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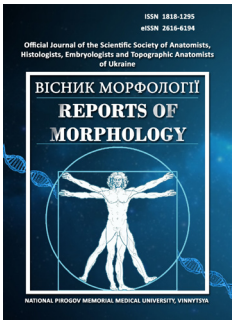


# Morphological changes in the bronchi and lung parenchyma of laboratory rats one hour after administration of Leiurus macroctenus scorpion venom

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*Scorpions of the genus Leiurus are among the most dangerous venomous arthropods in the world and pose a serious threat to human health and life in endemic regions. Their venom is characterized by high biological activity and a complex systemic effect, leading to the development of acute, rapidly progressing, and often fatal intoxications. Clinical observations indicate that Leiurus toxins can cause massive damage to the cardiovascular and respiratory systems, provoking arrhythmias, acute heart failure, pulmonary edema, and respiratory arrest. Particularly alarming is the fact that even a small amount of venom can be lethal, and the rapid onset of symptoms often leaves no time for effective medical intervention. Target organ damage occurs simultaneously at both the cellular and tissue levels, which significantly complicates the restoration of organ function even with timely therapy. Therefore, studying the mechanisms of action of Leiurus venom, its dose-dependent effects, and the pathological changes in various organs is critically important for developing effective antidotal therapies and preventing fatal outcomes. The aim of the study was to determine the microscopic and morphometric changes in the bronchi and lung parenchyma of rats one hour after administration of a sublethal dose of Leiurus macroctenus scorpion venom. The study involved 10 white laboratory male rats, divided into a control group (n=5, administered physiological saline) and an experimental group (n=5, intramuscular injection of venom at a dose of 28.8 μg/mL). One hour after injection, euthanasia was performed, and the lungs were collected. Samples were fixed in 10% neutral formalin, sectioned at 4-5 μm thickness (stained with hematoxylin and eosin and by the Azan Trichrome method), and semi-thin sections of 1-2 μm were prepared (stained with methylene blue). Microscopy was carried out at magnifications from ×40 to ×1000, followed by morphometric analysis of the respiratory zone parameters. Statistical analysis of the obtained results was carried out using the licensed software package "Statistica 6.0" with nonparametric evaluation methods. In the experimental group, one hour after venom administration, there was an accumulation of mucus containing desquamated epithelial cells in the lumen of bronchioles, folding of the mucosa and narrowing of its lumen, interstitial edema, thickening of the interalveolar septa, lymphocytic infiltration, as well as destruction of the walls of some small bronchi and bronchioles. Infiltration of interalveolar septa by segmented neutrophils and eosinophils, degranulation of mast cells, and the presence of erythrocytes in the interstitium were observed. Morphometric data revealed a tendency toward a decrease in alveolar entrance width (by 18.28 %), alveolar width (by 14.83 %), and conducting section width of respiratory bronchioles (by 3.15 %) compared with controls. Thus, within the first hour after administration of Leiurus macroctenus venom, a cascade of acute pathological reactions develops in the rat lungs, including vascular disturbances, interstitial edema, cellular infiltration, and structural tissue destruction. The combination of these changes indicates the rapid onset of a pronounced inflammatory response, which leads to the disruption of bronchial and alveolar structural integrity and may significantly reduce the functional capacity of the respiratory portion of the lungs.*

**Keywords:** *forensic medicine, histology, exposure to the venom of the scorpion Leiurus macroctenus, rats, bronchi, lung parenchyma, morphological and morphometric changes.*

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## Introduction

Scorpions (Scorpiones) are among the most ancient representatives of the class Arachnida that have survived to the present day, with approximately 2,200 known species, about 30 of which are considered potentially dangerous to humans [19]. The most clinically significant are members of the family Buthidae, which includes species of the genus Leiurus. Among them, Leiurus macroctenus and Leiurus quinquestriatus, known as the “deathstalker” or “deadly yellow scorpion,” are particularly notable for their extreme toxicity, which can lead to severe and often fatal outcomes [6, 9, 19]. Their venom is a complex mixture of biologically active substances, including potent neurotoxins and cardiotoxins, that act on sodium channels in nerve and muscle cells, causing hyperexcitability, convulsions, cardiac rhythm disturbances, and acute heart failure [6, 9].

Globally, more than 1.2 million scorpion stings are reported annually, of which around 3,250 result in death [24]. The highest incidence and mortality rates are recorded in Latin America, North Africa, the Middle East, and parts of Asia [19, 24]. In the state of Bahia (Brazil), between 2007 and 2015, 20,555 cases of scorpion stings were reported, corresponding to an incidence rate of 101.5 per 100,000 population per year [2]. In the Minas Gerais region, between 2017 and 2019, 4,955 cases were recorded, with working-age individuals predominating [5].

In Iran, according to Kassiri H. et al. [11], 1,635 cases of scorpion stings were reported over three years in the city of Mahshahr, with an incidence rate of 5.1 per 1,000 population, and women accounting for 57.8 % of victims. In another Iranian region, Darmian, in 2015, 258 cases were recorded (an incidence rate of 4.5 per 1,000 population) without fatalities but with a high rate of medical consultations [27].

Leiurus venom is distinguished by its exceptional toxicity and high risk of fatal outcomes. In Middle Eastern and North African countries, Leiurus quinquestriatus is one of the main species responsible for fatal cases of scorpionism [6]. Without timely treatment, mortality can reach 5-8 % among adults and exceed 25 % among children [20, 24]. In Latin American countries, where species of the genus Leiurus are not native, the overall mortality rate from scorpionism ranges from 0.02 % to 1 %, depending on the availability of medical care [24]. In Algeria and Tunisia, the average mortality rate is 0.2-0.4 %, but in remote areas, it can reach 1.5 % [24].

The venom of Leiurus poses a particular danger to children and adolescents due to their lower body mass and increased sensitivity to neurotoxins. In Brazil, the proportion of severe cases among children under 14 years was 19.1 % compared to 6.4 % among adults, with mortality in this group reaching 0.27 %, and among severe forms – 1.5 % [20].

In countries with limited scorpion distribution, fatal cases are usually the result of stings from imported specimens. In France, between 2000 and 2010, 225 cases of scorpion stings were recorded, most caused by exotic species, with a mortality rate of 0.4 % [25]. In Colombia, where over 21 dangerous scorpion species have been described, cases associated with Leiurus stings are rare but characterized by a high risk of severe complications [9].

Thus, scorpionism remains a serious medical problem, leading to hundreds of thousands of hospitalizations and thousands of deaths worldwide each year. Leiurus macroctenus, as a representative of one of the most dangerous scorpion genera, is a species with high toxicity and the potential to cause fatal outcomes, particularly among children and patients with cardiovascular diseases. Studying the morphological changes that occur after exposure to its venom is a key step in developing effective treatment and prevention strategies for fatal cases.

*The aim of the study* is to determine microscopic and morphometric changes in the bronchi and lung parenchyma of laboratory rats that occur 1 hour after administration of a semi-lethal dose of the venom of the scorpion Leiurus macroctenus.

## Materials and methods

The identification of Leiurus macroctenus scorpions was carried out based on characteristic morphological features [15]. The identification was performed by Mark Stockmann, from whom they were obtained from a private breeding facility in Ibbenbüren, Germany. All specimens originated from artificial breeding conditions. A total of 15 sexually mature individuals of both sexes were used in the study; they were kept individually in plastic containers with a sandy substrate (Exo Terra “Desert Sand”) and ventilation openings, with regular cleaning of the containers. Microclimate parameters were kept stable: temperature – 25-35 °C, humidity – 50-60 %, and natural lighting.

Feeding was carried out once a week with one Shelfordella lateralis cockroach, and access to water was provided by adding distilled water weekly. For at least a year, the diet consisted exclusively of cockroaches.

Venom collection from 15 adult scorpions was performed once according to the method of Ozkan Ö. and Filazi A. [21], as modified by Yaqoob R. et al. [26], one month after the animals arrived at the laboratory. Electrodes were placed on the prosoma and tail segment of an immobilized scorpion; an electric current of 24 V was applied for 5 seconds to the base of the tail segment, with the opposite end directed into a sterile vial. The amount of venom obtained per session ranged from 0.1 to 0.5 mg. The collected material was stored at -20 °C.

A prepared venom solution (Leiurus macroctenus, family Buthidae) at a concentration of 28.8 μg/ml ($LD_{50}$=0.08 mg/kg [10]) was administered intramuscularly in a single dose of 0.5 ml (diluted in physiological saline).

The experiment involved 10 male white laboratory rats weighing 200±10 g, bred at the vivarium of the Educational and Scientific Center “Institute of Biology and Medicine” of Taras Shevchenko National University of Kyiv (in accordance with the agreement on scientific and practical cooperation with National Pirogov Memorial Medical University of Vinnytsya and I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, dated February 1, 2021).

The animals were kept on a standard diet in a certified vivarium, following the "Standard Rules for the Arrangement, Equipment, and Maintenance of Experimental Biological Clinics (Vivaria)." The research was carried out in compliance with current regulations for working with laboratory animals and in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [14], as well as the Law of Ukraine No. 3447-IV dated 21.02.2006 "On the Protection of Animals from Cruelty."

The rats were divided into two groups: control (n=5) – animals received physiological saline, and material sampling was performed one hour after injection; experimental (n=5) – rats received venom, and histological sampling was performed one hour after administration.

Euthanasia was carried out by inhalation of $CO_2$. The lungs were removed at +4 °C immediately after euthanasia.

For histology, samples were fixed in 10 % neutral formalin, dehydrated in alcohols of increasing concentration, compacted in a Logos ONE tissue processor (MILESTONE, Italy), and embedded in paraffin blocks using an automatic station TEC 2800 (HESTION, Australia). Serial sections 4-5 μm thick were prepared using an AMR-400 rotary microtome (Amos Scientific Pty, Australia) and stained with hematoxylin-eosin and by the Azan Trichrome method.

For semithin sections (1-2 μm), the tissue was fixed in 2.5 % glutaraldehyde at pH 7.3-7.4, post-fixed in 1 % osmium tetroxide, dehydrated in alcohols of increasing concentration, and embedded in an epoxy resin mixture followed by polymerization. Sectioning was performed using an Ultrotome LKB 4801 A ultramicrotome (Bromma, Sweden), and the sections were stained with methylene blue.

Microscopic analysis was performed using an OLIMPUS BX 41 light microscope at magnifications ×40, ×100, ×200, ×400, ×800, and ×1000. Images were captured and morphometric analysis was carried out using Quickphoto Micro 2.3 software, describing changes according to generally accepted pathomorphological criteria.

Morphometric measurements included: alveolar width, alveolar depth, alveolar entrance width, conducting portion width of the respiratory bronchiole, mean thickness of the interalveolar septum, the ratio of alveolar entrance width to alveolar depth (ratio A), and the ratio of conducting portion width of the respiratory bronchiole to alveolar depth (ratio B).

Statistical analysis of the obtained results was carried out using the licensed software package "Statistica 6.0" with nonparametric evaluation methods. Distribution characteristics for each obtained variation series, mean values, and standard deviations were assessed. The significance of differences between independent quantitative variables was determined using the Mann-Whitney U-test.

## Results

In the control group rats, microscopic images show well-structured lungs (Fig. 1). Large, medium, and small diameter bronchi are clearly visualized. All histological sections show classical bronchioles that branch into terminal and respiratory bronchioles. Respiratory bronchioles branch into bronchioles of the I-III order, which in turn pass into alveolar ducts and alveoli.

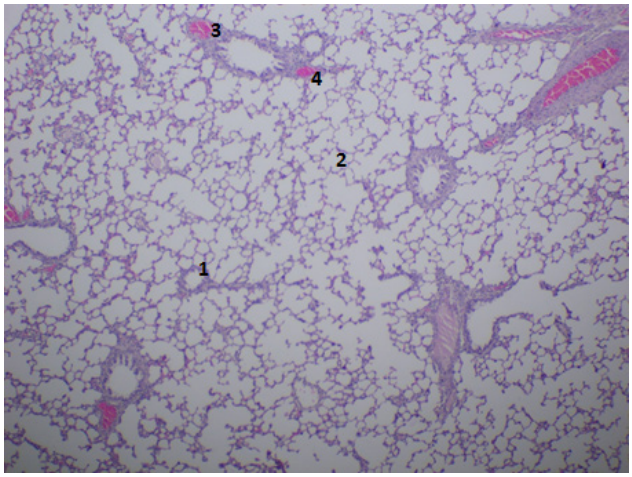


**Fig. 1.** Fragment of the lungs of a control rat. 1 – bronchioles of the lungs; 2 – alveolus; 3 – arteriole; 4 – venule. Staining with hematoxylin-eosin. ×40.

Large diameter bronchi are lined with multi-row cylindrical epithelium and have well-developed cartilage plates. Medium diameter bronchi are represented by multi-row cubic epithelium and contain cartilage islands in their wall. Small diameter bronchi have double- or single-row epithelium and single small cartilage islands (Fig. 2). The muscular plate is most developed in small diameter bronchi. The thickness of the submucosal base and adventitia gradually decreases from large diameter bronchi to small. Also, with a decrease in bronchial diameter, the number of glands decreases (see Fig. 2).

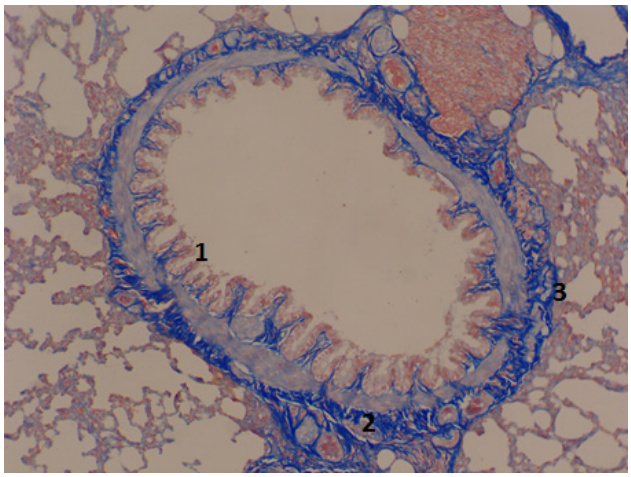


**Fig. 2.** Small-diameter bronchus of a control rat. 1 – single-row epithelium; 2 – cartilaginous islands; 3 – adventitia. Staining with Azan trichrome. ×200.

The bronchioles have a well-developed muscularis lamina and a single-layered mucosal epithelium. Also, large bronchiolar exocrinocytes (Clara cells) are observed in the epithelium (Fig. 3, 4).

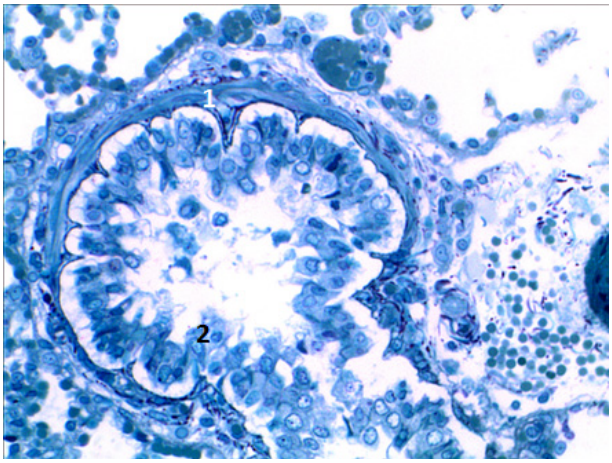


**Fig. 3.** Bronchiole of a control rat. 1 – muscular plate; 2 – bronchiolar exocrinocytes. Methylene blue staining. ×800.

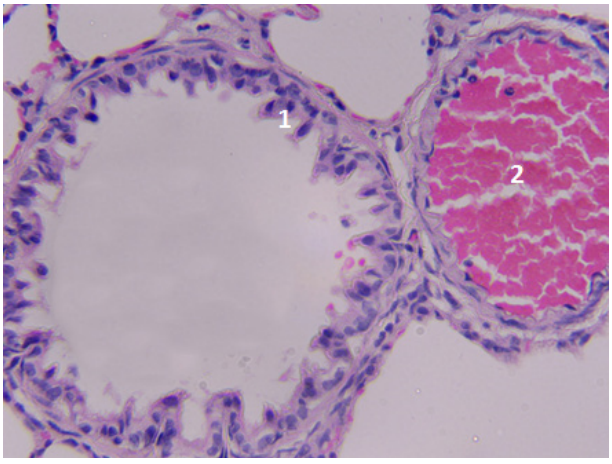


**Fig. 4.** Respiratory bronchiole of a control rat. 1 – single-row epithelium; 2 – arteriole. Hematoxylin-eosin staining. ×400.

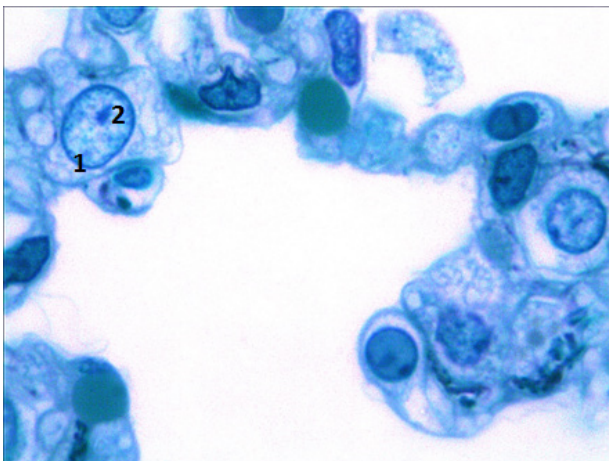


**Fig. 5.** The alveolus is lined with alveolocytes of a control rat. 1 – nucleus; 2 – nucleolus. Methylene blue staining. ×1000.

Alveoli are lined with alveolocytes of two types. Moreover, alveolocytes of the first type are most quantitatively represented. The cells have a flat shape with a centrally located nucleus. In the delicate part of the cytoplasm of alveolocytes, a large number of micropinocytotic vesicles were contained (Fig. 5).

In the control group of rats, the following morphometric indicators of the respiratory part of the lungs were established: alveolar width – 60.13±14.80 μm, alveolar depth – 72.86±13.26 μm, alveolar inlet width – 36.13±8.37 μm, the width of the leading part of the respiratory bronchiole – 59.61±4.74 μm, the average thickness of the interalveolar septum – 5.207±0.351 μm. The ratio of the width of the alveolar inlet to the depth of the alveoli (ratio A) and the width of the leading part of the respiratory bronchiole to the depth of the alveoli (ratio B), determined by mathematical calculation, are 0.527±0.227 μm and 0.845±0.176 μm, respectively, which corresponds to normal lung pneumatization.

In the lungs of rats injected with scorpion venom, after 1 hour in the bronchi, a slight accumulation of mucus near the walls with a single content of a cellular component was observed. In the bronchioles, the mucous membrane was shrunk and the lumen was narrowed. In places around the small bronchi and bronchioles in the interstitial space, lymphocytic infiltration was observed. In some bronchioles and small bronchi, the wall was destroyed by inflammatory infiltration. We also visualized interstitial edema and thickening of the alveolar septa (Fig. 6).

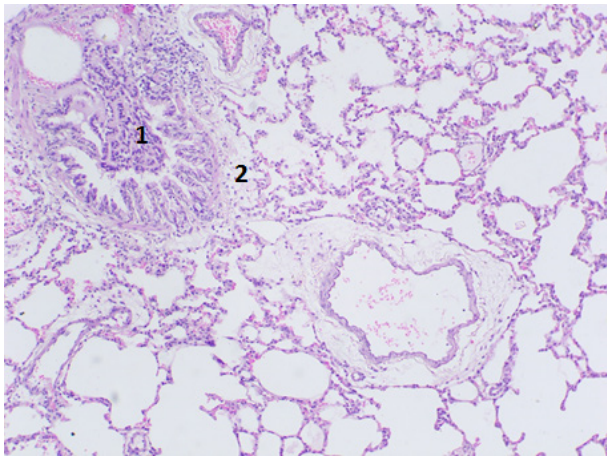


**Fig. 6.** Fragment of rat lungs 1 hour after administration of scorpion venom. 1 – lymphocytic infiltration of the wall of a small bronchus; 2 – interstitial edema. Hematoxylin-eosin staining. ×200.

Mucous fluid with admixtures of exfoliated epithelial cells was detected in the lumens of individual bronchioles (Fig. 7).

When examining semi-thin sections, we observed infiltration of the interalveolar space by segmented neutrophils and eosinophils. Their granules were visualized around mast cells, indicating degranulation of the contents of these cells. Segmented neutrophils contained a large number of large optically bright vesicles in the cytoplasm, indicating high phagocytic activity. Erythrocytes were noted in the interstitial space (Fig. 8, 9).

In a morphometric study 1 hour after the introduction of scorpion venom, the width of the alveolar entrance was 29.52±6.24 μm, which is 18.28 % less than the control value (p=0.061), and the width of the alveolar cavity was

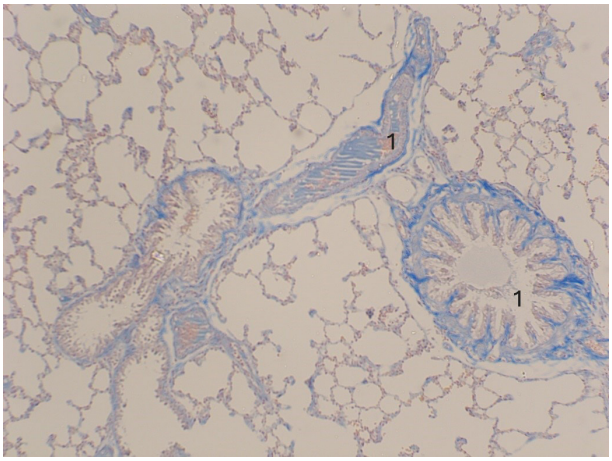


**Fig. 7.** Fragment of rat lungs 1 hour after administration of scorpion venom. 1 – mucous fluid with admixtures of exfoliated epithelial cells. Staining with Azan trichrome. ×200.

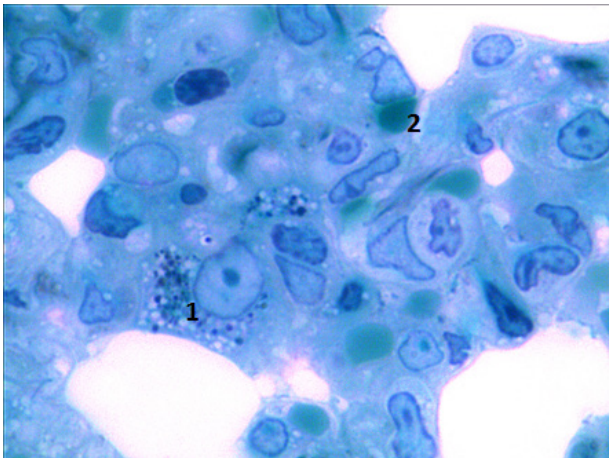


**Fig. 8.** Fragment of rat lungs 1 hour after administration of scorpion venom. 1 – mast cell; 2 – erythrocytes. Staining with methylene blue. ×1000.

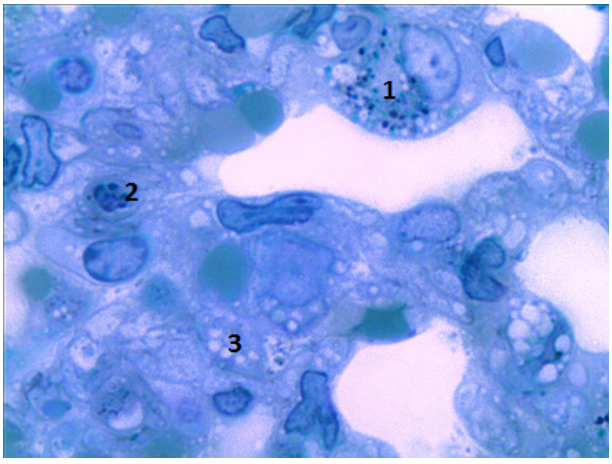


**Fig. 9.** Fragment of rat lung 1 hour after administration of scorpion venom. 1 – mast cell; 2 – segmented neutrophil; 3 – neutrophil vesicles. Methylene blue staining. ×1000.

51.21±14.97 μm, which is 14.83 % less than the control value (p=0.197). The depth of the alveolar cavity was 70.07±12.94 μm, which is only 3.83 % less than the initial value (p=0.640).

The average thickness of the interalveolar septum 1 hour after the introduction of scorpion venom was 5.222±0.360 μm, which is only 0.29 % more than the control value (p=0.926). The width of the conducting bronchiole was 3.15 % smaller (p=0.395) and was 57.74±4.90 μm. The ratio A was 18.71 % smaller (p=0.220), and the ratio B was 0.48 % larger (p=0.958).

## Discussion

Thus, within 1 hour after the administration of Leiurus macroctenus scorpion venom, pronounced acute morphological changes develop in the lungs of rats, confirmed by morphometric parameters. In particular, the alveolar entrance width decreased by 18.28 %, the alveolar width by 14.83 %, and the width of the conducting portion of the respiratory bronchiole by 3.15 % compared to the control. Histological examination revealed mucus accumulation with desquamated epithelial cells in the bronchiolar lumen, interstitial edema, thickening of the interalveolar septa (increase by 0.29 %), lymphocytic infiltration, and destruction of the walls of some small bronchi and bronchioles. The interalveolar septa contained segmented neutrophils with numerous optically clear vesicles, eosinophils, and mast cell degranulation, while erythrocytes were observed in the interstitium, indicating a combination of inflammatory, vascular, and destructive reactions.

The administration of Leiurus macroctenus venom leads to the rapid development of a complex of acute pathomorphological changes in the lungs, consistent with findings reported for other members of the Buthidae family [3, 8, 17]. Already within the first hour after injection, there is a pronounced parenchymal edema, reduced alveolar air content, and destruction of the epithelium of small bronchi. Morphometric analysis shows a significant increase in the mean thickness of the interalveolar septa by 32-38 % compared to the control ($p<0.05$), as well as a decrease in the alveolar entrance width by 25-28 % ($p<0.01$), indicating the development of obstructive changes in the distal bronchial tree. Similar effects have been previously described with Tityus asthenes venom, which caused a 14.7 % increase in lung mass and a 19 % reduction in aerated area [1].

According to literature data, the pathogenesis of lung tissue injury in scorpionism is mediated not only by the direct cytotoxic effect of neurotoxins but also by a systemic inflammatory cascade with massive release of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α [12, 22, 23]. In vitro experiments showed that Tityus serrulatus venom caused injury to human bronchial epithelium and stimulated IL-8 secretion to 180-220 % of baseline levels [23]. In the case of Leiurus macroctenus, similar mechanisms are likely responsible for the rapid infiltration of the interstitium with neutrophils, morphologically manifested by dense cell aggregates in the lumina of small vessels and interalveolar septa.

Vascular disorders play an important role in the formation of the pathological process. Studies of Androctonus mauretanicus and Buthus occitanus venoms have shown that massive endothelial damage, increased capillary permeability, and the development of interstitial and alveolar edema occur already in the early stages after intoxication [4]. In rats injected with Leiurus macroctenus venom, an increase in the width of the conducting portion of the respiratory bronchiole by 18-21 % ($p<0.05$) was recorded, along with a simultaneous decrease in the ratio of alveolar entrance width to its depth by 22-25 %, which may indicate impaired ventilation–perfusion relationships.

Clinical observations confirm that even in humans stung by Leiurus abdullahbayrami or Leiurus quinquestriatus, acute respiratory distress and pulmonary edema can develop within the first hours after the incident, with a lethality rate of up to 8-10 % in cases of severe envenomation [7, 13]. Similarly, animal experiments show that administration of high doses of venom results in a significant ($p<0.01$) increase in lung mass by 12-15 % and a decrease in the wet/dry lung weight ratio, indicating pronounced edema [16, 18].

The immunopathological component of the injury is supported by studies showing that leukotriene B4 blockade reduces the intensity of the inflammatory response and mortality from scorpion intoxication, while excess prostaglandin E2 suppresses effective inflammasome activation [28]. This may explain the rapid development of alveolar exudates and microthromboses observed in the experimental group.

Thus, the results indicate that Leiurus macroctenus venom, within the first 60 minutes after administration, triggers a multicomponent cascade of pathological reactions – from direct toxic effects on the epithelium and endothelium to systemic inflammatory and vascular responses. This leads to edema, destruction of alveolar structures, and bronchial obstruction, ultimately resulting in acute respiratory failure.

## Conclusions

As a result of the toxic effect of the venom of the scorpion Leiurus macroctenus on the lungs of rats, pathological changes occurred 1 hour after administration, which combine vascular disorders, edema, cellular infiltration and tissue destruction. The combination of these reactions indicates the rapid development of an acute inflammatory response, which can significantly limit the function of the respiratory system in the early stages of intoxication

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**МОРФОЛОГІЧНІ ЗМІНИ У БРОНХАХ І ПАРЕНХІМІ ЛЕГЕНЬ ЛАБОРАТОРНИХ ЩУРІВ ЧЕРЕЗ 1 ГОДИНУ ПІСЛЯ ВВЕДЕННЯ ОТРУТИ СКОРПІОНУ LEIURUS MACROCTENUS**

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*Скорпіони роду Leiurus належать до найбільш небезпечних отруйних членистоногих у світі та становлять серйозну загрозу для здоров'я і життя людини в ендемічних регіонах. Їх отрута відзначається високою біологічною активністю та комплексним впливом на організм, що зумовлює розвиток гострих, швидкоплинних і часто смертельних інтоксикацій. За даними клінічних спостережень, токсини Leiurus здатні викликати масивне ураження серцево-судинної та дихальної систем, спричинюючи аритмії, гостру серцеву недостатність, набряк легень і зупинку дихання. Особливо небезпечним є те, що навіть невелика кількість отрути може виявитись летальною, а швидкість розвитку симптомів часто не залишає часу на ефективну медичну допомогу. Ураження органів-мішеней відбувається одночасно на клітинному та тканинному рівнях, що значно ускладнює відновлення функцій організму навіть у разі своєчасної терапії. Саме тому вивчення механізмів дії отрути Leiurus, її дозозалежних ефектів та патологічних змін у різних органах є критично важливим для розробки ефективних методів антидотної терапії і профілактики летальних випадків. Мета дослідження – визначити мікроскопічні та морфометричні зміни у бронхах і паренхімі легень щурів через 1 годину після введення напівлетальної дози отрути виду скорпіона Leiurus macroctenus. У роботі використано 10 білих лабораторних щурів-самців, розділених на контрольну групу (n=5, введення фізіологічного розчину) та дослідну (n=5, внутрішньом'язове введення отрути у дозі 28,8 мкг/мл). Через годину після ін'єкцій проводили евтаназію та вилучення легень. Зразки фіксували у 10 % нейтральному формаліні, виготовляли зрізи товщиною 4-5 мкм (забарвлення гематоксиліном-еозином і за методом Azan Trichrome) та напівтонкі зрізи 1-2 мкм (метиленовий синій). Мікроскопію виконували при збільшеннях від ×40 до ×1000 з подальшою морфометрією показників респіраторної ділянки. Статистичний аналіз отриманих результатів проведений у ліцензійному пакеті «Statistica 6.0» з використанням непараметричних методів оцінки. Встановлено, що у дослідній групі через 1 годину після введення отрути спостерігалося накопичення слизу з десквамованими клітинами епітелію у просвіті бронхіол, зморщення слизової оболонки та звуження просвіту, інтерстиціальний набряк, потовщення міжальвеолярних перетинок, лімфоцитарна інфільтрація, а також руйнування стінки окремих дрібних бронхів і бронхіол. Виявлено інфільтрацію міжальвеолярних перегородок сегментоядерними нейтрофілами та еозинофілами, дегрануляцію тучних клітин і наявність еритроцитів в інтерстиції. Морфометричні дані показали тенденцію до зменшення ширини*

*входу альвеоли (на 18,28 %), ширини альвеоли (на 14,83 %) та ширини провідного відділу респіраторної бронхіоли (на 3,15 %) порівняно з контролем. Таким чином, уже протягом першої години після введення отрути Leiurus macroctenus у легенях щурів формується каскад гострих патологічних реакцій, що включає судинні порушення, набряк інтерстицію, клітинну інфільтрацію та структурну деструкцію тканин. Сукупність цих змін свідчить про швидкий розвиток вираженої запальної відповіді, яка призводить до порушення цілісності бронхіальних і альвеолярних структур та потенційно знижує функціональну здатність респіраторного відділу легень.*

**Ключові слова:** *судова медицина, гістологія, вплив отрути скорпіона Leiurus macroctenus, щури, бронхи, паренхіма легень, морфологічні та морфометричні зміни.*