 83.13% (p <0.05), in the fourth group by 37.35% (p <0.05), in contrast to the control data.

An increase in red blood cells was observed in all experimental groups. In the second group it was 18.85% (p <0.05), in the third group was14.74% (p <0.05), in the fourth group was 22, 8% (p <0.05). At the same time, the amount of hemoglobin also increased in the second group by 22.9% (p <0.05), in the third group by 15.20% (p <0.05), in the fourth group by 22% (p <0,05), in comparison with the results of the control group. It can be assumed that this increase in the number of erythrocytes is connected with a thickening of the blood due to excessive loss of body fluid, or increased formation of erythrocytes in the bone marrow as a result of oxygen deficiency. Perhaps, this can be explained by the hyperproduction of

erythropoietin in the defeat of kidney tissue and the resolution of the liver parenchyma. It is well known that erythrocytosis lead to an increase in blood viscosity, aggregation of uniform elements, microcirculation and the appearance of dystrophic changes in organs and tissues.

The parameters of the hematocrit remained within the norm, in comparison with the control data. So, the value in the second group increased by 4.11%, in the third group it decreased by 1%, in the fourth group it increased by 5.45%.

The mean erythrocyte volume increased in the second group by 6.8% ($p < 0.05$), in the third group by 5.08% ($p < 0.01$), in the fourth group by 5.75% ($p < 0.01$), although the mean hemoglobin concentration in the erythrocyte decreased in the second group by 2.38% ($p < 0.05$), in the third group by 5.33% ($p < 0.05$), in the fourth group by 6.09% ($p < 0.05$). An increase of this indicator may show the development of liver diseases, as well as a violation of bone marrow activity in severe leucocytosis, which is confirmed by the results of our studies.

The mean hemoglobin content in the erythrocyte decreased in the second group to 8.83% ($p < 0.05$), in the third group to 18.63% ($p < 0.05$), in the fourth group by 10.43% ($p < 0, 05$). Also, the mean and hemoglobin concentration in the erythrocyte in all the experimental groups decreased, in the second group to 2.38% ($p < 0.05$), in the third group to 5.33% ($p < 0.05$), in the fourth group to 6, 09% ($p < 0.05$) compared with the control data. This is possibly due to the development of absolute hypochromia of erythrocytes, as a result of a violation of iron assimilation.

Erythrocyte sedimentation rate decreased in the second group to 10.42% ($p < 0.01$), in the third group to 10% ($p < 0.01$), in the fourth group to 22.92% ($p < 0.05$). Decrease of ESR is probably due to a violation of protein synthesis in liver failure, as well as the violation of the columns formation, which is caused by the change in the form of red blood cells.

Conclusions. Thus, chronic combined intoxication of experimental animals with zinc, copper and arsenic salts led to the significant deviations from the norm of hematological parameters. In this case, the development of neutrophilic leucocytosis in groups of combined effect of zinc and copper, copper and arsenic salts, as a manifestation of poisoning, was observed. The revealed monocytosis in our experiments is the immune reaction of the organism to the action of heavy metals. Also, leucopenia in the group of combined intoxication with zinc and arsenic salts was detected. Because the number of erythrocytes increased, as well as their volume, this led to an increase in hemoglobin and may also indicate a violation of liver function. The increase in erythrocytes can also be connected with the activation of erythropoiesis with the increased erythropoietin formation, which is probably caused by a lack of oxygen, possible neoplasm in the kidneys and adrenal glands.

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МЫРЫШ, МЫС ЖӘНЕ МЫШЬЯК ТҰЗДАРЫНЫҢ ГЕМАТОЛОГИЯЛЫҚ КӨРСЕТКІШРІНІҢ ӨЗГЕРІСІНЕ ҚОСАРЛАСА СОЗЫЛМАЛЫ ӘСЕРІ

Аннотация. Осы зерттеуде мырыш, мыс және мышьяк тұздарымен қосарласа созылмалы әсері нәтижелерінде гематологиялық көрсеткіштердің өзгерістері берілді. Ағзаға улы заттар әрекеттерін бағалау өлшемдерінің бірі – гематологиялық көрсеткіштерді талдау болып саналады. Жүргізілген зерттеулер нәтижесінде мырыш пен мыс, мыс және мышьяк тұздарымен созылмалы қосарласқан улану кезінде лейкоциттер саны көбейіп, ал, мырыш және мышьяк тұздарымен улануы кезінде лейкоциттер саны төмендегені анықталды. Бірінші жағдайда лейкоцитоз және екінші жағдайда лейкопения дамиды. Сондай-ақ, барлық эксперименттік топтарда эритроциттердің, гемоглобиннің, эритроциттердің орташа көлемінің артуы көрсетіледі. Мақалада қан көрсеткіштерінің морфо-функционалдық өзгерістері ықтимал себептері анықталған және негізделген. Бұл ауыр металдар белгілі бір топтары әр түрлі іс-әрекеттерімен ерекшеленетінің көрсетеді.

Түйін сөздер: ауыр металдар, мырыш, мыс, мышьяк, қосарласа созылмалы улану, қан көрсеткіштері.

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ВЛИЯНИЕ ХРОНИЧЕСКОЙ СОЧЕТАННОЙ ИНТОКСИКАЦИИ СОЛЯМИ ЦИНКА, МЕДИ И МЫШЬЯКА НА ИЗМЕНЕНИЕ ГЕМАТОЛОГИЧЕСКИХ ПОКАЗАТЕЛЕЙ КРОВИ КРЫС

Аннотация. В статье представлены результаты исследования влияния хронических сочетанных отравлений солями цинка, меди и мышьяка на гематологические показатели крови. Поскольку одним из критериев оценки действия токсичных веществ на организм являются гематологические показатели и одним из объектов исследования, – индикаторы загрязнения окружающей среды на организменном уровне, были проведены эксперименты на животных с определением цитологического состава крови. В результате проведенных исследований выявлено, что при хронической сочетанной интоксикации солями цинка и меди, меди и мышьяка увеличивается количество лейкоцитов, а при интоксикации солями цинка и мышьяка снижается. Развиваются так называемые, лейкоцитоз – в первом случае и лейкопения – во втором. Также обнаружено увеличение содержания эритроцитов, гемоглобина, среднего объема эритроцитов во всех экспериментальных группах. Выявлены и обоснованы возможные причины изменений морфо-функциональных показателей крови. Выяснено, что определенные группы тяжелых металлов обладают различными токсическими действиями.

Ключевые слова: тяжелые металлы, цинк, медь, мышьяк, хроническая сочетанная интоксикация, показатели крови

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