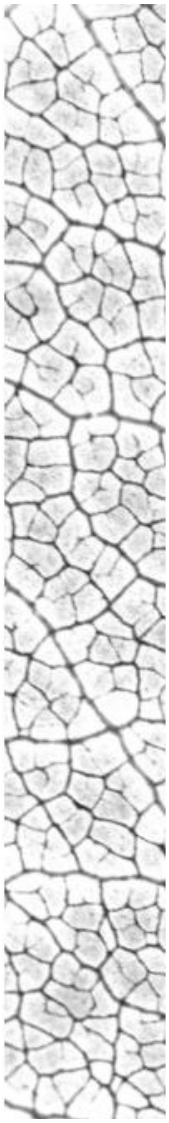

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Effect of lead on the viability of red blood cells and amniotic fluid cells of white rats

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Abstract
The unfavorable ecological state of the environment at present is as a result of man-made human activity. Among the main pollutants of the habitat at present time is recognized the lead, which has a polytropic effect on the organism. With the help of morphometric and statistical methods, the viability has been explored of red blood cells and amniotic fluid cells of control and experimental animals which are subjected to lead acetate toxicity. To identify the belonging of amniotic fluid cells to stem cells (SC), we carried out their cultivation in the special environment «Amniokar» of the company LLC PanEco Company, Russia. Studies have been carried out using an Axio Imager.M2 digital microscope (ZEISS, Japan) and a Countess ™ automatic cell counter (Invitrogen, USA). The effect of lead acetate led to the formation of aggregates of erythrocytes of blood of the type of «coin columns», to decrease in the concentration of living erythrocytes and their viability. The study of amniotic fluid under intoxication conditions has showed that only the squamous epithelium cells remained in the suspension which has been obtained as a result of centrifugation kept squamous cells only. Lead acetate leads to a decrease in the number of viable red blood cells in the blood, as well as to an increase in their aggregation properties.

Keywords: cerebellar cortex, cortex, lead acetate, nuclear cytoplasmic ratio (NCR)

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INTRODUCTION

The unfavorable ecological state of the environment at present is as a result of man-made human activity (Karrari et al. 2012, Kasimov and Vlasov 2018). Among the main pollutants of the habitat at present time is recognized the lead, which has a polytropic effect on the organism (Chiodo et al. 2004, Wang et al. 2005, Pillai 2012, Melnikova et al. 2013, Shubina and Dudenkova 2016, Shubina et al. 2018, Ryzewski et al. 2013, Eremenko 2013).

By means of method of colouring with theTrypan Blue and automatic cell counter the Countess ™ (Invitrogen, USA), has been determined the decrease of viability of red blood cells and amniotic fluid cells of white rats under the action of lead acetate. The obtained results can be made use in the process of rapid diagnosis of the effects of adverse factors on the organism in veterinary science and medicine.

RESEARCH METHODS

The animals were killed by decapitation under ether anesthesia with chloroform (1:1) in compliance with the principles of humanity as set out in the directives of the European Community (86/609/EES) and the Declaration of Helsinki and in accordance with the rules of carrying out the works using experimental animals.

The pubescent outbread albino male rats weighing 200-250 g were used as a biological test object.

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with the principles of humanity as set out in the directives of the European Community (86/609/EES) and the Declaration of Helsinki and in accordance with the rules of carrying out the works using experimental animals. The pubescent outbred albino male rats weighing 200-250 g have been used as a biological test object. In line with the research objectives, the animals have been divided into two groups. The control group of animals has been the male rats and the female rats (on the 18th day of pregnancy) contained on the common regime of the vivarium.

Experimental group included the males and the females that received within 7 days of oral acetate lead

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$Pb(CH_3COO)_2 \times 3H_2O$ in intermediate toxicity dose of 45 mg/kg/ per day.

The material of the study was blood, which has been collected from the heart with a syringe with an anticoagulant heparin (30 IU / ml of blood). After collecting the amniotic fluid (2.5 ml) from pregnant States), it has been determined the viability of red blood cells and amniotic fluid cells of white rats. To identify the belonging of the cells of the amniotic fluid to the stem cells, we have carried out cultivation) of isolated cell suspension in the Amniokar medium by the company PanEco Company LLC, Russia.The Amniokar medium contains DMEM contains the medium with modifications, 10% FCS (fetal calf serum), factors of cell growth in the optimal concentration for each part of FCS, hormones (Chestkov et al. 2014). female rats, it has been realized cell suspension with using a Centrifuge CM-6M centrifuge. (10 min, 220 g). Using the trypan blue staining method and an automatic Countess ™ cell counter (Invitrogen, United States), it has been determined the viability of red blood cells and amniotic fluid cells of white rats. To identify the belonging of the cells of the amniotic fluid to the stem cells, we have carried out cultivation) of isolated cell suspension in the Amniokar medium by the company PanEco Company LLC, Russia.

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The initial passage of 1.0 ml of cell suspension of amniotic fluid we have carried out in 7 ml of medium «Amniokar» in Petri's cups. The cultivation of the cells has been carried out in a climatic chamber at 37 °C for 10 days. On the 5th day, 1/2 volume of the culture medium has been diluted with the fresh Amniokar medium. The photography of blood products and colonies of amniotic fluid cells has been performed by means an Axio Imager.M2 digital microscope (ZEISS, Japan) with the software for image analysis AxioVision SE64 Rel. 4.8.3 and ZEN 2011 and an integrated digital camera with enlargement of 100×10. The resolution of the obtained images – 2048×1536 pixels.

Statistical processing of digital data was performed using the FStat and Excel program codes. Testing of statistical hypothesis was carried out by Student's t-test. When testing statistical hypotheses, the accepted significance points were $p \leq 0.05$.

RESULTS AND DISCUSSION

The influence of lead acetate on cell viability has been estimated by the following indicators:

1) total concentration of cell;
2) concentration of living cells;
3) concentration of dead cells;

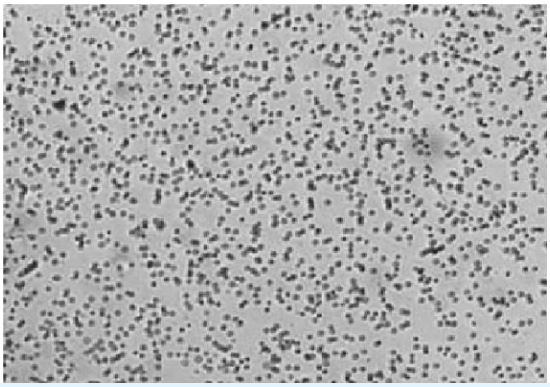

Fig. 1. Erythrocytes in the blood plasma of white rats (control). Colouring of trypan blue. Zooming 100×10

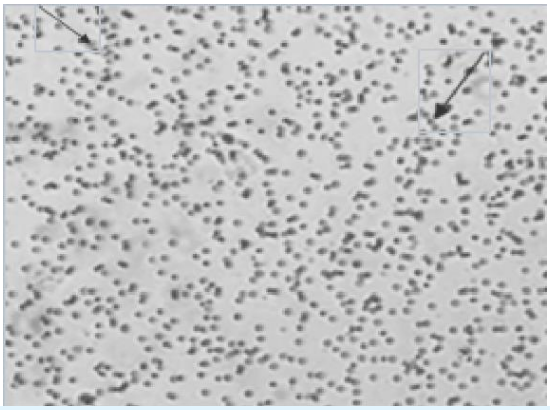

Fig. 2. Erythrocytes in the blood plasma of white rats (experiment). «Coin columns» are shown with the arrow the red blood cells Colouring of the rypan blue. Zooming 100×10

4) viability of cells (% of living cells of their total number).

The studied material we have painted with a mixture of saline solution and trypan blue (1:4) on a slide and examined with a Countess ™ automatic cell counter (Invitrogen, USA). Living cells trypan blue paints on the edges, the dead uniformly paints along the entire cell.

Statistical processing of digital data has been performed using the FStat and Excel programme codes. Testing of statistical hypothesis has been carried out by Student's t-test. When testing statistical hypotheses, the accepted significance points have been $p \leq 0.05$.

By installations of researches of red blood cells of control animals, it has been found that they are located freely in the thickness of the plasma, and they are not aggregated (**Fig. 1**).

After 7 days of action of lead acetate in the blood of experimental animals, it has been obtained the formation of aggregates of erythrocyte by the «coin columns» type (**Fig. 2**).

Morphometric studies have shown that in the experimental group of animals as compared with the control, there is an increase in the total concentration of red blood cells in the blood by 9.58% ($p \leq 0.01$). At the

Table 1. Quantitative and qualitative indicators of erythrocytes in the blood of male white rats ($x \pm s_x$)

Index	Control	Experiment
Total oncentration of red blood cells in the blood, $\times 10^9$/ml	4.28±0.15	4.69±0.17*
Concentration of living rythrocytes in the blood, $\times 10^9$/ml	3.86±0.13	2.60±0.11*
Concentration of dead erythrocytes in the blood, $\times 10^9$/мл	0.42±0.08	2.09±0.12**
Viability of red blood cells, %	90.17±2.25	55.45±1.79*

Note: * – p≤0.01 as compared to control animals; ** – p≤0.001 as compared to control animals

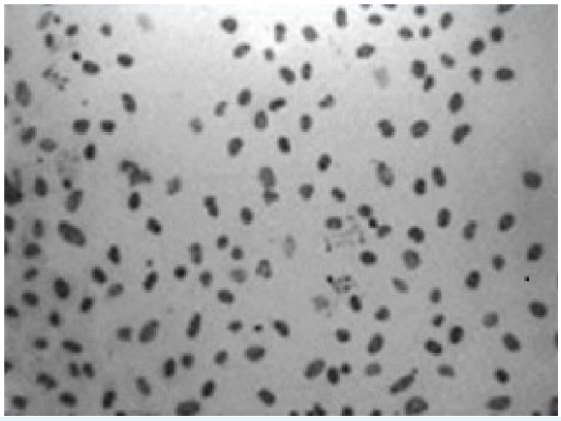

Fig. 3. Primary cultures of amniotic fluid cells consisting of squamous epithelial cells, ibroblast-like and small rounded cells. Colouring of the trypan blue. Zooming 100×10

Fig. 4. Primary cultures of amniotic fluid cells: a colony of cells the tenth day of cultivation in the Amniokar's medium. Zooming 100×10

same time, the concentrations of dead erythrocytes are increased almost 5 times (p≤0.001) as compared to their control and by 32.64%. (p<0.01) the concentration of living erythrocytes is decreased. Overall, in the experimental group it has been marked the decrease in the overall viability of red blood cells by 38.50% (p≤0.01) (**Table 1**).

Microscopic research of the cell suspension from the amniotic fluid of control animals has been shown that it consists of a heterogeneous population of cells, most of

Table 2. Quantitative and qualitative indices of the amniotic fluid cells of female white rats ($x \pm s_x$)

Index	Control	Experiment
Total concentration of amniotic fluid of cells, × 10^5 /ml	5.52±0.31	4.77 ± 1.01
Concentration of living cells of amniotic fluid, × 10^5 /ml	1.24±0.21	0.82 ± 0.01
Concentration of dead cells of amniotic fluid, × 10^5 /ml	4.28±0.54	3.95 ± 0.21
Viability of cells of amniotic fluid, %	23.09±4.01	14.32 ± 2.96

Note: * – p≤0.01 as compared to control animals; ** – p≤0.001 as compared to control animals

which represent cells of the flat epithelium, except for them are marked fibroblast-like and small rounded cells (**Fig. 3**), which is confirmed by previously obtained data in the study human amniotic fluid (Baranov 2007).

Cultivations in the Amniokar's medium for 10 days in Petri's cups allowed us to grow a colony of primary cells of the amniotic fluid (**Fig. 4**).

Morphometric studies have shown that in the experimental group of pregnant female rats, as compared with the control, happens decrease in the total concentration of amniotic fluid cells by 14.00% (p≤0.01), while the concentration of dead cells is increased by 66.13% (p≤0.001), is decreased the concentration of living cells by 7.81% (p≤0.01). It has been marked, that decrease as compared to the control of the overall viability of the amniotic fluid cells by 62.01%. (p≤0.01) (**Table 2**).

The blood system, as the internal environment of the organism, at the same time actively participating in maintaining its homeostasis, responds one of the first to external influences, including lead compounds (Shushkevich 2007).

At present time cells of amniotic fluid, their nature, life span, differentiation potential (pluripotency / multipotency) have not been precisely established. That is because that in the AF there are a variety of cells: from terminally differentiated to the SC. Furthermore, during pregnancy, the composition of the AF (its cellular component) may affect (De Coppi et al. 2008).

In our studies, an attempt has been made to establish the influence of lead acetate on the viability of red blood cells and amniotic fluid cells. As is known, the life span of highly specialized cells of multicellular organism is limited. Their death occurs naturally at the expense of processes of apoptosis (Logue et al. 2013).

As a result of impact of adverse factors, including lead acetate, pathological processes characteristic of necrosis are developed at the cellular level, and the number of viable cells in tissues and fluids of organism can significantly decrease (Bokara 2008).

The ascertainment of such a fact can be direct and accurate evidence of the negative impact of the studied factor on the organism. However, in the available literature, in the presence of various studies on the effect of lead and its salts on the cells and tissues of the organism, there is no data on the direct effect of lead on their viability. These emerged contradictions can be

explained by the fact that, until relatively of recently time, researchers had to determine the level of cell viability of liquid tissues and cultures under a microscope, which is a laborious operation, which does not always provide reliable data because of the small size of the objects of study and the characteristics of the study itself. The possibility of using modern equipment allowed us to solve this problem and ensured the reliability of results of our research.

Impact of acetate of lead on adult white rats at an average toxic dose of 45 mg/kg/day, after 7 days, causes as compared with the control: Increase by almost 5 times ($p \leq 0.001$) of the concentration of dead erythrocytes and by 32.64% ($p \leq 0.01$) decrease of the concentration of living erythrocytes. In general, the experimental group has been shown decrease in the overall viability of red blood cells by 38.50% ($P \leq 0.001$), and also to increase in their aggregation properties; Decrease of the total concentration of cells of amniotic fluid by 14.00% ($p \leq 0.01$), while the concentration of dead cells is increased by 66.13% ($p \leq 0.001$), the concentration of living cells decreases by 7.81% ($p \leq 0.01$). It has been marked, that decrease as compared with the control of the overall viability of cells of amniotic fluid by 62.01% ($p \leq 0.01$) that points to negative effect of lead acetate on the reproductive system.

CONCLUSION

Exposure to lead acetate in Mature white rats at an average toxic dose of 45 mg/kg/day. after 7 days causes compared to control

1. An increase of almost 5 times ($p \leq 0.001$) the concentration of dead red blood cells and a 32.64% ($p \leq 0.01$) decrease in the concentration of living red blood cells. In General, the experimental group showed a decrease in the overall viability of red blood cells by 38.50% ($p \leq 0.001$), as well as an increase in their aggregation properties.

2. Reduction of the total concentration of amniotic fluid cells by 14.00% ($p \leq 0.01$), while increasing the concentration of dead cells by 66.13% ($p \leq 0.001$), decreases the concentration of living cells by 7.81% ($p \leq 0.01$). A decrease of 62.01% in the total viability of amniotic fluid cells was observed in comparison with the control ($p \leq 0.01$), indicating a negative effect of lead acetate on the reproductive system.

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REFERENCES

Baranov VS (2007) Cytogenetics of human embryonic development. Sankt-Peterburg: 639.

Bokara KK, Brown E, McCormick R, Yallapragada PR, Rajanna S, Bettaiya R (2008) Lead-induced increase in antioxidant enzymes and lipid peroxidation products in developing rat brain. BioMetals 21(1): 9-16. https://doi.org/10.1007/s10534-007-9088-5

Chestkov VV, Tabakov, VYu, Schepkin YuV (2014) Amniocen – proliferative environment for mesenchymal cells. Advances in molecular Oncology 2: 74-75.

Chiodo LM, Jacobson SW, Jacobson JL (2004) Neurodevelopmental effects of postnatal lead exposure at very low levels. Neurotoxic. Teratol 26(3): 359-371. https://doi.org/10.1016/j.ntt.2004.01.010

De Coppi P, Soker S, Atala A (2008) Stem cells derived from amniotic fluid and placenta. Principles of Regenerative Medicine. Burlington. Elsevier Academic, 226-237.

Eremenko I.R (2013) The effect of lead intake to rats in the milk period on the concentration of lipids in the cerebral hemispheres and cerebellum in the prepubertal period of ontogenesis. Dal'nevostochnyj medicinskij zhurnal 3: 108–111.

Karrari PA, Mehrpour O, Abdollahi M (2012) A systematic review on status of lead pollution and toxicity in Iran; Guidance for preventive measures. Daru 20(1): 2-12. https://doi.org/10.1186/1560-8115-20-2

Kasimov NS and Vlasov DV (2018) Heavy metals and metalloids in urban soils of russian cities (according to the annual reports of rosgidromet). Moscow University Bulletin. Series 5. Geography 3:14-22.

Logue SE, Cleary P, Saveljeva S, Samali A (2013) New directions in ER stress-induced cell death. Apoptosis 18: 537-546. https://doi.org/10.1007/s10495-013-0818-6

Melnikova NA, Shubina OS, Dudenkova NA, Lapshina MV, Liferenko OV, Timoshkina OI (2013) A study of the viability of cells when exposed to lead acetate on the organism of rats. Modern problems of science and education, 5. Available from: http://www.science-education.ru/111-10588. Accessed on 19-08-2019

Pillai P, Pandya C, Bhatt N (2012) Biochemical and reproductive effects of gestational/lactational exposure to lead and cadmium with respect to testicular steroidogenesis, antioxidant system, endogenous sex steroid and cauda-epididymal functions. Andrologia 44(2): 92-101. https://doi.org/10.1111/j.1439-0272.2010.01109.x

Ryzewski BYa, Lebedko OA, Eremenko IR (2013) Influence of lead in the dairy period of ontogenesis on morphometric, histochemical and biochemical parameters of brain development. Far Eastern medical journal 4: 98-105.

Shubina OS and Dudenkova NA (2016) The effect of lead on the process of spermatogenesis in sex glands of male albino rats. Veterinary World 9 (10):1129-1134. https://doi.org/10.14202/vetworld.2016.1129-1134.

Shubina OS, Egorova MV, Dudenkova NA, Bardin VS (2018) Study of viability of stem cells of amniotic fluid of white rats. XXII international scientific and practical conference «Trends and innovations of modern science». Scientific Publishing Center Apriori, Krasnodar

Shushkevich NI (2007) Influence of lead on izmerenie blood counts. Vestnik RUDN. Ecology and life safety series 1: 49-53.

Wang WG, Wang YM, Feng LD (2005) Effect of plumbism on auditory brainstem response of children. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zh 12: 896-898. http://dx.doi.org/10.1016/j.bjorl.2016.12.004