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Study of the effect of cadmium, lead, zinc salts on the rat blood cells

Abstract. Changes were detected in the blood of rats caused by heavy metal compounds. The results show that the salts of heavy metals poisoning demonstrates in damage in the blood cells in the animal body and making weaker their immunity. As a result, various changes occur in animals. In addition, material was collected on immune depressive exposure to heavy metals in the body of rats 25, 50, 75, 100 times more compounds of zinc ions, lead and cadmium. Animal disorders were observed in the nervous system, including tearing, inflammation conjunctive sac and certain groups even with bleeding. Leukogram, which was made by the blood of rats, showed that the poisoning caused by the impact of three types of heavy salts, has led to significant changes in the health and behavior of the animals. When increasing by 100 times more poisoning three compound salts in animal organisms have been found strong signs of poisoning, finally, all experimental rats died within 5-6 days. It is important to note, animals poisoned by 75-100 times the maximum permissible concentration of heavy metal salts of the three compound salts have died. Leukogram of experimental rats showed that, in the blood of rats, poisoned by three salts, during neutrophilia and lymphopenia, leukopenia was found. Neutrophils are characterized by granulated toxicity and gipersigmatich core.

Key words: Rats, blood, neytrofil, heavy metals, leykogramma.

Introduction

Today the decrease and destruction of immune system of an organism are considered as important problems of modern physiology. In scientific-technical and industrial century, the increase of man-made and heavy metals exposure has an influence on psychological health of human. The response of an organism to environment's adverse factors is its increased defensive reaction and as a result changes of blood immunological indexes can be seen in physiological tendency of an organism [1; 2]. In a time of stress blood's leukogram and leukocytal index changes at one definite range that depends on organism's immunological demands [3; 4].

When affected by heavy metals human and animal organisms' adaptive abilities are decreased and organism's protective system is exhausted and weakened by exerting pressure on and disturbing organism's vital functional systems. To restore the individual and cumulative impacts of negative factors of metals such as zinc, lead and cadmium, the search for new biologically active compounds that determine the cellular mechanisms of immune-boosting properties and effects on the body is the basis for the research work. Industrial development of economy and agricultural chemistry led to a sharp increase in the number of transport vehicles in big cities in recent years and which overall leads to environmental pollution. Not only the production of chemical substances harm human body, but everyday use of household chemicals increase the risk of harm to human health [5].

In many countries, including Kazakhstan, the increased industrial development neglects the responsibility to nature protection and wastes like toxins, heavy metals, pesticides and other harmful substances lead to the environmental pollution. Adverse climatic factors interact with chemicals and further enhance the strength of their insalubrities [6; 7].

Many chronic diseases are considered to be the result of the negative influence of environmental factors on the human body. The occurrence of disease is due to decreased activity of immune system [8; 9].

If allowed concentration is increased, it may produce toxic effects in human and animal body. In all cases, when heavy metals enter the body of an organism it leads to development of various pathologies, gives rise to a distortion of defensive system, which leads to a decrease of organism's functional structures [10].

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The Republic of Kazakhstan is paying careful attention to water pollution. Research results show that rivers Irtysh and Ural are polluted [11]. In Semipalatinsk region groundwater pollution can cause an ecological disaster to the area of military airport. Three largest cities of Kazakhstan – Oskemen, Pavlodar, Semipalatinsk, where there are 900 water users, nonferrous metallurgical and chemical industries, engineering and oil-gas factories, food and water agricultures are provided with water from the artery of the country – river Irtysh. This river has always been polluted by copper, zinc and other metals, and water volume required for the population is 4.82 km^3 which is 20% of all water in the country. In Oskemen city the main sources of groundwater pollution are: lead, zinc, manganese, fluoride, cadmium. In addition, for the past time there is a lot of concern about Caspian Sea. For the flora and fauna of the water reservoir it is a universal geocenosis [12; 13].

The industrial metal ions apart from gas produced by vehicles are considered as separate wastes to the environment. It is known how heavy metal ions of the elements that are thrown into the environment are gradually collected and how they enter human body from the water and atmosphere. Then they accumulate in gastrointestinal tract for a long time which cause major changes in the activity of a single individual organs or body [14].

At the moment, there is a large amount of heavy metals in the environment. Every day human body is being exposed to heavy metals, the immunological and physiological influence of which is the main focus of the research works. But the cases of excess amount of metal ions in the immune system of animals and humans have not been studied. In this regard, in the course of poisoning by heavy metals the relevance and importance of the work will be substantiated.

During the experiment changes in blood cells of rats caused by excess amount of heavy metals will be determined. Tasks: to study the changes in rat blood's leukogram after 10-days exposure to 25, 50, 75, 100 times admissible concentration limit (ACL) of zinc, lead, cadmium salts; to study and observe general changes in rat's leukogram for 60 days following 20-days exposure to 50 times admissible concentration limit (ACL).

Materials and methods

In order to achieve goals and objectives faculty of Biology and Biotechnology in the Al-Farabi Kazakh National University provided in vivarium grown laboratory rats. Weight range was 220-250 g, rats were fed with standard food, adult rats were about 5-6 months, different sex, and in total of 50 rats. To determine the effect of salts of heavy metals toxic to laboratory rats were divided into 5 experimental groups. Each group of 10 rats.

Water with 25 times ACL of salts of heavy metals (mixture of zinc sulfate, lead acetate and cadmium chloride with concentrations of 125, 0.75, 0.025 mg/l respectively) was given to the animals in group 1.

Dose of 50 times ACL of salts of heavy metals (mixture of zinc sulfate, lead acetate and cadmium chloride with concentrations of 0.05, 1.5, 250 mg/l respectively) was given to the animals in group 2.

Dose of 75 times ACL of salts of heavy metals (mixture of zinc sulfate, lead acetate and cadmium chloride with concentrations of 0.075, 2.25, 375 mg/l respectively) was given to the animals in group 3.

Dose of 100 times ACL of salts of heavy metals (mixture of zinc sulfate, lead acetate and cadmium chloride with concentrations of 0.1, 3.0, 500 mg/l respectively) was given to the animals in group 4. After 10 days of poisoning the blood of rats was studied.

0.38 ml of 3% of acetic acid was poured into clean test tube and mixed with 0.02 ml of blood. White cell count techniques: counting chamber was filled with that solution. Then the biggest indicator is replaced [15]. The number of identified cells is calculated by using this formula:

$$X = \frac{B \cdot 4000 \cdot 20}{400}$$

X – number of leukocytes in 1mm^3 of blood; 1/4000 –volume of small square; dilution ratio is 20; The number of small squares is 400; B-25 number white blood cells on a large square. Mathematical data was analyzed using the program Excel Microsoft Office. All the statistical work and comparison of $p \leq 0.05$ *, ** $p \leq 0.01$, and *** $p \leq 0.001$ were done using Student (t) program. ANOVA was used to determine the accuracy of the method.

Results and discussion

Rats that were given salts of heavy metals with 25 times ACL did not show any immunological or physiological change that is physiological parameters like size, weight, appetite, skin and basic animal instincts are kept within normal index.

Groups of rats poisoned with a mixture of salts showed 3 times lower leukocyte indexes. It must be noticed that these groups of animals has sharp in-

crease in the number of segment and core neutrophils and there was a release of immature neutrophils into peripheral blood. Absolutely no eosinophilia was found. The number of basophils was for 5-6 times higher and the number of lymphocytes was also increased.

Groups of animals that were poisoned with the mixture of salts of heavy metals also showed low level of leukocytes. In particular the number of polynuclear cells: medullocells – up to 7% ($p < 0.05$), metamedullocells- up to 6% ($p < 0.05$). The number of segment core neutrophils increased and stick-core neutrophils increased by 5-6 times. No granulocyte white blood cells were found, but the number of agranulocyte white blood cells was up to 9% that is decreased by 4-5 times.

Leukogram of rat blood that were poisoned with all three salts heavily showed significant changes in blood. Because after poisoning the development of blood cells in the bone marrow has significantly changed. During lymphopenia and leucopenia, neutrophylesis was registered. Neutrophils were characterized as toxic and hyper segmented.

Leukogram of peripheral blood showed increased number of medullocells by 16% and metamedullocells by 4%. Stick-core neutrophils were 16% and segment core neutrophils were 62% but the number of lymphocytes has reduced comparing with normal level of leukocytes. However, monocytes and basophiles were lost (Table 1).

The result of poisoning animals by 50 times ACL showed no change in physiology and physical activity of the rats. Animal's appetite is normal, skin covering is smooth, conjunctiva is clean and all the basic instincts are reserved.

Poisoning groups of animals with all three salts of heavy metals resulted in reduced number of leukocytes. In particular the number of polynuclear cells: medullocells – up to 7% ($p < 0.05$), metamedullocells- up to 6% ($p < 0.05$). The number of segment core neutrophils increased and stick-core neutrophils increased by 5-6 times. No granulocyte white blood cells were found, but the number of agranulocyte white blood cells was up to 9% that is decreased by 4-5 times.

The number of leukocytes significantly reduced (Table 1). The leukogram of rat blood registered neutrophylesis along with lymphopenia and leucopenia. Neutrophils were characterized as toxic and hyper segmented.

The effects of poisoning animals by 75 times ACL were decreased appetite, disturbance of psychomotor response, lowered physical activity and other physiological changes. Also there were registered serious physiological changes. Rats were not paying any attention to the food, skin coverings are very dirty, and at some places there was no hair on the body, not clean conjunctive, red gums and swelling of the chin, swelled abdomen (Table 1).

Interestingly, lymphocytes were up to that is increased by 2-2.5 times. Moreover, there was a sharp decline in the total number of leukocytes, by 5-6 times. Lymphocytes and eosinophils count 2.0 ± 0.01 ($p < 0.05$), monocytes and basophiles were largely absent. Neutrophils were characterized as toxic and hyper segmented. In comparison to other groups the result of poisoning animals by 100 times ACL showed much more significant complications.

Animals poisoned with the mixture of salts after 5-6 trials began to die. The body of an animal was exposed to severe stress, no hair on some parts of the skin, tearing eyes, not clean conjunctiva and red swelled area around teeth some of them were bleeding, swelling of the chin. Total number of leukocyte decreased for 3-4 times compared to normal, whereas numbers of stick core and segment core neutrophils increased, immature granulocytes are decreased. The nuclei of neutrophils are hyper segmented. Also, in all poisoned groups of animals the red blood cells granulated with basophils. All animals with poisoning concentration of 75-100 times ACL died.

Poisoning with a mixture of heavy metals resulted in 1.5-fold increased neutrophil cells compared with the control (Table 1).

Three groups of animals poisoned with salts of heavy metals were observed: neutrophils number fell for 3.8 times.

Moreover, in blood of rats poisoned with heavy metals along with neutrophylesis and lymphopenia, leucopenia was registered. Neutrophils were characterized as toxic and hyper segmented.

Lymphocytes increased for 2.46 times, the size of immature lymphocytes increased for 1.5 times. Animals that were poisoned with concentration of 100 times ACL compared to control group showed a number of signs of heavy poisoning and which eventually died on 5^{th} and 6^{th} day. The study showed that significant changes in white blood cells and leukograms of poisoned animals were observed, and decrease of the immune system of animals was also noticed. As a result, there are different varieties of changes in animal body. The body of an animal was exposed to severe stress, no hair on some parts of the skin, tearing eyes, not clean conjunctiva and red swelled area around teeth, some of them were bleeding, swelling of the chin.

Table 1 – Total number of white blood cells and data of leukograms made by analyzing control group and groups where rats were poisoned with salts concentrations of 25, 50, 75, 100 times higher than ACL.

Concentration	Leukocytes	Medullocells	Metamedullo-cells	Neutrophils		Eosinophils	Basophils	Lymphocytes	Monocytes
				Stick core	Segment core				
	M± m	M± m	M± m	M± m	M± m	M± m	M± m	M± m	M± m
Control	11182.2±12.2	0 0	0 0	477.5±2.7 5.2±0.3	.5692.96±72.0 62.0±0.1	376.5±5.2 4.1±0.03	165.3±6.3 1.8±0.1	2020.4±6.1 22.0±1.9	560.1±4.2 6.1±0.5
25x ACL	9680.2±56,4	440.8±9.2 8.2±0.2	327.2±7.4 7.2±0.2	908.8±5.7 18.3±0.5	2356.4±13.2 70.2±3.1	113.6±1.7 4.1±0.03	0 0	568.4±0.4 12.3±0.9	0 0
50x ACL	3800.7±17,9	266.2±8.6 7.8±0.01**	228.0±1.8 6.5±0.2	570.0±5.2 15.3±0.3	2356.4±12.5 62.6±2.8	38.3±6.7 1.1±0.03*	0 0	342.2±2.1 9.0±0.2	0 0
75x ACL	1130.8±26,9	67.8±5.6 6.3±0.4	67.8±5.1 6.9±0.1	169.5±6.2 15.0±1.2	723.2±5.6 64.0±2.9	22.6±0.7 2.0±0.01**	0 0	79.1±2.7 7.1±0.8	0 0
100x ACL	0	0	0	0	0	0	0	0	0

Note – part–total number of cells in1μl of blood; the content of the cells is presented in %, the statistical reliability of results in comparison with control * p <0.05, ** p <0.01.

Leukograms of rats before and after poisoning with zinc, lead, cadmium salts were compared with each other, where at the beginning number medullocells was 0.5% but after it is increased up to 15% ($p < 0.001$) (Fig. 1, 2), the same with core segment neutrophils, which before poisoning were 23.5% and after increased up to 30% ($p < 0.01$), no immature neutrophils in peripheral blood were observed (Fig. 2).

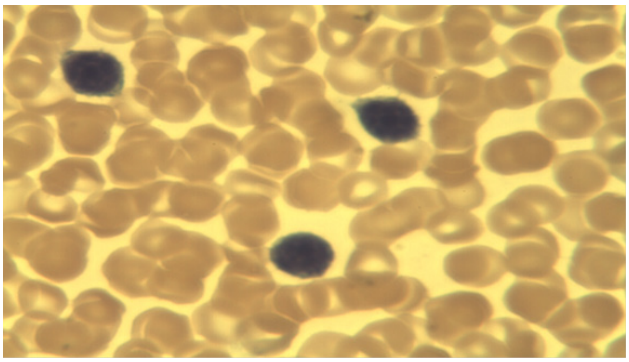

Figure 1 – Lymphocytes before poisoning

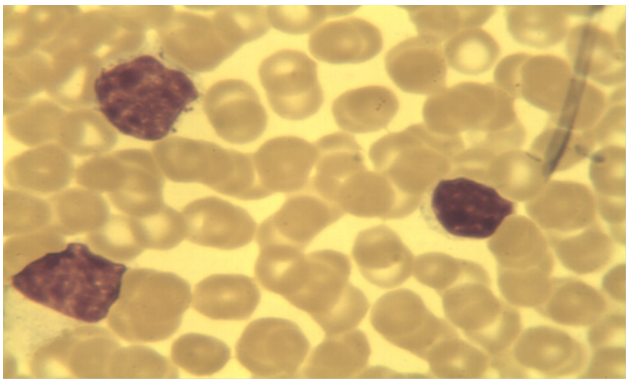

Figure 2 – Medullocells after poisoning with zinc, lead, and cadmium salts

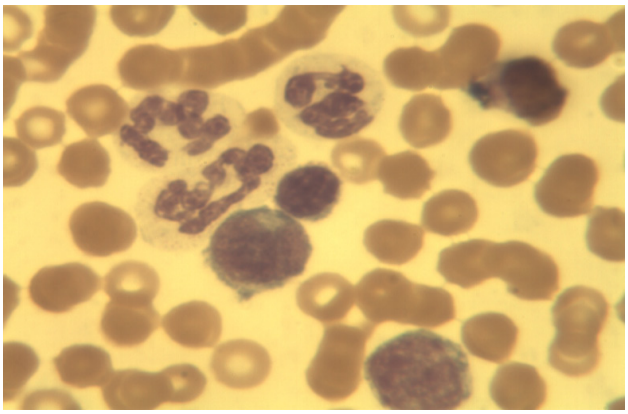

Figure 3 – Core segment neutrophils and monocytes after poisoning with zinc, lead, and cadmium salts

Therefore, cadmium salt decreases the number of lymphocytes, increases the number of immature lymphocytes and reduces activity of the immune system.

To conclude, zinc, lead and cadmium ions concentrations of 25, 50, 75, 100 times ACL hugely impact on immune system of rats which have an immunosuppressive effect on animals.

References

1. Velichkovsky B.T. Ecologiya cheloveka. V chem glavnayia problem Rossii? // Vest. RAMN. – 2002. – №9. – pp. 6-11.

2. Haytov P.M. Molecular-cellular physiology of the system of natural immunity // Physiology and pathology of the immune system. – 2005. – №6. – pp. 3-11.

3. Chereshnov V.A., Kevorkov N.N., Bahmetov B.A. Physiology of the immune system and ecology // Immunobiology. – 2001. – №3. – pp. 12-16.

4. Beskempirova K.B., Kaliyanova O.A. Hygienical estimation of contaminations of soil harmful substances in Eastern Kazakhstan area // Hygiene, epidemiology and immunobiology. – 2000. – № 3-4. – pp. 60-65.

5. Zasorin B.V., Iyrchenko V.I. Cue O.B. Immune system and its role in pathogeny disease of digestive tract at a contact with connections of heavy metals // Problems of medical ecology. Karaganda, 1995. – Vol. 2. – pp. 175-177.

6. Problems of contamination of environment and toxicology. – M.: Mir, 1993. – pp. 191.

7. Sabyrbek J.B. Influence of heavy metals on immune cells // World of Science. Almaty, 2009. – pp. 207-208.

8. Chigarkin A.V. Geoecology and conservancy of Kazakhstan. Almaty, Qazaq University, 2003. – pp. 350-356.

9. Baevsky R.M. Methodical ecological monitoring of population health. – Nukus, 1991. – pp. 65-68.

10. Panin M.S. Influence of technogenic factors and agrochemical activity of maintenance, migration of heavy metals in the “soil-plant” system // State and rational use of soils of the Republic of Kazakhstan. – Almaty, 1998. – pp.76-79.

11. Shubik V.M., Petlenko S.V., Smirnov B.C. Immunaya systema cheloveka v ekstremalnyh klimatoekologicheskih usloviyah // Immunodeficitnye sostoyanie (pod.red V.S. Smirnova, I.S. Freidlin). – SPb.: Izd-vo «Foliant», 2000. – pp. 237-289.

12. Stezhka V.A., Dmitruha N.N., Lampeka E.G. To the mechanism of material cummulation of

heavy metals in the organism of white rats // Occupational Health. – Kiev, 2001. – Issue 32. – pp. 219-230.

13. Kelina N.I., Bezruchko N.V. Estimation of influence of risk of environment factors on the organism of man: educational pasobie // Penza: Penz. tech, 2003. – 68 p.

14. Hudolei V.V. Ecological dangerous factors // Information of Almaty. "Science" 1996. – № 4. – pp. 74-78.

15. Ayzmana R.I., Dyikareva I.A. Practical employments on the course "Human and animal physiology". Novosibirsk: Sib. Univer., 2003. – pp.120.