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FEATURES OF MORPHOMETRIC PARAMETERS OF THE MYOCARDIUM AFTER RATS' EXPOSURE TO THE SCORPION *LEIURUS MACROCTENUS* VENOM

Yanchyshyn A.   Features of morphometric parameters of the myocardium after rats' exposure to the scorpion *Leiurus macroctenus* venom.


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ABSTRACT. Background. Toxic components of scorpion venom cause local symptoms and the development of severe neurological, hematological disorders, cardiovascular, respiratory, excretory systems, etc. Among the leading causes of death under these conditions are heart failure and pulmonary edema. Expanding the understanding of the effects of scorpion venoms will make it possible to establish pathogenetic mechanisms for developing certain complications and patterns of histological and biochemical changes in target organs. It can also be essential in developing treatment methods, prevention, and manufacturing medicines and antidotes. **Objective.** Morphometric analysis of changes in the structure of the rat heart wall and the course of hemodynamic processes in it in response to the effect of the venom of the scorpion *Leiurus macroctenus*. **Methods.** Experimental studies were conducted on 60 male rats (180 g \pm 3 g), which were injected intramuscularly with 0.5 ml of the venom solution (28.8 mg/ml) (LD₅₀=0.08 mg/kg). Morphometry of digital images was performed in the Fiji: ImageJ program. The width of cardiomyocytes and the percentage of myocardial area occupied by blood were determined. **Results and conclusion.** Against the background of the action of the scorpion venom *Leiurus macroctenus*, a statistically significant increase in the width of cardiomyocytes and the area of hemorrhage zones in the myocardium is observed; these characteristics are more pronounced over time. Cardiomyocytes showed signs of pathological changes already in the first hour of the venom action, when, morphometrically, the difference from the control group was still impossible to register. In the case of cardiomyocyte sizes, if in the first hour after the venom administration, the increase in their width was not observed in the experimental group. The width was already significantly more significant in the third hour than in the control group. The area of the vessels increased due to the aggregation in their lumens and near the walls of formed blood elements, the perfusion of the vessels themselves and edema of the middle and outer membranes. The area of hemorrhagic zones outside the vessels was determined by the increase in permeability, or even the destruction of their walls, which led to diapedesis and passive penetration of formed blood elements into the surrounding tissues.

Key words: venom, scorpions, myocardium, morphometry, rats.

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Introduction

Poisoning due to scorpion stings is a current medical problem that poses a threat to the health and life of people, especially children and the elderly with a complicated medical history [1]. The epidemiology of scorpionism worldwide is impressive. Every year, about 1.2-1.5 million people suffer from the bites of these animals, which leads to 2600 deaths. Until recently, it was believed that poisoning due to scorpion stings is characteristic mainly of poorly developed tropical and subtropical countries, namely Africa, India, the Middle East, Mexico, and Latin America. However, it is now well known that their usual distribution area has significantly expanded, so fatal cases are also recorded in countries with a high standard of

living [2, 3].

Currently, there are over 1500 different species of scorpions, of which 50 are venomous subspecies [4, 5, 6, 7, 8, 9]. It has been established that the toxic components of their venom cause not only local symptoms but also the development of severe neurological, hematological disorders, disorders of the cardiovascular, respiratory, excretory systems, etc. Among the leading causes of death under these conditions are heart failure and pulmonary oedema [10]. Proteins are essential structural components of their venom and have extremely toxic properties [11, 12, 13, 14, 15]. Even though more than 800 peptides have been identified to date, the composition of scorpion venom is considered poorly studied. In addition, it

can vary significantly even within the species; therefore, the features of biological action, impact on target organs, and morphological changes in body systems are variable. Therefore, a detailed study of the structural elements of scorpion toxins is a promising direction in toxicology, molecular biology, morphology, etc. Expanding the understanding of the effects of scorpion venoms will make it possible to establish pathogenetic mechanisms for developing certain complications and patterns of histological and biochemical changes in target organs. It can also be essential in developing treatment methods, prevention, and manufacturing medicines and antidotes.

In recent years, pronounced changes in climatic conditions have been recorded, which can lead to the appearance in specific territories of animals, including scorpions, that are not typical for a particular area. In addition, the creation of artificial conditions for breeding predatory, poisonous animals that pose a significant threat to the life and health of living organisms is currently relevant. That is why the importance of the problem of poisoning due to bites of predatory animals, their consequences, and practical and, most importantly, timely treatment is growing.

The above data indicate the need for an in-depth study of the structure, biological action, and functional activity of toxins of various species of scorpions since a significant proportion of them remains unknown. In addition, it is essential to deepen knowledge in this field since every year, scientists discover new representatives of these animal species. Thus, *Leiurus macroctenus*, belonging to the Buthidae family, was found recently. Toxicological studies of the composition of its venom are minimal, and the specifics of the impact on the victims' bodies are entirely unknown. Based on this, the importance and timeliness of our study on the study of morphometric indicators of the hearts of rats exposed to the venom of the scorpion *Leiurus macroctenus* are undeniable.

The study aims to perform a morphometric analysis of changes in the structure of the rat heart wall and the course of hemodynamic processes in response to the effect of the scorpion venom *Leiurus macroctenus*.

Materials and methods

Experimental studies were conducted on 60 male rats (180 g \pm 3 g), which were injected intramuscularly with 0.5 ml of venom solution (28.8 mg/ml) (LD₅₀=0.08 mg/kg) dissolved in saline (0.9%) [16]. The control group (13 rats) was injected with only 0.5 ml of saline (0.9%).

In this study, absolute and relative variables were selected that reflect the potential myocardial response to the toxic effect of scorpion venom. To assess these changes over time, measurements were performed on digital images of histological preparations of tissues removed one and three hours after the corresponding venom entered the body. Morphometry of digital images was performed in the Fiji: ImageJ program. The width of cardiomyocytes was de-

termined (higher accuracy of measurements was ensured by working with images at a magnification of x200 and using preparations stained using the unique Azan Trichrome method; the data were compared between the intact and two experimental groups, which differed in the duration of the poison; in each of the two groups, measurements were performed in 16 fields of view (5 random cardiomyocytes in each); as a result of this approach, 80 measurements were obtained for each group) and the percentage of the myocardial area occupied by blood (since the rate of the area is a relative indicator, measurements were performed both in micrometers and in pixels to calculate it; the percentage ratio between the regions filled with red blood cells and the surrounding tissues was calculated; 20 digital images were analyzed for each group: one control and two experimental; the obtained indicators were statistically processed in Excel, in (Graphs were also constructed based on these measurements to better illustrate the results obtained).

Results and discussion

At the beginning of our analysis, we assessed the normality of the distribution of the data obtained in the control and four experimental groups, which showed that the graph that displayed it did not correspond to a Gaussian curve. In connection with such results, the non-parametric Mann-Whitney test was used to assess the reliability of the increase in the size of cardiomyocytes in the experimental groups relative to the control and the differences between them. Statistical analysis of the data on the width of cardiomyocytes showed a significant increase in the size of heart cells relative to animals not exposed to scorpion venom in all experimental groups, except for the group with the shortest duration of its exposure - one hour (Fig. 1). If for the control group the average value of this indicator is 21.22 μ m (first quartile 19.44 μ m; third quartile 22.73 μ m), then for rats one hour after exposure to *Leiurus macroctenus* poison, it is 20.44 μ m (first quartile 17.84 μ m; third quartile 22.88 μ m).

In general, the smaller average width of cardiomyocytes in this group is only evidence that the toxic effect on the proteins of their cytoplasm did not have enough time to manifest itself at the cellular level. In particular, the loss of transverse striation of muscle fibers in the rat heart, noted already an hour after inoculation, indicates a toxic effect of the poison on the proteins actin and myosin, which determine the striation of muscle fibers, or on auxiliary proteins that help in their attachment to the cytoskeleton and the organisation of organelles in the cytoplasm. The destruction of such finely constructed interactions between proteins sooner or later inevitably leads to the stratification of structures in the cytosol, their disorganisation and expansion.

At the same time, the cardiomyocytes of the hearts of rats from the control group, although they were slightly (statistically unreliable) more expansive than those in the group after one hour of exposure to the poison, had pronounced transverse striations and

even intercalated discs, which stood out as thickenings between neighboring cells. Thus, despite the morphometric data, with a careful assessment of the results of the morphological description, we can say that there were no intracellular changes in the sarcoplasm of cardiomyocytes that were not exposed to toxins from scorpion venom. In the experimental

groups, at later times after the introduction of the poison, a statistically significant increase in the size of heart muscle cells was noted as a result of changes in sarcolemmal proteins, which appeared already in the first hour of exposure to the poison but were not observed in the control group.

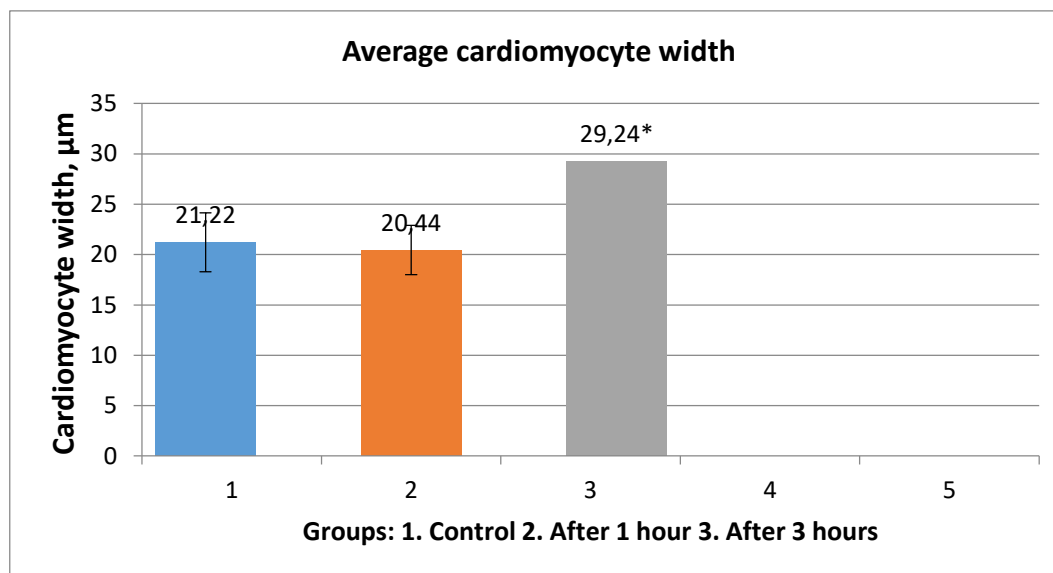


Fig. 1. Average value of cardiomyocyte width in the control group and experimental groups with different durations of action of *Leiurus macroctenus* venom. * - difference from the control and experimental groups 1 hour after venom administration is significant at $p \leq 0.01$; ** - difference from the experimental group 3 hours after venom administration is significant at $p \leq 0.01$.

Another critical aspect for comparing the results of quantitative assessment of the width of cardiomyocytes is the dispersion of data relative to their average value. In the control group, this indicator is $2.34 \mu\text{m}$; in the experimental group, after one hour of exposure to the poison, it is $3.8 \mu\text{m}$. This indicates the presence of significantly smaller and significantly larger cardiomyocytes in width, which does not affect the average indicator of their size, but shows the presence of single cells, which already at such an early stage show signs of edema and other hypertrophic processes, which will be more clearly manifested in later stages of the poison. However, the presence of a certain number of narrower than usual cardiomyocytes affected the average value of the width of these cells in this group.

Suppose we return to the morphological description of the rat myocardium in the first hour after the poison enters the body. In that case, we cannot ignore other evidence of intracellular changes, which will later be reflected in the quantitative characteristics of its muscle cells. The blurred contours of the cardiomyocyte nuclei in this group indicate the early stages of developing edema processes, which cause moderate hypertrophy of only single cells. The homogenisation of the cytoplasm confirms the destructuring of actin-myosin interactions, which, on the one hand, are manifested by the loss of striation and, on the other hand, indicate the further development of intracellular edema.

And suppose this edema, the most apparent

cause of an increase in the size of any cell, including cardiac cardiomyocytes, is a wholly expected cause of such a significant difference for more extended periods of exposure to the poison. In that case, it is interesting to note the statistically significant increase in the size of heart cells relative to the control in the “next” experimental group – only three hours after the start of the poison. However, this result correlates with the data of morphological studies of heart tissues conducted in this group.

Three hours after exposure to *Leiurus macroctenus* venom, the width of cardiac muscle cells in rats is already $29.24 \mu\text{m}$ (first quartile $26.50 \mu\text{m}$; third quartile $31.22 \mu\text{m}$) (Fig. 1). This is significantly greater not only in the control group but also in the rats one hour after the venom was administered.

An examination of the results of the morphological study of the heart tissues of rats from this group explains such a dramatic increase in their size. After all, the wave-like modification of the shape of these cells intensified, which confirmed the further development of edema. The sarcoplasm of cardiomyocytes became more eosinophilic and somewhat enlightened, the first morphological sign of fluid penetration into the cell and oedema of the cytosol. Interestingly, at this stage, edema, as the leading cause of the increase in cell size, had already reached the nuclei of cardiomyocytes, which contained numerous vacuoles, presumably with fluid. Vacuolisation of the nuclei confirms the deep stage of edema of cardiomyocytes, which significantly increased their size and

width. If one hour after the introduction of the poison, the actin-myosin interactions underlying striation were only beginning to be disturbed, here the striation was no longer determined. This speaks in favor of the loss of spatial structuring of motor proteins, which determine the intracellular organisation of muscle fibers.

A review of the sources also helped to understand the origins of such an increase in cell size. As is known, the action of toxic substances causes a deterioration in local blood circulation precisely at the third hour after the poison's impact on the organ, which, in turn, leads to a decrease in the level of Oxygen in the cardiac muscle, which is morphologically manifested in the appearance of vacuoles in the cytosol at earlier stages of damage and the nucleus at later ones [17]. The expansion of the lumen between the membranes of the smooth endoplasmic reticulum and the swelling of lysosomes primarily determines vacuolisation of the cytoplasm. These single-membrane organelles are responsible for counteracting and neutralising toxins that enter the cytosol through the membrane.

The set of morphological features we have presented confirms the most acute manifestation of the processes of destruction of the surface apparatus, intracellular edema and death of rat cardiomyocytes on the third day after the introduction of the poison, which was morphometrically reflected in a statistically significant increase in the size, namely the width of these cells, not only relative to the control group, but also relative to the groups with earlier periods of exposure to the poison.

Another probable mechanism of the action of scorpion venom on cardiomyocytes, and, accordingly, their morphometric characteristics, is myocardial ischemia, caused primarily by impaired microcirculation in its tissues [18]. In studies on the effect of scorpion venom on the heart muscle, impaired blood

flow in the coronary vessels is separately noted [19]. That is why the second parameter on which we focused our attention is the state of the vessels of the myocardial microcirculatory bed in response to the action of scorpion venom.

We needed to quantitatively assess such processes in the vessels as their expansion, filling with blood, violation of the integrity of their walls and hemorrhages, which are logical consequences of the action of scorpion venom toxins on the heart. For this purpose, we chose a threshold method of assessing the area of a specific color in a digital image by screening out other spectrum elements. The use of this method of quantitative analysis became possible due to the contrast of rat heart preparations when stained with Azan trichrome [20], because the blue color of cardiomyocytes and connective tissue elements of the extracellular matrix contrasted sufficiently with the intense red blood cells, so that it was possible to screen out all areas except the zones occupied by blood for further assessment of their absolute area and fate relative to all other structures on the section.

As is known, the vessels deliver the poison to the surrounding tissues, and their wall is the first barrier to the poisons' toxins. In addition, as already mentioned, the vessels are the main factor in ischemia, one of the characteristic effects of scorpion venom on the heart of its victim.

It is quite characteristic that, according to the results of our morphometric studies, all experimental groups demonstrated a statistically significant increase in the area occupied by red blood cells relative to the control group starting from the first hour of the poison's action (Fig. 2, 3). Such results correlate with the literary sources' data and the morphological study of myocardial tissues in these groups.

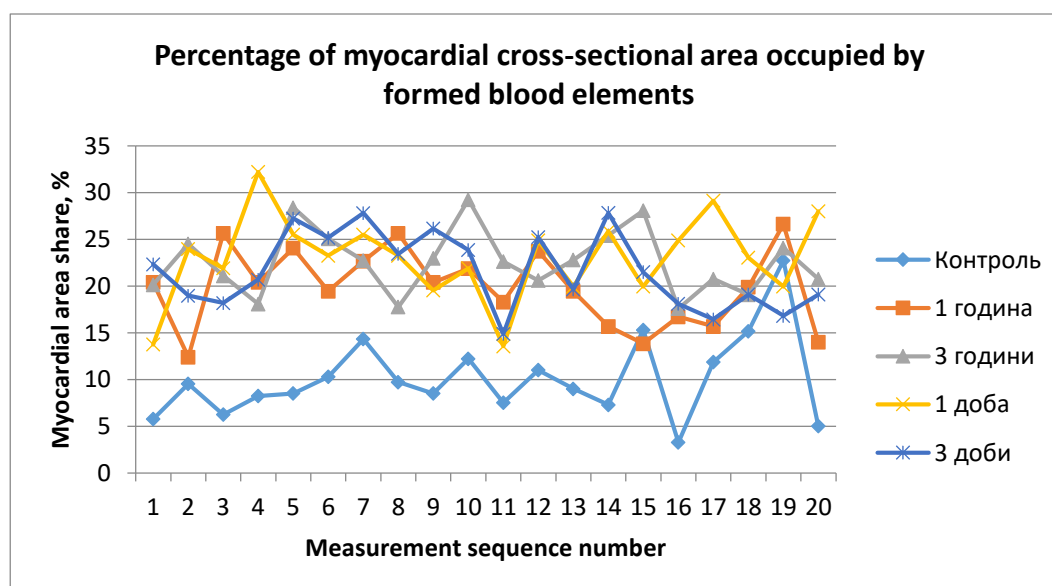


Fig. 2. Individual indicators of the proportion of myocardium filled with formed blood elements in the control and experimental groups with different durations of exposure to the venom of the scorpion *Leiurus macroctenus*.

Many studies on the effects of scorpion venom on the myocardium identify three main factors of cardiac dysfunction: adrenergic myocarditis, toxic myocarditis and myocardial ischemia [21]. These acute effects develop within the first hour after the venom enters the body [22]. In our case, after just one hour, the proportion of the myocardium filled with red

blood cells in the experimental group was significantly higher than in the control group. If in the intact group, this figure was 10.08% (first quartile 7.5%; third quartile 12.0%), then for the group whose animals were exposed to *Leiurus macroctenus* venom for one hour, it was 19.84% (first quartile 16.45%; third quartile 22.95%).

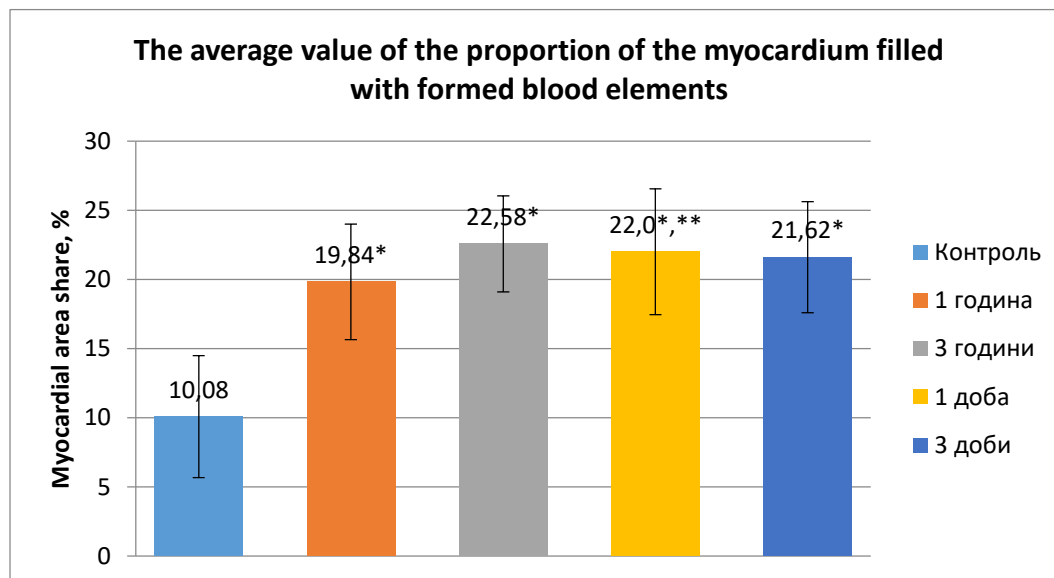


Fig. 3. Average value of the percentage of myocardium filled with blood in the control and experimental groups with different scorpion venom *Leiurus macroctenus* action durations. * - difference from the control group is significant at $p \leq 0.01$; ** - difference from the experimental group of animals 1 hour after venom administration is significant at $p \leq 0.05$.

Let's turn to the data of the histological analysis of the state of the blood vessels in the heart's muscular wall in the control group. We can note the initial characteristics, which in the future, with an increase in the total time of exposure of the poison to the heart, will be modified more and more deeply. In particular, it is necessary to pay attention to the state of endothelial cells in the vessels of the microcirculatory bed. Here, endothelial cells were located on the basement membrane tightly to each other and did not show signs of detachment. Tunica media and tunica adventitia also demonstrated the absence of oedema in smooth muscle and loose connective tissues. It is also essential to pay attention to the internal contents of the vessels because the processes of erythrocyte agglomeration and whole blood significantly affect the cross-sectional area of the vessels. In the intact group, the lumens of the blood vessels demonstrated only the presence of red blood cells, without any hints of clumping or coagulation.

In turn, in our morphological analysis of the state of the microcirculatory bed in the experimental group, where rats were exposed to poison for one hour, attention is drawn to the presence of significant diapedetic hemorrhages and the presence of red blood cells outside the vessels - they infiltrated between the fibers of the heart muscle. Thus, the exit of erythrocytes outside the microcirculatory bed could not but affect the increase in the proportion of the myocardium filled with blood.

Another factor that increased this indicator was the expansion of the venous vessels of the heart muscle due to their entire blood supply. As is known, the wall of venous vessels is devoid of a developed framework of elastic fibers and membranes, so it does not resist a change in shape and stretching in response to blood stasis. Such vessel stretching, accompanied by increased diapedesis, was also confirmed by increased gaps between endothelial cells on the basal membrane. Venous vessel hyperextension was determined by whole blood, as evidenced by the aggregated accumulations of erythrocytes noted by us both in the lumens of the vessels and attached to their walls. The walls themselves were already characterised by desquamation of endothelial cells from the basement membrane and their invagination at such an early stage.

Let's look at the dynamics of changes in the myocardial microcirculatory bed at the third hour after the onset of the poison. We note a slight further increase in the area of hemorrhage, which nevertheless does not significantly differ from the indicators at the first hour after poisoning (Fig. 3). Here, the proportion of the area of the sections filled with blood elements was on average 22.58% (first quartile 20.48%; third quartile 24.65%).

Morphologically, this group revealed areas of hemorrhage, while morphometric assessment did not show a significant increase in their area compared to animals after one hour of exposure to the poison. This

does not indicate the absence of further development of pathological processes in and around the vessels because we observed increased infiltration of immune cells through their walls and clumps of erythrocytes stuck together in their lumens. Simply deepening these processes did not significantly affect the increase in the area of the vessels. Nevertheless, there was fullness of blood and expansion of the vessels, and the divergence of endothelial cells from each other, which was noted even in the first hour of exposure to the poison, continued to intensify, in places resulting in the detachment of these cells from the basement membrane and their desquamation into the lumen of the vessels. Such changes in the lining of the vessel wall cannot but increase diapedesis and the outflow of fluid from the vessels, which was morphologically reflected in the accumulation of formed blood elements outside the vessels and edema in the matrix, respectively.

Conclusion

According to the results of the morphometric analysis of the data, we can speak of a statistically significant increase in the width of cardiomyocytes and the area of hemorrhage zones in the myocardium; these characteristics became more pronounced over time. In particular, cardiomyocytes morphologically showed signs of pathological changes already in the first hour of the poison's action, when morphometrically, the difference from the control group was still

impossible to register. In the case of cardiomyocyte sizes, if an increase in their width was not observed in the first hour after the poison's administration in the experimental group, the width was already significantly more significant in the third hour than in the control group. In the statistical analysis of the fraction of the area of the sections occupied by red blood cells in or outside the vessels, a significant difference from the control group according to the Mann-Whitney criterion was observed already from the first hour of the poison's action. The area of the vessels increased due to aggregation of formed blood elements in their lumens and near the walls, engorgement of the vessels themselves, and edema of the tunica media and tunica adventitia. The area of hemorrhagic zones outside the vessels was determined by increased permeability or even destruction of their walls. This led to diapedesis and passive penetration of formed blood elements into the surrounding tissues.

Prospects for further development are related to the study of histological changes in the myocardium of rats exposed to the venom of the scorpion *Leiurus macroctenus* at later stages of the experiment.

Information on conflict of interest

No potential or apparent conflicts of interest are associated with this manuscript at publication, and no conflicts of interest are anticipated.

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Янчишин А. Особливості морфометричних показників міокарда після впливу на щурів отрути скорпіонів *Leiurus macroctenus*.

РЕФЕРАТ. Актуальність. Токсичні компоненти отрути скорпіонів зумовлюють появу не лише місцевих симптомів, але і розвиток важких неврологічних, гематологічних розладів, порушень діяльності серцево-судинної, дихальної, видільної систем, тощо. Серед основних причин смерті за даних умов є серцева недостатність та набряк легень. Розширення уявлень щодо впливу отрут скорпіонів надасть змогу встановити патогенетичні механізми розвитку тих, чи інших ускладнень, закономірностей гістологічних та біохімічних змін в органах-мішенях, а також може відіграти важливу роль у розробці методів лікування, профілактики, виготовлення лікарських засобів і протиотрути. **Мета.** Морфометричний аналіз змін структури стінки серця щурів та перебігу гемодинамічних процесів в ній у відповідь на вплив отрути скорпіонів *Leiurus macroctenus* **Методи.** Експериментальні дослідження проводили на 60 щурах-самцях щурів (180 г±3 г), яким внутрішньом'язово вводили 0,5 мл розчину отрути (28,8 мг/мл) (LD50=0,08 мг/кг). Морфометрія цифрових зображень проводилась у програмі Fiji:ImageJ. Визначали ширину кардіоміоцитів та відсоток площі міокарду, зайнятий кров'ю. **Результати та підсумок.** На тлі дії отрути скорпіонів *Leiurus macroctenus* статистично достовірно спостерігається зростання ширини кардіоміоцитів та площі зон крововиливів у міокарді, ці характеристики виявляються все яскравіше з плином часу. Кардіоміоцити проявляли ознаки патологічних змін вже на першу годину дії отрути, коли морфометрично відмінність від групи контролю ще неможливо було зареєструвати. У випадку розмірів кардіоміоцитів, якщо на першу годину після введення отрути зростання їх ширини у експериментальній групі не відмічалось взагалі, то на третю ширину вже була достовірно більшою відносно групи контролю. Площа судин зростала через агрегацію в їх просвітах та біля стінок формених елементів крові, повнокрів'я самих судин та набряки середньої та зовнішньої оболонок. Площа ж геморагічних зон поза судинами визначалась підвищенням проникності, або навіть руйнуванням їх стінок, що призводило до діapedезу і пасивного проникнення формених елементів крові в оточуючі тканини.

Ключові слова: отрута, скорпіони, міокард, морфометрія, щури.