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MICROSCOPIC ORGANISATION OF THE MYOCARDIUM OF EXPERIMENTAL RATS 1 AND 3 HOURS AFTER EXPOSURE TO THE VENOM OF THE SCORPION LEIURUS MACROCTENUS

Yanchyshyn A. ✉ Microscopic organisation of the myocardium of experimental rats 1 and 3 hours after exposure to the venom of the scorpion Leiurus macroctenus.
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ABSTRACT. Background. Animal venom toxins are characterised by significant variability in structure and biological effects. They are usually represented by proteins, numerous peptides that have undergone restructuring over many years of evolution, which influenced the mechanisms and features of their effect on the victim's body. Scorpion venom can cause myocardial damage, initiating many pathogenetic mechanisms. After bites from these animals, the development of myocardial infarction due to coronary spasms is quite often recorded. **Objective.** Study of microscopic changes in the heart of mature rats 1 and 3 hours after exposure to the venom of the scorpion Leiurus macroctenus. **Methods.** Experimental studies were conducted on 60 male rats (180 g±3 g), which were injected intramuscularly with 0.5 ml of venom solution (28.8 mg/ml) (LD_{50}=0.08 mg/kg). Heart samples of animals from all groups were taken for microscopic examination. Histological preparations of the heart were stained with hematoxylin and eosin, azan trichrome and iron hematoxylin. **Results and conclusion.** Administration of Leiurus macroctenus scorpion venom to rats resulted in the smoothing and homogenisation of cardiac muscle fibres and their moderate hypertrophy. Diapedetic haemorrhages and pronounced lymphohistiocytic infiltration were observed. After 3 hours of the experiment, the deepening of morphological changes in the rat myocardium was detected. Wave-like deformation of muscle fibres and their pronounced oedema was noted. Edematous changes, lymphocytic infiltration, and haemorrhages characterised the stroma of the cardiac muscle. Manifestations of desquamation of the endothelial lining of the vascular wall and perivascular lymphohistiocytic infiltration were observed.
Key words: venom, scorpions, myocardium, lymphohistiocytic infiltration, rats.

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Introduction

Venomous animals are known to be distributed globally and represented by many biological species. Many of them belong to the kingdom Animalia [1, 2]. Venomous animals include both vertebrates and invertebrates. In their bodies, they constantly or periodically contain substances that are toxic to representatives of other animal species or humans who become victims of their bites. It has been established that even small doses of these compounds cause disorders of normal functioning and, in severe cases, death [3, 4, 5, 6]. Some species of venomous animals have special venom glands that produce the venom itself, while others accumulate toxic components from the external environment in various tissues of the body [7, 8, 9].

Animal venom toxins are characterised by significant variability in structure and biological effects [10]. They are usually represented by proteins, numerous peptides that have undergone restructuring over many years of evolution, which influenced the mechanisms and features of their effect on the victim's body [11, 12, 13]. These toxins use ion channels, receptors, and enzymes as targets while exhibiting extraordinary efficiency and selectivity of action [14, 15, 16]. Animal venoms are natural sources of biologically active molecules that exert various pharmacological effects [17, 18, 19]. They are classified according to their origin - snake, scorpion, spider venoms or according to the features of their action on the

victim's body - neurotoxins, hemo-vasotoxins, cardiotoxins, cytotoxins, etc. [20, 21, 22]. They are usually aqueous solutions containing many components of predominantly protein nature. The venom of one species of animal sometimes contains several hundred different toxins. As a rule, it includes enzymes (phospholipases, proteases, oxidases), proteins without enzymatic activity (for example, disintegrins), peptides, and metal ions [23, 24, 25]. Such a complex composition of animal venoms is the reason for the impact on all body systems and the development of severe clinical poisoning.

In modern conditions, scorpions significantly expand the territory of their existence, spreading even to those areas of the globe that were not previously characteristic of them. About thirteen families of these animals have been established, most of which are dangerous to humans. Their venom consists of salts, biogenic amines, nucleotides, enzymes (phospholipases, hyaluronidase, L-amino acid oxidases, metalloproteinases, serine proteases), peptides with low molecular weight [26]. These compounds affect almost all systems of the victim's body. They are also characterised by their extraordinary pharmacological properties, which allow them to use certain toxins for therapeutic purposes [27, 28].

Scorpion venom can cause myocardial damage, initiating many pathogenetic mechanisms. After bites from these animals, the development of myocardial infarction due to coronary spasm is quite often recorded [29, 30, 31]. It has been established that poisoning with scorpion toxins is associated with the release of vasoactive inflammatory and thrombogenic peptides and amines, such as histamine, bradykinin, serotonin, thromboxane, leukotrienes, which induce coronary artery spasm, facilitate platelet aggregation processes and the development of thrombosis. The venom can have a direct cardiotoxic effect with the development of myocarditis (toxic and adrenergic).

The study aimed to study microscopic changes in the hearts of mature rats 1 and 3 hours after exposure to Leiurus macroctenus scorpion venom.

Materials and methods

Experimental studies were conducted on 60 male rats (180 g±3 g), which were injected intramuscularly with 0.5 ml of a solution of poison (28.8 mg/ml) (LD_{50}=0.08 mg/kg) dissolved in saline (0.9%) [32]. The control group (13 rats) was injected with only 0.5 ml of saline (0.9%).

For microscopic examination, heart samples of animals from all groups were taken. The pieces were fixed in a 10% formalin solution for 1 day. Then, they were dehydrated in alcohols with increasing concentration and embedded in paraffin blocks. Histological preparations of rat hearts were stained with hematoxylin and eosin, azan trichrome, and iron hematoxylin. Histological preparations were studied using an SEO SCAN light microscope and photographed using a Vision CCD Camera with an image output system from histological preparations.

Results and discussion

The introduction of Leiurus macroctenus scorpion venom into rats was accompanied by morphological changes in the myocardium of the animals. In particular, the first changes were detected already 1 hour after venom injection. Loss of transverse striation of muscle fibres was observed in the myocardium, barely noticeable in individual fields of view. Cardiac muscle fibres were characterised by moderate hypertrophy and appeared as wave-like structures (Fig. 2). The cytoplasm of contractile cardiomyocytes was homogenised. The nuclei of these cells had a predominantly oval (elongated) shape and contained condensed chromatin, which was concentrated marginally. One or two nucleoli were noted in them. Due to oedema in individual cardiomyocytes, the nuclei contours were indistinct and bizarre (Fig. 1).

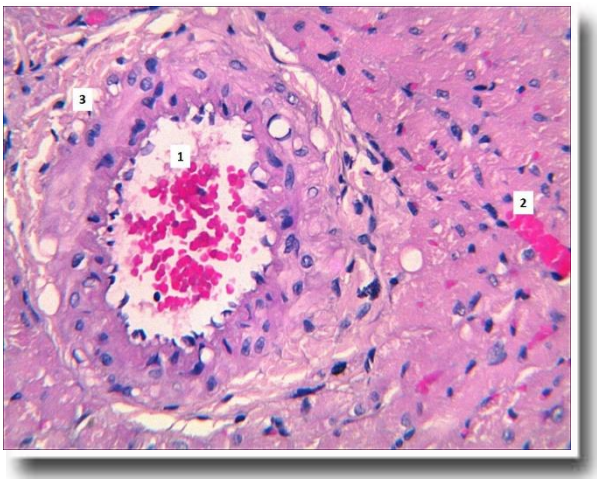

Fig.1. Histological changes in the rat heart 1 hour after exposure to the venom of the scorpion Leiurus macroctenus. The lumen of the myocardial blood vessel (1), the focus of haemorrhage (2), and the loosening of the outer membrane of the vessel wall (3). Staining with hematoxylin and eosin. ×200.

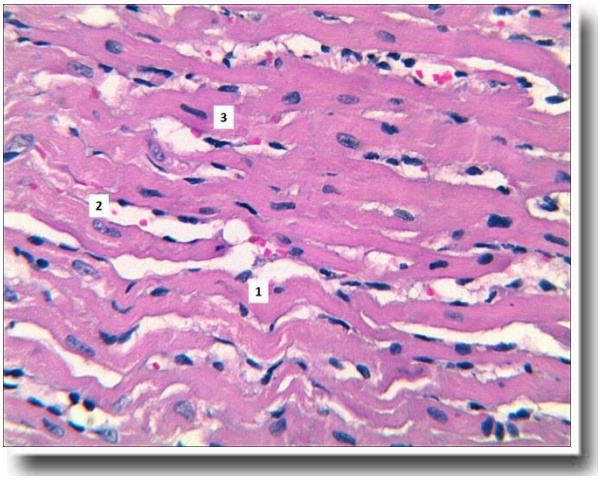

Fig. 2. Microscopic organisation of the rat heart 1 hour after exposure to the venom of the scorpion Leiurus macroctenus. Wave-like deformation of myocardial fibres (1), nuclei of contractile cardiomyocytes (2), and myocardial muscle fibres (3). Staining with hematoxylin and eosin. ×200.

The spaces between the myocardial fibres were significantly expanded, showing the interstitial connective tissue oedema. Myosatellite cells were visible between the muscle fibres. The nuclei of the latter had an elongated shape and contained condensed chromatin. Significant diapedetic haemorrhages and accumulation of erythrocytes between the myocardial fibres were observed in all fields of view. The venous vessels of the heart muscle were markedly full-blooded, with conglomerates of erythrocytes in their lumens and adhesion of the latter to the vessel walls. Increased spaces distinguished the endothelial lining of the veins between the cells. The nuclei of the endothelial cells were oval and hyperchromic. The smooth myocytes of the tunica media of the vein walls were characterised by moderate oedema and contained rounded or elongated nuclei. Loosening and fibrilisation of the adventitia of the vessels were noted. Fibrin threads, foci of desquamation of endothelial cells from the basement membrane, and their invagination are also visible in the lumens of blood vessels. A characteristic histological finding under these conditions was pronounced lymphohistiocytic perivascular infiltration (Fig. 4). Numerous lymphocytes, macrophages, and occasionally plasma cells were observed around the myocardial vessels. Leukocyte and lymphohistiocytic infiltration was also noted in the conducting system of the rat heart (Fig. 2).

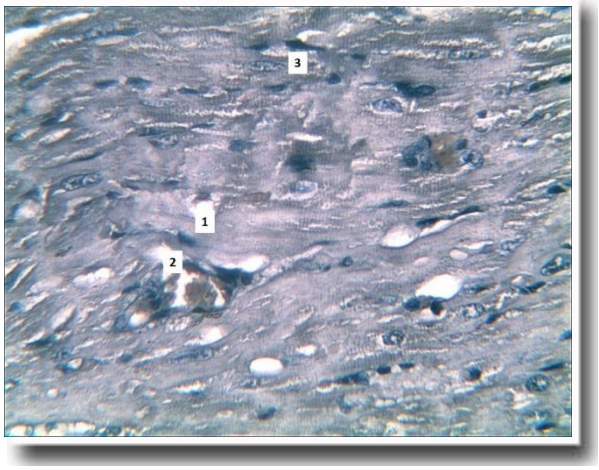

Fig. 3. Histological changes in the rat heart 1 hour after exposure to the venom of the scorpion Leiurus macroctenus. Muscle fiber (1), blood vessel lumen (2), contractile cardiomyocytes (3). Staining with iron hematoxylin. ×200.

Three hours after the start of the experiment, microscopic studies of the rat myocardium revealed deepening morphological changes. This period was characterised by increased wave-like deformation of the heart muscle fibres. They were distinguished by significant oedema. The sarcoplasm of contractile cardiomyocytes was eosinophilic and somewhat enlightened. The nuclei of these cells increased in size, mainly were elongated, contained numerous vacuoles, and contained 1-2 nucleoli. Chromatin was distinguished because it formed lumps localised directly under the karyolemma. It should be noted that vacuolisation and an increase in the volume of the nuclei of contractile cardiomyocytes indicate their oedema due to the influence of toxins from the venom of the scorpion Leiurus macroctenus. The striation of the muscle fibres of the myocardium under these conditions was not determined. Significant expansion of the spaces between the cardiac muscle fibres and edematous changes in the stroma were noted. Moderate lymphocytic infiltration was observed in the interstitium. In addition, areas of haemorrhage were detected. Signs of apoptotic changes in the nuclei were noted in some contractile cardiomyocytes (Fig. 5, 6).

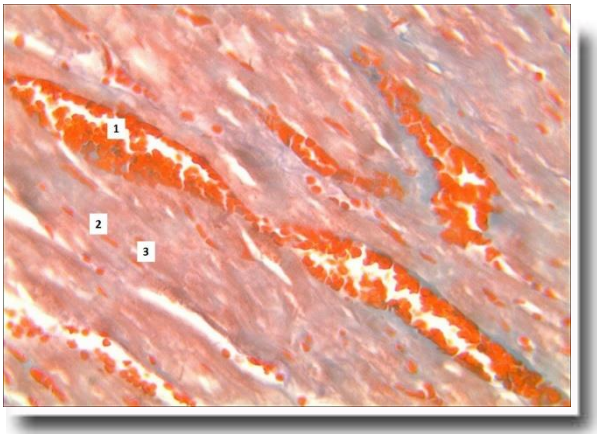

Fig. 4. Histological organisation of the rat heart 1 hour after exposure to the venom of the scorpion Leiurus macroctenus. The lumen of the myocardial blood vessel (1), muscle fibres (2), and contractile cardiomyocytes (3). Azan trichrome staining. ×200.

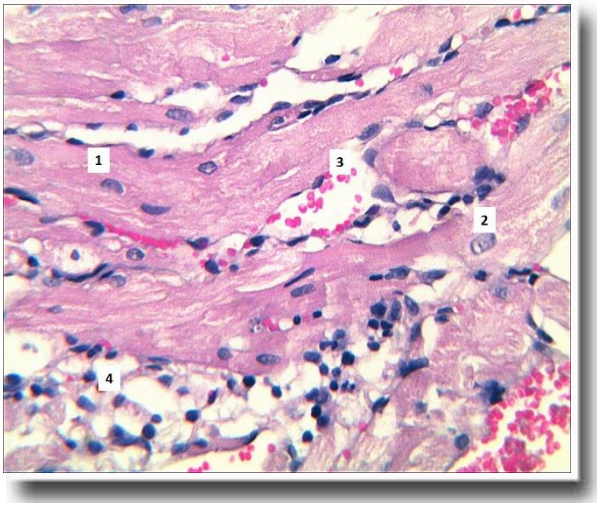

Fig. 5. Microscopic changes in the rat heart 3 hours after exposure to the venom of the scorpion Leiurus macroctenus. Hypertrophied myocardial muscle fibres (1), nuclei of contractile cardiomyocytes (2), areas of haemorrhage (3), oedema and lymphohistiocytic infiltration (4). Hematoxylin and eosin staining. ×200.

Leading cardiomyocytes also underwent structural changes under conditions of acute intoxication with the venom of the scorpion Leiurus macroctenus. They increased in size; their shape was indefinite. The

cytoplasm of leading cardiomyocytes had a homogenised structure. The cell nuclei were shifted mainly to the poles. They were both hypochromic and hyperchromic and contained dispersed chromatin. The karyoplasm was sometimes lightened, with signs of oedema, and vacuolated.

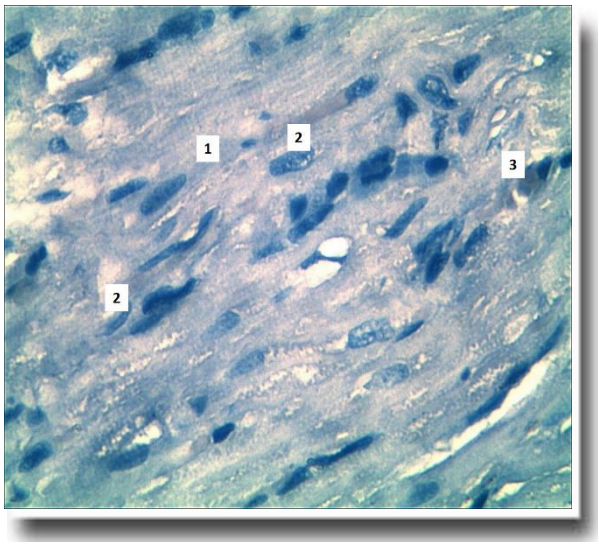

Fig. 6. Histological organization of the rat heart 3 hours after exposure to the venom of the scorpion Leiurus macroctenus. Contractile cardiomyocytes (1), nuclei of contractile cardiomyocytes (2), lumen of the myocardial blood vessel (3). Staining with iron hematoxylin. ×200.

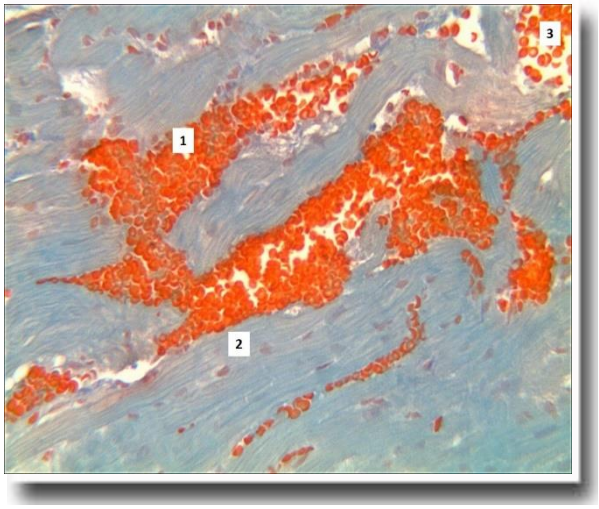

Fig. 7. Photo-optical changes in the rat heart 3 hours after exposure to the venom of the scorpion Leiurus macroctenus. Accumulation of erythrocytes in the myocardial stroma (1), myocardial muscle fibres (2), and lumen of the myocardial blood vessel (3). Azan trichrome staining. ×200.

Photo-optical studies of the rat heart after 3 hours of the experiment also revealed specific changes in the typical organisation of the heart muscle vessels. It is worth noting the increased lymphohistiocytic infiltration of the vessel walls, compared with the group of animals after 1 hour from the beginning of the experiment. The lumens of the myocardial blood vessels were dilated and full-blooded. They revealed erythrocyte sludge, stasis, and fibrin threads. The endothelial layer of the inner membrane of the vessels was discontinuous; the gaps between the cells increased significantly, which probably increased the degree of haemorrhage. The nuclei of the endothelial cells had an elongated shape, were hyperchromic, and contained condensed chromatin. The endothelium was sometimes exfoliated from the basement membrane, and initial manifestations of cell desquamation into the lumen of the vessels were observed. Lymphohistiocytic perivascular infiltration was noted (Fig. 5, 7).

Conclusion

Administration of Leiurus macroctenus scorpion venom to rats resulted in smoothing and homogenising cardiac muscle fibres and their moderate hypertrophy. Diapedetic haemorrhages and pronounced lymphohistiocytic infiltration were observed.

After 3 hours of the experiment, morphological changes in the rat myocardium deepened. Wave-like deformation of muscle fibres and their pronounced oedema were noted. Edematous changes, lymphocytic infiltration, and haemorrhages characterised the cardiac muscle stroma. Manifestations of desquamation of the endothelial lining of the vascular wall and perivascular lymphohistiocytic infiltration were observed.

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

There are no potential or apparent conflicts of interest related to this manuscript at the time of publication and are not anticipated.

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Янчишин А. Мікроскопічна організація міокарда експериментальних щурів через 1 та 3 години після впливу отрути скорпіонів *Leiurus macroctenus.*

РЕФЕРАТ. Актуальність. Токсини тваринних отрут характеризуються значною варіабельністю структури та біологічних ефектів. Зазвичай вони представлені білками, чисельними пептидами, що піддавались перебудовам протягом багатьох років еволюції, які вплинули на механізми та особливості впливу їх на організм жертви. Отрута скорпіонів може викликати ураження міокарда, ініціюючи низку патогенетичних механізмів. Після укусів цих тварин доволі часто реєструють розвиток інфаркту міокарда внаслідок коронарного спазму. **Мета.** Вивчення мікроскопічних змін серця зрілих щурів через 1 та 3 години після впливу 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). Для мікроскопічного дослідження забирали зразки серця тварин всіх груп. Фарбування гістологічних препаратів серця здійснювали гематоксиліном та еозином, азан трихром і залізним гематоксиліном. **Результати та підсумок.** Введення щурам отрути скорпіонів *Leiurus macroctenus* призводило до згладжування та гомогенізації волокон серцевого м'язу, їх помірної гіпертрофії. Відмічали появу діапедезних крововиливів, виражену лімфогістіоцитарну інфільтрацію. Після 3 годин проведення експерименту виявляли поглиблення морфологічних змін міокарда щурів. Відмічали хвилеподібну деформацію м'язових волокон, їх виражений набряк. Строма серцевого м'язу характеризувалась едематозними змінами, лімфоцитарною інфільтрацією, геморагіями. Спостерігали прояви десквамації ендотеліального вистилення судинної стінки, периваскулярну лімфогістіоцитарну інфільтрацію.

Ключові слова: отрута, скорпіони, міокард, лімфогістіоцитарна інфільтрація, щури.