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The State Of Hematopoiesis Of A Bird Under The Influence Of Various Doses Of Zinc Chloride.

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ABSTRACT

The systematic effect of heavy metals on the poultry organism causes pathological changes in the animal organism, leading to metabolic disorders, the structure of organs and tissues, etc. Hemopoiesis cells are considered “critical” populations when exposed to adverse environmental factors. By increasing the dose of zinc chloride to 60 mg/kg of mass, we established deeper changes in the red bone marrow and peripheral blood in chickens. Organelles were often completely destroyed or vacuolated, and in the cytoplasm, heterogeneous inclusions or vacuoles were determined instead. In erythroblastic cells, ribosomes and polyribosomes were destroyed, mitochondria, the cytoplasm was homogenized, chromatin was destroyed in the nuclei, and the nuclei were often deformed. In the field of view, the number of reticulocytes increased. The results of the study showed that zinc poisoning of chickens even within 1-3 days leads to damage to bone marrow cells. In the cytoplasm of the hematopoietic and stromal cells of the bone marrow, mostly signs of destructive processes are revealed, which increase as the duration and dose of exposure increases. Zinc mostly causes impairment of morphofunctional structures in erythroblasts and mature cells of other bone marrow cell lines.

Keywords: hematopoiesis, bird, zinc chloride, heavy metals.

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INTRODUCTION

The intensive development of industry in recent years has led to the fact that the problem of environmental pollution and the survival of mankind in these conditions has become a central problem of our time and has affected all spheres of human activity. In a number of cases, technological processes have escaped control, resulting in a rapid accumulation of substances uncharacteristic for the biosphere (radionuclides, heavy metals and other toxicants) [1, 2].

According to some authors [3], ions of such heavy metals as zinc, cadmium, lead, mercury, chromium, copper, nickel, molybdenum and others, with an increased content in the environment, can inhibit the vital activity of many living organisms and induce non-enzymatic breaking of chemical bonds in the DNA molecule, leading to one and double-stranded breaks with the formation of chromosomal aberrations, affecting the synthesis of DNA in the cell and causing repression of the genome[4, 5].

At the same time, metals such as zinc and copper are found in the environment of any industrial city. Toxic metals trapped in the body are distributed unevenly in it. The first blows are taken by the main organs of excretion (liver, kidneys, lungs, skin). In particular, once in the liver, they can undergo various changes, even with a favorable outcome for the body, which contributes to their neutralization and excretion through the kidneys and intestines. If these mechanisms no longer work, then heavy metals accumulate in the body of humans and animals [6].

Agriculture, including poultry farming is one of the most effective, highly profitable and promising livestock sectors, as unlike other industries it does not have seasonality and takes the leading place in satisfying the population with food during the year [7]. Successful implementation of the target program for the development of this sector depends on the maximum utilization of the potential of highly productive cross-country agricultural animals [8].

As a result of environmental problems in the environment (soil, water, air basin, feed), the incidence and mortality of agricultural and wild animals increases, and their productivity decreases. Systematic exposure to toxic substances causes pathological changes in the body of animals, leads to disruption of metabolism, immunological status, neurohumoral systems, organ and tissue structure, etc. [9].

As is known, the blood system and organs of hemopoiesis are the most sensitive, so-called "critical systems". In some cases, changes in the blood occur when the body is exposed to relatively small doses of substances and may be the only diagnostic indicators of diseases and their consequences.

Based on this, further improvement of approaches to the assessment of bone marrow hematopoiesis should occur taking into account the accumulation of information about the impact of exogenous factors on the biosystems and the ultrastructure of organs and body systems in a state of functional norms and pathology.

In this regard, the purpose of the study was to study the effect of zinc on the hematopoiesis of agricultural birds.

MATHERIALS AND METHODS

The studies were carried out under the conditions of the experimental biological clinic (vivarium) of the Orenburg State University. Animal studies were carried out in accordance with the "Rules for carrying out work using experimental animals" (annex to the order of the Ministry of Health of the USSR of 12.08.1977, No. 755) and according to the recommendations of VNITIP (2002).

A total of 210 ten-day broiler chickens were selected, of which 3 groups (n = 30) were formed (Table 1). The difference between the groups consisted of the level of exchange energy and the content of regulated trace elements in the diet.

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Table 1: Scheme of the study

Group	Accounting period, age, days
	2-60
control	-
I experienced	intraperitoneally single dose 40 mg / kg zinc chloride
II experienced	intraperitoneally single dose of 60 mg / kg of zinc chloride

Poultry slaughter (VNITIP, 2000) was carried out at the beginning and 10, 20, 30, 40 days of experimental studies with ether raush anesthesia followed by the formation of red bone marrow samples.

After the end of the preparatory period, the bird of the 1st experimental group was transferred to an "no mineral" diet. Broilers of the 2nd experimental group received a diet with an exchange energy content of 13.2 MJ/kg NE, not standardized for microelements. The third test group is the main diet with an exchange energy content of 13.4 MJ/kg SW with the addition of a mineral premix, compiled according to the recommendations of VNITIP (2004), including the salts of manganese, iron, copper, cobalt, iodine and selenium.

Feeding of the experimental bird was carried out 2 times a day. The bird was poultry without restrictions. The microclimate in the room was in accordance with the requirements of ONTP-4-88.

EXPERIMENTAL

In acute experiments, intraperitoneally, zinc chloride was additionally administered at doses of 40 mg/kg and 60 mg/kg of animal weight(Table 1).

The statistical processing of the received material was carried out using the generally accepted methods using the Excel application from the Office XP and Statistica 10.0 software packages, including the determination of the arithmetic mean (x), the standard error of the mean (Sx). In order to identify statistically significant differences, the Student's test was used, the similarity (difference) between the mean values in all groups was the Kraskel-Wallis method, discriminant analysis was used to classify individuals according to the influence of various factors.

For an ultrathin study, the bone marrow was fixed in a 2.5% solution of glutaraldehyde on phosphate buffer followed by hourly fixation with 1% OsO_4 solution on the same buffer. After treatment with a saturated solution of uranyl acetate on 70% ethanol, the material was dehydrated in the alcohols of the ascending fortress and enclosed in epon. Ultrathin sections were examined on a transmission electron microscope JEM-100 CX II (JEOL, Japan).

One day after the zinc intoxication of chicks, even in a dose of 40 mg/kg, signs of pronounced structural changes were revealed in the cells of the bone marrow. Stromal bone marrow reticular cells underwent dystrophic changes. In the cytoplasm, single swollen mitochondria with collapsing cristae and finely vacuolated mitochondrial matrix, vesicles and vesicles were determined. In the nuclei, chromatin was partially destroyed, although the shape of the nuclei did not change, remaining round or somewhat elongated.

In macrophages of the bone marrow, the cytoplasm was inhomogeneous, with a large number of different electro-density of large and small round inclusions, numerous vesicles. Most organelles in the cytoplasm of macrophages were not visible. Perinuclear spaces in the cells were expanded. Heterochromatin in the nuclei of macrophages was degraded, in the karyoplasm it was defined as rounded and fuzzy dark islets. In most macrophages the processes of cytoplasm as such were absent. Cells took a round shape, indicating a decrease in their functional activity. The cytoplasm was overloaded with residual bodies. Sometimes in the erythroblast islets, among the erythroblasts, macrophages were identified with a devastated light cytoplasm containing single scraps of collapsed organelles.

In erythroblasts and erythrocaryocytes, the destruction of ribosomes and polyribosomes, single mitochondria, often cytoplasm was homogenized. The cells were either elongated, or had a different outline instead of a rounded shape in the norm. In many nuclei of erythroblastic cells, heterochromatin was detected in large amounts, which strongly thickened along the inner side of the karyolemma. In individual erythrocaryocytes, the nuclei diminished in size, compacted, and underwent karyopicnosis. In the bone marrow there was an increase in the number of basophilic erythrocaryocytes, nuclear-free reticulocytes, and a decrease in the hemoglobinized forms of erythrocytes.

In the promontocytes organelles in the cytoplasm were partially retained, but in the chromatin nucleus it was somewhat homogenized, the shape of the nucleus changed, and small protrusions of the karyoemma appeared in the cytoplasm. In some cells of the monocytoid series organelles were destroyed in the cytoplasm, as a result, it was vacuolized, or a pronounced edema of the perinuclear space was detected, while part of the organelles was preserved.

Lymphocytes with almost complete preservation of the nucleus had a wide cytoplasm with pronounced dystrophic changes in the form of destruction of intracellular organelles, enlightenment and expansion of its sites.

After 3 days after the zinc intoxication of chickens at a dose of 40 mg/kg, the destructive processes in the cells of the bone marrow increased. Both hematopoietic cells and bone marrow stromal cells underwent dystrophic changes. The most pronounced morphological changes in cells consisted in the complete destruction of organelles, in the formation of heterogeneous lipid inclusions instead of them, in the pronounced vacuolization of the cytoplasm, in the strong expansion of the perinuclear space.

These changes were more concerned with the mature forms of the hematopoietic cells. The figures of mitosis were not determined. There were promonocytes with a relatively normal ultrastructure. In their cytoplasm, short channels of the GER, rounded dark mitochondria, many ribosomes and polyribosomes, various vesicles and vesicles were detected, small round lysosomes were identified, and the Golgi plate complex was well pronounced. It should be noted that in most cells, pathological changes concerned nuclear material. Even in cells with a preserved cytoplasmic structure, heterochromatin often disappeared, the structure of the nucleoli varied, they assumed bizarre forms and sometimes dissolved in the karyoplasm (Fig. 1).

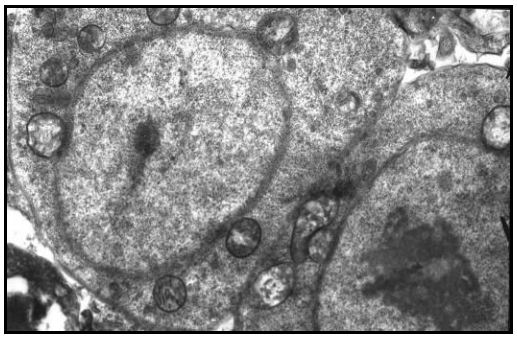

Figure 1: Changing the structure of nuclear material in the cells of the chicken bone marrow after 3 days after zinc intoxication in a dose of 40 mg / kg. Electronogram. Magnification×10000.

Studying the effect of zinc chloride on the cells of the bone marrow of non-native mice in similar doses, [10] revealed signs of development of apoptosis. We have such signs in the chickens are not established.

With the increase in zinc concentration after 1 day, dystrophic changes in bone marrow cells were revealed: they are approximately the same as in the chickens group with intoxication in a dose of 40 mg/kg every other day.

Three days after intoxication in a dose of 60 mg/kg of mass destructive changes of hematopoietic and stromal cells of the bone marrow increased. Organelles were often completely destroyed or vacuolated, and

heterogeneous inclusions or vacuoles were identified in the cytoplasm instead of them. Similar data were obtained in mammals in a dose of 40 mg/kg [11]. In erythroblastic cells, ribosomes and polyribosomes were degraded, mitochondria, the cytoplasm was homogenized, chromatin was destroyed in the nuclei, the nuclei were often deformed. The number of reticulocytes increased in the field of vision.

After three-day zinc intoxication, in most bone marrow cells, regardless of the range to which they belong, various changes in the structure of the nucleus were noted: sometimes this manifested itself in the condensation of chromatin in the nucleus; sometimes, on the contrary, in the destruction of chromatin and the clarification of the nucleus, the separation of the filamentous component of the nucleolus, the destruction of the nucleoli. Cells often determined karyopicnosis - shrinkage of the nucleus (Figure 2).

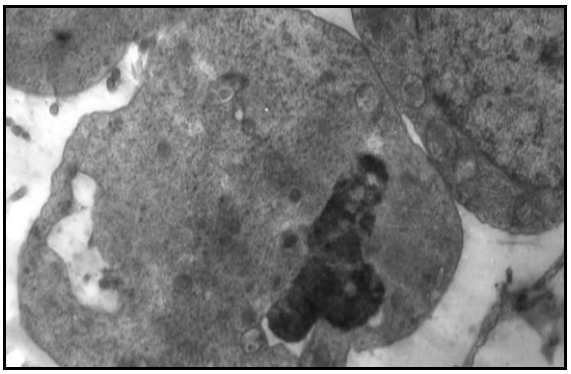

Figure 2: Karyopicnosis in the bone marrow of a chicken 3 days after zinc intoxication in a dose of 60 mg/kg. Electronogram. Magnification ×10000

By the fifth day in the animals of the first test group, the maximum content of the total number of red bone marrow cells was 46.36% higher than the background values ($p < 0.001$); In the future, this index decreased to 56.67% ($p < 0.001$) up to 45 days, and by the end of the study it was noted that it increased to control values. In the second test group, the total cell count reached a maximum on the seventh day, which is 5.26% higher than that in intact poultry; By the end of the 45th day, we detected a sharp decrease in them by a factor of 2.0 ($p < 0.001$), and by the end of the second month this indicator is at the level of 59.65% of the control ($p < 0.01$).

If we talk about the change in the cellular composition of various hematopoiesis, the picture is as follows: in the first test group, the day after the exposure, the concentration of all cells in the marrow punctate was at the level of background values, in the second - a sharp decrease in the number of cells lymphoid (at 14,2 times, $p < 0.001$) and reticuloendothelial (9.3 times, $p < 0.001$) groups; the increase was noted only in the cells of the granulation group (by 21.25%, $p < 0.01$), all other cells were below the control values by 15.93 - 55.40% ($p < 0.05$).

Later the following changes occur in the number of cells of different groups: the concentration of the cells of the granulation group in both experimental groups varies in the same way as the total number of cells; the content of erythroid cells in the first group changes wavy with the maximum peak on the seventh day (56.62% higher than the background values, $p < 0.001$) and the minimum - by 45th (28.54%, $p < 0.01$); in the second group throughout the whole experiment, below the values in intact animals and by the 60th day is 1.169 ± 0.0060 million in 1 mm3, which is 43.48% lower than the control indices ($p < 0.001$).

The absolute content of cells of the lymphoid group in both the first and second test groups was lower than in intact birds throughout the study, and to its end in the first group was 59.21%, and in the second group - 31.40% of control animals ($p < 0.001$).

In the first week of the life of the chickens, the number of cells of the monocyte group increased in the first group to 0.151 ± 0.0018 million/mm^3, and in the second to 0.121 ± 0.0011 million/mm^3 ($p < 0.001$), by the 15th day a sharp decrease to 0.047 ± 0.0005 and 0.045 ± 0.0007 million/mm^3, respectively ($p < 0.001$), and

by the 60th day there was a restoration of their quantity, however, these values did not reach the initial values.

One day after exposure to zinc chloride in animals of both the first and second experimental groups, the main parameters of the peripheral blood remain at the level of background values. In the following week, we detected an increase in red blood cells and hemoglobin in both groups and leukocytes in the second ($p < 0.001$); up to 15 days the number of platelets increases and exceeds the control values by 19.11% ($p < 0.01$). At the end of the study, the concentration of erythrocytes is below the values for intact birds in the first test group by 14.29% ($p < 0.05$), by 28.57% - in the second ($p < 0.01$); the hemoglobin content approaches the initial values; platelets - in the first test group at the control level, in the second trial group - by 26.65% lower ($p < 0.01$); of the leukocytes was lower by 17.41% in the first test group ($p < 0.01$) and 34.30% in the second ($p < 0.001$).

From the side of the leukocyte formula with increasing dose we found the following changes: a significant decrease in the concentration of eosinophils, basophils, monocytes, lymphocytes and an increase in stab-and segmented pseudo-eosinophils was observed.

RESULTS AND DISCUSSION

Thus, the results of the study showed that zinc poisoning of chickens even within 1 to 3 days leads to damage to bone marrow cells. In the cytoplasm of the hematopoietic and stromal cells of the bone marrow, the signs of destructive processes are revealed mostly, which increase with the duration and dose of the exposure. In this case, the structure of the cell nuclei often changes in the bone marrow. Identifying morphological signs indicate a decrease in the transcription of ribosomal RNA genes in bone marrow cells. In the bone marrow, the number of basophilic erythrocaryocytes increases and the number of hemoglobinized forms of erythrocytes decreases. Zinc mostly causes disturbances in morpho-functional structures in erythroblasts and mature cells of other bone marrow cell lines. With an increase in the dose of zinc chloride, we have established more profound changes in the red bone marrow and peripheral blood in the bird. Zinc chloride affects the cells of monocytic and megakaryocytic groups.

Showed that *in vitro* and *in vivo* zinc compounds in significant concentrations induced the appearance of chromosomal aberrations in human and animal lymphocytes. However, the entry of zinc into cells at concentrations significantly higher than physiological levels (> 200 mg/l) promotes the growth of transplanted tumors and carcinogenesis, and at concentrations below 7 mg/l Zn inhibits carcinogenesis and tumor growth. [12]

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Zinc chloride in doses of 40 mg/kg and 60 mg/kg during acute exposure causes accumulation in the red bone marrow of mature cells with signs of destructive processes in the nucleus and cytoplasm. With an increase in dose, cells with signs of apoptosis appear. The concentration of monocytic cells decreases and the megakaryocytic group increases ($p < 0.01$).

CONCLUSION

Zinc chloride in doses of 40 mg/kg and 60 mg/kg during acute exposure causes accumulation in the red bone marrow of mature cells with signs of destructive processes in the nucleus and cytoplasm. With an increase in dose, cells with signs of apoptosis appear. The concentration of monocytic cells decreases and the megakaryocytic group increases ($p < 0.01$).

Ethical approval: All applicable international guidelines for the care and use of animals were followed

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REFERENCES

[1] Gupta DK, Chatterjee S, Datta S, Voronina AV, Walther C. Radionuclides: accumulation and transport in plants. In: Reviews of Environmental Contamination and Toxicology 2016;Springer, Cham, 241:139-160.
[2] Hegazy AK, Faisal M, Alatar AA, Kabiel HF, Emam MH. Induced mutagenesis and genotoxicity by accumulated radionuclides in some edible plants cultivated in black sand soil detected by RAPD and SDS-PAGE. Fresenius Environ Bull 2015; 24:343-354.
[3] Xiong J, Liu X, Cheng QY, Xiao S, Xia LX, Yuan BF, Feng YQ. Heavy metals induce decline of derivatives of 5-methycytosine in both DNA and RNA of stem cells. ACS ChemBiol 2017; 12(6):1636-1643.
[4] Anke M, Groppel B, Krause U, Arnhold W, Langer M. Trace element intake (zinc, manganese, copper, molybdenum, iodine and nickel) of humans in Thuringia and Brandenburg of the Fed. Rep. of Germany. Journal of trace elements and electrolytes in health and disease 1991: 5(2):69-74.
[5] Lebedev SV, Rakhmatullin SG, Sizova EA, Kwan OV. The elemental status of the body of broiler chickens on the background of various nutrient security. Proceedings of the Orenburg State Agrarian University 2008; 4:10-15. (In Russian)
[6] Mudgal V, Madaan N, Mudgal A, Singh RB, Mishra S. Effect of toxic metals on human health. The Open Nutraceuticals Journal 2010; 3(1):94-99.
[7] Steinfeld H, Wassenaar T, Jutzi S. Livestock production systems in developing countries: status, drivers, trends. Rev Sci Tech 2006; 25(2):505-516.
[8] Von Braun, J., & Kennedy, E. T. (1994).Agricultural commercialization, economic development, and nutrition.Published for the International Food Policy Research Institute [by] Johns Hopkins University Press.
[9] Apaydın, F. G., Baş, H., Kalender, S., &Kalender, Y. (2016). Subacute effects of low dose lead nitrate and mercury chloride exposure on kidney of rats. Environmental toxicology and pharmacology, 41, 219-224.
[10] Karzakova, L.M. (2005). Zinc deficiency induces FAS-mediated apoptosis. Medical Immunology, 7 (4).
[11] Fedorina, T. A., Nadeev, V. P., Chabaev, M. G., &Yakhin, A. Ya.(2013). Histological structure of internal organs when feeding chelates of copper, iron, manganese, zinc and selenium. Proceedings of the Nizhnevolzhskyagrouniversity complex: science and higher professional education, 1 (2-1 (30)).
[12] Cai, L., & Cherian, M. G. (1996). Adaptive response to ionizing radiation-induced chromosome aberrations in rabbit lymphocytes: effect of pre-exposure to zinc, and copper salts. Mutation Research/Genetic Toxicology, 369(3-4), 233-241.