DOI 10.26724/2079-8334-2021-2-76-243-247
UDC 611.843.018:615.212.7]:615.225

Yu.R. Soguiko[1], Ye.V. Paltov, V.B. Fik, Z.Z. Masna, M.Ye. Kowalska, R.P. Krynytskyi, Yu.Ya. Kryvko[1]
Danylo Halytsky Lviv National Medical University, Lviv
[1]Andrei Krupynskyi Lviv Medical Academy, Lviv

CHANGES IN THE RETINA WITH TEN-WEEK OPIOID EXPOSURE WITH A SIMULTANEOUS FOUR- WEEK VASODILATOR CORRECTION

e-mail: dr.kowalska.marta@gmail.com

The purpose of our study was to establish pathomorphological manifestations of angio- and neuroretinopathy because of chronic exposure to therapeutic doses of opioid analgesic and possibilities of simultaneous correction of these changes with the help of pentoxifylline at the late terms of destructive changes in retinal layers. This was achieved by using histological methods of retinal imaging. In the future, the results of the study will allow forming a pathomorphological base with clinical recommendations for the unacceptable simultaneous application of opioids and pentoxifylline. The above mentioned will give an opportunity to access the most optimal time limits for which there is a characteristic set of pathomorphological manifestations of the initial phenomena of angioneuroretinopathy in the retinal layers, which is preferably exposed to corrective effect.

Key words: retina, opioid, late terms, combined effect, rats.

Ю.Я. Согуйко, Є.В. Пальтов, В.Б. Фік, З.З. Масна, М.Є. Ковальська, Р.П. Криницький, Ю.Я. Кривко

ДИНАМІКА ПАТОМОРФОЛОГІЧНИХ ЗМІН У ШАРАХ СІТКІВКИ ПРИ ДЕСЯТИТИЖНЕВОМУ ВЖИВАННІ ОПІОЇДНОГО АНАЛЬГЕТИКА З ПАРАЛЕЛЬНОЮ ЧОТИРЬОХТИЖНЕВОЮ КОРЕКЦІЄЮ ПЕНТОКСИФІЛІНОМ В ЕКСПЕРИМЕНТІ

Метою нашого дослідження було вивчення патоморфологічних проявів ангіо- та нейроретинопатії в результаті хронічного впливу терапевтичних доз опіоїдного знеболюючого та можливості одночасної корекції цих змін за допомогою пентоксифіліну на пізніх термінах деструктивних змін шарів сітківки. Ця мета була досягнута за допомогою гістологічних методів візуалізації сітківки. Надалі результати дослідження дозволять сформувати патоморфологічну базу з клінічними рекомендаціями щодо неприпустимого одночасного застосування опіоїдів та пентоксифіліну. Вищезазначене дасть можливість встановити найбільш оптимальні часові межі, для яких існує характерний набір патоморфологічних проявів початкових явищ ангіонейроретинопатії в шарах сітківки, який переважно піддається коригуючому ефекту.

Ключові слова: сітківка, опіоїд, пізні терміни, комбінований ефект, щури.

The work is a fragment of the research project "*Morphofunctional features of organs in pre- and postnatal periods of ontogenesis, under the influence of opioids, food supplements, reconstructive surgery and obesity*", *state registration No. 0120U002129.*

The data from the European Monitoring Centre for Drugs and Drug Addiction testify that the number of people in the European region using drugs at least once a year is about 275 million people, which is equivalent to approximately 5.6 % of the world population aged 15 to 64. Specific gravity of mental and behavioral disorders because of psychoactive substances (PAS) in the general class F incidence in 2016 was 60.1 %, 115,170 people, or 270.4 per 100,000 population (in 2015 – 58.4 %) [1, 11].

In the structure of mental disorders, mental and behavioral disorders have the first place due to the PAS (dispensary and preventive supervision groups). It is 41.7 %, or 694,928 people, 1,631.6 per 100,000 population [1].

The fight against drugs has been going on for decades and proves that forceful methods of struggle are ineffective. Punitive drug policy is directed primarily against those who use drugs, not against those who sell and distribute them. This, in turn, is a factor of the social aggravation, increasing the level of corruption in law enforcement authorities and courts. The result of such repressive drug policies is the growth of the illegal drug market and the number of drug addicts, who do not seek medical assistance and social protection who being afraid of persecution [5, 6].

According to the latest biobehavioral studies, the estimated number of people who use injecting drugs was 317,000 (in the government-controlled territory of Ukraine), among whom the number of only opioid users was 200,661 people (63.3 % of the total number), 38,674 people – stimulant users (12.2 % of the population), 77,665 people practiced mixed use of drug substances (24.5 %) [8].

In recent years, in Ukrainian and foreign sources there have become increasingly common publications dealing with structural changes in organs under the influence of opioid drugs in experimental

research **[3].** In particular, data on pathology of structures of the organ of vision, concerning the iris-corneal angle are covered in scientific journals [9, 12–15].

A number of questions concerning the manifestations of opioid angio and neuroretinopathy during the correction of experimental opioid effects remains open. In particular, the issue of the smoothness of growth and depth of angio and neuroretinopathies and manifestations in the layers of retina at the late terms with the simultaneous exposure to opioid and performing medication correction during the last four weeks with ten-week opioid use in experiment has not been fully clarified yet.

Taking into consideration the above mentioned, we believe that this study is topical both in terms of experimental morphology and in terms of practical ophthalmology.

The purpose of the study was to investigate the corrective influence of pentoxifylline in order to correct pathological angioretinopathic changes in the layers of retina with a ten-week experimental opioid effect.

Materials and methods. 24 mature, outbred, white male rats, weighing 160–255g aged 4.5–7.5 months old were the material of the study. The initial dose of nalbuphine (1 ml of solution contains nalbuphine hydrochloride dihydrate in terms of nalbuphine hydrochloride anhydrous 10 mg, Rusan Pharma Ltd., India) during the first two weeks was 0.212 mg/kg, for the next two weeks (2^{nd}–4^{th} weeks) – 0.225 mg/kg, the next (4^{th}–6^{th} weeks) – 0.252 mg/kg, the next (6^{th}–8^{th} weeks) – 0.260 mg/kg, and during 8^{th}–10^{th} weeks – 0.283 mg/kg. Thus, the conditions for chronic opioid exposure were created [10].

From the 42^{nd} day, continuing to administer the opioid, pentoxifylline (1 tablet contains pentoxifylline 200 mg; PJSC "Pharmaceutical Company" Ukraine) injections were added in parallel, one time per day daily at the same time (10–11 a.m.) until the end of the 70^{th} day. The dose of pentoxifylline was 2.857 mg/kg. By the end of the 10^{th} week, the material was collected for microscopic examination of the retina.

The animals were divided into three groups. The 1^{st} group of animals received nalbuphine for 70 days intramuscularly at the same time. The 2^{nd} group was the control and received injections of saline solution intramuscularly for 70 days at the same time. The 3^{rd} group by the end of the 42^{nd} day received for 28 days until the end of the 70^{th} day in parallel with the opioid pentoxifylline. All animals were kept in vivarium conditions. Retention, care, marking and other manipulations were carried out in compliance with the provisions of "The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [Strasbourg, 1985], "General Ethical Principles for Experiments on Animals", approved by the First National Congress on Bioethics [Kyiv, 2001], Law of Ukraine No. 3447 – IV "On the Protection of Animals Against Cruelty". The Commission on Bioethics of Danylo Halytsky Lviv National Medical University found that the performed scientific study met the ethical requirements according to the Order of the Ministry of Health of Ukraine No. 231 of 01.11.2000 (Protocol No. 10 of 26.12.2011), (Protocol No. 2 of 20.02.2012). Before sampling the intravital material, the animals were sacrificed by intraperitoneal administration of sodium thiopental (at the rate of 25mg/1kg). The eyeballs of rats obtained by the method of post-mortem enucleation with further consideration of maintaining the topographic ratio of the eye membranes were used as the material for the microstructural study. Histological sections were made with a thickness of 5–7μm. Histological preparations were prepared according to the conventional method using hematoxylin, eosin and azan dyes by Heidenhain [13]. Microscopic examination and photographing of the preparations were performed using an MBI–1 microscope and a digital camera Nicon D 3100.

Results of the study and their discussion. After ten weeks of experiment in rats that were administered opioids for six weeks, and subsequently for four weeks continued to administer opioid with parallel administration of pentoxifylline, structural changes were progressively increasing. The cytoplasm of the pigment epithelium (I) is swollen, stained in pink-purple color, when stained with azan in light pink color, sometimes grainy. The nuclei of the pigment epithelium are basophilic, mostly rounded, in individual cells oval. Bruch's membrane is holistic, homogeneous, swollen in some places, slightly colored in light blue. The choroid vessels are dilated, full of blood. In some vessels the erythrocytes agglutinated, there are present leukocytes. Most vessels are slightly expanded; their lumen is free of blood. The wall of the arterioles is swollen. Swelling and endothelial proliferation sites also observed. Necrotic changes of the pigment epithelium observed near the vessels in which there are pronounced circulatory disorders, as shown in fig. 1.

The appendages of the photoreceptor cells of the photosensory layer (II) are represented by narrow long strands of pink color. In separate areas, the photosensory layer is slightly narrowed. In some areas, the single nuclei of the photoreceptor cells are moving to the photosensory layer. In some places, a slight illumination of the outer segments of photoreceptors and cytoplasm of bipolar and horizontal cells is observed.

The outer limiting membrane (III), formed by flat thick adhesive contacts between the photoreceptors and the outer appendages of the Müller cells, in some areas is viewed as a pink line with a purple tint. The outer nuclear layer (IV) – in the central part of the retina is located in 11–14 rows of nuclei, and near the dentate line – 4-6 rows of nuclei of photoreceptor cells.

There are pycnotic nuclei of photoreceptor cells in some places. Between he nuclei of the photoreceptor cells appear gaps**.** In the peripheral areas of the retina between the nuclei of photoreceptor cells, the gaps increase sharply and become significant. Small vacuoles are present in the cytoplasm of some photoreceptor cells (near the nucleus).

Outer plexiform layer (V) is slightly thinner in some areas. The main substance of the outer plexiform layer is acidophilic, light gray with a pale purple hue, contains a fine gray and violet granularity, sometimes enlightened. Nuclei of photosensor cells (sticks) sometimes are moving in the outer zone of the outer plexiform layer. The nuclei of cells of inner nuclear layer are also moving in some places to outer plexiform layer. The clarification of the main substance occurs next to the inner nuclear layer.

The inner nuclear layer (VI) is of unequal thickness. The nuclei of nerve cells located in this layer of unequal shape and size are placed in 4-5 rows, and in some areas in 3 rows. The first row of nuclei are nuclei of horizontal cells, which are large enough, rounded, and lightly colored with single grains of condensed chromatin. The nuclei of these cells do not form a continuous row and are located on the border with the outer plexiform layer. The nuclei of bipolar cells are located a bit deeper in 2-3 rows. The nuclei of these cells are smaller than the nuclei of horizontal cells, mostly rounded, and more intensively colored, with numerous intensively colored grains of chromatin. In places where there are no nuclei of horizontal cells, the nuclei of bipolar cells border on the inner part of the outer plexiform layer. Between the nuclei of bipolar cells, there are located vertically elongated nuclei of Müller cells. Sometimes the nuclei of these cells are triangular and slightly darker (more intensive) stained with hematoxylin than the rounded light nuclei of bipolar cells. The innermost row of the inner nuclear layer bordering on the inner plexiform layer is the row of nuclei of amacrine cells. There are individual nuclei of the inner nuclear layer in the pycnosis state (reduced in volume, intensely basophilic), as it can be seen in fig. 2.

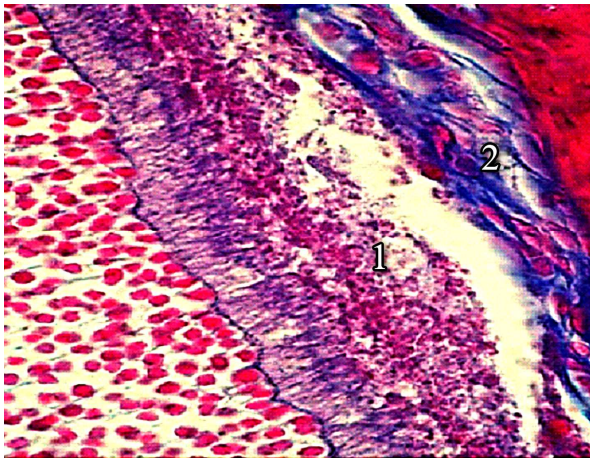

Fig**.** 1**.** The retina of a rat. Administration of nalbuphine and pentoxifylline at the end of the 10th week. Azane. Inc. x 1000. 1 – the area of destruction of the peripheral part of the sticks and cones; 2 – necrosis of the pigment epithelium.

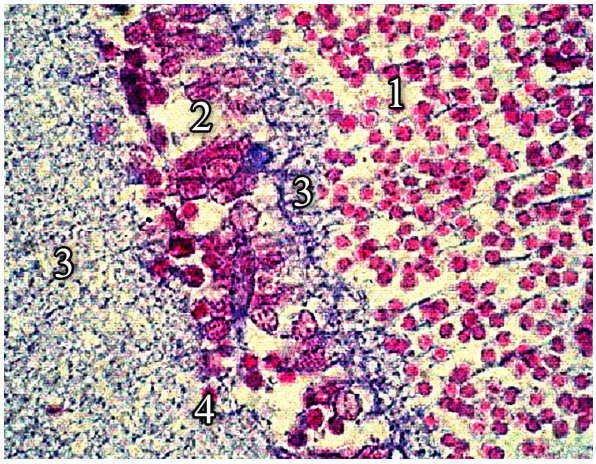

Fig. 2. The retina of a rat. Administration of nalbuphine and pentoxifylline at the end of the 10th week. Azane. Inc. x 1000. 1 – increasing the gaps between nuclei of photoreceptor cells; 2 – karyopyknosis of cells of the inner nuclear layer; 3 – clarification of the main substance of outer and inner plexiform layers; 4 – transposition of cells of the inner nuclear layer into the inner plexiform layer.

There are areas where clarification and vacuolation of the cytoplasm of bipolar and amacrine cells are observed, as well as the clarification of the main substance of the inner nuclear layer. Small gaps appear between the nuclei of the inner nuclear layer. The inner retinal layer (VII), formed by the dendrites of the ganglionic multipolar neuron, the appendages of amacrine and bipolar cells, contains synapses of bipolar cells with ganglionary, as well as the contacts of the dendrites of ganglion cells with the appendages of amacrine cells. Amacrine and interplexiform cells also interact within this layer. Its main substance is clarified, swollen, light pink in color, reticulated, fine-grained. Single lymphocytes and nuclei of Müller cells appear in the inner retinal layer. Ganglionic layer (VIII) is represented predominantly by one or rarely two rows of nuclei. The nuclei of the ganglion cells are mostly rounded, sometimes oval, and contain a clearly visible rounded bulk nucleolus. The cytoplasm of many ganglion cells is quite wide, slightly basophilic. There are mass areas where the distances between the nuclei of ganglion cells increase. The

central part of the individual nuclei is clarified; the chromatin is located near the periphery of the nuclear envelope. There are the nuclei of the ganglion cells, which become irregular in shape and shrunken. The main substance of the ganglionic layer is clarified, especially near the layer of nerve fibers. There are single lymphocytes in the ganglionic layer. Lumens of venous vessels are expanded, overflown with blood. In some places, there is uneven swelling, clarification and vacuolation of the main substance of the ganglionic layer. The wall of the vessels, especially the arterioles is thickened, unevenly swollen. The vascular endothelium is swollen, in some places proliferation of endothelial cells is observed, as shown in fig. 3.

The layer of nerve fibers (IX) is sometimes unevenly swollen. Its main substance is slightly basophilic, quite often clarified, and vacuolated. The distances between the ganglion cells with mass phenomena of pycnosis are increased, as shown in fig. 4. The vessels located at the border of the layer of nerve fibers and the inner boundary layer are expanded, overflown with blood. The wall of the vessel is irregularly thickened, swollen, as it can be seen in fig. 3.

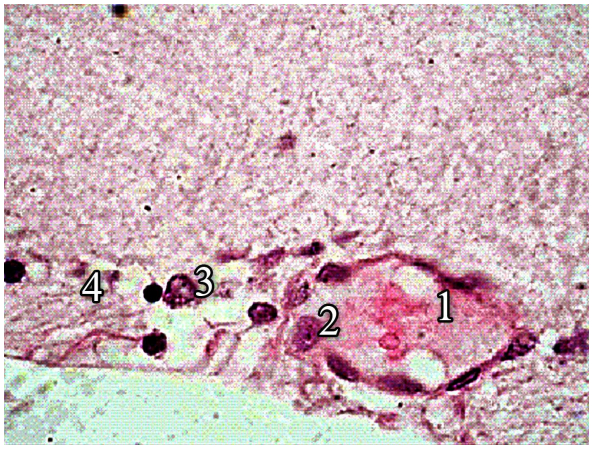

Fig. 3**.** The retina of a rat. Administration of nalbuphine and pentoxifylline at the end of the 10th week. Hematoxylin and eosin. Inc. x 1000. 1.– expansion of the lumen of the vein at the border of the ganglionic layer and the layer of nerve fibers; 2.– swelling of endotheliocytes; 3.– clarification of the cytoplasm of the ganglion cells; **4.**– vacuolation of the main substance of the layer of nerve fibers and the ganglionic layer.

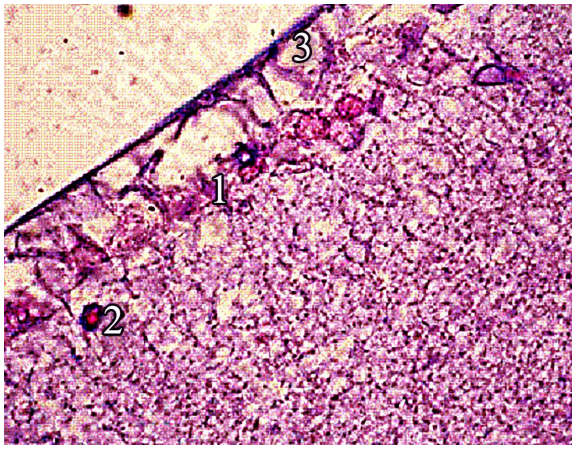

Fig**.** 4. The retina of a rat. Administration of nalbuphine and pentoxifylline at the end of the 10th week. Azane. Inc. x 1000. 1.– increasing of the distance between the ganglion cells; 2.– karyopicosis of the ganglion cells; 3.– vacuolation of the main substance of the layer of nerve fibers.

The inner limiting layer (X) (inner limiting membrane) lays a layer of nerve fibers and separates the retina from the vitreous body. On histological specimen stained with hematoxylin-eosin, this layer is represented by basophilic homogeneous fibers; when stained with azan, this layer is represented by clearly visible violet collagen fibers and the main substance of gray-violet in colour.

Taking into account that during the development of angioneuroretinopathic changes the main etiopathological factors are peripheral blood flow disorders based on the development of oxidative stress, we used trental, which has the ability to improve blood flow at the level of hemomicrocirculation, affects platelet aggregation, decreases blood viscosity **[**9, 12-15].

In our study of 24 adult white male rats, we found that a combination of drugs (nalbuphine and pentoxifylline) during the correction of opioid changes to stabilize dyscirculatory changes in the retina is undesirable. This combination of drugs in the later stages of correction led to a more progressive increase in pathological changes in the retinal layers based on improved blood supply and contributed to a clearer swelling in the retinal layers. This is due to parallel processes: on the one hand, the attempt to improve blood supply, and on the other hand, as a result of improved blood supply, the transport of opioids to the retinal layers in the vascular networks from the retinal pool increased. As there is no research in this area in the available professional Ukrainian and foreign literature, it is impossible to conduct a comparative analysis of the results.

In scientific works [2, 7] after 2 weeks of nalbuphine administration on 144 adult white male rats with subsequent cessation of opioid administration changes in the structure of the vascular membrane of the eyeball are insignificant and associated with the rearrangement of cells and their organelles in the direction of intensive endothelial repair and the basement membrane of the links of the hemomicrocirculatory tract, the epithelium of the ciliary processes, cellular and non-cellular elements of the iris and the vascular membrane itself. After 4 weeks, and even more so 6 weeks of nalbuphine administration, despite the 2-week discontinuation of the drug, there are irreversible deep destructive changes in the vascular membrane of the eyeball.

Based on experimental studies, it can be confirmed that the correction of angioneuroretinopathies with late opioid exposure should be carried out after the complete abolition of opioids.

Conclusions

1. As a result of such medication combination, a progression of necrotic changes in the photoreceptor and among cells of the inner nuclear layer was revealed in the retina of a rat after ten weeks of opioid administration with accession of pentoxifylline at the end of the sixth week. Vacuolar dystrophy of the cells of the inner nuclear layer is also increasing. The transposition of nuclei of photoreceptor cells and cells of the inner nuclear layer into the outer plexiform layer is progressing. The main substance of the ganglionic layer and the layer of nerve fibers is clarified. In general, the increase of swelling processes affecting all layers of the retina is occurring.

2. According to the above-mentioned changes, it can be concluded that the combination of these preparations (nalbuphin and pentoxifylline) is unacceptable during correction of opioid changes with the aim of stabilization of the dyscirculatory changes in the retina. Such medication combination at late terms of correction has caused a progressive increase in pathological changes in the retinal layers has contributed more distinct swelling processes, as a result of progression of dyscirculatory changes in links of vascular nets from the retinal basin.

References

1. Balakirieva OM, Bondar TV, Pryimak YuIu. [ta in.] Kurinnia, vzhyvannia alkoholiu ta narkotychnykh rechovyn sered pidlitkiv, yaki navchaiutsia: poshyrennia i tendentsii v Ukrayini : Za rezultatamy doslidzhennia 2015 roku v ramkakh mizhnarodnoho proektu «Yevropeyske opytuvannia uchniv shchodo vzhyvannia alkoholiu ta inshykh narkotychnykh rechovyn ESPAD» K. Polihrafichnyi tsentr «Foliant». 2015;200 s. Rezhym dostupu: https:.www.unicef.org/ukraine/ukr/ESPAD-ForWEB.pdf. [in Ukrainian]

2. Horalskyi LP, Khomych VT, Kononskyi OI. Za redaktsiieiu Horalskyi LP. Osnovy histolohichnoyi tekhniky i morfofunktsionalni metody doslidzhen u normi ta pry patolohiyi. Navchalnyi posibnyk. Vyd. III, vypravlenne i dopovnene. Zhytomyr: Polissia. 2015: 286 s. [in Ukrainian]

3. Markazova L, Linskyi I, Baranenko O. Analiz dynamiky poshyrenosti ta zakhvoriuvanosti na rozlady psykhiky i povedinky vnaslidok uzhyvannia psykhoaktyvnykh rechovyn v Ukrayini za period 1990–2014 rokiv. Psykhiatriya, nevrolohiya ta medychna psykholohiya 2017; 4.(1 (7)): 52–58. [in Ukrainian]

4. Markozova, L. Prychyny ta zakonomirnosti kooperatyvno-konkurentnoyi vzaiemodiyi staniv zalezhnosti riznoho pokhodzhennia. Psykhiatriya, nevrolohiya ta medychna psykholohiya, 2016;3(2 (6)):58–61. [in Ukrainian]

5. Mironova NV. Psihologicheskaya adaptatsiya i samoaktualizatsiya u muzhchin s alkogolnoy zavisimostyu na raznykh stadiyakh remissii. Istoricheskaya i sotsialnaya obrazovatelnaya mysl. 2013;3:19–23. [in Russian]

6. Novytskyi IIa, Yakymiv NIa, Yerokhova OM, [ta in.] Toksychne urazhennia zorovykh nerviv vnaslidok tryvaloho pryiomu levomitsetynu na tli narkotychnoyi zalezhnosti vid kodterpinu. Oftalmol. zhurnal. 2012; 3: 43–45. [in Ukrainian]

7. Patent №76565 Ukraina. Sposib modeliuvannia khronichnoho opioidnoho vplyvu. Paltov YeV, Fik VB, Vilkhova IV, Onysko RM, Fitkalo OS, Kryvko YuIa; zaiavnyk i patentovlasnyk Lvivskyi natsionalnyi medychnyi universytet imeni Danyla Halytskoho. – opubl. 10. 01. 2013, Biul. №1. [in Ukrainian]

8. Pinchuk II, Petrychenko OO, Kolodiezhnyi OV. [ta in.]. Zakhvoriuvanist i poshyrenist rozladiv psykhiky ta povedinky vnaslidok vzhyvannia psykhoaktyvnykh rechovyn sered uchasnykiv antyterorystychnoyi operatsiyi v Ukrayini za pershe pivrichchia 2016 roku. Arkhiv psykhiatriyi. 2016; 22(4 (87)):11–14. [in Ukrainian]

9. Fitkalo OS. Monitorynh poshyrenosti rozladiv psykhiky ta povedinky vnaslidok vzhyvannia psykhoaktyvnykh rechovyn sered naselennia m. Lvova ta Lvivskoyi oblasti. 2013. Medychna osvita. 105–108 [in Ukrainian]

10. Yakimiv NYa. Morfologicheskaya kharakteristika struktur raduzhno-rogovichnogo ugla krys na raznykh srokakh deystviya i na rannikh srokakh posle otmeny eksperimentalnogo opioidnogo vliyaniya. Oftalmologiya. Vostochnaya Yevropa. 2014; 2: 89–97. [in Russian]

11. Yakymiv NIa, Kryvko YuIa. Mikrostrukturna kharakterystyka raiduzhno-rohivkovoho kuta ochnoho yabluka shchuriv pry opioidnomu vplyvi. Svit medytsyny ta biolohiyi. 2013; 4:120–124. [in Ukrainian]

12. Yakymiv NIa. Ultrastrukturna kharakterystyka struktur raiduzhno-rohivkovoho kuta ochnoho yabluka shchuriv na 35-u ta 42-u dobu opioidnoho vplyvu. Svit medytsyny ta biolohiyi. 2014; 2:185–188. [in Ukrainian]

13. Yakymiv NIa. Ultrastrukturna kharakterystyka struktur raiduzhno-rohivkovoho kuta ochnoho yabluka shchuriv na 7-u, 14-u, 21-u, 28-u dobu opioidnoho vplyvu. Ukrainskyi morfolohichnyi almanakh. 2014; 2:28–31. [in Ukrainian]

14. Diskovskyi I, Pidvalna U, Popyk P. The method of mathematical analysis of angioarchiotectonics of organs in normal and pathological state. XII International Congress of Medical Sciences: Book of Abstracts. Sofia. 2013: 67 p.

15. Mateshuk-Vatseba L, Pidvalna U, Kost A. Peculiarities of vascular tunic microstructure of the white rat eyeball under the effect of opioid. Romanian Journal of Morphology and Embryology. 2015; 56 (3): 1057–1062.

Стаття надійшла 10.05.2020 р.