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Gunas V.I.
National Pirogov Memorial Medical University, Vinnytsya

MICROSCOPIC AND MORPHOMETRIC CHANGES IN THE PULMONARY MICROVASCULATURE OF LABORATORY RATS THREE HOURS AFTER ADMINISTRATION OF LEIURUS MACROCTENUS SCORPION VENOM

e-mail: freekozak1@gmail.com

Three hours after administration of Leiurus macroctenus scorpion venom, pronounced structural and functional disturbances of the microcirculatory bed develop in the lungs of laboratory rats. Among the microscopic changes, marked congestion of small-caliber vessels predominates, combined with their narrowing caused by edema of both the vascular walls and the stromal component of the lung. In addition, a pronounced inflammatory reaction is observed, characterized by massive leukocyte migration with a predominance of segmented neutrophils and activation of basophils in the perivascular space. Disruption of vascular wall integrity is accompanied by various microscopic alterations, including structural disorganization, stratification, and the development of perivascular edema. In most fields of view, pulmonary tissue demonstrates foci of hemorrhage and fibrin thrombi formation in small-caliber vessels. Morphometric analysis confirms a statistically significant decrease in the internal diameter of arterioles, capillaries, and venules ($p=0.004$, $p=0.007$, and $p=0.0002$, respectively) compared with the control group. The overall spectrum of identified microscopic changes indicates the development of acute inflammatory and thrombohemorrhhagic disturbances of blood circulation in the small pulmonary vessels in response to the toxic effect of Leiurus macroctenus scorpion venom.

Key words: pulmonary microvessels of laboratory rats, the effects of Leiurus macroctenus scorpion venom, morphological and morphometric changes.

Гунас В.І.

МІКРОСКОПІЧНІ ТА МОРФОМЕТРИЧНІ ЗМІНИ В МІКРОСУДИНАХ ЛЕГЕНЬ ЛАБОРАТОРНИХ ЩУРІВ ЧЕРЕЗ ТРИ ГОДИНИ ПІСЛЯ ВВЕДЕННЯ ОТРУТИ СКОРПІОНУ LEIURUS MACROCTENUS

Через три години після введення отрути скорпіона Leiurus macroctenus у легенях лабораторних щурів формуються виражені структурні та функціональні порушення мікроциркуляторного русла. Серед мікроскопічних змін на перший план виходить виражене повнокрів'я судин дрібного калібру у поєднанні з їх звуженням, зумовленим набряком як їх оболонок так і стромального компоненту легені. Окрім цього виявлено виражену запальну реакцію, що характеризується масивною міграцією лейкоцитів із переважанням сегментоядерних нейтрофілів та активацією базофілів у периваскулярному просторі. Порушення цілісності судинної стінки супроводжується різноманітними мікроскопічними змінами, зокрема, її дезорганізацією, розшаруванням та розвитком периваскулярного набряку. У тканині легені в більшості полях зору спостерігаються вогнища з формуванням крововиливів та фібринових тромбів у судинах дрібного калібру. Морфометричний аналіз підтверджує виявлене достовірне зменшення внутрішнього діаметра артеріол, капілярів і венул ($p=0.004$, $p=0.007$ та $p=0.0002$ відповідно) порівняно з контролем. Сукупність виявлених мікроскопічних змін свідчить про розвиток гострих запально-тромбогеморагічних порушень з боку циркуляції крові у дрібних судинах легень у відповідь на токсичну дію отрути скорпіона Leiurus macroctenus.

Ключові слова: мікросудини легень лабораторних щурів, вплив отрути скорпіона Leiurus macroctenus, морфологічні та морфометричні зміни.

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Scorpionism is a global problem that primarily affects regions such as North Africa, the Middle East, and Latin America. Approximately 1.5 million cases of scorpion envenomation are reported annually, of which more than 2,600 result in death. These outcomes are largely attributable to the presence of peptide neurotoxins in scorpion venom that selectively interact with voltage-gated Na^+, K^+, and Ca^{2+} channels, leading to a variety of clinical manifestations, the most severe being acute heart failure and pulmonary edema [2]. It is important to note that in many of these regions, access to both general and specialized medical care for scorpion stings is often limited. Furthermore, many researchers question the accuracy of statistical data, as patients frequently do not seek medical attention or may be unable to identify the sting specifically as caused by a scorpion [13].

The situation is further complicated by the increasing frequency of human encounters with scorpions in their natural habitats. This trend is driven both by the expansion of scorpion distribution ranges due to climate change, which renders more territories environmentally suitable, and by human settlement in areas inhabited by scorpions [5]. Consequently, regions that previously did not experience scorpionism must now be prepared for the potential emergence of such cases.

Iran is one of the countries that has long been significantly affected by scorpionism. Epidemio-

logical studies conducted in East Azerbaijan Province during 2022–2023 identified 3,154 scorpion sting cases requiring medical attention. Most victims were working-age men (31-40 years), and the number of cases was nearly equally distributed between urban and rural areas. Scorpion stings were most frequently recorded between midnight and 2:00 a.m., and the overall incidence rate was 7.8 cases per 10,000 population [1]. In Ecuador, more than 70,000 cases were registered between 2021 and 2024, with an average annual incidence of approximately 100-120 cases per 100,000 population [14].

At the same time, scorpion venom represents a valuable source for the development of novel therapeutic agents. More than 2,000 bioactive components have been identified in scorpion venoms, exhibiting antitumor, antimicrobial, and immunomodulatory activities, many of which are currently being investigated in clinical research for potential drug development [2]. Promising findings have been reported for venom-derived peptides from Scorpio maurus palmatus in experimental mouse models of lung cancer. These studies demonstrated that at concentrations of 2-8 μM, the venom significantly reduced the viability of A549 lung cancer cells and induced apoptosis in a dose-dependent manner. Importantly, tumor volume reduction occurred without significant systemic toxicity [7].

Considering both the growing incidence of scorpionism and the перспективу therapeutic application of scorpion venoms, understanding the morphological effects of scorpion venom on key target organs is of critical importance.

The purpose of the study was to investigate microscopic and morphometric changes in the pulmonary microvessels of laboratory rats three hours after administration of a sublethal dose of Leiurus macroctenus scorpion venom.

Materials and methods. To obtain the venom, 15 sexually mature Leiurus macroctenus scorpions of both sexes, bred in captivity and identified by Mark Stockmann from his private breeding facility, were used. The scorpions were maintained for at least one year on a standardized diet consisting of one Shelfordella lateralis cockroach per week, with access to distilled water. Housing conditions were standardized: all specimens were kept in plastic containers filled with sand, ventilated through openings in the boxes, at a constant temperature of 25–35 C, humidity of 50–60 %, and under natural lighting conditions. Venom extraction was performed using the method of Ozkan O. and Filazi A. [10], modified by Yaqoob R. et al. [15] (immobilization of the scorpion followed by placement of electrodes on the prosoma and metasoma; an electric current of 24 W was applied for 5 seconds to the base of the metasoma). Milking was performed once, with an interval of at least one month after arrival from the breeding facility. The amount of venom obtained ranged from 0.1 to 0.5 mg. Immediately after collection, the venom was stored at -20 °C. The venom was administered intramuscularly to rats as a single dose calculated according to an LD50 of 0.08 mg/kg established in a previous study [6].

The venom was administered to 10 white male laboratory rats weighing 200 g (±10 g), bred in the vivarium of the Educational and Scientific Center “Institute of Biology and Medicine” of Taras Shevchenko National University of Kyiv, in accordance with the “Standard Rules for the Arrangement, Equipment, and Maintenance of Experimental Biological Clinics (Vivaria).” The experiments were conducted in compliance with current regulatory documents governing the use of experimental animals and in accordance with the principles of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes,” as well as the Law of Ukraine of February 21, 2006 No. 3447-IV “On Protection of Animals from Cruel Treatment and Ethical Norms and Rules for Work with Laboratory Animals.”

The rats selected for the study were randomly divided into two groups of five animals each. The first group served as the control, receiving physiological saline, with tissue sampling performed three hours after injection. The second group was experimental, receiving the venom, with tissue sampling performed three hours after administration. The rats were euthanized by carbon dioxide inhalation. Lung extraction was performed at 4°C immediately after euthanasia.

For histological examination, lung tissues were fixed in a 10 % formalin solution immediately after removal. Dehydration was carried out in graded alcohols followed by tissue processing using a Logos ONE tissue processor (MILESTONE, Italy), and embedding in paraffin blocks using an automated embedding station TEC 2800 (HESTION, Australia). Thin sections (4-5 μm) were obtained using a rotary microtome AMR-400 (Amos Scientific Pty, Australia). Sections were stained with hematoxylin-eosin and by the Azan Trichrome method.

For semithin sections (1-2 μm), lung samples were fixed in 2.5 % glutaraldehyde solution (pH 7.3-7.4), post-fixed in 1 % osmium tetroxide, dehydrated in graded alcohols, and embedded in epoxy resin with subsequent polymerization. Semithin sections were prepared using an Ultrotome LKB 4801 A ultramicrotome (Bromma, Sweden) and stained with methylene blue.

Microscopic examination of histological specimens was performed using an OLIMPUS BX 41 light microscope at magnifications ranging from ×40 to ×1000. Image visualization and morphometric analysis were conducted using the Quickphoto micro 2.3 morphometric software. The morphometric parameters assessed included the internal diameter of arterioles, venules, and capillaries.

Statistical analysis of the obtained results was performed using the licensed software package "Statistica 6.0" with the application of nonparametric evaluation methods. The significance of differences between independent quantitative variables (mean values and standard deviation) was determined using the Mann-Whitney U test.

Results of the study and their discussion. Three hours after administration of Leiurus macroctenus scorpion venom, the pulmonary microvessels of laboratory rats were congested but somewhat narrowed due to edema of the tunica media and tunica adventitia.

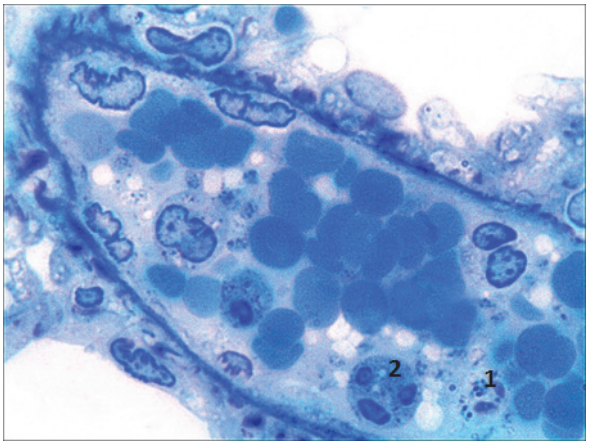

Fig. 1. Fragment of rat lung three hours after administration of Leiurus macroctenus scorpion venom. 1 – leukocytes; 2 – segmented neutrophil. Methylene blue staining. Magnification ×1000.

Examination of semithin sections revealed a large number of leukocytes within the lumina of the microcirculatory vessels. These cells were located along the walls of the capillaries and migrated into the interstitial space through interendothelial gaps. Among the leukocytes, segmented neutrophils were the most numerous (Fig. 1).

At this time point after scorpion venom administration, edema increases in the lungs, particularly within their stromal component, leading to collapse of the walls of arterioles, capillaries, and venules, and in some areas to obliteration of their lumina (Fig. 2).

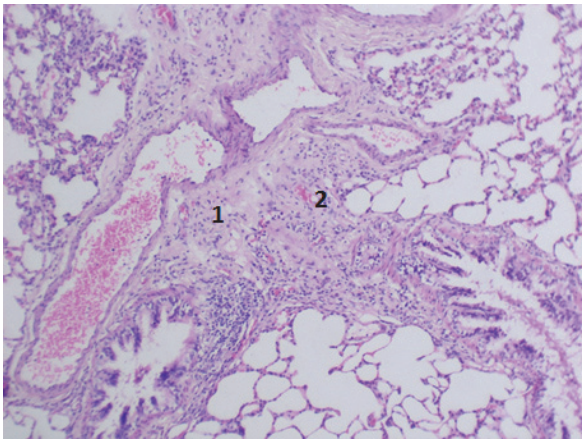

Fig. 2. Fragment of rat lung three hours after administration of Leiurus macroctenus scorpion venom. 1 – stromal edema; 2 – thrombus in an arteriole. Hematoxylin-eosin staining. Magnification ×40.

Areas of hemorrhage into the alveolar sacs and alveolar ducts are also observed. In addition, fibrin thrombi are detected in the alveolar capillaries and pulmonary arterioles (Fig. 3).

Examination of the perivascular space revealed numerous degranulating basophils. The vascular wall was frequently stratified, with signs of edema and, in some areas, a corrugated appearance. Adjacent to the vessel walls, isolated, irregularly shaped, shrunken cells lacking nuclei were observed (Fig. 4).

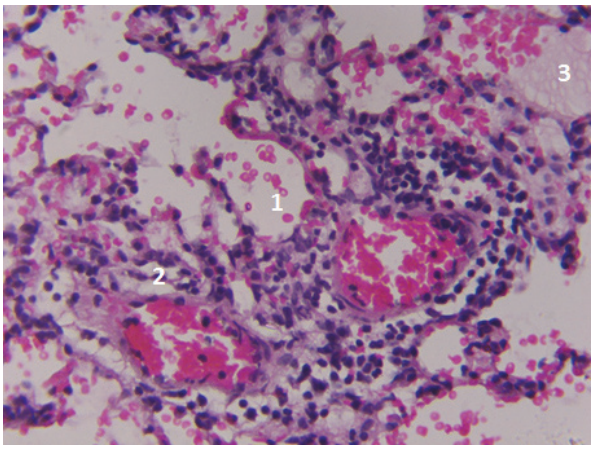

Fig. 3. Fragment of rat lung three hours after administration of Leiurus macroctenus scorpion venom. 1 – dilation of alveoli with destructive changes in their walls; 2 – collapse of alveoli; 3 – fluid accumulation. Hematoxylin-eosin staining. Magnification ×400.

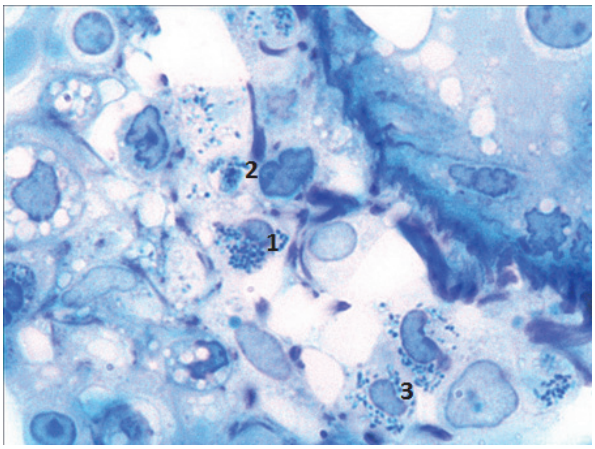

Fig. 4. Fragment of rat lung three hours after administration of Leiurus macroctenus scorpion venom. 1 – degranulating basophil; 2 – eosinophil; 3 – basophil. Methylene blue staining. Magnification ×1000.

Morphometric analysis of the vessels of the microcirculatory bed demonstrated a statistically significant decrease in the internal diameter of arterioles (33.07±4.55 μm) by 19.07 % (p=0.004), capillaries (11.20±3.30 μm) by 32.33 % (p=0.007), and venules (40.91±2.51 μm) by 24.16 % (p=0.0002) compared with the control values (40.86±4.95 μm, 16.55±4.78 μm, and 53.94±5.11 μm, respectively).

The cardiovascular and respiratory systems are the primary targets of scorpion venom. In clinical cases of scorpionism, pulmonary edema is observed in 7-46 % of patients, and cardiac arrest occurs in approximately 7 % of cases. One clinical case report describes the development of toxic myocarditis, cardiogenic shock, and pulmonary edema following a scorpion sting [8].

From a pathophysiological perspective, one initiating mechanism is the venom-induced systemic inflammatory response, which leads to infiltration of lung tissue by neutrophils and other inflammatory cells, followed by tissue damage. In an experimental study in mice, blockade of TLR4 resulted in a significant reduction in peripheral neutrophilia, decreased neutrophil degranulation in lung tissue, and lower levels of oxidative stress markers [9].

In the study by Darkaoui B. et al., the effects of the venoms of Androctonus mauretanicus and Buthus occitanus on mouse lungs were investigated [4]. Both external and internal changes were assessed dynamically at 30, 60, and 120 minutes after experimental venom administration. It was established that Androctonus mauretanicus exhibited a significantly lower LD50 compared to Buthus occitanus (300±25 μg/kg and 875±20 μg/kg, respectively), indicating greater toxicity, and mice receiving Androctonus mauretanicus venom demonstrated more pronounced external and internal manifestations. Clinically, dyspnea appeared after 60 minutes and progressed over time. Microscopically, lung tissue showed dilation of alveolar spaces and alveolar destruction accompanied by massive hemorrhages, which were most prominent at 120 minutes. Round-cell infiltration, predominantly macrophages, was already evident at 60 minutes.

In another study, the effects of Androctonus australis hector venom were examined. Microscopic analysis of lung samples revealed thickening of alveolar walls and marked round-cell infiltration consistent with acute lung injury. Evans Blue staining demonstrated increased permeability of the alveolar–capillary membrane. The number of neutrophils in the bronchoalveolar compartment was more than three times higher than in the control group. Focal hemorrhages were also observed [11].

A generalized review of the literature on scorpion venom effects indicates that the main pathomorphological changes in lung tissue include increased capillary permeability, pulmonary edema, activation of neutrophils and macrophages, and the release of pro-inflammatory cytokines such as TNF-α and IL-6. Depending on the venom type, different patterns of leukocytic infiltration may occur, although neutrophilic infiltration is most common, while alveolar wall thickening and interstitial edema remain consistent findings. Ultimately, these changes impair gas exchange and reduce pulmonary function, potentially leading to fatal outcomes [12].

Studies have also examined the effects of other animal venoms on the lungs. For example, Azevedo E. et al. investigated the impact of Crotalus durissus cascavella snake venom in mice, assessing changes at 1, 3, 6, 12, 24, and 48 hours after administration. Within the first hours, tachypnea with pronounced abdominal breathing was observed, and morphologically there was thickening of interalveolar septa, vascular stasis, and round-cell infiltration. At 3 hours, these findings were accompanied by marked peribronchial infiltration, emphysema, and atelectasis. Concurrently, elevated levels of TNF and IL-6 were detected [3].

Overall, the findings obtained in the experimental study of Leiurus macroctenus venom are consistent with data reported in other studies. However, the thrombus formation observed in the present study may represent a specific feature characteristic of this particular scorpion species' venom.

Limitations. This study focuses on the effect of the venom of the scorpion Leiurus macroctenus. At the same time, existing data in the literature show that the structure of the venom can vary depending on the scorpion's sex and the type of milking. Our results are limited by this because scorpions of both sexes were used for milking, and electrical stimulation was used to induce milking. It is also worth noting that the injection was administered intramuscularly, which also affects the morphological picture and its intensity.

Conclusions

Three hours after administration of the venom of the scorpion Leiurus macroctenus, pronounced acute damage of the microcirculatory bed develops in the lungs of laboratory rats, accompanied by congestion in the veins and capillaries. Pronounced edema of the vascular wall and stroma was detected, as a result of which narrowing of the lumen of vessels of different types and calibers occurred and, in some places, obliteration of their lumen. Regarding round-cell infiltration, it is represented predominantly by the leukocytic component with a predominance of segmented neutrophils and their migration into the interstitium. A significant number of degranulating basophils were detected in the perivascular space. The morphological changes are also accompanied by hemorrhages into the alveoli, destructive changes of the interalveolar septa, accumulation of fluid in their lumen, and formation of fibrin thrombi. Morphometrically, a significant decrease in the internal diameter of arterioles by 19.07 %, capillaries by 32.33 %, and venules by 24.16 % was confirmed, which indicates that rapid development of severe inflammatory-ischemic and thrombotic disturbances takes place in the lungs.

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Conflict of interest. The authors have no conflicts of interest to declare.

ORCID: Gunas I.V. https://orcid.org/0000-0003-4260-2301.

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