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HISTOLOGICAL AND MORPHOMETRIC CHANGES IN THE PULMONARY MICROVASCULATURE OF RATS ONE DAY AFTER ADMINISTRATION OF LEIURUS MACROCTENUS SCORPION VENOM

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One day after administration of *Leiurus macroctenus* venom, pronounced histological changes were observed in the pulmonary microvasculature of rats, characterized by altered vascular blood filling and remodeling of the vessel walls. The arterioles and venules demonstrated thickening of the tunica media and tunica adventitia, marked perivascular edema, and infiltration by round inflammatory cells. In some vessels, narrowing or partial collapse of the lumen was observed, while adjacent vessels in certain microscopic fields exhibited pronounced congestion and erythrocyte stasis. Perivascular hemorrhages were also detected in some areas of the pulmonary stroma. Morphometric analysis confirmed a consistent reduction in the internal diameter of all examined components of the microcirculatory bed. The most pronounced changes were observed in the capillaries, whose diameter decreased by 44.51 % ($p=0.0019$), followed by the arterioles and venules, whose diameters decreased by 35.61 % and 35.00 %, respectively ($p=0.0002$), compared with the control group. These findings indicate the development of pronounced disturbances in pulmonary microcirculation as early as the first day following exposure to *Leiurus macroctenus* scorpion venom.

Key words: pulmonary microvessels of laboratory rats, micromorphometric changes one day after exposure to *Leiurus macroctenus* scorpion venom.

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ГІСТОЛОГІЧНІ ТА МОРФОМЕТРИЧНІ ЗМІНИ В СУДИНАХ МІКРОЦИРКУЛЯТОРНОГО РУСЛА ЛЕГЕНЬ ЩУРІВ ЧЕРЕЗ ДОБУ ПІСЛЯ ВВЕДЕННЯ ОТРУТИ СКОРПІОНА LEIURUS MACROCTENUS

Через одну добу після введення отрути *Leiurus macroctenus* у легенях щурів спостерігаються виражені гістологічні зміни мікроциркуляторного русла, що характеризувалися порушенням кровонаповнення та ремоделюванням судинних стінок. З боку артеріол та венул виявлені такі зміни як потовщення середньої й зовнішньої оболонок, виражений периваскулярний набряк та інфільтрацію круглоклітинними елементами білої крові. У деяких судинах виникало звуження або часткове запусніння просвіту, що межувало в деяких полях зору з судинами з вираженими ознаками повнокров'я та явищем стазу еритроцитів. У деяких ділянках стромы виявляли периваскулярні крововиливи. Проведений морфометричний аналіз підтвердив наявність системного явища зменшення внутрішнього діаметра всіх досліджених ланок мікроциркуляторного русла. Найбільш виражені зміни спостерігали з боку капілярів, діаметр яких зменшувався на 44,51 % ($p=0,0019$), за якими далі слідували показники діаметру артеріол і венул, які зменшувалися на 35,61 % та 35,00 % відповідно ($p=0,0002$) порівняно з контролем. Отримані результати свідчать про розвиток виражених порушень з боку легеневої мікроциркуляції вже протягом першої доби після початку дії отрути скорпіона *Leiurus macroctenus*.

Ключові слова: мікросудини легень лабораторних щурів, мікоморфометричні зміни через добу після впливу отрути скорпіона *Leiurus macroctenus*.

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Scorpionism is a globally widespread phenomenon occurring on all continents except Antarctica, although the distribution of medically important scorpion species is uneven. The regions of North Africa, the Middle East, the Arabian Peninsula, and Iran are among those most severely affected in terms of the hazards associated with scorpionism.

Analysis of the literature on this topic has shown that, in Saudi Arabia, for example, 28 scorpion species have been recorded, some of which belong to the notorious family Buthidae, which includes a considerable number of the most venomous scorpion species in the world. In particular, the genus *Leiurus*, which is investigated in the present study, belongs to this family. As expected, despite the country's relatively small population and low population density, the number of scorpion stings remains high; according to the review, approximately 14,500 cases occur annually. The authors of the review also

emphasize the high cost of treatment for scorpion stings. On average, treatment costs amount to slightly more than USD 1,000 for mild cases and almost USD 5,000 for severe cases [1].

Another country substantially affected by scorpionism is Iran. In this country, scorpions of the genera *Hemiscorpius* and *Androctonus* are among the most medically important and pose a considerable health hazard. The distribution of scorpionism cases across the country is uneven, with the highest prevalence reported in the southwestern and western provinces. A meta-analysis demonstrated an incidence rate of 92.7 per 100,000 population per year (95 % CI: 68.4-117.0), with extremely high heterogeneity ($I^2=98.9\%$), supporting the aforementioned regional variability. An important aspect of this publication is that the authors identify anthropogenic factors as a key contributor to the high prevalence of scorpionism, particularly climate

change and the expansion of human settlements into previously uninhabited areas [9]. Among the previously mentioned regions of Iran, the Andimeshk area in the southwest of the country deserves particular attention, as it has one of the highest rates of scorpionism in Iran. Between 2012 and 2016, the prevalence of scorpion stings in this area was 9.1 per 1,000 inhabitants. Among the scorpions most commonly found in this region, researchers collected 850 specimens belonging to three families: Buthidae, Scorpionidae, and Hemiscorpiidae. The predominant species were *A. crassicauda* and *H. Lepturus* [10]. In Qom Province, located in central Iran, 395 cases of scorpionism were recorded over a nine-year period, further emphasizing the regional differences in the frequency of human contact with scorpions [12].

An analysis covering the period from 2019 to 2024 at Şanlıurfa Harran University Hospital in Turkey showed that 1,041 cases of scorpionism were registered, including one fatal case. The authors of the study analyzed the age and sex of the patients as well as the anatomical sites of the stings. The mean age of the affected individuals was 25.61 ± 18.50 years, and scorpion stings were more frequently observed in females, who accounted for 61.47 % of cases. Patients aged 1-17 years constituted the largest age group among those affected (39.38 %). As in most other studies, the highest number of cases was recorded during the summer months (63.4 %). The extremities were the most common sites of injury, accounting for 29.5 % of cases [8].

However, scorpions of the New World should not be overlooked. In Central and South America, scorpionism remains a relevant medical problem despite considerable advances in its treatment since the 1960s, as indicated by data from Mexican researchers [3]. In their analytical study, the authors identified several important patterns that deserve attention. The first is the marked decline, in fact a dramatic reduction, in mortality from scorpion stings in Mexico since 1995, when improved treatment approaches began to be implemented. Indeed, the investigation and development of effective treatments for scorpion envenomation remain a priority, and morphological studies using animal models often represent an initial stage in the search for such therapeutic approaches. The second aspect identified by the authors was the uneven distribution of scorpionism cases within the country. Despite the decline in the number of cases, approximately 300,000 cases per year still result in patients seeking medical care, and their frequency is approximately 20 times higher in the Central and Pacific Coast regions than in the northern and southern coastal regions of Mexico. Both climatic and sociodemographic factors are considered possible explanations for this difference [3].

Thus, research into the effects of scorpion venom remains a relevant and important medical and social issue.

The purpose of the study was to investigate histological and micromorphometric changes in the arterial and venous microvessels of the lungs of laboratory rats one day after administration of *Leiurus macroctenus* scorpion venom.

Materials and methods. The venom was obtained from captive-bred *Leiurus macroctenus* scorpions of both sexes. The scorpions were identified and bred at a private breeding facility owned by Mark Stockmann (Germany). Venom extraction was performed according to the method described by Ozkan O. and Filazi A. [11], as modified by Yaqoob R. et al. [15]. Venom was collected once, after complete acclimatization of the scorpions in Ukraine (at least one month after transportation). The animals were maintained under standard conditions, with free access to water, and were fed *Shelfordella lateralis* cockroaches. The typical amount of venom obtained per extraction ranged from 0.1 to 0.5 mg. Immediately after collection, the venom was transferred to a sterile vial and stored temporarily at -20 °C. Subsequently, the venom was administered as a single intramuscular injection at a dose based on the previously determined LD50 value of 0.08 mg/kg [6].

The study was conducted on 10 white 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 No. 3447-IV of February 21, 2006, "On the Protection of Animals from Cruel Treatment," and the ethical standards and regulations governing work with laboratory animals.

The rats were divided into two groups: a control group (n=5), which received physiological saline, with tissue sampling performed three hours after injection; and an experimental group (n=5), which received the venom, with tissue sampling performed 1 day after administration. Prior to tissue collection, the rats were euthanized by carbon dioxide inhalation. Lung tissue was collected immediately after euthanasia at 4 °C.

The collected lung samples were subsequently subjected to histological examination. For this purpose, lung samples were fixed in a 10 % formalin solution, followed by dehydration in graded alcohols and tissue processing using a Logos ONE tissue processor (MILESTONE, Italy). The samples were then embedded in paraffin blocks using a TEC 2800 automated embedding station (HESTION, Australia). Thin sections (4-5 μ m) were obtained using an AMR-

400 rotary microtome (Amos Scientific Pty, Australia). The resulting sections were stained with hematoxylin and eosin (H&E) and using the Azan trichrome method. For the preparation of semithin sections (1-2 μm), lung samples were fixed in a 2.5 % glutaraldehyde solution (pH 7.3-7.4), post-fixed in 1 % osmium tetroxide, dehydrated in graded alcohols, and embedded in epoxy resin, followed by polymerization. Semithin sections were prepared using an Ultratome LKB 4801 A ultramicrotome (Bromma, Sweden). The sections were stained with methylene blue.

All sections were examined using an OLYMPUS BX41 microscope at magnifications ranging from $\times 40$ to $\times 1000$. Image visualization and morphometric analysis were performed using QuickPHOTO MICRO 2.3 morphometric software. The morphometric parameters assessed included the internal diameters of arterioles, venules, and capillaries.

Statistical analysis was performed using the licensed Statistica 6.0 software package and nonparametric statistical methods. Differences between independent quantitative variables,

expressed as mean values and standard deviations, were assessed using the Mann-Whitney U test.

Results of the study. One day after administration venom, pronounced structural and microcirculatory alterations were observed in the lungs of laboratory rats, involving both the vascular walls and the surrounding pulmonary tissue. These changes were characterized by narrowing and partial collapse of small vessels, blood stasis, hemorrhages, perivascular edema and inflammatory infiltration, accompanied by a significant reduction in the internal diameters of the vessels of the pulmonary microcirculatory bed.

One day after administration of *Leiurus macroctenus* scorpion venom, the pulmonary microvessels of laboratory rats exhibited both blood-filled and collapsed lumens. The muscular and adventitial layers of the arterioles were thickened due to edema (Fig. 1).

In some areas of the lungs, extensive hemorrhages were detected as a result of destruction of the arteriolar walls. Thickening of the tunica media and tunica adventitia was observed in the venules (Fig. 2).

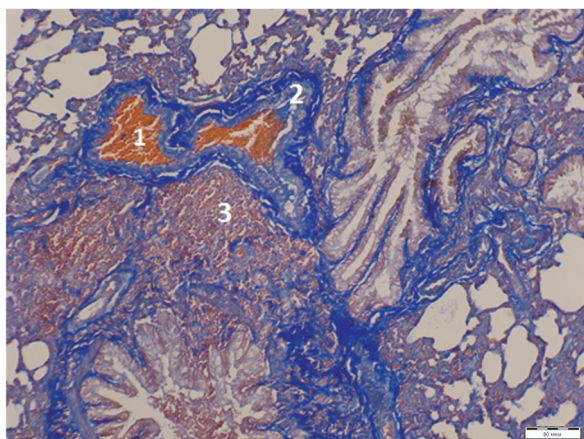


Fig. 1. Fragment of rat lung one day after administration of scorpion venom. 1 – blood-filled lumen of an arteriole; 2 – thickened muscular and adventitial layers of the arteriole; 3 – consolidation of lung tissue. Azan trichrome staining. Magnification $\times 200$.

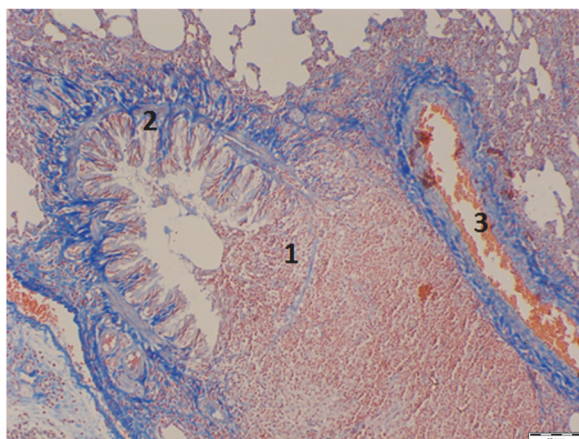


Fig. 2. Fragment of rat lung one day after administration of scorpion venom. 1 – hemorrhage; 2 – bronchiole; 3 – venule. Azan trichrome staining. Magnification $\times 200$.

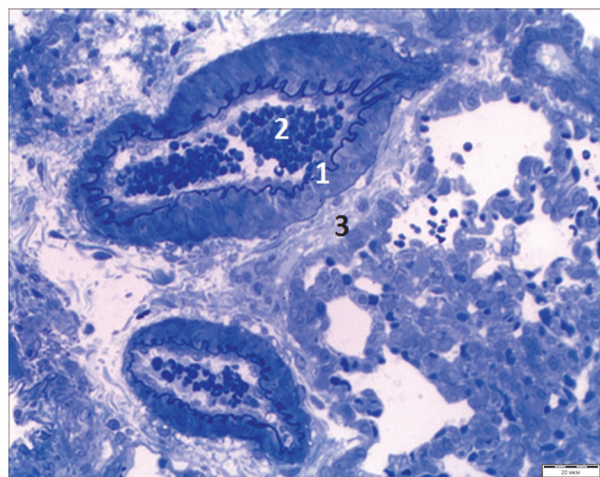


Fig. 3. Fragment of rat lung one day after administration of scorpion venom. 1 – internal elastic membrane; 2 – erythrocytes; 3 – edema of the interstitial space. Methylene blue staining. Magnification $\times 400$.

Examination of semithin lung sections one day after administration of scorpion venom revealed narrowing of the arterial lumens due to contraction of the internal elastic membrane and bundles of smooth muscle cells in the tunica media. The appearance of connective tissue in their walls was also observed. The arteriolar lumens were blood-filled with signs of stasis. At the same time, partial collapse of blood vessels was observed. Thickening of the perivascular tissues was noted due to infiltration by macrophages, leukocytes and lymphocytes, as well as edema of the surrounding connective tissue (Fig. 3).

Morphometric analysis of the vessels of the microcirculatory bed one day after administration of scorpion venom showed that, as at the previous study time points, the most pronounced changes occurred in the internal diameter of the capillaries ($9.183 \pm 2.210 \mu\text{m}$), which significantly decreased by 44.51 %

($p=0.0019$) compared with the control. The reductions in the internal diameters of the arterioles ($26.31 \pm 3.86 \mu\text{m}$) and venules ($35.06 \pm 4.05 \mu\text{m}$) were almost identical, decreasing by 35.61 % ($p=0.0002$) and 35.00 % ($p=0.0002$), respectively, compared with the control values.

Discussion. Cases in which pulmonary involvement becomes the predominant manifestation of scorpionism are not uncommon. Moreover, the lungs are considered one of the target organs, along with the central nervous and cardiovascular systems. In affected patients, arterial blood gas analysis reveals metabolic acidosis with severe hypoxemia, indicating type 1 respiratory failure, as well as elevated blood levels of the creatine kinase-MB isoenzyme. The pronounced pulmonary edema that accompanies most such cases results from reduced alveolar fluid clearance, which in turn develops due to downregulation of sodium-potassium ATPase channels in the alveolar epithelium [2].

The hemorrhages detected in the pulmonary stroma of rats during microscopic examination are in good agreement with the findings of a methodologically similar study performed using *H. lunatus* scorpion venom. The authors of that study also evaluated morphological changes in the heart; however, microscopic examination revealed differences only in the lungs, where diffuse hemorrhages were observed. It is important to note that these pronounced microscopic changes developed rapidly. Unfortunately, biochemical analyses were performed only on cardiac tissue samples, which does not allow conclusions to be drawn regarding the mechanisms underlying such marked pulmonary changes [4]. At the same time, the study by Thumtecho S. et al. [13] focused specifically on explaining the effects of scorpion venom on the lungs, particularly on the microcirculatory bed. The current concept of toxin-induced lung injury directly suggests that the pulmonary microcirculation may represent one of the principal targets of the systemic effects of venom. Due to the highly developed pulmonary vascular bed and its large surface area, vascular reactions, secondary hemodynamic alterations, and inflammatory responses associated with scorpion venom exposure may be particularly pronounced morphologically in the lungs.

At the same time, it should be noted that morphological examination alone cannot distinguish between the direct morphological effects of venom and the secondary changes that subsequently develop, including hemodynamic and inflammatory alterations. For this purpose, isolated cell cultures may be used, as demonstrated in the study by Issaad N. et al., in which the toxic effects of *Androctonus australis* hector scorpion venom were investigated directly in cultured A549 alveolar epithelial cells. The results demonstrated dose-dependent impairment of cellular integrity, particularly of the cell membrane, alterations of the actin cytoskeleton, and reduced migratory capacity, providing direct evidence of the toxic effects of scorpion venom on pulmonary cells [7]. A similar direct effect of venom on the microvasculature may also exist, although this hypothesis requires additional targeted investigation.

In our study, microscopic examination of tissue sections also revealed round-cell inflammatory infiltration and perivascular edema, which are consistent with findings reported for the venoms of *Tityus zulianus* and *Tityus discrepans*. That study confirmed that scorpion venoms can activate mechanisms of neutrophil oxidative stress. Excessive production of reactive oxygen species causes cellular injury and may therefore contribute to the observed edema and the previously described hemorrhages, particularly in the presence of marked inflammatory infiltration [5].

It is also important to emphasize that our study was performed using whole scorpion venom. This is methodologically important when the aim is to assess the actual rather than the potential pharmacological effect of the venom, since individual venom components may exert effects that differ from, or even oppose, those of the venom as a whole. A similar phenomenon was described by Xu L. et al. [14], where an isolated scorpion venom polypeptide, in contrast, suppressed the immune response and reduced pulmonary injury.

The findings obtained in the present study substantially expand the theoretical understanding of the specific effects of scorpion venom, particularly considering the almost complete absence of studies evaluating venom-induced changes at later time points.

Conclusion

One day after administration of *Leiurus macroctenus* venom, pronounced structural and microcirculatory disturbances develop in the lungs of rats, combining vasoconstrictive, congestive, inflammatory and hemorrhagic changes. These alterations include narrowing and partial collapse of small vessels, blood stasis, thickening and edema of the vascular walls, perivascular inflammatory infiltration, and hemorrhages associated with disruption of the microvascular wall. Morphometric analysis confirmed a significant reduction in the internal diameters of arterioles, venules and, most prominently, capillaries. The greatest decrease observed in the capillary diameter indicates that the capillary component of the pulmonary microcirculation is particularly sensitive to the effects of scorpion venom. Overall, the findings demonstrate marked involvement of the pulmonary microvascular bed during the later stages of experimental envenomation.

Prospects for further research – determining morphological changes resulting from the action of the investigated poison at other time intervals.

References

1. Alhamoud MA, Al Fehaid MS, Alhamoud MA, Alzoayed MH, Alkhalifah AA, Menezes RG. Scorpion stings in Saudi Arabia: an overview. *Acta Bio Medica: Atenei Parmensis*. 2021 Sep 2;92(4):e2021273. doi: 10.23750/abm.v92i4.11550
2. Bompelli N, Reddy R, Deshpande A. Scorpion bite-induced unilateral pulmonary oedema. *BMJ Case Reports CP*. 2018 Apr 5;2018:bcr-2018. doi: 10.1136/bcr-2018-224476
3. Chippaux JP, Celis A, Boyer L, Alagón A. Factors involved in the resilience of incidence and decrease of mortality from scorpion stings in Mexico. *Toxicon*. 2020 Dec 1;188:65-75. doi: 10.1016/j.toxicon.2020.10.011
4. Costal-Oliveira F, Guerra-Duarte C, Oliveira MS, Castro KL, Lopes-de-Sousa L, Lara A, Gomes ER, Bonilla C, Guatimosim S, Melo MM, Chávez-Olórtegui C. Cardiorespiratory alterations in rodents experimentally envenomed with *Hadruroides lunatus* scorpion venom. *Journal of Venomous Animals and Toxins including Tropical Diseases*. 2016 Jul 15;23(1):2. doi: 10.1186/s40409-016-0076-5
5. El-Benna J, Hurtado-Nedelec M, Gougerot-Pocidalo MA, Dang PM. Effects of venoms on neutrophil respiratory burst: a major inflammatory function. *Journal of Venomous Animals and Toxins including Tropical Diseases*. 2021 Jun 28;27:e20200179. doi: 10.1590/1678-9199-JVATITD-2020-0179
6. Gunas V, Maievskiy O, Raksha N, Vovk T, Savchuk O, Shchypanskyi S, Gunas I. Study of the Acute Toxicity of Scorpion *Leiurus macroctenus* Venom in Rats. *The Scientific World Journal*. 2024 Jul;(1):9746092. doi: 10.1155/2024/9746092.
7. Issaad N, Ait-Lounis A, Laraba-Djebari F. Cytotoxicity and actin cytoskeleton damage induced in human alveolar epithelial cells by *Androctonus australis* hector venom. *Toxin reviews*. 2018 Jan 2;37(1):67-74. doi: 10.1080/15569543.2017.1320806
8. Kafadar H, Erin N, Demir U, Buyukaslan H. Scorpion Stings: Epidemiological, Clinical, and Forensic Medical Evaluation Using University Hospital Data. *Revista da Sociedade Brasileira de Medicina Tropical*. 2025 Dec 15;58:e0208-2025. doi: 10.1590/0037-8682-0208-2025
9. Nasirian H. Scorpion sting stratification in Iran: A systematic review of epidemiological patterns, determinants, and climate change impacts. *International Journal of Biometeorology*. 2026 Jul;70(7):209. doi: 10.1007/s00484-026-03269-3
10. Nejati J, Saghaipour A, Rafinejad J, Mozaffari E, Keyhani A, Abolhasani A, Kareshk AT. Scorpion composition and scorpionism in a high-risk area, the southwest of Iran. *Electronic physician*. 2018 Jul 25;10(7):7138. doi: 10.19082/7138
11. Ozkan O, Filazi A. The determination of acute lethal dose-50 (LD50) levels of venom in mice, obtained by different methods from scorpions, *Androctonus crassicauda* (Oliver 1807). *Acta Parasitol. Turcica*. 2004 Jan;28(1):50-3.
12. Saghaipour A, Jesri N, Noroozi M, Mostafavi R. Epidemiologic status of scorpion sting in Qom, Iran 2004-2013. *Archives of Hygiene Sciences*. 2017 Mar 10;6(2):145-51. doi: 10.29252/ArchHygSci.6.2.145
13. Thumtecho S, Suteparuk S, Sitprija V. Pulmonary involvement from animal toxins: the cellular mechanisms. *Journal of Venomous Animals and Toxins including Tropical Diseases*. 2023 Sep 18;29:e20230026. doi: 10.1590/1678-9199-JVATITD-2023-0026
14. Xu L, Zhang Y, Dai Q, Lin N, Guan T, Song X, Hong S. Scorpion venom polypeptide governs alveolar macrophage M1/M2 polarization to alleviate pulmonary fibrosis. *Tissue and Cell*. 2022 Dec 1;79:101939. doi: 10.1016/j.tice.2022.101939
15. Yaqoob R, Tahir HM, Arshad M, Naseem S, Ahsan MM. Optimization of the conditions for maximum recovery of venom from scorpions by electrical stimulation. *Pakistan journal of zoology*. 2016 Jan;48(1):265-9.

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

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