DOI 10.29254/2077-4214-2023-1-168-325-330
UDC 591.481.3

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MORPHOLOGICAL CHANGES OF THE PINEAL GLAND OF RATS UNDER CHRONIC INTOXICATION WITH LEAD AND CORRECTION WITH SODIUM SELENITE

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Pollution of the environment with lead is an important ecological problem of today, which negatively affects the health of the population and leads to desynchronosis, violation of protective and compensatory mechanisms, adaptation processes and the occurrence of diseases. The pineal gland is one of the organs of the central endocrine system that ensures adaptation and maintains the body's homeostasis. The purpose of the study was to study the morphological changes of the pineal gland under the conditions of intoxication with lead acetate and correction with sodium selenite.

The study was conducted on 18 male Wistar laboratory rats, which were divided into three groups. Animals of the 1st group were kept under normal vivarium conditions without the influence of additional factors. Animals of the 2nd group were simulated lead acetate intoxication daily for 30 days by intragastric administration at a dose of 0.3 mg/kg. For 30 days, animals of the 3rd group were simulated lead acetate intoxication and intraperitoneally administered sodium selenite (Na2SeO3) at the rate of 0.2 mg/kg. The animals were removed from the experiment on the next day after the last administration of lead acetate.

Histological examination of the pineal gland of experimental animals after a 30-day intoxication with lead acetate revealed signs of swelling and structural rearrangement of cells in the peripheral zone of the parenchyma. Morphological changes were due to the presence of a large number of pinealocytes with an increased volume of optically transparent cytoplasm and the presence of cysts of various sizes filled with a light optically transparent liquid, which indicates the development of hydropic dystrophy. As it approached the central part of the parenchyma, the volume of the cytoplasm of pinealocytes and cysts decreased, which was confirmed by the results of morphometric measurements. The nuclei of light pinealocytes, which were at different stages of necrosis, also underwent morphological changes. It was determined that sodium selenite reduces the toxic effect of lead acetate on pinealocytes, promotes the restoration of the cellular composition of the parenchyma, synthetic and secretory activity of pinealocytes, reduces the signs of their edema and the manifestation of dystrophic-necrotic changes.

Key words: *rats, pineal gland, lead, selenium, dystrophy*

Connection of the publication with planned research works.

The present work is part of the research topic of the Department of Anatomy, Clinical Anatomy, Operative Surgery, Pathomorphology and Forensic Medicine of the Petro Mohyla Black Sea National University of the Ministry of Education and Culture of Ukraine «The influence of ecologically dangerous factors on the mechanisms of development of diseases of civilization and their correction with physiologically active substances», state registration number 0122U002033.

Introduction.

The rapid development of heavy and chemical industry led to a progressive increase in the level of environmental pollution and a rapid deterioration of the ecological situation in Ukraine and the world, which was further complicated by the influence of military actions [1]. Thus, the detonation of shells, the use of artillery weapons, aerial bombs and rockets led to the release of a significant amount of heavy metals into the air and their impact on the body, causing a number of toxic effects, the development of pathological processes and the emergence of environmentally dependent diseases. One of the most widespread and toxic heavy metals present in the environment is lead and its salts. It is known that lead intoxication is accompanied by signs of desynchronosis, violation of protective and compensatory mechanisms, adaptation processes, which leads to the occurrence of diseases [2]. One of the organs of the central endocrine system, which is actively involved in adaptation processes and ensures the maintenance of homeostasis and the start of the stress reaction, is the pineal gland [3]. However, insufficient attention has been paid to the histological changes of the pineal gland under the conditions of exposure to lead. The issue of pharmacological correction of pathological conditions of the pineal gland caused by the harmful effects of lead remains unresolved.

According to the literature, it is known that the ability to neutralize the harmful effects of heavy metal salts is inherent in selenium compounds, in particular sodium selenite [4, 5, 6], since selenium is an important cofactor of key enzymes involved in providing antioxidant protection [7, 8]. The mechanism of the protective effect of selenium is due to its interaction with salts of heavy metals with the subsequent formation of biologically inactive selenides, which accumulate in the form of insoluble compounds [5, 9]. In modern literary sources, the histopathological changes of various organs under the conditions of intoxication with salts of heavy metals and correction with selenium are described in sufficient detail. However, there are no works devoted to changes in the morphology of the pineal gland under conditions of chronic lead intoxication and selenium correction, which prompted us to conduct our own research.

The aim of the study.

To study the morphological changes of the pineal gland under conditions of chronic intoxication with lead acetate and correction with sodium selenite.

Object and methods of research.

The study was conducted on 18 adult, sexually mature male Wistar rats with a body weight of 200-240 g. The animals were kept in standard vivarium conditions under natural lighting and had free access to food and drinking water. The experimental animals were divided into 3 groups: a control group and two experimental groups. The animals of the (control) group were kept under normal vivarium conditions without the influence of additional factors. The animals of the second group were simulated lead acetate intoxication daily for 30 days by intragastrically administration at a dose of 0.3 mg/kg [10]. Rats of the third group were simulated lead acetate intoxication for 30 days and injected with sodium selenite (Na_2SeO_3) in order to correct the intoxication syndrome at the rate of 0.2 mg/kg of body weight [8, 11]. Sodium selenite was dissolved in 0.9% NaCl solution and administered intraperitoneally [5].

To study the morphological changes in the pineal gland, rats were removed from the experiment on the next day after the last administration of lead acetate by decapitation under thiopentane anesthesia at the rate of 25 mg/kg of body weight intraperitoneally. After the decapitation procedure was completed, the brains of the animals were removed and fixed in a 10% solution of neutral formalin. After isolated of the pineal gland, dehydration was carried out in alcohols of increasing concentration, after which the material was embedded in paraffin, sections with a thickness of 4-6 μm were made on a rotary microtome of the semi-automatic type «Microm» (Germany) and stained with hematoxylin-eosin according to the standard method. After the sections were embedded in Canadian balsam, the preparations were studied under a microscope brand «Carl Zeiss» (Germany) at a magnification of objective lens x20, ×40 and ocular lens x10. Photo documentation of the research results was made using a Canon digital SLR camera.

The number of pineal cells and cysts in the field of view of the microscope (objective ×20, binocular ×10) was counted on the histological preparations of the pineal gland. The counting of the investigated structures was performed by analogy with Goryaev's counting chamber. When counting cells/cysts, Yegorov's rule was used, namely, they were counted in the field of view and along the border of the upper and right sectors, while the studied structures located along the lower and left border of the field of view of the microscope were not taken into account. In order to obtain statistically significant results, the number of cells/cysts in 10 fields of view of the microscope was determined.

Morphometric measurements were carried out using a micrometer eyepiece screw type MOV 1-16 at magnification (x40). The area of cysts, nucleus and cytoplasm was calculated using the formula: $S=\pi rR$, where: r is a small radius; R is a large radius. Large and small diameters of cysts, nucleus and cytoplasm of light and dark pineal cells were measured. Since the nucleoli had a rounded shape, their area was determined using the formula for calculating the area of structures having the shape of a circle: $S=\pi r^2$. The nuclear-cytoplasmic index was calculated according to the formula: NCI = Sn/Sc, where: Sn is the area of the pinealocyte nucleus, Sc is the area of the cytoplasm.

All the obtained data were recorded and subjected to statistical analysis, using generally accepted methods of variational statistics, namely the Student's t-test. Mathematical and statistical processing of the obtained numerical results took place on a personal computer using the standard software «STATISTICA 6» for computer equipment with the Windows operating system. For all indicators, arithmetic mean values, standard errors of the arithmetic mean, and mean square deviation were calculated, which are indicated in the work by the corresponding symbols M±m and σ. The difference was considered reliable when the difference in numerical parameters between the intact and experimental series was at a level of at least $p<0.05$.

All stages of the research were carried in accordance with the general principles of work with experimental animals in accordance with the following standards: Convention on Bioethics of the Council of Europe (1997); European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, General Ethical Principles of Animal Experiments, approved by the First National Congress of Ukraine on Bioethics (2001); Law of Ukraine «On Protection of Animals from Cruelty» (2006) and other international agreements and current national legislation in the field of medical and biological research.

Research results and their discussion.

During the histological study of histology specimens of the pineal gland of intact animals, it was established that the pineal gland had a typical structure. From the outside, the pineal gland is covered with a thin capsule of connective tissue. Numerous membranes separated the parenchyma of the pineal gland into lobes of different sizes. Connective tissue membranes contained blood and lymphatic vessels. The content of the lobules was represented by pinealocytes of two types: light and dark. Light pinealocytes were characterized by a rounded or oval shape and light cytoplasm. Nuclei and nucleoli were very well visualized in light cells. It should be noted that light cells were the most numerous. Dark cells differed from light ones by significantly smaller morphometric parameters, their nuclei always looked intensely basophilic due to the high content of heterochromatin, which made it impossible to identify nucleoli. It was established that the percentage of light cells was 84.12±0.32% of the total number of cells, and the percentage of dark cells was 15.88±0.68%. The number of pineal cells in one field of view of the microscope was 224.6±8.7 cells. Another type of cells that belong to astrocytic glia and, unlike pinealocytes, perform a supporting function are glial cells. Gliocytes on the preparations were diffusely located in the parenchyma.

During histological examination of the pineal gland of experimental animals after 30-day intoxication with lead acetate, significant deviations in the morphology of the pineal gland were observed compared to animals of the control group. We found signs of edema in the peripheral zone of the parenchyma, as well as changes in the structure of pineal cells in the peripheral region **(fig. 1)**.

Morphological changes were due to the presence of a large number of pinealocytes with an increased volume of optically transparent cytoplasm and the presence of cysts of various sizes filled with light optically transparent liquid. Thus, if in the control group of animals the number of cysts in one field of view of the microscope was 8.2±3.4, then in the group of animals ex-

posed to lead intoxication the number of cysts increased to the level of 32.6±5.8 ($p<0.05$). It should be noted that the light-colored pinealocytes of the peripheral region of the parenchyma of the organ looked swollen due to an increase in morphometric parameters, mainly due to an increase in the area of the cytoplasm **(fig. 2)**. It was determined that the average values of the cytoplasm area of light pinealocytes under physiological conditions were 51.89±1.72 μm^2. On the next day after the last administration of lead acetate, the average area of the cytoplasm of light cells of the peripheral region of the parenchyma was 83.54±6.37 μm^2, which exceeded the indicator of the control group by 60.99% ($p<0.05$).

Changes in the density of placement of pinealocytes in the parenchyma of the gland were also revealedIt was found that the number of pineal cells in one field of view of the microscope decreased relative to the intact and amounted to 176.5±10.6 ($p<0.05$) cells. The decrease in the density of the location of pinealocytes can be explained by the presence of a large number of cells with an increased volume of cytoplasm and cysts, due to which the distance between neighboring cells increased. It was established that the accumulation of pinealocytes with an increased volume of cytoplasm and large cysts was observed mainly in the subcapsular and peripheral zones of the parenchyma of the pineal gland. As it approached the central part of the parenchyma, the volume of the cytoplasm of pinealocytes and cysts decreased. It was found that the average area of the cytoplasm of light cells of the central zone of the parenchyma was 66.62±3.45 μm^2, which is less than the average area of the cytoplasm of the cells of the peripheral zone of the pineal gland by 20.25% ($p<0.05$). The average area of cysts in the peripheral region was 61.46±1.82 μm^2, which was 25.61% higher than the average area of cysts in the central region, which was 45.72±2.39 μm^2 ($p<0.05$). It should be noted that the number of cysts also decreased compared to the peripheral zone of the pineal gland. In the peripheral zone, the number of cysts was 26.0±7.2, and in the central zone – 6.6±3.8. It was found that in some cases, vacuolization of the cytoplasm was accompanied by the rupture of the plasmolemma, as a result of which the lobe was completely filled with cytoplasmic fluid, in which the released nuclei floated which lost their structure and underwent either partial or complete lysis. The detected morphological changes indicate the development of hydropic dystrophy, which turns into necrosis with the formation of cysts. The presence of a large number of vacuolated cells and cysts in the peripheral zone of the parenchyma indicates a delay in hormone secretion.

Upon further study of the epiphysis sections, it was found that the light-stained nuclei of pineal cells were concentrated in the peripheral regions compared to the pinealocytes located in the central areas of the parenchyma. The degree of basophilia of light

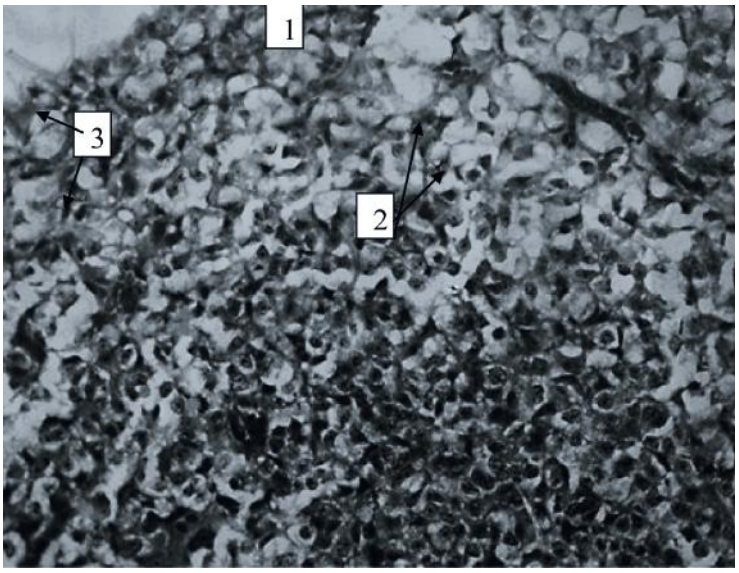

Figure 1 – A fragment of the pineal gland of a rat after a 30-day intoxication with lead acetate. Staining with hematoxylin and eosin. Magnification ×200. Symbols: 1 – edema of the peripheral zone of the parenchyma; 2 – light pinealocytes with vacuolated cytoplasm; 3 – cysts.

cell nuclei is determined by the different content of heterochromatin and indicates different functional activity of the cells. It should be noted that in vacuolated pinealocytes, the nuclei had an oval or rounded shape and occupied either central positions or were pushed by the vacuole to the periphery of the cell. There were nuclei of light pinealocytes that were at different stages of mitosis. We found pinealocytes with signs of karyorrhexis and karyolysis, which are manifested by a decrease in the size of the nucleus, discoloration of the nuclear substance and nucleolus, and the formation of «shadows» of the nuclei with their subsequent dissolution. Most of the nuclei were in a state of karyorrhexis, which is evidenced by a decrease in their average area.

Based on the results of the morphometric measurements, it was established that the average area of the nuclei and nucleoli of the peripheral and central regions also differed from the similar indicators of the control

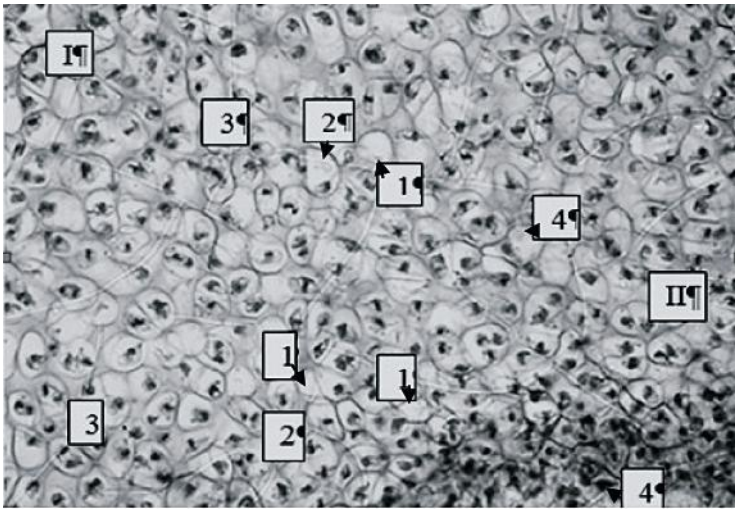

Figure 2 – Fragment of the peripheral zone of the rat pineal gland after 30-day intoxication with lead acetate. Staining with hematoxylin and eosin. Magnification ×400. Symbols: I – subcapsular zone: 1 – cyst; 2- hydropic dystrophy; 3 – cells at different stages of mitosis; 4 – lysis of nuclei and nucleoli of light pinealocytes; II – zone close to the central part of the parenchyma of the pineal gland: 1 – decrease in the size of cysts; 2 – decrease in the volume of cytoplasm of light cells; 3 – mitosis; 4- glial cell.

Table – Changes in the average morphometric parameters of pinealocytes under the conditions of intoxication with lead acetate and correction with sodium selenite (M±m)

Group	Cytoplasmic area (μm²)	Cell nucleus area (μm²)	Nucleoli area (μm²)
Light pinealocytes			
Control	51,89±1,72 n=50	22,76±0,62 n=50	3,08±0,14 n=50
Lead acetate intoxication (peripheral zone)	83,54±6,37* n=50	18,55±1,38* n=50	2,52±0,23* n=50
Lead acetate intoxication (central zone)	66,62±3,45* n=50	23,38±0,46 n=50	3,10±0,19 n=50
Correction with sodium selenite	60,79±2,35*/# n=50	20,94±0,53*/# n=50	3,02±0,41 n=50
Dark pinealocytes			
Control	29,88±0,93 n=30	14,76±0,47 n=30	Not defined
Lead acetate intoxication (peripheral zone)	28,35±0,27 n=30	13,62±0,53 n=30	Not defined
Lead acetate intoxication (central zone)	29,13±1,06 n=30	13,11±0,69 n=30	Not defined
Correction with sodium selenite	31,27±0,86/# n=30	15,78±0,38# n=30	Not defined

Notes: * significantly with control (p<0.05) # significantly with a group of animals exposed to lead acetate intoxication (peripheral zone) (p<0.05)

group. It was established that the average area of the nucleus of light cells of the peripheral region decreased by 18.5% (p<0.05), the area of the nucleolus by 18.19% (p<0.05) **(table)**. By similar measurements, it was established that the average parameters of light pinealocytes in the central zone of the pineal parenchyma do not undergo significant changes compared to the control. Thus, the average area of the nucleus increases by 2.72%, and the area of the nucleolus by 0.65%.

It is necessary to note changes in the ratio between dark and light pinealocytes compared to the control group of animals. It was determined that the number of light cells increased by 8.95%, and the number of dark cells decreased by the corresponding percentage in animals that were under the conditions of 30-day intoxication with lead acetate. It was established that the percentage of light cells in the experimental group was 93.07±1.18%, and the percentage of dark cells decreased to the level of 6.93±0.36. The detected changes in the ratio of cells indicate the transformation of dark cells into light, which is a manifestation of adaptation to maintain the synthesizing function of the organ. The morphometric parameters of dark cells did not undergo significant changes relative to the control group, but tended to decrease **(table)**.

Histological analysis of the pineal gland of experimental animals exposed to lead acetate and corrective therapy with sodium selenite for 30 days showed a decrease in the intensity of morphological changes compared to a group of rats that were not exposed to the corrector. A decrease in the number of light pinealocytes by 3.84% and their number approaching the level of the intact group was revealed. It was established that the number of light pineal cells was equal to 89.23±1.38%, and the number of dark cells increased to 10.77±0.86%. An increase in the total number of pinealocytes in each of the studied fields of view of the microscope was also noted. Thus, if the number of pinealocytes in the field of view of the microscope was 176.5±10.6 in the rats that were under the conditions of intoxication, then in the group of animals that were under the conditions of correction, the number of cells increased to 202.1±6.8, approaching according to the control indicators, which indicates the reparation processes.

The protective effect of sodium selenite is also indicated by a decrease in the number of cysts and vacuolated cells, which is a sign of the normalization of hormone secretion **(fig. 3)**. It was found that the number of cysts in the peripheral area decreases by 45% compared to the group that was under the conditions of lead acetate intoxication and is 14.3±2.9. The average size of cysts in the peripheral region decreased by 32.85% compared to the first experimental group and amounted to 41.27±1.36 μm² (p<0.05). The number and sizes of cysts in the central zone of the parenchyma practically did not change compared to the group of rats that were not exposed to selenium. The number of cysts was 7.1±2.5 and the average area was 42.68 ± 1.86 μm².

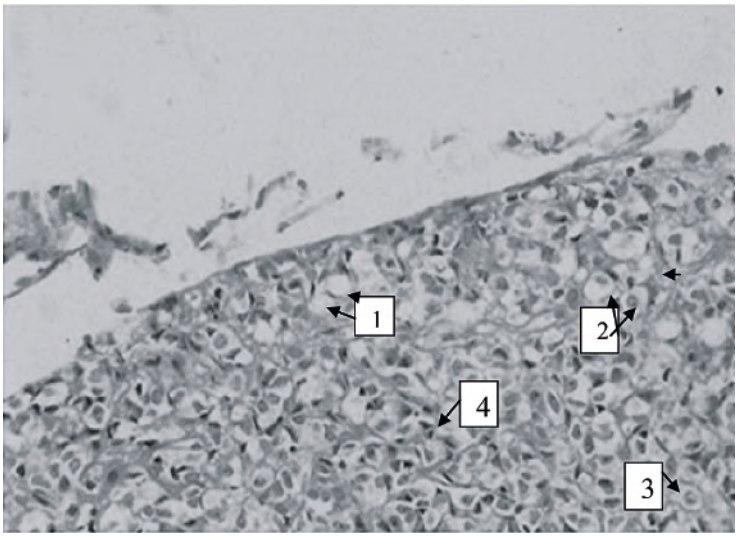

Figure 3 – Fragment of rat pineal gland parenchyma after 30-day intoxication with lead acetate and correction with sodium selenite. Staining with hematoxylin and eosin. Magnification ×200. Symbols: 1 – cysts; 2- cells with an increased volume of cytoplasm; 3 – lysis of nuclei and nucleoli of light pinealocytes: 4 – dark pinealocytes.

The data obtained during the morphometric analysis of pineal cells of this group indicate an increase in the functional and synthetic activity of light pinealocytes compared to light cells of the peripheral region of animals that were under intoxication conditions. It was found that the average area of the nucleus of light pinealocytes increased by 8.00% (p<0.05), while the cytoplasm, on the contrary, decreased by 27.23% (p<0.05). The obtained digital data are close to the control indicators **(table)**. It should be noted that the light pinealocytes of both the peripheral and the central zone of the pineal gland of animals of this group almost do not differ in the degree of vacuolization. Comparing the data of the morphometric indicators of dark cells with the similar indicator of the intact group, we found a tendency to increase the average area of the cytoplasm and nucleus and a prob-

able increase in the parameters relative to the first experimental group **(table)**.

Conclusions.

Chronic exposure to lead acetate leads to a morphological restructuring of the peripheral and subcapsular region of the parenchyma of the pineal gland, which is manifested by a decrease in the number of pinealocytes, the appearance of cysts, vacuolization of the cytoplasm of varying degrees, and damage to the plasmalemma membranes. The identified features indicate an increase in dystrophic and dystrophic-necrotic changes. Sodium selenite reduces the toxic effect of lead acetate on pineal cells, helps to restore the synthetic and secretory activity of pinealocytes, reduces signs of edema and dystrophic changes, which is confirmed by a decrease in the number of cysts by 45%, their average area by 32.85% ($p<0.05$), a change in the morphometric indicators of pinealocytes and their approach to the control indicators, as well as an increase in the number of cells by 14.56%, which indicates the restoration of the cellular composition of the parenchyma. Sodium selenite is a potentially useful agent for the pharmacological correction of pathological changes in the pineal gland caused by chronic exposure to lead acetate.

Prospects for further research.

In the future, it is planned to investigate the condition of the vascular bed of the epiphysis during intoxication with heavy metal salts and correction of sodium selenite.

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МОРФОЛОГІЧНІ ЗМІНИ ЕПІФІЗУ ЩУРІВ ЗА УМОВ ХРОНІЧНОЇ ІНТОКСИКАЦІЇ СВИНЦЕМ ТА КОРЕКЦІЇ СЕЛЕНІТОМ НАТРІЮ

Пшиченко В. В., Черно В. С., Чеботар Л. Д., Юлевич О. І., Шевчук Н. П.

Резюме. Бурхливий розвиток промисловості призвів до прогресуючого збільшення рівня забруднення навколишнього середовища та впливу на організм значної кількості важких металів. Свинець та його солі є одним із найбільш поширених і токсичних важких металів присутніх у довкіллі. Інтоксикація свинцем супроводжується ознаками десинхронозу та порушенням адаптаційних процесів. Оскільки епіфіз активно залучається до адаптаційних процесів і забезпечує підтримання гомеостазу, метою дослідження було вивчення морфологічних змін епіфіза за умов інтоксикації ацетатом свинцю та корекції селенітом натрію.

Дослідження проведено на 18 щурах-самцях лінії Wistar вагою 200-240 г. Тварини I (контрольної) групи перебували за звичайних умов віварію без впливу додаткових факторів. Тваринам II групи моделювали інтоксикацію ацетатом свинцю щоденним упродовж 30 діб інтрагастральним уведенням у дозі 0,3 мг/кг. Тваринам III групи протягом 30 діб моделювали інтоксикацію ацетатом свинцю та вводили внутрішньоочеревинно селеніт натрію (Na_2SeO_3) з розрахунку 0,2 мг/кг.

Як показав аналіз отриманих результатів, спостерігається виражений, токсичний вплив ацетату свинцю на морфологію епіфізу, що супроводжується перебудовою периферичного та підкапсулярного регіону паренхіми органу, внаслідок розвитку гідропічної дистрофії та некрозу світлих клітин. Виявлено, що селеніт натрію забезпечує зменшення токсичного впливу ацетату свинцю на пінеалоцити, сприяє відновленню синтетичної та секреторної активності клітин, зменшує ознаки набряку та дистрофічних змін. На захисний вплив селеніту натрію також вказує збільшення загальної кількості пінеалоцитів та наближення їхньої кількісного вмісту до показників контролю, що свідчить про відновлення клітинного складу паренхіми епіфізу та зниження проявів некрозу. Селеніт натрію є потенційно корисним засобом для фармакологічної корекції патологічних змін епіфізу, обумовлених хронічних впливом ацетату свинцю.

Ключові слова: щури, епіфіз, свинець, селен, дистрофія.

MORPHOLOGICAL CHANGES OF THE PINEAL GLAND OF RATS UNDER CHRONIC INTOXICATION WITH LEAD AND CORRECTION WITH SODIUM SELENITE

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Abstract. The rapid development of industry has led to a progressive increase in the level of environmental pollution and the impact of a significant amount of heavy metals on the body. Lead and its salts are one of the most common and toxic heavy metals present in the environment. Lead intoxication is accompanied by signs of desynchronization and disruption of adaptation processes. Lead intoxication is accompanied by signs of desynchronization and disruption of adaptation processes. Since the pineal gland is actively involved in adaptation processes and ensures the maintenance of homeostasis, the purpose of the study was to study the morphological changes of the pineal gland under the conditions of intoxication with lead acetate and correction with sodium selenite.

The research was conducted on 18 male Wistar rats weighing 200-240 g. Animals of the 1st (control) group were kept under normal vivarium conditions without the influence of additional factors. Animals of the II group were simulated lead acetate intoxication daily for 30 days by intragastric administration at a dose of 0.3 mg/kg. For 30 days, animals of the III group were simulated lead acetate intoxication and intraperitoneally administered sodium selenite (Na_2SeO_3) at the rate of 0.2 mg/kg.

As the analysis of the obtained results showed, there is a pronounced, toxic effect of lead acetate on the morphology of the pineal gland, which is accompanied by the restructuring of the peripheral and subcapsular region of the parenchyma of the organ, due to the development of hydropic dystrophy and necrosis of light cells.

It was found that sodium selenite reduces the toxic effect of lead acetate on pinealocytes, promotes the restoration of synthetic and secretory activity of cells, reduce signs of edema and dystrophic changes. The protective effect of sodium selenite is also indicated by the increase in the total number of pinealocytes and the approximation of their quantitative content to the control indicators, which indicates the restoration of the cellular composition of the pineal parenchyma and the reduction of the manifestations of necrosis. Sodium selenite is a potentially useful agent for the pharmacological correction of pathological changes in the pineal gland caused by chronic exposure to lead acetate.

Key words: rats, pineal gland, lead, selenium, dystrophy.

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Conflict of interest:
The Authors declare no conflict of interest.

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A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

Received 20.08.2022
Accepted 26.01.2023