ADAPTIVE MORPHOFUNCTIONAL REARRANGEMENTS IN THE ADULT RATS ADENOHYPOPHYSIS AFTER LONG-TERM EXPOSURE OF HEAVY METAL SALTS

Hryntsova Nataliia [1], Hodorova Ingrid[2], Romanyuk Anatoly[1], Kravets Alexander[1], Rybarova Silvia[2], Lovasova Kvetuse[2], Mihalik Josef[2], Pavliuk-Karachevtseva Andriana[2], Vecanova Janka[2], Kolesar Dalibor[2], Bolekova Andriana[2], Flesarova Slavka[2]

[1]Department of Pathological Anatomy, Sumy State University, Medical Institute, Rimsky Korsakov 2, Sumy, 40007, Ukraine
[2]Department of Anatomy, LF UPJS, Kosice, Srobarova 2, Kosice, Slovak Republik

Abstract

Important environmental problem of some northern regions of Ukraine is the accumulation of heavy metal salts in the soil, water and air, which is observed in various combinations depending on the region and causes adverse effects on population's health. The central link, in particular the pituitary gland, is involved in triggering a stress response, limiting its further development preventing adverse effects on the body. The study of morphofunctional rearrangements in the pituitary gland of adult male rats under the influence of heavy metal salts complex on the body remains a relevant aspect of modern morphology. The experiment was performed on 12 white mature male rats weighing 200-250g at the age of 7-8 months, which were divided into 2 groups (control and experimental). The experimental group included rats, which after 90 days of use of a heavy metals salts complex (zinc, copper, iron, manganese, lead and chromium) for 30 days used ordinary drinking water. General morphological (histological, morphometric), mmunohistochemical, biochemical and statistical research methods were used. Results showed a decrease in the linear parameters of the adenohypophysis, there was a significant increase in the value of fibrous connective tissue component in the stroma, increased collagenization of large vessels, significant diffuse stromal edema and edematous processes in the gland parenchyma persisted. Measurements revealed an increase in the number of chromophobes and at the same time a decrease in the number of chromophilic basophils compared to control animals, the presence of cysts in the parenchyma was found out. number of morphological features still indicated the development of adaptive and compensatory processes in the adenohypophysis (vascular plethora decreased, significantly improved the rheological properties of blood, an increase in the number of adenocytes with increasing expression of hsp90 in their cytoplasm). However, despite the positive dynamics of adaptive processes, it should be noted that the 30-day period of adaptation is insufficient for complete recovery of the organ.

Keywords: pituitary gland, heavy metals, hypoxia

INTRODUCTION

Environmental contamination with some metalloids and heavy metals raises concern due to well known adverse effects on health (1). There is growing interest in the possible health threat posed by endocrine-disrupting chemicals (EDCs), which are substances in our environment, food, and consumer products that interfere with hormone biosynthesis, metabo-

Corresponding author: Nataliia Hryntsova; e-mail: natalia.gryntsova@gmail.com

©2022 Hryntsova N. et al.
This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 License (https://creativecommons.org/licenses/by-nc-nd/4.0/)

lism, or action resulting in a deviation from normal homeostatic control or reproduction. Endocrine disruptors have effects on neuroendocrinology (2). They mimic natural hormones, inhibit the action of hormones, or alter the normal regulatory function of the endocrine system (3).

Anterior pituitary hormones are central for the body homeostasis (4), are involved in triggering a stress response, limiting its further development preventing adverse effects on the body. In the modern scientific literature there is evidence of the effects on the pituitary gland of certain heavy metals salts. The effect of cadmium Cd, chromium Cr VI and arsenic As through drinking water on the hypothalamic-pituitary system of rats was studied.

Cabilla et al. in their study found out that heavy metals accumulated in hypothalamus and pituitary gland and decreased pituitary cell viability and prolactin release mostly by generation of reactive oxygen species, since it was partially prevented by antioxidant treatment. In the pituitary, they increased lipid peroxydation and the expression of several oxidative stress markers. Cell death was mainly due to caspase-dependent apoptosis. Cd stimulated the production of low levels of nitric oxide which exerted cytoprotective actions. These results showed that these heavy metals display deleterious actions in hypothalamic-pituitary physiology by altering hormone release and promoting cell death (1). he anterior pituitary gland can be a target of Cr VI toxicity in vivo and in vitro, thus producing a negative impact on the hypothalamic-pituitary-gonadal axis and affecting the normal endocrine function (4). ther US data showed a positive association between lead and testosterone levels in males, and cadmium and FSH levels in perimenopausal women (5,6). In China, a recent study found positive associations between lead and testosterone levels in men and between lead and FSH and LH levels in postmenopausal women (7). Lead exposure can affect the FSH level in postmenopausal women. Further studies are needed to evaluate the effects of low-dose long-term exposure to heavy metals on sex hormones (8).

Today, an important environmental problem of some northern regions of Ukraine is the accumulation of heavy metal salts (zinc, chromium, lead, manganese, copper and iron) in the soil, water and air, which is observed in various combinations depending on the region and causes adverse effects on population's health (9).

The purpose of the study was to elucidate the morphofunctional rearrangements the structural component changes in the pituitary gland of mature rats after the long-term influence of heavy metal salts complex (zinc, copper, iron, manganese, lead and chromium) on the body.

MATERIALS AND METHODS

The experiment was performed on 12 white mature male rats weighing 200–250 g at the age of 7-8 months, which were divided into 2 groups (control and experimental). The control group included rats that were kept in vivarium conditions similar to those of experimental animals. At the same time, a constant temperature regime was observed, as well as a natural day/night regime. As food, the control animals received granular mixed fodder, drinking – ordinary drinking water. The study was conducted in the autumn-winter period. Animals were kept and manipulated in accordance with national and international bioethics standards. The experimental group included rats, which after 90 days of use of heavy metals salts complex: zinc (ZnSO4 7H2O) – 5 mg / l, copper (CuSO4 5H2O) – 1 mg / l, iron (FeSO4) - 10 mg / l, manganese (MnSO4 5H2O) – 0.1 mg / l, lead (Pb (NO3) 2) – 0.1 mg / l and chromium (K2Cr2O7) – 0.1 mg / l, for 30 days used ordinary drinking water.

Groups of experimental animals were removed from the experiment after previous thiopental anesthesia (at the rate of 30–40 mg / 10 g of body weight) on the 120th day of the experiment (Protocol No. 8 of 17/11/2020 of the Bioethics Commission of Sumy State University). The subject of the study was the pituitary gland of experimental and control animals. For morphological studies of the pituitary gland, the organ was removed, histological preparations were made, stained with hematoxylin-eosin and Mason-Goldner were stained according to the original method (10). The absolute number of different types of adenocytes was counted in a grid by experimenters in various random fields of the adenohypophysis view (at least 5 fields from each control and experimental animal).

To calculate the level of expression of receptors for antibodies, used a semi-quantitative method. Determination of the expression of the heat shock protein marker 90 (Hsp90) was performed using an antibody panel "Thermo scientific", USA: rabbit polyclonal antibodie to the Hsp90 protein with a titer of 1:200 according to the manufacturer's recommendations. Evaluation of Hsp90 marker expression was performed by the number of stained nuclei and cytoplasm of gland cells. The result was expressed as a percentage and evaluated on a scale in the case of a positive reaction: low positive (1 point), moderately positive (2 points) and strongly positive (3 points) reaction, taking into account the number of cells and the intensity of their color. The number of HSP90-positive cells was counted in a grid by experimenters in various random fields of view of the adenohypophysis (at least 10 fields from each control and experimental animal).

The functional state of the pituitary was evaluated by determining the adrenocorticotropic hormone ACTH (pg / ml) in the serum of peripheral blood of experimental animals (by the ELISA method). A set of reagents from the Siemens was used on the Immulite 1000 Siemens Healtheare Global Immune Chemiluminescent Analyzer. General morphological analysis was performed using a light optical microscope Zeiss Primo Star with lenses x4, x10, x40, glasses 7 and 10. For the morphometric study of micropreparations, the “SCPR-2017-Zen 2 lite” software was used with photo-documentation of the results by means of “axiocam ERC 5S Zeiss” digital camcorder. Statistical processing of the obtained data was performed by parametric method of variation statistic using the software package STATISTIKA v. 10 (Stat Soft Inc., USA). Data are presented as the mean (X) ± standard deviation (SD), using the Student's t test. The error probability of less than 5% ($p \leq 0.05$) was considered sufficient.

RESULTS

Thirty days of adaptation to long-term exposure to heavy metals salts complex did not cause significant positive changes in most structural components of the experimental animals. The length and width of the pituitary gland decreased by 16.2% ($P > 0.05$) and 15% ($P < 0.05$, $t = 2.28$), respectively, compared with control animals. The thickness of the pituitary capsule significantly decreased compared to control animals by 59.6% ($P < 0.001$, $t = 6.36$), but significantly increased the value of the fibrous component of connective tissue in the stroma of the gland (Table 1).

Significant diffuse stroma edema (Mason-Goldner trichrome staining) and edematous processes in the gland parenchyma continued. The intertrabecular spaces were sharply expanded. Venous plethora was preserved, and adventitia of large vessels underwent collagenization processes (Fig. 1). At the same time, the state of rheological properties of blood has significantly improved. Blood coagulation processes have practically disappeared. Capillaries remained significantly full-blooded, in the peripheral areas of the gland they were still visualized violations of the rheological properties of blood and formation of "coin

Table 1 Indicators of experimental and control rats adenohypophysis after adaptation to long-term exposure to heavy metal salts complex (X ± SD).

Indicator	Series of animals	
	Control rats (n=6)	Experimental rats (n=6)
Pituitary length, mm	7.92±0.83	6.64±0.59
Pituitary width, mm	4.26±0.19	3.63±0.2*
Capsule thickness, μm	3.52±0.27	1.42±0.19***
Vessels area, μm²	215.83±5.96	224.3±7.96
Chromophobic cells	57.46 ± 2.58	65.15 ± 0.43 *
Chromophilic acidophiles	31.28 ± 1.71	30.2 ± 0.59
Chromophilic basophils	11.26 ± 0.06	4.65 ± 0.18 ***
ACTH (pg / ml)	591.0±1.83	319.5 ± 3.17***

Note: * p≤0.05, *** p≤0.001

columns". Vessels with local disorders (increased permeability) of the vascular wall and infiltration of a small number of erythrocytes into the extravascular space were found in some areas, with the formation of single small hemorrhages. At the same time, perivascular and pericellular edema are absent. There was an increase in the area of vascularization of the adenohypophysis compared with animals in the 90-day period of the experiment.

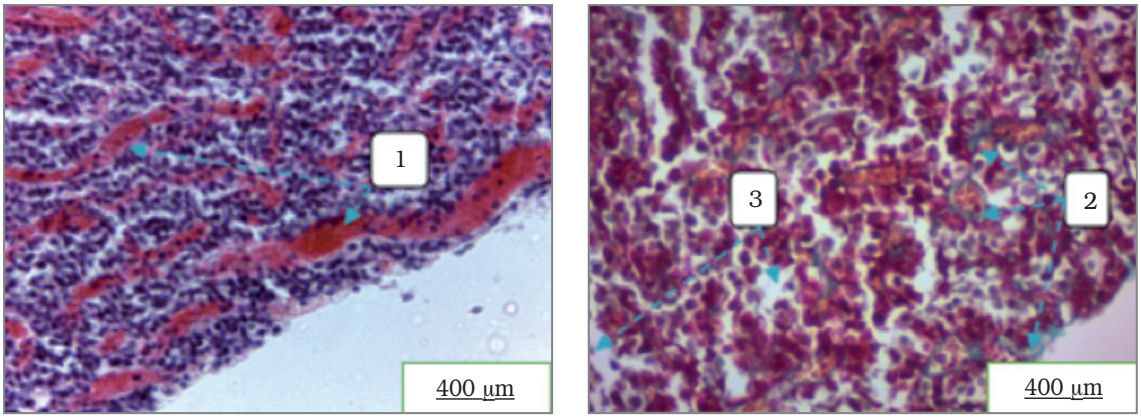


Fig. 1. Morphological rearrangements in the experimental rat pituitary gland under the condition of 30-days adaptation to heavy metals salts: 1 – venous pleural effusion; 2 – edema tissue trabeculae; 3-collagenization of the vessel walls. Staining: A – by hematoxylin-eosin; B – by Mason-Goldner.

As in the previous period of the experiment, significant edema of connective tissue trabeculae and vascular plethora led to further discomplexation of epithelial trabeculae, violation of their histoarchitectonics. Endotheliocytes continued to show signs of significant edema, their hyperchromic and hypertrophied nuclei protruding sharply into the lumen of full-blooded vessels. The area of the lumen of the vessels increased by 3.9% (P> 0.05) relative to the control animals, which can be considered a compensatory phenomenon (Table 1). In our opinion, the most obvious feature of this term of the experiment is cyst formation. Subcapsular and peripheral areas of the gland had a moderate number of cysts of various sizes (from small multiple to single large and very large). The shape of the cysts varied from round to oval. The wall of large cysts consisted of flat adenocyte cells and was filled with light oxyphilic content (Fig. 2).

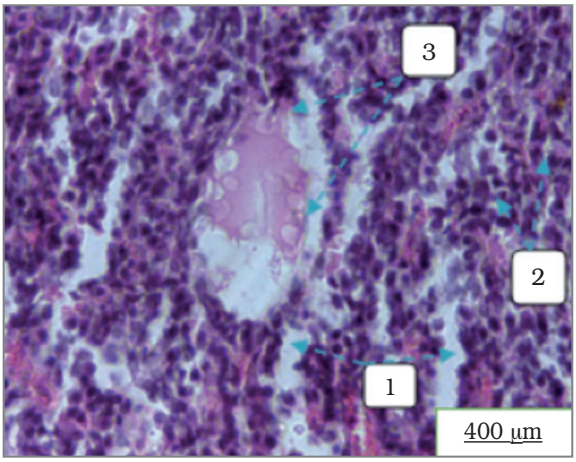


Fig. 2. Morphological rearrangements in the experimental rats pituitary gland under the condition of 30-days adaptation to heavy metals salts: 1 – edema of connective tissue trabeculae; 2 – discomplexation of cellular trabeculae; 3 – cyst. Staining: by hematoxylin-eosin.

Despite the 30-day recovery period after exposure to heavy metal salts, no significant recovery of adenohypophysis cell composition was observed in experimental rats. Thus, in the epithelial trabeculae of experimental animals there was an increase in the number of chromophobes and at the same time a decrease in the number of chromophilic basophils compared with control animals. However, groups of basophilic adenocytes were found in some isolated areas of the adenohypophysis (Fig. 3).

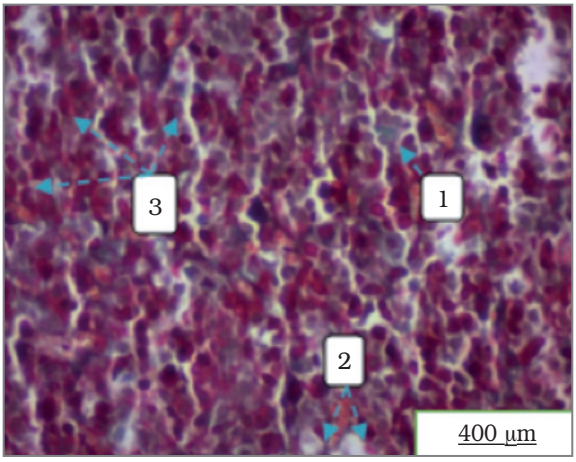


Fig. 3. Cellular composition of the experimental rats adenohypophysis after the condition of 30-day readaptation to heavy metal salts: 1 – chromophilic basophils; 2 – chromophobes; 3 – chromophilic acidophiles. Staining: by Mason Goldner.

The number of basophils decreased by 58.7% (P <0.001, t = 34.84), the number of acidophiles was lower by 3.4% (P> 0.05) compared with control animals. The number of chromophobes in males was higher by 14.4% (P <0,05, t = 2,94) compared with control animals (Table 1).

Thirty days after the end of exposure to heavy metal salts, morphologically, 2 types of cells were detected in the parenchyma. In the peripheral area, subcapsular groups of adenocytes with signs of vacuolation of the cytoplasm, balloon dystrophy. The nuclei of such cells were often enlightened and with chromatin margination. Often such groups of cells were located together with cysts. However, the vast majority of parenchymal cells had hypochromic cytoplasm, homogeneous hyperchromic elongated nuclei, with increasing amounts of heterochromatin in them. The nucleoli in the nuclei of cells of both types were not contoured. A small percentage of cells showed signs of apoptosis.

Adaptive changes in the adenohypophysis were characterized by an increase in the level of Hsp90 expression in the cytoplasm of cells to moderate (2 points) and strongly positive (3 points) levels. Hsp90-positive cells were diffusely located in the parenchyma, had a high level of staining (++ and +++). The number of low positive 1 adenocytes decreased by 69.05% (P <0.001, t = 431.562) in the adenohypophysis of experimental animals compared to control animals. The number of adenocytes moderately positive 2 HSP90 increased by 34% (P <0.001, t = 7.21), in the adenohypophysis of experimental rats compared to control animals. The number of strong positive 3 HSP90 adenocytes increased by 57.14%(P <0.001, t = 35.49) in the adenohypophysis of experimental rats compared to control animals (Tabl.2).

Table 2 Expression level of HSP90 in adenohypophysis adenocytes of control and experimental rats after adaptation to long-term exposure to heavy metal salts complex (X ± SD).

HSP90 expression	Control group total number 6 animals	Experimental group total number 6 animals
Low positive 1	69.05±0.16	–
Moderately positive 2	31.91±0.82	42.75±1.26***
Strong positive 3	–	57.14±1.61***

Note: * p≤0.05, *** p≤0.001

Particularly high levels of expression to Hsp90 were found in the cytoplasm of cells located perivascularly (Fig. 4).

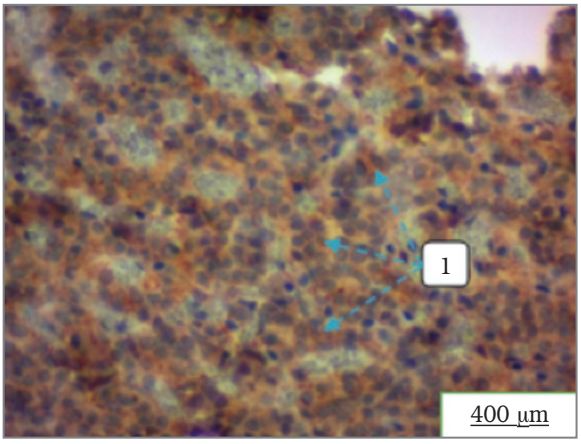


Fig.4. Expression of hsp90 in the cytoplasm of experimental animals adenocytes under the condition of 30-day readaptation to 90-day exposure to heavy metal salts: 1 – high level of hsp90 expression in the adenocytes cytoplasm. Immunohistochemical study of hsp90 expression.

The level of ACTH in the serum of experimental animals decreased by 46% (t = 74,17424, p <0,001) relative to control animals (Table 1).

DISCUSSION

Thirty days of readaptation to long-term exposure to heavy metals salts did not cause significant positive changes in most structural components of the experimental animal adenohypophysis. There were signs of weakening of the compensatory adaptation processes functional activity in the body, as evidenced by mutually conditioned morphological changes of the stromal and parenchymal components. At these period of adaptation to the exposure of heavy metals salts we revealed a decrease in the linear parameters of the adenohypophysis, but there was a significant increase in the value of fibrous connective tissue component in the stroma, increased collagenization of large vessels. According to the authors, this indicated the development of alternative changes due to organ hypoxia. Hypoxic phenomena accompanying vascular disorders caused an increase in the proliferative activity of stromal fibroblasts and their production of fibrous connective tissue component (11,12).

As in the previous period of the experiment (90th day of the experiment), significant diffuse stromal edema and edematous processes in the gland parenchyma persisted, which undoubtedly negatively affected the trophism and the course of regenerative processes in the adenohypophysis and histoarchitectonics of the gland. Morphological signs of adenocytes indicated slow recovery processes in their structure: the cytoplasm of some adenocytes had signs of vacuolation, balloon dystrophy, which indicated their vacuolar degeneration. There was no significant improvement in the functional activity of adenocytes: cells with enlightened nuclei, chromatin margination, nucleoli were absent and a small number of cells with signs of apoptosis. ACTH levels decreased compared to control animals.

Despite the thirty days adaptation period after exposure to heavy metal salts, no significant recovery in the cellular composition of the experimental rats adenohypophysis was observed. An increased number of chromophobes was accompanied by the decreased number of chromophilic basophils compared to control animals. Phenomenon of a decrease in the number of chromophilic cells can be explained by the occurrence of an "emergency" in the body due to the increased level of tropical hormones secretion by the body in the previous terms of the experiment. Conclusion correlates with the opinion of a number of authors (13,14).

However, groups of basophilic adenocytes were found in some isolated areas of the adenohypophysis. This can be explained by the mosaic of morphological changes of the gland – the alternation of dystrophic, necrotic areas of the parenchyma with intact, which can be explained by the manifestations of the law of intermittent activity of functioning structures (13). It can be assumed that the complex of salts of heavy metals negatively affected the morphofunctional parameters of the adenohypophysis, in particular by activating the processes of lipid peroxidation and oxidative stress. This, in turn, led to a change in hormone release and contributed to cell death by caspase-dependent apoptosis, which correlates with the opinion of some authors. (1, 4).

One of the clear morphological features of this term of adaptation is the presence of cysts in the parenchyma, the formation of which can be explained by delayed excretion of hormones from the adenohypophysis due to disruption of transmembrane transport and insufficient time of adaptation.

However, a number of morphological features still indicated the development of adaptive and compensatory processes in the adenohypophysis aimed at leveling the stress response and a number of hypoxic phenomena caused by the previous exposure to heavy metal salts (15). Thus, vascular plethora decreased, morphometric indicators of vascular area approached those of control animals. There was an increase in the area of vascularization of the

adenohypophysis and significantly improved the rheological properties of blood. However, the condition of the vascular wall in terms of its permeability and the condition of endothelial cells improved only partially. The rather bright adaptive rearrangements in the adenohypophysis of experimental animals include an increase in the number of adenocytes with increasing expression of hsp90 in their cytoplasm. This fact can be considered one of the mechanisms of cellular and organ defense. According to the literature, the production of heat shock protein (HSP) cells make these cells more resistant to further extreme conditions, developing resistance to further stress (16). After all, the renaturation of proteins damaged during stress is an integral part of stress resistance. Hsp90 has been shown to play an important role in protein quality control by directing damaged proteins to 26S proteosomes for degradation or to other chaperones (particularly Hsp70) for renaturation (16). Under stressful conditions, leading to the accumulation of proteins in the cells with disturbed conformation, Hsp90, it is believed, partially switch to their refolding (17). This fact indicates the participation of Hsp90 in the active processes of adenohypophysis cells adaptation to prolonged exposure to heavy metal salts.

CONCLUSIONS

Thus, a comprehensive study of the adenohypophysis structural components of experimental animals with thirty days of adaptation to long-term consumption of heavy metal salts indicate a number of adaptive and regenerative morphofunctional changes aimed at reducing the stress of adaptive processes on the part of the adenohypophysis. However, despite the positive dynamics of adaptive processes, it should be noted that the thirty days period of adaptation is insufficient for complete recovery of the organ.

REFERENCES

1. Cabilla JP, Ronchetti SA, Duvilanski BA. effects induced by chromium VI, cadmium and arsenic exposure on hypothalamus-pituitary physiology. Biocell 2016; 40(1):15-18.
2. Diamanti-Kandarakis E, Bourguignon J-P, Linda C. Giudice LC., Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement. Endocrine Reviews 2009; 30(4):293–342.
3. Sikka, Suresh C, Wang R. Endocrine disruptors and estrogenic effects on male reproductive axis. Asian Journal of Andrology 2008; 10(1): 134-145.
4. Quinteros FA, Poliandri Ariel HB, Machiavelli LI, Cabilla JP, Duvilanski BH. In vivo and in vitro effects of chromium VI on anterior pituitary hormone release and cell viability. 2007. Toxicology and Applied Pharmacology 2007; 218(1): 79-87.
5. Kresovich JK, Argos M, Turyk ME. Associations of lead and cadmium with sex hormones in adult males. Environ Res 2015; 142:25-33.
6. Gallagher CM, Moonga BS, Kovach JS. Cadmium, follicle-stimulating hormone, and effects on bone in women age 42–60 years, NHANES III. Environ Res 2010;110(1):105-11.
7. Chen C, Wang N, Zhai H, Nie X, Sun H, Han B, et al. Associations of blood lead levels with reproductive hormone levels in men and postmenopausal women: results from the SPECT-China Study. Sci Rep 2016; 6:37809.
8. Tae-Woo L, Dae HK, Ji Y R. The effects of exposure to lead, cadmium and mercury on follicle-stimulating hormone levels in men and postmenopausal women: data from the Second Korean National Environmental Health Survey (2012–2014). Ann Occup Environ Med. 2019; 31(21):1-9.
9. Hryntsova NB, Timakova OO, Romanyuk AM. Morphofunctional reconstructions of the epiphysal-parathyroide axis structural components of rats in the period of readaptation after prolonged exposure to heavy metals. Problems of Endocrine Pathology. 2020; 4: 106–114.

10. Hryntsova N.B., Romanyuk A.M., Lindin M.S. (2021) A method of manufacturing histological preparations of the rats pituitary gland for experimental morphological studies. Patent for utility model 149052 /13.10.21 of the State Register of Patents of Ukraine for utility models.
11. Sukhovey YG, Kostolomova EG, Unger IG, Akuneeva TV, Aptekar IA. Model of the effect of hypoxia on the cellular component and synthesis of extracellular matrix components in fibroblast culture. Russian Journal of Immunology. 2019; 22(2-2): 939- 941.
12. Redka OG. Morphofunctional changes in the adenohypophysis-thyroid system under conditions of prolonged exposure to pesticide intoxication. Scientific works of the Ukraine Medical Sciences Academy National Congress of Gerontologists and Gerontology. 2010: 152.
13. Korniykova IP. Morphofunctional transformations of the pituitary gland under the influence of hyperhydration disorders of water-salt metabolism. Abstr. PhDr. (Med.). Lugansk, Ukraine, 2012.
14. Bolshakova OV. Morphological characteristics of the adenohypophysis in lead intoxication and correction of changes with -tocopherol. Universum: Medicine and pharmacology. 2016; 8(30).
15. Zaichenko GV, Mishchenko OJ., Sharifov HS, Gordienko AD. The effect of peach leaf extract (Persica vulgaris) on the state of the rats hypothalamic-pituitary-adrenal system under conditions of chronic immobilization stress. Problems of endocrine pathology. 2019; 2: 89-97.
16. Nikitin KD. Heat shock proteins: biological functions and prospects for application. Clinical oncohematology. 2008; 1(2):125-130.
17. Lindin MS. Morphogenesis of infiltrative ductal carcinoma of the mammary gland in the minds of dowkill fermentation with salts of important metals. Sumy: Sumy State University; 2015.

Acknowledgment: The work was carried out within the framework of a scholarship of the Government of the Slovak People's Republic (2020) at the Department of Anatomy of the LF UPJS, Kosice, Šrobarova 2, Kosice, SR.

Received: May, 17, 2022
Accepted: June, 12, 2022