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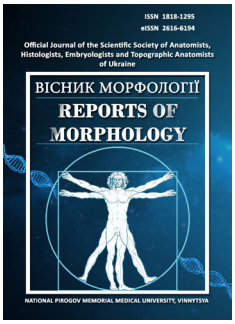

Microscopic and morphometric changes in the bronchi and lung parenchyma of laboratory rats three hours after administration of Leiurus macroctenus scorpion venom

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Scorpionism is a widespread phenomenon occurring in various regions of the world and poses particular danger to elderly individuals and children. Despite numerous cases of scorpion venom exposure in humans, only a limited number of publications describe the clinical picture, especially regarding respiratory system involvement. Even fewer studies address morphological changes in the lungs or bronchi of deceased individuals or laboratory animals, particularly at different time intervals after a scorpion sting. These gaps at the morphological level, even with a relatively well-studied pathogenetic mechanism of scorpion venom action, do not allow for a complete understanding of its toxic effects. Only through comprehensive investigation of the venom is it possible not only to develop effective antivenoms but also to explore its potential as a promising therapeutic agent. The aim of the study was to determine microscopic and morphometric changes in the bronchi and lung parenchyma of rats three hours after administration of Leiurus macroctenus scorpion venom. Ten male rats maintained under vivarium conditions were used and divided into two groups of five animals each: group 1 (control) received physiological saline, and group 2 received scorpion venom at a dose of 28.8 μg/ml. Three hours after administration, the animals were euthanized and the lungs were collected for histological examination. For microscopic and morphometric analysis, 4-5 μm sections (stained with hematoxylin-eosin and Azan Trichrome) and semi-thin sections of 1-2 μm (stained with methylene blue) were prepared. Microscopy was performed at magnifications ranging from ×40 to ×1000 using an OLIMPUS BX 41 microscope. Morphometric analysis was conducted using Quickphoto Micro 2.3 software. Statistical analysis of the results was performed using the licensed software package "Statistica 6.0" with nonparametric methods. Three hours after venom administration, pronounced inflammatory changes developed in the lungs of experimental animals, manifested by massive lymphocytic-neutrophilic infiltration, most intense around medium- and small-diameter bronchi and bronchioles, up to partial destruction of their walls, fragmentation of muscle bundles, and adventitial edema. The interalveolar space was dominated by basophils, eosinophils, and macrophages, indicating a marked inflammatory response. In the parenchyma, areas of distelectasis alternated with emphysematous regions, where focal destruction of interalveolar septa and fluid accumulation were observed. Morphometric analysis demonstrated a decrease in alveolar width, alveolar entrance width, and alveolar depth (p=0.112, p=0.008, and p=0.174, respectively), as well as a statistically significant narrowing of the conducting portion of respiratory bronchioles (p=0.045). A tendency toward redistribution of air between the alveolar and bronchiolar compartments was identified, reflected histologically by changes in morphometric ratios. The obtained data indicate the development of acute inflammatory-destructive lung injury with impaired ventilatory function already at early stages after intoxication. The combination of observed microscopic changes suggests the formation of structural remodeling of the lung parenchyma as early as three hours after venom administration in laboratory animals.

Keywords: *morphology, scorpion venom, Leiurus macroctenus, rats, bronchi, lung parenchyma, morphological changes, morphometric changes.*

Introduction

Climate-driven shifts in environmental processes have led to an expansion of the distribution range of scorpion species that are dangerous to humans. Thus, scorpions of the genus Tityus, whose range is represented in South America, have shown that over 20 years, in a number of regions the number of scorpionism cases increased by 20 %, and in some regions of Argentina by 30-50 %. According to scientists, this was a consequence of an increase in regional temperature by 1-1.5 °C. In particular, in Argentina a trend toward an increase in scorpionism cases specifically in cities has been noted, which poses an additional hazard [2]. In particular, Tityus carrilloi, which was previously considered endemic to Argentina, has already spread into Paraguay, representing a serious challenge to that country's health care system, as the venom of this scorpion has pronounced neurotoxic activity [4].

Similar results were obtained in another study, where scientists predict that by 2070 the distribution range of the scorpion T. metuendus in Brazil will increase, posing a danger to the Indigenous peoples of this country, who usually suffer from scorpionism incidents 2.4 times more often and have a child mortality rate 10 times higher [7]. The use of artificial intelligence to create a predictive model of scorpion distribution in Brazil supplemented and confirmed these data as well [8].

Globally, the number of scorpion stings has reached 1 million per year, with rates of 100-300 cases per 100,000 population in certain regions. Case fatality is estimated at 0.2-0.5 % [3].

A particular concern is the fact that scorpions even within the same region can have different venom composition, which is explained by substantial proteomic diversity. This further complicates the management of scorpionism cases, given the frequent unavailability of antivenom in rural areas [5].

Morocco is one of the countries that suffers considerably from scorpionism. From 2021 to 2024, 2,206 cases were recorded there, of which 41 were fatal. Notably, most cases were recorded at home [10]. Data from Algeria show that scorpion stings are also increasing there: the annual number of cases averages 585.8 per 100,000 population. In 2019, the highest rate was recorded 694.8 per 100,000. Over the observation period from 2018 to 2024, 10 deaths were recorded [23].

In Brazil, the number of cases is steadily increasing from 66,986 in 2014 to 170,616 in 2023. Predictive models show that the number of scorpionism cases will rise to 274,246 in 2033. According to the authors, a key role in this is played by the scorpion species Tityus serrulatus, individuals of which can survive 400 days without food and are capable of reproducing without the participation of males [21].

Thus, scorpionism is a widespread and dangerous phenomenon, because scorpion venom varies markedly by species and is often a mixture of neurotoxins, cardiotoxins, nephrotoxins, and hemolytic toxins that affect different organ systems [25]. There is a need for morphological studies of venom effects on the main target organs, which will allow assessment of the possibility of developing appropriate pharmacotherapy.

The aim of the study was to determine microscopic and morphometric changes in the bronchi and lung parenchyma of rats three hours after administration of Leiurus macroctenus scorpion venom.

Materials and methods

The study was conducted on 10 white laboratory rats weighing 200±10 g, which were bred and maintained in a certified vivarium in accordance with the "Standard Rules for the Arrangement, Equipment, and Maintenance of Experimental Biological Clinics (Vivaria)" (vivarium of the Educational and Scientific Center "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv) on a standard diet with free access to water and a 12-hour light cycle. The animals were divided into two groups of 5 animals each. Group 1 – control group, which received physiological saline. Group 2 – experimental group, which received a single intramuscular injection of Leiurus macroctenus scorpion venom solution at a concentration of 28.8 μg/ml in a dose of 0.5 ml (venom diluted in physiological saline) calculated according to LD50=0.08 mg/kg for this species [12]. Three hours after venom administration, the rats were euthanized by CO_2 inhalation. The lungs were removed at +4 °C immediately after euthanasia.

Venom was obtained by milking 15 sexually mature Leiurus macroctenus scorpions bred in captivity, which had been maintained for one year on a standard diet of Shelfordella lateralis cockroaches with free access to distilled water. The scorpions were kept under standard conditions at a temperature of 25-35 °C, humidity of 50-60 %, and natural lighting, with adequate ventilation provided in the terrariums. The milking procedure was performed once according to the method of Ozkan Ö. and Filazi A. [20] as modified by Yaqoob R. et al. [27], by applying an electrode to the prosoma and tail segment of an immobilized scorpion, with an electric current of 24 V applied for 5 seconds. The collected venom was stored at -20 °C.

The study was carried out in accordance with current regulations governing work with laboratory animals and in compliance with 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 dated 21.02.2006 "On the Protection of Animals from Cruelty".

Part of the lung samples was fixed in 10 % neutral formalin, followed by dehydration in alcohols of increasing concentration, processing using a Logos ONE tissue processor (MILESTONE, Italy), and embedding in paraffin blocks using an automatic station TEC 2800 (HESTION, Australia). Serial sections 4-5 μm thick were then prepared using an AMR-400 rotary microtome (Amos Scientific Pty, Australia). The sections were stained with hematoxylin-

eosin and by the Azan Trichrome method. Another portion of the samples was fixed in 2.5 % glutaraldehyde solution (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. Semi-thin sections 1-2 μm thick were prepared from these samples. The sections were cut using an Ultratome LKB 4801 A ultramicrotome (Bromma, Sweden) and stained with methylene blue.

Section analysis was performed using an OLIMPUS BX 41 light microscope at magnifications ×40, ×100, ×200, ×400, ×800, and ×1000. Image acquisition and morphometric analysis were carried out using Quickphoto Micro 2.3 software. The morphometric parameters studied included alveolar width, alveolar depth, alveolar entrance width, width of the conducting portion 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 the conducting portion width of the respiratory bronchiole to alveolar depth (ratio B).

Statistical analysis of the obtained results was performed using the licensed software package “Statistica 6.0” with nonparametric evaluation methods. Distribution characteristics of each 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 lungs of rats administered Leiurus macroctenus scorpion venom, massive areas of lymphocytic-neutrophilic infiltration are observed three hours after injection. These changes are most pronounced around medium- and small-diameter bronchi and bronchioles, in some areas leading to partial destruction of their walls. Foci of lipocyte accumulation are also noted, predominantly located near sites of inflammatory infiltration. In the muscular layer of certain bronchi, disruption of its continuous structure is observed due to fragmentation of muscle bundles. In addition, marked adventitial edema is present in these bronchi (Figs. 1, 2).

In the interalveolar space, basophils and eosinophils predominate, containing numerous granules of varying optical density. Basophils exhibit abundant cytoplasm filled with large transparent vesicles and optically dense granules, and their nuclei are bilobed. Eosinophils also possess bilobed nuclei, although cells with bean-shaped nuclei are observed. Their cytoplasm is densely packed with numerous small granules. Macrophages are also identified, with cytoplasm completely filled with relatively large transparent phagolysosomes, indicating high phagocytic activity. Some of these cells lack nuclei, while others show reduced nuclei, which may suggest incomplete phagocytosis (Fig. 3).

Areas of lung tissue consolidation are observed due to collapse of alveolar walls. At the same time, regions of alveolar dilation are noted, followed by destructive changes in their walls and accumulation of fluid (Fig. 4).

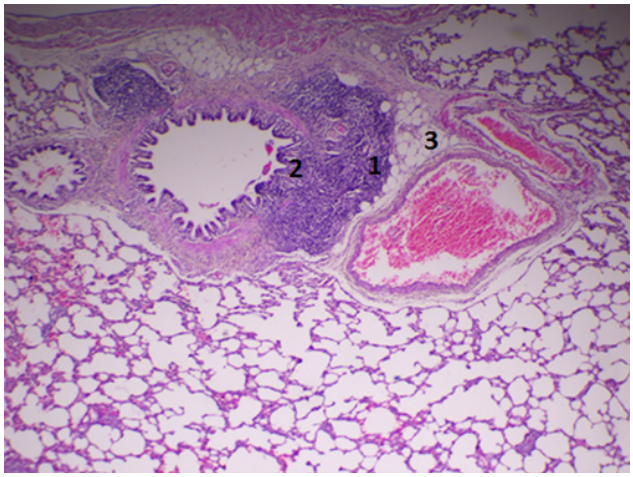

Fig. 1. Fragment of rat lung three hours after administration of Leiurus macroctenus scorpion venom. 1 – lymphocytic-neutrophilic infiltration; 2 – destroyed bronchiolar wall; 3 – lipocytes. Hematoxylin-eosin staining. Magnification ×40.

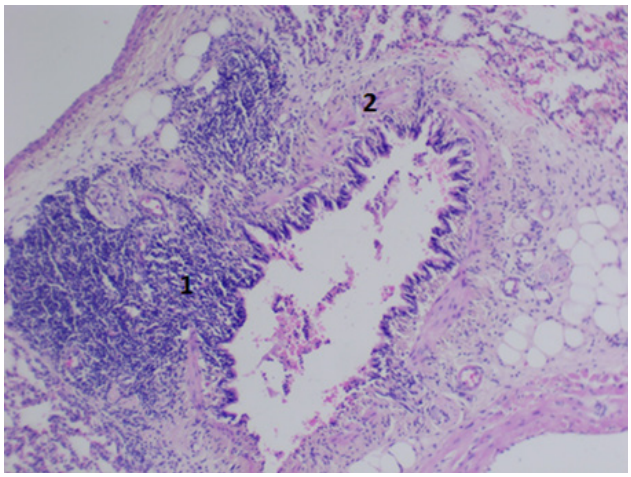

Fig. 2. Fragment of rat lung three hours after administration of Leiurus macroctenus scorpion venom. 1 – lymphocytic–neutrophilic infiltration; 2 – fragmented muscular layer of the bronchus. Hematoxylin-eosin staining. Magnification ×40.

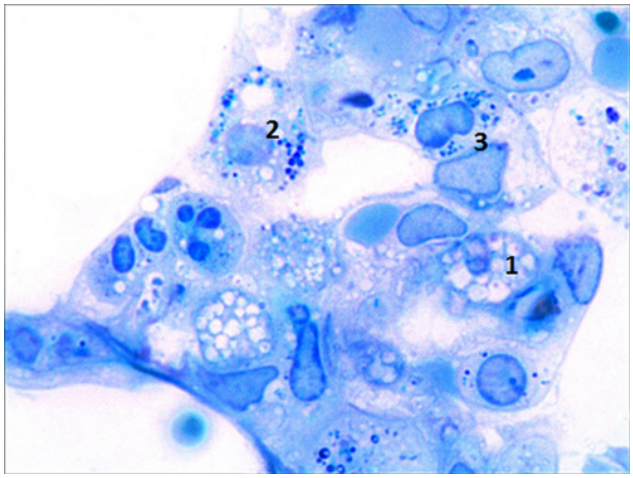

Fig. 3. Fragment of rat lung three hours after administration of Leiurus macroctenus scorpion venom. 1 – macrophage with phagolysosomes; 2 – eosinophil; 3 – basophil. Methylene blue

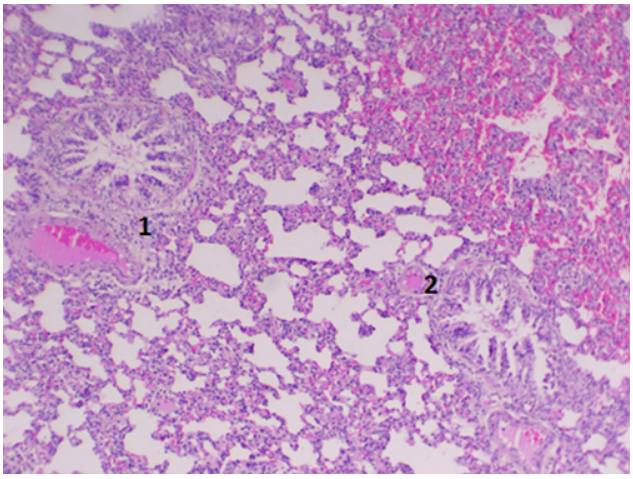

Fig. 4. Fragment of rat lung three hours after administration of Leiurus macroctenus scorpion venom. 1 – stromal edema; 2 – arteriole. Hematoxylin-eosin staining. Magnification ×40.

Morphometric analysis of the specimens demonstrated progression of a general trend toward redistribution of air between the alveoli and the conducting portion of the respiratory bronchioles. This was reflected by a decrease in alveolar width to 47.29±11.31 μm, alveolar entrance width to 27.50±4.01 μm, and alveolar depth to 68.72±9.50 μm, which were 21.35 % (p=0.112), 23.89 % (p=0.008), and 5.68 % (p=0.174) lower than control values, respectively. The mean thickness of the interalveolar septum was only 0.67 % lower (p=0.406) than in the control group. The width of the conducting portion of the bronchiole three hours after venom administration was 55.22±4.39 μm, which was statistically significantly 7.36 % lower than the control value (p=0.045).

The ratio of alveolar entrance width to alveolar depth (ratio A) and the ratio of the conducting portion width of the respiratory bronchiole to alveolar depth (ratio B) three hours after venom administration were 0.407±0.082 and 0.817±0.125, respectively, which were 22.77 % (p=0.112) and 3.31 % (p=0.940) lower compared to the control group.

Discussion

Thus, three hours after administration of Leiurus macroctenus scorpion venom, progressive edema was observed in the rat lungs, predominantly affecting the pulmonary stroma, along with infiltration of the interstitial space by inflammatory cells, which may impair tissue trophism and gas exchange processes. A continuing trend toward redistribution of air between the alveoli and the conducting portion of the respiratory bronchioles was noted, manifested by decreased alveolar width and entrance depth, consolidation of lung tissue, and collapse of alveolar walls. At the same time, areas of alveolar dilation were observed, accompanied by subsequent destructive changes of their walls and accumulation of fluid.

Studies describing lung morphology under the action of animal venoms are few in number, and they often concern snake venoms [1, 9, 13]. Thus, observation of groups of mice injected with the venom of the snake Crotalus durissus cascavella at a dose of 50 μg/kg showed different microscopic patterns at 1, 3, 6, 12, 24, and 48 h after administration. After 1 h, thickening of the interalveolar septa and marked vascular congestion were observed, with the appearance of a round-cell infiltrate, which by 3 h had already extended to the interalveolar septa with the formation of emphysema and disatelectasis. After 6 h, the infiltration decreased, but other changes persisted or progressed, and bronchial changes appeared which at 24-48 h caused bronchiolar spasm. Hemorrhages also appeared [1]. Other authors also studied the venom of this snake species, but administered it at a lower dose, 3.0 μg/kg. They found agreement between the macroscopic picture, manifested by impaired respiratory function, and the histological picture of lung injury acute parenchymal damage with a combination of atelectasis, emphysema, hemorrhages, round-cell infiltrate, edema, and vascular stasis [9].

A study of the effects of Vipera berus berus and Vipera berus nikolskii venom on lung morphometric parameters found that, compared with the control group (rats injected with saline), the mean vascular area in the lung increased 1.31-fold relative to intact animals, while the mean “respiratory compartment” index decreased to 0.8. The relative areas of disatelectasis, atelectasis, and emphysematous changes increased 6.03-, 7.15-, and 2.0-fold, respectively, compared with controls [16, 17].

Administration of Pseudechis papuanus venom at a dose of 35 μg after 40-60 min causes pulmonary edema in mice with massive accumulation of “hyaline” (proteinaceous) material in the alveolar spaces. At the same time, administration of the venom at a dose of 15 μg does not lead to the appearance of any microscopic changes in the lungs [26].

Comparison of morphological changes after administration of the venoms of the scorpions Tityus serrulatus and T. bahiensis at a dose of 200 μg/kg showed that in both cases laboratory animals exhibited marked hemorrhages, an inflammatory reaction, and an increased number of white blood cells in the bronchi and bronchioles [19].

A histological study of the effects of two Moroccan scorpion venoms showed that there is a correlation between the severity of the pathomorphological manifestations of venom action and its LD_{50}. At different time intervals, the authors noted dilation of alveolar spaces and destruction of alveolar structures in experimental animals [6].

Only isolated studies address the morphological impact of Leiurus macroctenus scorpion venom. In particular, available results concern changes in the adrenal glands of rats. One day after venom administration, pronounced changes in adrenal structure were detected, with hemorrhages and vascular congestion in this organ, indicating a systemic effect of the venom of this scorpion species [15]. Same microscopic changes were observed during kidneys investigation. Three hours after administration of Leiurus macroctenus venom intensive polymorphonuclear infiltration and disruption of blood vessels were found. Tubular necrosis appears in

kidneys tubular system [18].

A systematic review of 12 autopsy cases after scorpion stings was conducted by a team of researchers, who found that although the changes were nonspecific, they were uniform: the lungs showed signs of marked blood congestion and edema, which microscopically manifested as hemosiderophages in the alveoli, atelectasis, disatelectasis, edema (eosinophilic proteinaceous material), and subpleural emphysema [11].

A study of the effect of Tityus serrulatus scorpion venom on the bronchial epithelial cell line BEAS-2B showed that exposure to the venom at concentrations of 10 and 50 μg/mL for 1, 3, 6, and 24 h reduced cell viability, and cell death occurred through necrosis rather than apoptosis, as indicated by cytometry results [22].

Androctonus australis hector scorpion venom, when administered to laboratory animals at a dose of 10 μg/20 g, causes acute lung injury with involvement of alveolar macrophages and neutrophils (in particular, around the bronchi), occurring against a background of areas of edema and hemorrhages [24].

At the same time, bee venom, conversely, may have a positive effect on the morphological structure of the lungs. An experimental study showed that its venom can block IL-13-induced upregulation of MUC5AC [14].

Overall, comparison of data on the effects of scorpion venoms with the obtained results showed partial overlap in the morphological picture. The differences are likely the result of both different experimental models and species-specific features of venom composition.

Conclusions

1. Three hours after administration of Leiurus macroctenus venom, acute inflammatory-destructive lung injury develops in experimental animals, combining pronounced round-cell infiltration, structural damage to bronchi of various calibers, and reactive changes in the parenchyma.

2. Morphometric analysis demonstrated a reduction in the examined alveolar parameters, including statistically significant narrowing of the conducting portion of the respiratory bronchioles (p=0.045) and the alveolar entrance width (p=0.008), indicating impaired ventilatory balance in the studied lung samples.

3. The identified tendency toward redistribution of air between the alveolar and bronchiolar compartments reflects early functional alterations accompanying structural remodeling of lung tissue under the toxic influence of Leiurus macroctenus venom as early as three hours after exposure.

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МІКРОСКОПІЧНІ ТА МОРФОМЕТРИЧНІ ЗМІНИ У БРОНХАХ І ПАРЕНХІМІ ЛЕГЕНЬ ЛАБОРАТОРНИХ ЩУРІВ ЧЕРЕЗ ТРИ ГОДИНИ ПІСЛЯ ВВЕДЕННЯ ОТРУТИ СКОРПІОНУ LEIURUS MACROCTENUS

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Скорпіонізм є поширеним явищем, що зустрічається в різних регіонах світу, та несе особливу небезпеку для осіб похилого віку та дітей. Незважаючи на численні випадки дії отрути скорпіонів на організм людини, незначна кількість публікацій описують клінічну картину, особливо з боку дихальної системи у таких пацієнтів. Ще менше робіт присвячені опису морфологічних змін легень чи бронхів у померлих осіб чи лабораторних тварин, зокрема в різні часові рамки після укусу скорпіона. Дані прогалини на морфологічному рівні, навіть при досить добре вивченому патогенетичному шляху дії отрути скорпіона, не дозволяють мати повне розуміння картини токсичного впливу даної речовини. Лише за умов всебічного дослідження отрути можливе в подальшому не тільки створення протиотрути до неї але і застосування як перспективного лікарського засобу. Мета дослідження – визначити мікроскопічні та морфометричні зміни у бронхах і паренхімі легень щурів через 3 години після введення отрути виду скорпіона Leiurus macroctenus. Для дослідження використано 10 щурів самців, що утримувалися в умовах віварію і були розділені на 2 групи по 5 осіб у кожній: група 1 – щури контрольної групи, яким вводили фізіологічний розчин та щури групи 2 – яким вводили отруту скорпіона у дозі 28,8 мкг/мл. Через 3 години після введення проводили евтаназію щурів з вилученням легень на гістологічне дослідження. Для проведення мікроскопічного і морфометричного дослідження виготовлялися 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 на мікроскопі OLIMPUS BX 41. Морфометричний аналіз виконували з допомогою програмного забезпечення Quickphoto Micro 2.3. Статистичний аналіз отриманих результатів проведений у ліцензійному пакеті «Statistica 6.0» з використанням непараметричних методів оцінки. Через 3 години після введення отрути у легенях дослідних тварин формуються виражені запальні зміни, що проявляються масивною лімфоцитарно-нейтрофільною інфільтрацією, найбільш інтенсивною навколо бронхів середнього, малого діаметрів та бронхіол, аж до часткового руйнуванням їх стінок, фрагментації м'язових пучків та набряку адвентиції. У міжальвеолярному просторі переважають базофіли, еозинофіли та макрофаги, що також свідчить на користь вираженої запальної реакції. У паренхімі ділянки дисателектазів чергуються з ділянками емфіземи, де місцями спостерігається деструкція міжальвеолярних

перегородок та накопичення рідини. При морфометричному дослідженні зафіксовано зменшення ширини альвеол, їх входу та глибини (p=0,112, p=0,008 та p=0,174 відповідно), а також статистично значуще звуження провідного відділу респіраторних бронхіол (p=0,045). Виявлено тенденцію до перерозподілу повітря між альвеолярним та бронхіолярним відділами, що відображено при гістологічному дослідженні змінами співвідношень морфометричних показників. Отримані дані свідчать про розвиток гострого запально-деструктивного ураження легень з порушенням вентиляційної функції вже у ранні терміни після інтоксикації. Сукупність виявлених мікроскопічних змін вказує на формування структурної перебудови легеневої паренхіми у відповідь на токсичну дію отрути вже на 3 годину після її введення лабораторним тваринам.

Ключові слова: *морфологія, отрута скорпіона, Leiurus macroctenus, щури, бронхи, паренхіма легень, морфологічні зміни, морфометричні зміни.*

Author's contribution

Gunas V. I. – research, methodology and writing of the original draft, conceptualization, formal analysis, software.
Maievskyi O. Ye. – supervision.
Skoruk R. V. – review writing and editing.
Kyrychenko V. I. – review writing and editing.
Bandurka N. M. – validation.
Sorokoumov V. P. – data visualization.
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