ATJ, 2022, №4, 164-169

AZƏRBAYCAN TİBB JURNALI
AZERBAIJAN MEDICAL JOURNAL

DOI: 10.34921/amj.2022.4.026

Sikora K.A.[1], Lındina Yu.N.[2], Hiryavenko N.İ.[1], Avuah E.V.[1], Abdul-Rahman T.[1], Lındin N.S.[1], Sikora V.V.[1], Romanyuk A.N.[1]

SİÇOVUL BALALIĞINDAN ESTROGEN VƏ PROGESTERON RESEPTORLARININ EKSPRESSİYASINA AĞIR METALLARIN TƏSİRİ

[1]Sumı Dövlət Universitetinin Patoloji anatomiya və [2]Morfologiya kafedraları, Sumı, Ukrayna

Xülasə. *Məqalədə ağır metalların (AM) siçovul balalığından estrogen (ER) və progesteron (PR) reseptorlarının ekspressiyasına təsirini və E vitamininin mühafizəedici effektini araşdırmaq məqsədilə aparılmış tədqiqat haqqında məlumat verilmişdir.*

Bu məqsədlə dişi siçovullar 90 gün ərzində ağır metal birləşmələri ilə (sink, mis, dəmir, manqan, qurğuşun və xrom) suyun təsirinə uğradılmışdır. Təcrübə aparılan heyvanlar 3 qrupa bölünmüşdür: I qrup – adi içməli su verilən heyvanlar; II qrup – peroral yolla vaxtaşırı AM-lə zəngilləşdirilmiş su içirdirilənlər; III qrup – AM-lə birgə E vitamini alan heyvanlar. Estrogen və progesteron reseptorlarının immunkimyəvi metodla tədqiqi üçün heyvanların balalıq toxumasının kəsikləri "Ultra Vision Quanto Detection System HRP DAB Chromogen" sistemi ilə boyadılmışdır.

Tədqiqat göstərmişdir ki, II və III qrup heyvanların balalığının stroma və endometriumundan ER kontrol qrupdakına nisbətən az ekspressiya edilir və bu, hüceyrələrin boyaq maddəsini zəif qəbul etməsi ilə təzahür edir. Analoji vəziyyət (zəif boyanma və boyanmış hüceyrələrin azlığı) miometriumda da müşahidə edilir. Tədqiqatdan aydın olmuşdur ki, endometriumun stromadan, epitel qişasından və boylama əzələ liflərindən progesteron reseptorlarının ekspressiyası azalsa da, həlqəvi əzələlərdə belə dəyişiklik baş vermir. Bundan əlavə, III qrupda (AM-lə birgə E vitamini alan heyvanlar) estrogen və progesteron reseptorlarının ekspressiyası II qrupun heyvanlarındakından (yalnız ağır metallar alanlardan) fərqli olmuşdur.

Açar sözlər: *balalıq, ağır metallar, reseptorlar, estrogen, progesteron*

Ключевые слова: *матка, тяжелые металлы, рецепторы, эстроген, прогестерон*

Key words: *uterus, heavy metals, receptors, estrogen, progesterone*

Sikora K.[1], Lyndina Y.[2], Hyriavenko N.[1], Awuah A.W.[1], Abdul-Rahman T.[1], Lyndin M.[1], Sikora V.[1], Romaniuk A.[1]

HEAVY METALS EFFECT ON ESTROGEN AND PROGESTERONE RECEPTORS EXPRESSION IN THE RAT UTERUS

[1]Department of Pathology and [2]Department of Morphology, Sumy State University, Sumy, Ukraine

The results of the study present the effect of heavy metals (HMs) on estrogen (ER) and progesterone (PR) receptors expression in the rat`s uterus and the possible protective effect of vitamin E treatment.

Female rats were exposed to heavy metal salts (zinc, copper, iron, manganese, lead, and chromium) via contaminated water for 90 days. Animals were assigned to three Groups: control animals (Group I) that received ordinary drinking water; animals (Group II) that were orally administered with HMs substances given with a reference range; and rats (Group III) were administered with HMs and treated with vitamin E. The Ultra Vision Quanto Detection System HRP DAB Chromogen with primary antibodies to ER and PR were used for the immunohistochemical research.

The results of the study showed a decrease of ER expression (weaker staining with a smaller proportion of stained cells) in the stroma and epithelium of the endometrium from animals of experimental groups II (HMs exposure only) and III (HMs exposure with vitamin E treatment) compared to the control. A similar tendency (weak staining and an insufficient number of stained cells) was found in the uterus myometrium. The study showed a reduction (weak staining with an insufficient number of stained cells) of PR expression in the endometrium stroma and epithelium and longitudinal muscular layer, while its level in the circular muscle cells remained unchanged. Furthermore, a significant difference in the ER and PR expression was observed in the endometrial epithelium and longitudinal muscular layer in Group III compared to Group II.

The pathology of the uterus, the largest organ of the female reproductive system in humans and vertebrates, remains an actual problem. Despite the progress achieved in improving diagnostic methods and treatment tactics, diseases with non-specific symptoms of the course remain neglected in animals and humans. This applies to oncological, inflamematory and non-inflammatory processes in the uterus, especially those of mysterious origin [1-3]. This includes pathological lesions of the female reproductive system organs caused by the action of exogenous environmental pollutants (chemical substances or elements exogenous to the organism). It is well known that there is a clear etiological and pathological link between controlled (smoking, drugs, alcohol, chemical food additives, chemotherapy, etc.) and uncontrolled (living in urbanized locations with an unfavorable ecological background) exogenous agents of influence. [3-7]. Moreover, the latter has a more global and massive nature and is accompanied by high risks of worsening the quality of life and unpredictable consequences for health [5-9].

The top place among dangerous environmental pollutants is occupied by heavy metals (HMs). Their adverse effect on health occurs through the release of artificial chemicals and/or the disposal of wastes by polluting the water we drink, the air we breathe, and the soil in which plants grow [7]. Taking into account the fact that HMs are able to external and internal influence, they can be toxic to cells, tissues and organs homeostasis and perturb physiological processes (endogenous metabolism and molecular mechanisms), leading to detrimental pathological outcomes in all organisms in general [7, 10, 11]. To date, it is well known that xenobiotic influence may induce changes in uterine sexual health and lesions in different organs. However, the information on sex hormone imbalance and signaling pathways in the uterus varies and generally depends on the timing of HMs intoxication. At the same time, much attention is paid to the search for natural biological compounds that could protect the body from exogenous influence [9-13]. The response of estrogen (ER) and progesterone (PR) expression in the uterus to pollutants differ from that of other target organs, and they may serve as indicators of the deleterious effects of environmental pollution on reproductive health.

Therefore, the aim of this research was to study the effect of HMs on estrogen (ER) and progesterone (PR) receptors expression in the rat`s uterus and the possible protective effect of vitamin E treatment.

Materials and methods. Experimental design involved the administration of heavy metals by contaminated water that was supplied in a drinking bottle for oral exposure for 90 days by female rats. The list of six common HMs was dissolved in ordinary water in the following concentrations: zinc ($ZnSO_4 \times 7H_2O$) – 5 mg/l, copper ($CuSO_4 \times 5H_2O$) – 1 mg/l, iron ($FeSO_4$) – 10 mg/l, manganese ($MnSO_4 \times 5H_2O$) – 0.1 mg/l, lead ($Pb(NO_3)_2$) – 0.1 mg/l, and chromium ($K_2Cr_2O_7$) – 0.1 mg/l. At the same time, alpha-tocopherol (vitamin E) was used as a prophylactic compound at an average daily dose (2,02 mg, considering species' characteristics). Based on this, twenty-four female rats, weighing 221.7±17.1 g, were randomly assigned to three groups (8 rats per Group) in this study: control animals (Group I) that received ordinary drinking water; animals (Group II) that were orally treated with HMs substances; and rats (Group III) were administrated with HMs and vitamin E (via the oral gavage technique). Animals were kept in polypropylene cages with individual ventilation and were maintained under environmentally controlled laboratory conditions of temperature (22°C±1°C), relative humidity (55±5%), and light/dark (12 hours) cycle. The rats had *ad libitum* access to standard pellets and water. The experiment has been conducted in the European Community Guide for the Care and Use of Laboratory Animals guidelines, ethical and responsible manner and is in full compliance with all relevant codes of experimentation (institutional and national) and legislation. This study was approved by the Bioethics Committee of the Medical Institute of Sumy State University (No. 2/10 from 10.10.2019).

Upon expiration of the experiment duration, the experimental animals were euthanized by CO2 inhalation followed by cervical dislocation and their uteruses were immediately exposed to a low abdominal midline incision. The uteruses were collected, fixed in 10% neutral buffered formalin for 24 h, dehydrated (in ascending grades of ethanol 70–96%), and then embedded in the paraffin block (4% wax) for later use. Formalin-Fixed Paraffin-Embedded tissue specimens were sectioned using a rotary ultramicrotome Shandon Finnesse 325 (Thermo Scientific, USA) and distributed onto glass slides (Thermo Scientific, USA). For the immunohistochemical assay, the Ultra Vision Quanto Detection System HRP DAB Chromogen (Thermo Fisher Scientific, USA) with primary antibodies to ER (E115 clone) and PR (SP2 clone) were used in accordance with the technique presented in the preliminary report [14]. The ER and PR expression levels were evaluated in the nuclei of the luminal and

glandular endometrial epithelium, endometrial stroma, and myometrium. The immunostaining was quantified on five randomly selected fields in each compartment. Immunoreactivity of ER and PR was evaluated, graded, and scored by a combined score based on nuclear staining intensity and percentage of cell staining on the principle of the standard IRS scoring system. The immunohistochemical staining of the percentage of positive cells was estimated as 0 – negative (0%); 1 – low (1–30% of positive cells); 2 – moderate (31–70% of positive cells); 3 – high (71–100% of positive cells). The immunostaining intensity of specifically stained cells was also scored based on the following grades: 0 – negative staining; 1 – weak positive staining; 2 – moderate positive staining; 3 – strong positive staining. An immunohistochemical score was calculated for each case by the sum of the proportion of percentage and intensity rating as follows: –, 0 points; +, 1–2 points; ++, 3–4 points; +++, 5–6 points. Two independent pathologists additionally evaluated the immunohistochemical study with the following discussion of the results. The immunohistochemical analysis was evaluated with the Zeiss Axio Primo Star microscope, Zeiss AxioCam ERc 5s digital camera, and ZEN 2 (blue edition) software package (Germany). The computer vision system used for the evaluation of pathomorphological images is a generally recommended technique and was carried out to avoid errors in the assessment of visual fields [15]. All values were tested for the Kolmogorov-Smirnov normality criterion and were analyzed by Student's t-test (unpaired) through the computer software Graph Pad Prism, version 6.0 (San Diego, CA, USA) to determine the level of significance. A p-value < 0.05 was considered to be statistically significant.

Results. Expression of ER and PR in the uterine of control animals was represented by positive nuclear staining of luminal and glandular epithelial cells, in endometrial stroma and myometrium (strong staining with a sufficient proportion of stained cells). Strongly positive staining of cells for the corresponding female sex hormones was visualized in almost all epithelium cells. At the same time, ER and PR immunoreactivity of the endometrial stroma was heterogeneous (located singly or grouped). In the myometrium, the indicators of the generation of hormone receptors were at a high level but lower compared to the endometrium.

On the 90th day of the experiment, the levels of ER and PR expression in the uterus of rats in Group II and Group III showed changes in the immunohistochemical pattern (Fig. 1). A significant overall decrease in staining intensity and expression levels of ER and PR expression compared to control levels was found (Table 1).

Thus, the level of ER expression in the stroma, luminal and glandular epithelium of the endometrium sharply decreased (weaker staining with a smaller proportion of stained cells) in animals of Group II and animals of Group III, compared to the control. A similar tendency (weak staining and an insufficient number of stained cells) was found in the uterus myometrium of Group II animals. However, a difference in the expression of ER in the uterus depending on the compartment was also established between Group II and Group III. In the endometrial epithelium and longitudinal muscular layer in animals of Group III the immunostaining and cells number were less changed compared to Group II.

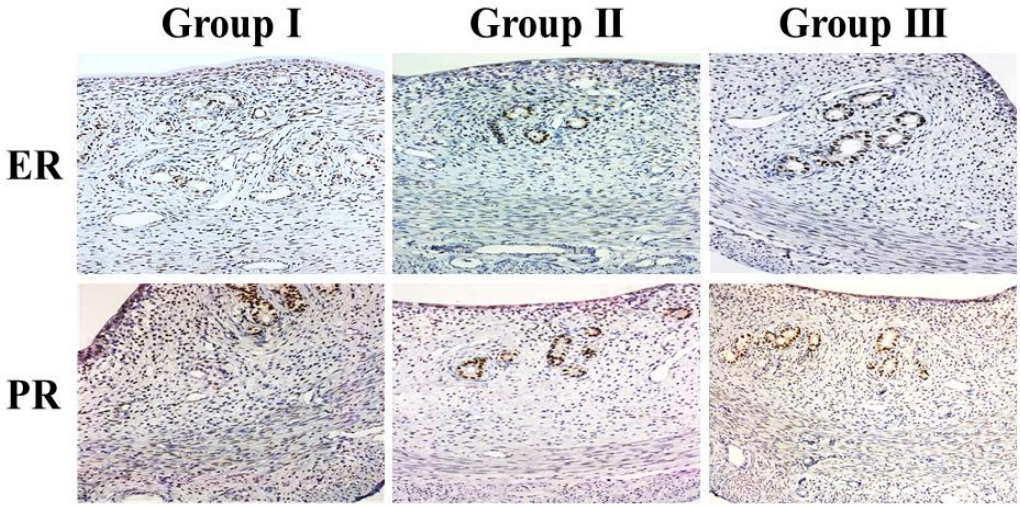

Fig. 1. Rat uterus. Immunohistochemical staining assay of ER and PR expressions in Group I, Group II and Group III. Chromogen – diaminobenzidin; nuclei were counterstained with Mayer's hematoxylin. Magnification:×200

Table 1. *ER and PR expression levels in the uterus of rats*

	Endometrium				*Myometrium*			
	Epithelium		*Stroma*		*Circular layer*		*Longitudinal layer*	
	ER	**PR**	**ER**	**PR**	**ER**	**PR**	**ER**	**PR**
Group I	+++	+++	++	++	++	++	++	++
Group II	+*	++*	+**	+*	+/++	++	+*	+*
Group III	++*#	+++#	+*	+*	++	++	++#	++

Note: significant difference between Groups, * $p < 0.05$; ** $p < 0.01$; # $p < 0.05$. * – compared to Group I;
– compared to Group II

At the same time, changes in PR expression in the rat uterine tissue from experimental groups II (HMs exposure only) and III (HMs exposure with vitamin E treatment) were less pronounced, compared to the ER study. The study of PR expression also showed a decrease in both the immuno-staining proportion of percentage and intensity in the endometrial epithelium and stroma (weak staining with an insufficient number of stained cells), like in the longitudinal muscular layer in experimental groups, compared to the control. However, a significant difference in the PR expression was observed in the endometrial epithelium against the treatment background in Group III compared to Group II.

Discussions. It has long been known that the most preferred experimental biomodel among vertebral animals is rats. This especially applies to their similarity of the structure and functions of the female reproductive system with humans to determine the molecular peculiarities of various pathological processes [14]. That is why the analogous studies' findings are essential in understanding the role of HMs as a negative exogenous factor in the pathophysiological pathways related to uterus diseases, possible complications and prognosis.

One of the important prognostic factors of the uterus state is its receptors' sensitivity to sex hormones. Estrogen and progesterone are steroid hormones that have an important role in the growth and differentiation of the reproductive tissue, fertility, and maintaining organism development in general [16]. The ovaries secrete these hormones (in the frame of the hypothalamic-pituitary-ovarian axis) and can easily be determined in serum for screening. They are well-known regulators of ER and PR expression in the stromal-epithelial interactions of the rat uterus, which provides the cyclic changes in the uterine tissues during the estrous cycle via binding to specific intracellular receptors in the target cells. It is important to note that PR exists in A and B isoforms when ER exists in α, β and γ forms [14,16-17].

A careful analysis of literature data showed a link between various female ER and PR expressions and sensitivity to organism exposure by HMs as agonists or antagonists. In a series of cases, some metals in medium and high doses cause a decrease in the levels of sex hormones in rats: aluminum suppresses the production of testosterone, estrogen and progesterone; cobalt interferes with the secretion of progesterone; depending on the dose, cadmium induct of estrogen and progesterone-sensitive uterine gene expression; etc. Also, the action of the metal may depend on its concentration and ability to accumulate in the body. Indeed, high levels of zinc and nickel can cause a decrease in estrogen production, whereas low levels have the opposite effect. On the other hand, it is believed that other metals such as cadmium, vanadium, iron, chromium, lead, nickel, and copper can be linked to ERα by mimicking the function of estrogen and lead to increasing cases of inflammation, hyperplasia or tumor progression. Moreover, over time, such endocrine fluctuations are accompanied by a loss of sensitivity of receptors of the target

cells to the corresponding hormones and a decrease in fertility [16,18,19].

The results demonstrate changes in ER and PR expression levels in the uterus after 90 days of heavy metals exposure. Thus, a significant decrease in receptors expression to both female sex hormones was shown mainly in the endometrium (as for epithelial as stromal compartment). At the same time, ER and PR expression in the myometrium changed only in the longitudinal muscular layer of Group II and Group III. It is important to note the positive effect of vitamin E administration as a protector. The vitamin E treatment did not completely stabilize the expression level changes. However, its effect had a significant difference in PR expression in the endometrial epithelium, the same for ER expression in the longitudinal layer of the myometrium.

Moreover, the reduction of hormonal expression is observed not only when xenobiotics affect the organism but also during such lesions as inflammation and cancer progression. Lastly, it is important to note that loss of differentiation in cancer progression is also often conditioned by the participation of HMs in carcinogenesis, which may indicate their role in common complex cell signaling pathways involved in gynecologic disorders processes [1, 16, 20]. That is why the ER and PR indicators of the uterus and level of sex hormones in serum can serve as specific indicators of the effect of HMs on the body for predicting probable consequences and developing preventive protocols. Much additional work will be required before a complete understanding of this phenomenon between the dependence of receptor sensitivity of sex hormones and HMs exposure. These findings are essential in understanding the presence and role of metals in the ER and PR expression in the female reproductive system and its pathology. This will also stimulate further investigations in this field.

Summarizing, long-term administration by HMs on the organism contributes to the reduction of the ER and PR expression in the uterus of rats. In contrast, the vitamin E treatment showed a protective effect against HMs exposure and decreased their effect on both ER and PR expression in the uterus of female rats. The ER and PR expression variation in endometrium and myometrium had different tendencies.

Funding: This research has been performed with financial support by the Ministry of Education and Science of Ukraine [Grant № 0121U100472] and the research theme of the Department of Pathology of Sumy State University [Grant № 0119U100887].

Conflicts of Interest: The authors report no conflict of interest.

REFERENCES

1. Mleko M., Pitynski K., Pluta E., et al. Role of Systemic Inflammatory Reaction in Female Genital Organ Malignancies - State of the Art // Cancer Manag Res. 2021 Jul 9;13:5491-5508. doi: 10.2147/CMAR.S312828.
2. Lytvynenko M.V., Narbutova T.Y., Vasylyev V.V., Gargin V.V. Indicators of proliferative activity of endometrium in women with immunodeficiency // Azerbaijan Medical Journal. 2021;2:53-60. doi: 10.34921/amj.2021.2.008
3. AlAshqar A., Reschke L., Kirschen G.W., Borahay M.A. Role of inflammation in benign gynecologic disorders: from pathogenesis to novel therapies // Biol Reprod. 2021 Jul 2;105(1):7-31. doi: 10.1093/biolre/ioab054.
4. Lytvynenko M., Narbutova T., Vasylyev V., Bondarenko A., Gargin V. Morphofunctional changes in endometrium under the influence of chronic alcoholism // Georgian Med News. 2021;(315):160-164.
5. Lytvynenko M., Bocharova T., Zhelezniakova N., Narbutova T., Gargin V. Cervical transformation in alcohol abuse patients // Georgian Med News. 2017;(271):12-17.
6. Klyuchko K.O., Gargin V.V. Influence of neoadjuvant chemoradiotherapy for locally advanced cervical cancer // Pol Merkur Lekarski. 2020;48(288):406-409.
7. Manisalidis I., Stavropoulou E., Stavropoulos A., Bezirtzoglou E. Environmental and Health Impacts of Air Pollution: A Review // Front Public Health. 2020 Feb 20;8:14. doi: 10.3389/fpubh.2020.00014.
8. Avilova O., Shyian D., Marakushin D., Erokhina V., Gargin V. Ultrastructural changes in the organs of the immune system under the influence of xenobiotics // Georgian Med News. 2018;(279):132-137.
9. Avilova O., Marakushin D., Nakonechna O., Gargin V. Microscopic features of the spleen under the influence of laproxides // Georgian Med News. 2018;(Issue):163-167.
10. Romaniuk A., Lyndin M., Lyndina Y., et al. Changes in the Hematopoietic System and Blood Under the Influence of Heavy Metal Salts Can Be Reduced with Vitamin E // Turk Patoloji Derg. 2018;34(1):73–81. doi: 10.5146/tjpath.2017.01412.

11. Romaniuk A., Sikora V., Lyndin M., et al. The features of morphological changes in the urinary bladder under combined effect of heavy metal salts // Interv Med Appl Sci. 2017;9(2):105–11. doi: 10.1556/1646.9.2017.2.09.
12. Mosavat M., Mohamed M., Ooi F.K., et al. Histological changes of female reproductive organs subjected to different jumping exercise intensities and honey supplementation in rats // PeerJ. 2019 Sep 10;7:e7646. doi: 10.7717/peerj.7646.
13. Romaniuk A., Sikora V., Lyndina Yu., et al. Effect of heavy metals on the readaptive processes in the urinary bladder // Bangladesh Journal of Medical Science. 2019;18(1):100–6. doi: 10.3329/bjms.v18i1.39558
14. Sikora K., Lyndin M., Hyriavenko N., et al. Morphological features of the rat uterus // Pol Merkur Lekarski. 2021;49(294):420-425. PMID: 34919085.
15. Gargin V., Radutny R., Titova G., et al. Application of the computer vision system for evaluation of pathomorphological images // 2020 IEEE 40th International Conference on Electronics and Nanotechnology, ELNANO 2020 - Proceedings; 2020:469-473 doi: 10.1109/ELNANO50318.2020.9088898.
16. Mohammad H.S., Hossein M.A., Amani R., et al. Subchronic effects of different doses of Zinc oxide nanoparticle on reproductive organs of female rats: An experimental study // Int J Reprod Biomed. 2019;17(2):107-118. Published 2019 Mar 19. doi:10.18502/ijrm.v17i2.3988
17. Lytvynenko M.V., Narbutova T.Ye., Oliynyk N.N., et al. Estrogen and progesterone receptors in endometrial, cervical and breast tissues in immunodefficient conditions // Azerbaijan Medical Journal. 2021;3:70-77.
18. Höfer N., Diel P., Wittsiepe J., Wilhelm M., Degen G.H. Dose- and route-dependent hormonal activity of the metalloestrogen cadmium in the rat uterus // Toxicol Lett. 2009 Dec 15;191(2-3):123-31. doi: 10.1016/j.toxlet.2009.08.014.
19. Lukacinova A., Benacka R., Sedlakova E., Lovasova E., Nistiar F. Multigenerational lifetime low-dose exposure to heavy metals on selected reproductive parameters in rats // J Environ Sci Health A Tox Hazard Subst Environ Eng. 2012;47(9):1280-7. doi: 10.1080/10934529.2012.672132.
20. Lytvynenko M.V., Gargin V.V. Triple-negative transformation of the endometrium, cervix and mammary gland on the background of immunodeficiency // Medicni perspektivi. 2022;27(2):119-124

Сикора К.А.[1], Лындина Ю.Н.[2], Гирявенко Н.И.[1], Авуах Э.В.[1], Абдул-Рахман Т.[1], Лындин Н.С.[1], Сикора В.В.[1], Романюк А.Н.[1]

ВЛИЯНИЕ ТЯЖЕЛЫХ МЕТАЛЛОВ НА ЭКСПРЕССИЮ РЕЦЕПТОРОВ ЭСТРОГЕНА И ПРОГЕСТЕРОНА В МАТКЕ КРЫС

[1]Кафедра патологической анатомии и [2]Кафедра морфологии Сумского государственного университета, Сумы, Украина

Резюме. В статье представлены результаты исследования проведённые с целью изучения влияния тяжелых металлов (ТМ) на экспрессию рецепторов эстрогена (ЭР) и прогестерона (ПР) в матке крыс и возможного защитного эффекту витамина Е.

Самки крыс подвергались воздействию солей ТМ (цинка, меди, железа, марганца, свинца и хрома) через загрязненную воду в течение 90 дней. Животные были разделены на три группы: контрольные животные (группа I), получавшие обычную питьевую воду; животные (группа II), которым перорально вводили ТМ в референтном диапазоне; крысы (группа III), которым вводили ТМ и витамин Е. Для иммуногистохимического иследования использовали систему Ultra Vision Quanto Detection System HRP DAB Chromogen с первичными антителами к ЭР и ПР.

Результаты исследования показали снижение экспрессии ЭР (более слабое окрашивание с меньшей долей окрашенных клеток) в строме и эпителии эндометрия у животных II (воздействие только ТМ) и III (воздействие ТМ с обработкой витамином Е) экспериментальных групп по сравнению с контрольными параметрами. Аналогичная тенденция (слабая окраска и недостаточное количество окрашенных клеток) обнаружена в миометрии матки. Исследование показало снижение (слабое окрашивание с недостаточным количеством окрашенных клеток) экспрессии ПР в строме эндометрия, эпителии и продольном мышечном слое, в то время как его уровень в клетках циркулярной мускулатуры оставался неизменным. Кроме того, значительная разница в экспрессии ЭР и ПР наблюдалась в эпителии эндометрия и продольном мышечном слое в группе III, по сравнению с группой II.

Author for correspondence:
Vladyslav Sikora – Associate professor at the Department of Pathology, Sumy State University, Sumy, Ukraine
E-mail: v.sikora@med.sumdu.edu.ua