DOI 10.26724/2079-8334-2023-4-86-198-202
UDC 616.4;612 43;61.0422.062;57

Yu.B. Ismayilov, T.A Salimli[1], A.T.Ismayilova, Z.Sh.Iskandarova, G.K.Jafarova
Scientific Research Center of Azerbaijan Medical University, Baku, Azerbaijan
[1]ASATID named after A. Aliyev, Baku, Azerbaijan

THE EFFECT OF LEAD ACETATE ON THE NEURO-IMMUNO-ENDOCRINE REGULATION OF THE ORGANISM AND THE NUMBER OF HEMATOLOGICAL MARKERS

e-mail: mic_amu@mail.ru

Changes in the cell composition of the blood of rats that occurred under the effect of toxic stress caused by lead acetate have been studied. The blood cells of the control group animals corresponded to the physiological norm because they were kept under optimal conditions. However, the number of blood cells in the rats exposed to the toxic effect caused by lead acetate changed sharply. The toxic stress condition changes the number of specific and non-specific cells that provide the immune response of the organism in different directions. Depending on the duration of the toxic stress caused by lead acetate, there are various directional changes in the number, percentage composition, and distribution density of hematological indicators in the blood, and the mechanisms that ensure the homeostasis and hemostasis of the body are disturbed. Therefore, it is important to conduct research aimed at developing ways to correct toxic stress caused by intensive environmental pollution.

Key words: extreme condition, stress, heavy metal, monoamine, hormone, immunoreactivity

Ю.Б. Ісмайлов, Т.А Салімлі, А.Т. Ісмайлова, З.Ш. Іскандарова, Г.К. Джафарова

ВПЛИВ АЦЕТАТУ СВІНЦЮ НА НЕЙРО-ІМУНО-ЕНДОКРИННУ РЕГУЛЯЦІЮ ОРГАНІЗМУ ТА ЧИСЛО ГЕМАТОЛОГІЧНИХ МАРКЕРІВ

Вивчено зміни клітинного складу крові щурів, що виникають під дією токсичного стресу, спричиненого ацетатом свинцю. Клітини крові тварин контрольної групи відповідали фізіологічній нормі, оскільки утримувалися в оптимальних умовах. Однак кількість клітин крові у щурів, які зазнали токсичної дії ацетату свинцю, різко змінилося. Стан токсичного стресу змінює кількість специфічних і неспецифічних клітин, що забезпечують імунну відповідь організму у різних напрямках. Залежно від тривалості токсичного стресу, викликаного ацетатом свинцю, відбуваються різноспрямовані зміни кількості, відсоткового складу та щільності розподілу гематологічних показників у крові, порушуються механізми, що забезпечують гомеостаз та гемостаз організму. Тому важливо проводити дослідження, створені задля розробку способів корекції токсичного стресу, викликаного інтенсивним забрудненням довкілля.

Ключові слова: екстремальні стани, стрес, важкий метал, моноамін, гормон, імунореактивність.

The study of various aspects of the unified neuro-immuno-endocrine system is one of the urgent issues of experimental medicine. The nervous, immune, and endocrine systems act interconnected and ensure the normal functioning of the body [10, 15]. These three systems maintain the stability of the body's internal environment by “serving the triangle of homeostasis” [9, 11, 12].

There are different opinions about the mechanisms of pathologies that arise from the negative impact of the environment on the neuroendocrine and immune systems [2, 3]. When heavy metals enter living organisms, they act as toxic agents with a wide range of effects in the environment for an extended period of time [6]. The most dangerous elements for human health are considered lead, cobalt, cadmium, mercury, etc. [3, 6, 12–15]. These elements are not only highly toxic but also have a high ability to accumulate in the body, and when they move from one environment to another, they have a toxic effect by changing their chemical state. Inadequate amounts of heavy metals entering the body, not only cause toxic stress, they can also lead to various pathologies, including damage to the central nervous, endocrine, and immune systems [13].

As a result of intense environmental pollution caused by these agents, a number of new diseases appear [1–3, 5–7, 15]. Thus, serious functional disorders develop in the body, and a person becomes vulnerable to toxic stress due to relevant optimal conditions. A positive correlation was detected between blood lead levels and immune cells in preschool children living near heavy metal waste areas [7]. The latest WHO reports show that now more than 25 % of diseases are caused by environmental factors, including heavy metals.

The blood system, which plays an important role in homeostasis, is characterized by a certain stability, and its cellular composition shows high sensitivity to the influence of endogenous and exogenous factors. On the other hand, the human body can respond to the negative effects of various factors of the environment by the mechanism of adaptive functional regulation and the change of the cell composition of the blood system [3, 4, 5, 8, 11]. When the body performs defense reactions, formed

elements of the blood play a major role, providing specific and non-specific cellular immunity to the body. Moreover, hemopoiesis under the influence of toxicants has been poorly studied and the obtained data are contradictory. The authors conducting this research correctly noted that the background of long-term and intense toxic stress was the main reason for the unsatisfactory course of various diseases [1, 7, 10, 15].

Since the changes in the number of hematological markers under the influence of heavy metals have not been comprehensively studied, it is important to carry out research in this direction. Thus, it is critical to develop methods for the prevention and correction of the disturbance of interactions between disorders of the neuro-immuno-endocrine system and hematological markers in people living under the influence of toxic stress.

The purpose of the study was to assess the changes in the neuro-immuno-endocrine system and hematological markers under the toxic stress caused by lead acetate in rats.

Materials and methods. The study was performed on 35 female rats of the Wistar line weighing 250±8 g. The animals were divided into 7 groups (5 rats in each group) and kept in the vivarium in special cages of 75x40x40 cm size under standard laboratory conditions following sanitary-hygienic rules. The research was conducted following the principle of the International Bioethical Committee Declaration of the European Union (Strasbourg, March 18, 1986) on the protection of animals used for experiments and other scientific purposes and the rules approved by the Ethical Commission of the AMU (protocol No. 1, 3.09.20).

The 1st group of rats was in an intact state (control), the 2nd, 3rd, and 4th groups of animals were per os administered a 0.4 % lead acetate solution (per 100 g of live weight) for 7, 14, and 21 days, respectively [13]. In the 5th, 6th, and 7th groups of rats, 7, 14, and 21 days after the administration of the same dose, respectively, the administration was ceased for 7, 14, and 21 days. Then, the recovery process was detected. The same amount of the physiological solution was given to the 1st group of animals. In order to investigate the effect of toxic stress caused by lead acetate on the dynamics of hematological parameters, after the 7th, 14th, and 21st days of the experiment, as well as 7, 14, and 21 days after stopping the administration, the rats were decapitated in the morning according to modern recommendations [12] and hematological examination was performed. The number of leukocytes (WBC), lymphocytes (LYM), middle-sized cells (MID), granulocytes (GRA), erythrocytes (RBC), platelets (PLT), and hemoglobin (RBC); the percentage composition of lymphocytes (LYM,%), middle-sized cells (MID, %) and granulocytes (GRA, %); hematocrit (HCT) level, mean corpuscular (MCV) volume, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW–SD), percentage distribution of erythrocyte cells by size (RDW–CV), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), and percentage of platelets to large cells (P–LCR) were determined. Examinations of hematological markers in the blood were carried out using the automatic RAYTO (RT–7600)–AUTO Hematology Analyzer RAYTO-RT–7600 made in China using a disposable vacuum blood collection tube containing EDTA.

The statistical significance of the differences was calculated using Microsoft Office Excel software applications, according to the Student's t-test and the nonparametric Wilcoxon-Mann-Whitney U-test. The statistical difference was considered significant at a value of $P<0.05$.

Results of the study and their discussion. According to the results, the weight of the animals exposed to toxic stress caused by lead acetate decreased dramatically throughout the experiment compared to the control group. This decrease was greater at the end of the experiment. The neurochemical balance of the dopaminergic and serotonergic systems in the hypothalamus and blood was disturbed and hormonal activity changed its direction, the body's immune reactivity weakened due to the toxic effect of lead acetate . The toxic stress caused by the effect of lead acetate drastically changed the level of humoral immune system markers in the body. The effect of lead acetate was found to cause deep pathologies in the neuro-immuno-endocrine system.

Such changes in the neuro-immuno-endocrine system also affected hematological indices. Thus, the results of the performed study revealed that compared to the control, after the 7th day of lead acetate administration, the number of leukocytes in the blood increased by 33.0 % ($p>0.05$), but after the 14th day, it decreased by 26.7 % ($p<0.01$), and after the 21st day of the experiment, it increased sharply to 141.53 % ($p<0.001$). After the toxic effect of lead acetate was stopped, the number of leukocytes in the blood increased by 83.3 %, 163.1 %, and 182.2 % ($p<0.001$), respectively (Table 1).

Table 1

Dynamics of changes in the number of hematological markers under the effect of lead acetate in the blood of rats

Statistic indices / Hemato-logical indices	Control	Lead acetate administration period			Period after stopping lead acetate administration		
		Days of experiments					
		7	14	21	7	14	21
	M±m	M±m	M±m	M±m	M±m	M±m	M±m
WBC(10^9 /L)	6.96±0.45	9.25±0.09^	5.10±0.60**	16.81±1.68*	12.75±1.46^	18.31±1.06*	19.63±1.70*
LYM(10^9 /L)	3.74±0.3	5.58±0.10*	5.58±0.18 * *	7.36±0.52*	8.68±0.16*	6.92±0.18*	13.26±1.40*
MID(10^9 /L)	0.66±0.62	0.85±0.05	0.56±0.09	1.19±0.21**	0.86±0.16	1.03±0.06×	1.67±0.16*
GRA(10^9 /L)	2.68±0.62	3.22±0.17	2.84±0.39	5.88±0.27^	3.66±0.72	11.12±0.26*	5.95±0.40^
LYM(%)	56.86±2.71	60.42±1.07	52.64±1.12	40.4±2.25**	65.26±4.97 *	40.28±1.09* *	67.80±0.86 *
MID(%)	8.96±1.16	8.70±0.37	5.46±0.27 **	7.28±0.12	6.64±0.22	6.54±0.39	7.46±0.12
GRA(%)	34.4±6.14	32.9±1.44	38.14±2.74	49.16±0.70**	27.54±3.14	57.42±2.29^	26.9±0.31
RBC(10^12 /L)	7.12±0.16	6.95±0.25	5.68±0.32^	5.72±0.26^	7.20±0.26	6.75±0.11	8.06±0.14^
HGB(g/L)	133±3.18	124.0±2.92	107.4±4.80^	100.0±1.52*	119.4±0.93^	116.8±1.88^	152.8±1.28*
HCT(%)	37.7±0.86	34.9±0.54	30.66±1.00*	28.54±0.87*	34.28±0.54^	33.14±0.94^	43.5±0.44*

Note: Significant at the level of P*<0.001 ;P^<0.01 ; P* * <0.05

Thus, toxic stress changed the number of leukocytes in the blood in two phases.

In rats exposed to lead acetate, the number of middle cells in the blood compared to the control decreased only after the 14th day of the experiment, as did leukocytes. On other days, the number of middle cells in the blood increased. On the contrary, the number of lymphocytes in the blood increased during the entire experiment.

Certain regularities were also observed in the number of granulocytes in the blood. Although its number slightly increased on the 7th and 14th days compared to the control, after the 21st day, this number increased sharply and became equal to 119.5 %, $p<0.05$. In the groups of animals where the administration of lead acetate had stopped, the number of granulocytes increased reliably on the 14th and 21st days (315.2 % and 122.0 %) but not on the 7th day (increased by 36.6 %).

Compared to the control, the percentage of lymphocytes in the experimental groups increased by only 6.3 % after the 7th day of the experiment, but decreased between 7.4 % and 18.6 % on the 14th and 21st days, respectively. In the groups of animals where the administration of lead acetate had stopped, although it decreased by 29.2 % ($p>0.05$) after the 14th day, it increased by 14.8 % after the 7th day, and by 19.2 % after the 21st day ($p>0.05$).

Although the percentage content of middle-sized cells decreased slightly at the beginning of the toxic stress period compared to the control, it decreased by 39.1 % on the 14th day. However, after the 21st day, the percentage of MID cells increased by 43.1 % ($p>0.05$).

In contrast, the percentage of MID cells in the blood decreased by 25.9 %, 27.0 %, and 16.7 % in the groups of animals where the administration of lead acetate had stopped.

Certain regularities were also found in the percentage composition of granulocytes. Thus, in the experimental groups, it decreased slightly on the 7th day compared to the control but increased by 11.1 % after the 14th day, and by 19.7 % on the 21st day ($p>0.05$). Certain changes occurred also in the groups after stopping the exposure to lead acetate.

Thus, the percentage composition of granulocytes decreased by 19.9 % and 21.7 % ($p>0.05$) on the 7th and 21st days, respectively. However, after the 14th day, its percentage composition in the blood increased sharply and became equal to 67.1 % ($p>0.05$).

Due to toxicity, the number of erythrocytes in the blood decreased by 2.4 %, 20.2 %, and 24.8 % $p>0.05$ during the experiment. In the groups where the administration of lead acetate had stopped, the number of erythrocytes in the blood changed weakly on the 7th and 14th days. In contrast, on the 21st day, this parameter was 13.1 % more than the control.

Similar regularities were present in the hemoglobin concentration. Corresponding changes were also observed in the level of hematocrit. Thus, the hematocrit level decreased during all experimental days compared to the control. Only 15.4 % improvement was achieved in the group 21 days after stopping lead acetate administration, $p<0.001$ (Figs. 1, 2).

The mean corpuscular hemoglobin volume did not significantly differ from the control in almost all groups (either during or after lead acetate administration).

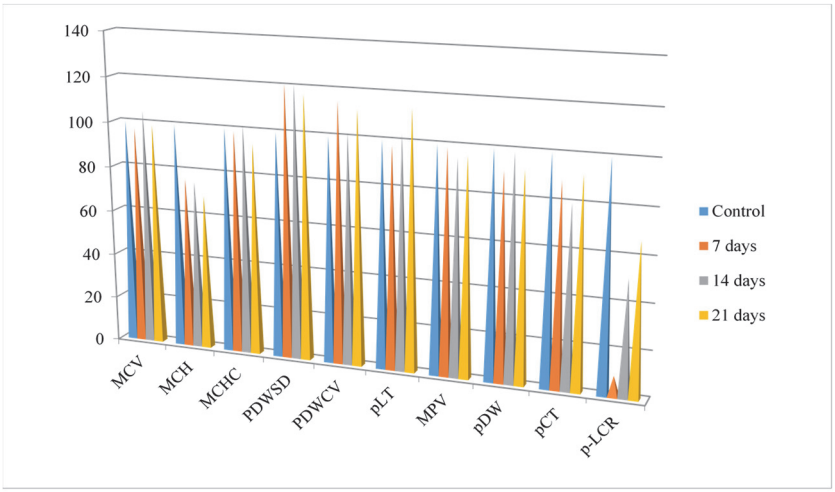

Fig. 1. The dynamics of changes in the number of some hematological markers (%) in the blood of rats under the influence of lead acetate. Note: Significant at the level of P<0.001

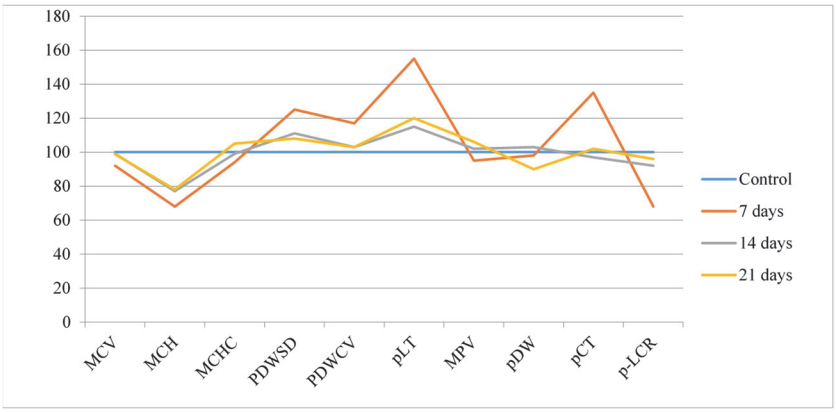

Fig. 2. The dynamics of changes (%) in the number of some hematological markers in the blood of the rats after stopping the exposure to lead acetate

Note: Significant at the level of P<0.001

Different results were obtained in the mean corpuscular volume. Compared to the control, during the periods of 7, 14, and 21 days of lead acetate administration, the mean corpuscular volume in the blood decreased by 23.5 %, 25.2 %, and 30.7 %, respectively ($p<0.05$). Its reduced level was not restored in the groups where the administration of lead acetate had stopped.

Slight changes in the concentration (volume) of the average corpuscular hemoglobin were observed. Thus, compared to the control, this concentration practically changed weakly during and after the period of toxic stress.

In addition, certain regularities were detected in the difference between the smallest and largest erythrocytes in the blood sample (the standard deviation of erythrocytes–RDW–SD). Compared to the control, this parameter increased in all experimental groups. Although the percentage distribution of erythrocyte cells by size (RDW–CV) in comparison with the control did not change practically on the 14th day of the experiment, it increased slightly (14.8 % and 12.0 %) after the 7th and 21st days. Although this parameter increased (16.8 %) on the 7th day of the experiment in the group where the administration of lead acetate had stopped, it practically did not change on the other days of the experiment.

Compared to the control, during the administration of lead acetate, the number of platelets in the blood practically did not change on the previous days of the experiment, and on the 21st day, it increased to a certain extent and equaled 13.6 % ($p<0.01$). In the groups where the administration of lead acetate had stopped, compared to the control, the number of platelets increased according to the days: 55.3 %; 14.6 %, and 19.7 %, $p<0.05$. However, the average volume of platelets and the distribution density of platelets changed little in practically all groups and no significant differences were obtained.

Certain regularities were detected in PCT. Compared to the control, during the entire period of administration of lead acetate, the PCT in the blood decreased (11.2 %, 19.7 %, and 7.7 %). However, compared to the control, in the groups where the administration of lead acetate had stopped, this parameter increased by 34.8 % after the 7th day of the experiment, but the following days it practically did not change.

Compared to the control, during the administration of lead acetate, the percentage of platelets to large cells in the blood decreased by 81.3 % on the 7th day of the experiment, by 49.4 % on the 14th day of the experiment, and by 33.4 % on the 21st day. However, in the groups where the administration of lead acetate had stopped although the percentage of platelets to large cells decreased by 32.3 % compared to the control on the 7th day after the period of lead acetate administration, it changed slightly in the following days of the experiment.

Our experiments revealed that since intact animals were kept under standard laboratory conditions according to sanitary and hygienic rules, the blood cells corresponded to the physiological norm [1]. However, in rats exposed to toxic stress, the number of peripheral blood cells, the number of specific and non-specific cells that provide the immune response of the organism, and the activity of inflammatory and anti-inflammatory interleukins changed in different directions throughout the experiment [3].

In the process of evolution, mechanisms are formed that ensure homeostasis and hemostasis of the body against stress effects of various origins. Because of the acute impact of the toxic factor on the composition of blood cells, the body's homeostasis is disrupted as special mechanisms mobilize the induction of hemopoiesis under physiological and extreme conditions [2]. Such an extreme situation changes the functions of various physiological processes, mobilizes defense mechanisms, and leads to the development of a general adaptation syndrome. However, depending on the intensity of the toxic stress affecting the organism, due to certain changes in the neuro-immuno-endocrine functions, the immune reactivity weakens, and the hemostasis of the body as well as the stability of the homeostasis is disturbed. Thus, the toxic effect of lead acetate is a stress that produces a noticeable reaction in the direction of the cellular composition of the whole blood. The biochemical reciprocity in the activity of monoamines changes during and after the toxic stress effect caused by lead acetate [11]. Due to this effect, the neuro-immuno-endocrine system of the body is characterized by deep pathologies. It is appropriate to investigate ways of correcting the central regulation mechanism of such pathology, considering that one of the important functions of cytokines, which are central regulators of homeostasis, is to ensure the regulated communication of the immune, endocrine, and nervous systems [9]. However, under toxic stress, since there are disturbances in this connection mechanism, the body's neuro-immuno-endocrine regulation mechanism is completely disrupted. According to a group of authors, heavy metal toxicity affects innate and adaptive immunity, immune response, and cytokine production [8, 15].

Conclusion

Thus, the activity of hormones and immune markers in the blood changes depending on the duration of the toxic stress caused by lead acetate because it disrupts the mechanism of interaction between monoaminergic systems. The mechanisms that maintain the homeostasis and hemostasis of the body are disturbed due to various directional changes in the number, percentage composition, and distribution density of hematological indices in the peripheral blood. Therefore, it is appropriate to carry out studies aimed at developing methods of correcting the toxic stress caused by xenobiotics. It is also critical to examine the immune and hormonal-mediator status of people exposed to toxic substances in heavy metal-contaminated areas.

References

1. Arzhakova LI, Garmayeva DK, Dmitriyeva TI, Fedorova AI, Belolyubskaya DS, Afanasyeva OG, et al. Pokazateli periphericheskoy krovi pri eksperimentalnom kholodovom vozdeystvii. Yakut Medikal Journal. 2018; 2: 12–14. doi: 10.25789/YMJ.2018.62.03/22 [in Russian]

2. Bulgakova SV, Romanchuk NP. Immunnyy gomeostaz: novaya rol mikro- I makroelementov, zdorovoy mikrobioty. Bulletin of Science and Practice 2020; 6 (10): 206–233. https://doi.org/10.33619/2414-2948/59 [in Russian]

3. Dmukhal'skaya YeB. Sostoyaniye immunnoy sistemy u krys raznogo vozrasta pri deystvii tverdykh metallov i raundapa. Universum: khimiya i biologiya : elektron. nauchn. zhurn. 2018; 9 (51). URL: https://7universum.com/ru/nature/archive/item/6327 [in Russian]

4. Korneva YeA. Puti vzaimodeystviya nervnoy i immunnoy sistem: istoriya i sovremennost, klinicheskoye primeneniye. Meditsinskaya immunologiya. 2020; 22(3): 405–418. https//doi.org/10.15789/1563-0625PON,-1974 [in Russian]

5. Romanchuk PI. Zdorovaya mikrobiota i naturalnoye funktsionalnoye pitaniye: gumoralnyy i kletochnyy immunitet. Bulletin of Science and Practice. 2020; 6(9):127–166. doi: 10.33619/2414-2948/58/14 [in Russian]

6. Cao J, Xu X, Zhang Y, Zeng Z. Increased memory Tcell populations in Pb-exposed children from an e-waste-recycling area. Sci Total Environ. 2018 Mar, p.616–617. doi:10.1016/j.scitotenv.2017.10.220

7. Doeselaar L, Yang H, Bordes J, Brix L, Engelhardt C, Tang F, et al. Chronic social defeat stress in female mice leads to sex-specific behavioral and neuroendocrine effects. Stress. 2021 Mar;24(2):168–180. doi: 10.1080/10253890.2020.1864319.

8.Ebrahimi M, Khalili N, Razi S, Keshavarz-Fathi M, Khalili N, Rezaei N. Effects of lead and cadmium on the immune system and cancer progression. J Environ Health Sci Eng. 2020 Feb 17;18(1):335–343. doi: 10.1007/s40201-020-00455-2.

9.Engert V, Linz R, Grant JA. Embodied stress: The physiological resonance of psychosocial stress. Psychoneuroendocrinology. 2019 Jul; 105:138–146. doi: 10.1016/j.psyneuen.2018.12.221.

10. Jalsrai A, Biswas A, Suslov NI, Martin JV. Neuropsychopharmacological profile of Astragalus membranaceous var. mongholicus. Journal of Traditional Chinese Medical Sciences, 2019, 6(3): 254–262. https://doi.org/10.1016/j.jtcms.2019.08.002

11. Huo J, Dong A, Niu X, Dong A, Lee S, Ma C, et al. Effects of cadmium on oxidative stress activities in plasma of freshwater turtle Chinemys reevesii. Environ Sci Pollut Res Int. 2018 Mar;25(8):8027–8034. doi: 10.1007/s11356-017-1139-z.

12. Ismayilov YB, Ismayilova AT, Jafarova QK, Salimli TA, Garayeva SQ. The effect of heavy metal on the hormonal-mediator status. Karadeniz 1. Uluslararası multidisipliner çalışmalar konqresi, Giresun. 2019; 39–44.

13. Rafique S, Gillani SS, Nazir R. Lead and Cadmium Toxic Effects on Human Health: A Review. J Nutr Food Sci. 2021; 11: 459 p.

14.Shatornaya VF, Nefyodova OO, Nefyodov OO, Kolosova II, Major VV, Kuznetsova OV, et al. Cardiogenesis changes after the plumbic acetate impact in rats under the correction conditions in the experiment**.** World of Medicine and Biology. 2020; 4(74): 219–223. doi:10.26724/2079-8334-2020-4-74-219-223

15. Veiga-Fernandes H, Artis D. Neuronal-immune system cross-talk in homeostasis. Science. 2018 Mar 30;359(6383):1465–1466. doi: 10.1126/science.aap9598.

Стаття надійшла 25.11.2022 р.