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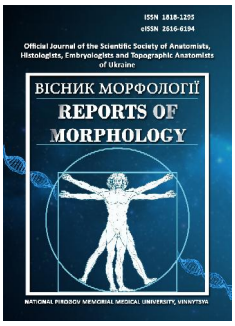


# Morphological state of lungs of rats under the influence of Vipera berus berus venom

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*About 5.4 million snake bite cases are registered worldwide every year. About half of them cause 81,000-138,000 deaths or disabled 400,000 people. Despite this, this problem is neglected in many countries in Asia, Africa and Latin America. It is believed that death from snakebites is associated with poverty since the lack of proper access to medical facilities in such sections of the population causes the development of fatal complications. The purpose of the study is to study the morphological state of rats' lungs under exposure to Vipera berus berus venom. Experimental studies were carried out on white, non-linear male rats. Animals were conditionally divided into control and experimental groups, ten individuals each. Experimental rats were injected intraperitoneally with a semi-lethal dose ($LD^{50}$) (1.576 mg/g$^{-1}$) of Vipera berus berus venom in a physiological solution. Animals of the control group were injected intraperitoneally with only a physiological solution. Rats were removed from the experiment 24 hours after exposure to the poison and anaesthetized by cervical dislocation. Lung tissue samples were taken for microscopic examination. Fixation of the material and preparation of paraffin blocks were carried out according to generally accepted methods. Staining of histological preparations of the lungs was carried out with hematoxylin and eosin, according to Masson, and the PAS+"Hale" reaction was carried out according to the Mowry method. Histological preparations were studied using an SEO SCAN light microscope. The toxic effect of Vipera berus berus venom caused significant changes in the vascular, stromal and parenchymal components, which was confirmed by histological, histochemical and morphometric data. In the lungs of experimental animals, the vascular bed is primarily restructured, forming coagulopathies and thrombosis. The development of DIC syndrome and haemorrhages accompanies an increase in the permeability of the vessel wall. Inflammatory phenomena are found in the bronchi and respiratory departments' walls. There is a remodelling of the respiratory department components, with the formation of significant areas of dis- and atelectasis and zones of emphysema, which is confirmed morphometrically.*

**Keywords:** *vipers, lungs, poison, inflammation, rats.*

## Introduction

About 5.4 million cases of snakebites are registered around the world every year. About half of them cause 81,000-138,000 deaths or disabled 400,000 people. Despite this, this problem is neglected in many countries in Asia, Africa and Latin America. It is believed that death from the bites of snakes and other animals with poisonous glands, in particular scorpions, is associated with poverty since the lack of proper access to medical facilities in such sections of the population causes the development of fatal complications [7, 12, 14, 15]. However, changes in climatic conditions and human settlement of territories endemic to these species of animals lead to the fact that the risk groups expand significantly and include not only low-income residents of these regions [8, 13, 19, 38]. The consequences of snake bites in children and pregnant women are especially severe. In the first case, the immaturity of the immune system and low body weight compared to adults becomes the cause of the development of severe complications [1, 9, 21]. In the second case, as a result of disorders of the coagulation system and, accordingly, haemorrhages or thrombosis, it is possible to terminate pregnancies at different times [17, 23, 28].

The venom produced by snakes consists of a significant variety of components, among which the largest share are proteins and peptides with different molecular weights [18, 22, 25]. Most of the components have a synergistic effect, which causes the rapid effect of toxins on the victim's body [6, 20, 31]. Snide venom leads to significant structural changes in the cardiovascular, respiratory, excretory, endocrine, immune, and nervous systems [4, 5, 33].

Today, scientists worldwide actively study the components of snake toxins [24, 30, 30]. First of all, this is explained by the wide range of effects of the poison on the victim's body, as well as the possibilities of using it for medical purposes and making antidotes. The toxins of snakes from different families vary considerably [27, 29, 34]. However, it was established that many structural elements are identical for all snakes [36]. In Europe, the most common vipers are Vipera berus berus and Vipera berus nikolskii, which determines our exceptional attention to the study of the toxins of these types of snakes and the specifics of their effect on the body of victims [2, 26, 37].

To date, no data describe the full range of histological changes in the lungs during bites of poisonous snakes. Few available studies do not reveal the main pathogenetic links in developing one or another complication from the respiratory system under these conditions. In particular, there is a complete lack of data in the literature regarding the effect of Vipera berus berus viper toxins on the structure and functions of the respiratory system, which explains the importance of our study.

*The purpose of the study* is to study the morphological state of rats' lungs under exposure to Vipera berus berus venom.

## Materials and methods

Experimental studies were carried out on white, non-linear male rats. For preliminary acclimatisation, the animals were kept for seven days in the animal husbandry of the Taras Shevchenko National University of Kyiv and later in laboratory conditions in compliance with temperature and light regimes [10]. Rats received standard chow and water ad libitum. The National Institutes of Health Guidelines performed all experiments for the Care and Use of Laboratory Animals and the European Council Directive of November 24, 1986, on the Care and Use of Laboratory Animals (86/609/EEC). The research was approved and confirmed by the bioethics commission of the NSC "Institute of Biology and Medicine" of Taras Shevchenko Kyiv National University (protocol No. 2 dated 08.19.2021).

Vipera berus berus venom was obtained from V. N. Karazin Kharkiv National University. Freeze-dried native venom was stored at -20 °C and dissolved in saline immediately before the experiment.

The animals were conditionally divided into a control and an experimental group of 10 individuals. Experimental rats were injected intraperitoneally with a semi-lethal dose (LD50) (1.576 mg/g-1) of Vipera berus berus venom in a physiological solution. Animals of the control group were injected intraperitoneally with only a physiological solution. Rats were removed from the experiment 24 hours after exposure to the poison and anaesthetized by cervical dislocation.

Lung samples of animals of all groups were taken for microscopic examination. The pieces were fixed in a 10 % formalin solution for one day. Next, the pieces were dehydrated in alcohols of increasing concentration and embedded in paraffin blocks. Histological preparations of lungs were stained with hematoxylin and eosin, according to Masson (to detect collagen fibres), and the PAS+"Hale" reaction was performed according to the Mowry method (detection of glycoproteins and glycosaminoglycans in the intercellular substance of connective tissue) [16]. Histological preparations were studied using an SEO SCAN light microscope and photo-documented using a Vision CCD Camera with a system of image output from histological preparations.

Morphometric studies were carried out using the visual analysis system of histological preparations. Images from histological preparations were displayed on a computer monitor using a MICROmed SEO SCAN microscope and a Vision CCD Camera. Morphometric studies were conducted using SEO programs ImageLabBio, ImageJ and STATISTICA 10.0. The research was carried out in the specified terms of the experiment in preparations stained with hematoxylin and eosin.

Morphometrically, the relative share of blood vessels, bronchi, lymphoid tissue and the respiratory department was determined, in which the relative share of lung tissue with unchanged histostructure, atelectasis, disatelectase, and emphysematously changed lung tissue was measured.

The obtained digital material was processed using variational statistics using the Student's t-test. Arithmetic mean values (M), arithmetic mean errors (m), variation coefficients, and mean square deviations were calculated. Changes were considered reliable at $p \leq 0.05$.

## Results

Histological examination of the lungs of experimental animals after the bite of the viper Vipera berus berus showed that significant destructive and inflammatory changes of all constituent components occur in the organ. Hemotoxins provoked hemodynamic disorders with the remodelling of vessel walls, in particular, increased permeability, which leads to swelling of the wall, disorganisation of the amorphous component and fibres, and perivascular, interstitial and peribronchial connective tissue. An increase in the number of glycoproteins in their composition is confirmed by pronounced PAS-positive properties and redistribution and an increase in the number of sulfated glycosaminoglycans with Hale-positive properties (Fig. 1).

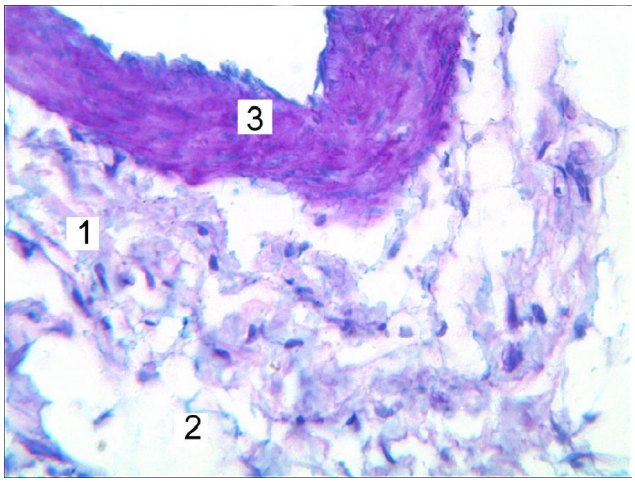


**Fig. 1.** Histochemical changes in a white rat's lung under exposure to the viper Vipera berus berus. Collagen fibres of perivascular connective tissue with weakly expressed PAS-positive properties (1) and intensively expressed "Hale" positive properties of glycosaminoglycans (2), arterial media (3). Staining according to the Mowry method. x400.

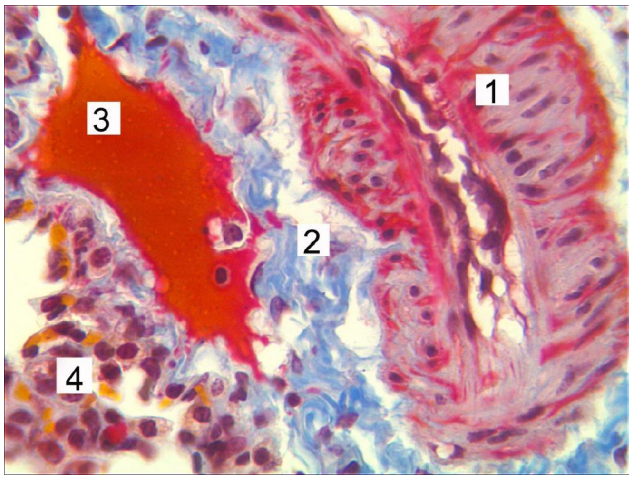


**Fig. 2.** Microscopic changes in a white rat's lung under viper Vipera berus berus exposure. Swollen artery media (1), disorganisation of adventitia fibres (2), and perivascular accumulation of fibrin (3). Staining by the MSB (OKH) method. x400.

**Table 1.** The ratio of structural components of animal lungs after exposure to steppe viper venom (M±m).

| Indicator | Intact group | Vipera berus berus |
|---|---|---|
| Vessels, % | 4.821±0.213 | 6.33±0.252* |
| Bronchi, % | 5.472±0.264 | 12.24±0.46* |
| Lymphoid tissue, % | 6.751±0.232 | 15.05±0.55* |
| Respiratory department, % | 82.96±3.84 | 66.38±2.89* |

**Note:** * - values significantly differ from the indicators of the control group of animals (p<0.001).

The toxic effect of Vipera berus berus venom provokes choalugopathy, manifested histologically by stasis, erythrocyte sludge, and blood clots. Blood circulation disorders are accompanied by the accumulation of fibrin intra- and extravascularly, which confirms the development of DIC syndrome (Fig. 2).

Morphometrically, a significant increase in the average value of the vascular area was established by 1.31 times (p<0.001) relative to the value of the intact group. The average values of the bronchi and lymphoid tissue area also increased progressively by 2.24 and 2.23 times (p<0.001) relative to the average values of the intact group. Accordingly, the average indicator of the respiratory department decreases significantly and is 0.80 relative to the intact value (Table 1).

In the lungs' respiratory department, the alveoli's wall is mainly thickened due to oedema and infiltration by histo- and leukocyte series cells. Extensive dys- and atelectasis areas and emphysematously changed lung tissue zones are revealed (Fig. 3).

It was morphometrically investigated that under the conditions of exposure to Vipera berus berus viper toxins in the lungs of experimental animals, the relative proportions of dys- and atelectasis, emphysematously changed lung tissue increased reliably by 6.03, 7.15, and 2.00 times (p<0.001) relative to the parameters of the intact group. Accordingly, the average value of lung tissue with unchanged histostructure, which is 0.31 relative to the value of the intact group, progressively decreases (Table 2).

Alteration of vessel walls is also manifested by diapedesis of formative elements into alveolar lumens. Activation of inflammatory processes in the respiratory tract of the lungs is characterised by the presence of inflammatory local conglomerates of macrophages, lymphocytes, and neutrophils, mainly in the loci of atelectasis (Fig. 4).

In the areas of haemorrhages, the presence of macrophages with brightly "Hale"-positive lumps of hemosiderin, which results from pathological accumulation and breakdown of haemoglobin in erythrocytes, was

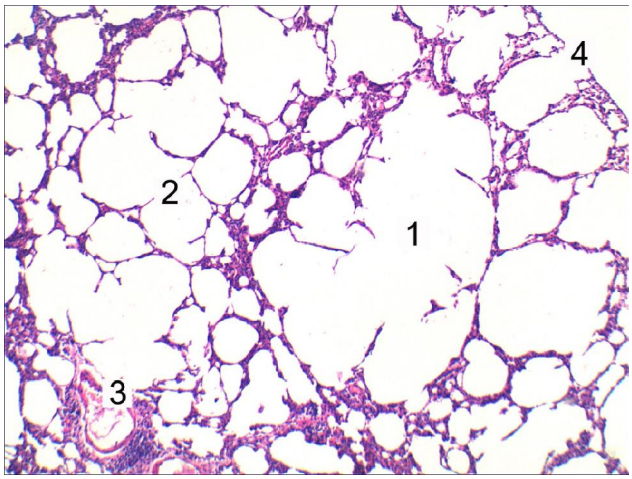


**Fig. 3.** Microscopic changes in a white rat's lung under viper Vipera berus berus exposure. Emphysematously changed alveoli (1), thin interalveolar septa (2), destructively changed vessel (3), and thin pleura (4). Staining with hematoxylin and eosin. x 100.

**Table 2.** The ratio of the structural components of the respiratory department of the lungs of animals under exposure to the poison Vipera berus berus (M±m).

| Groups | Indicators | | | |
|---|---|---|---|---|
| | The relative proportion of lung tissue with unchanged histostructure, % | Relative share of atelectasis, % | The relative proportion of dysatelectases, % | The relative share of emphysematous changed lung tissue, % |
| Intact group | 82.82±3.91 | 3.552±0.151 | 5.280±0.241 | 8.352±0.393 |
| Vipera berus berus | 26.06±1.25* | 25.38 ±1.23* | 31.85±1.49* | 16.71±0.81* |

**Note:** * - values significantly differ from the indicators of the control group of animals (p<0.001).

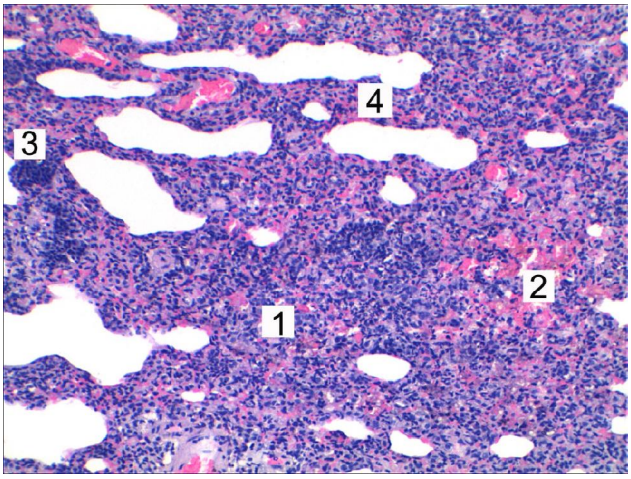


**Fig. 4.** Histological changes in a white rat's lung under exposure to the viper Vipera berus berus. Zones of atelectasis (1), diapedesis diffuse haemorrhages (2), local leukocyte infiltrates (3), thickened, infiltrated alveolar walls (4). Staining with hematoxylin and eosin. x100.

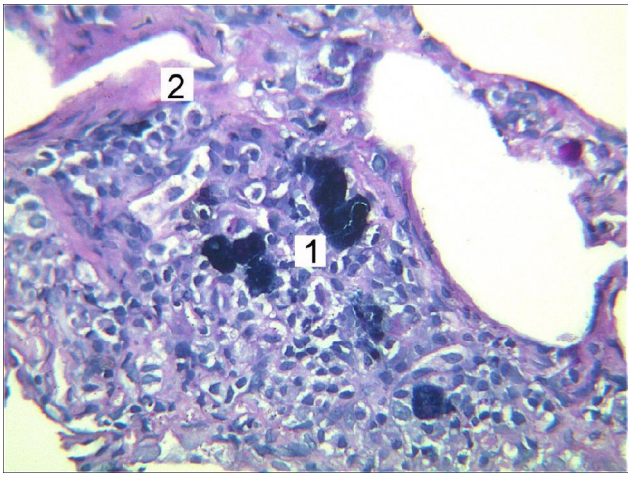


**Fig. 5.** Histochemical changes in a white rat's lung under exposure to the viper Vipera berus berus. Macrophages with an accumulation of brightly "Hale"-positive hemosiderin grains in the areas of atelectasis (1), moderately PAS-positive reticular fibres of the stroma (2). Staining according to the Mowry method. x400.

detected histochemically (Fig. 5).

Bronchi are characterised by wall damage with alteration of all membranes. In most fields of view, in the parenchyma of the lungs and mainly in the wall of the bronchi, voluminous inflammatory infiltrates in which lymphocytes and macrophages are detected (Fig. 6).

Bronchial obstruction due to bronchospasm is observed in part of the bronchi, not only of small bronchi but also of

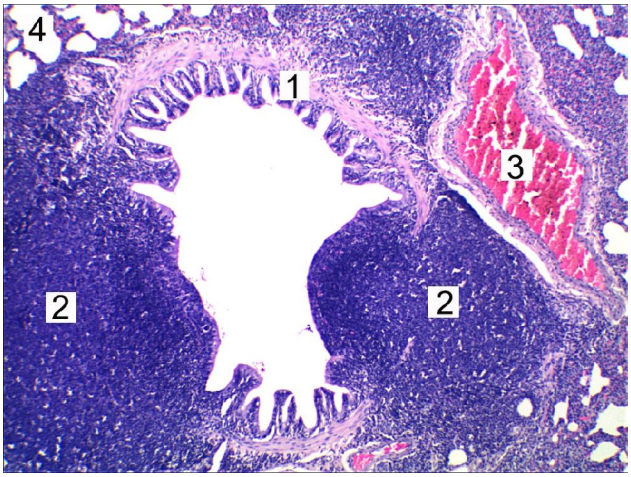


**Fig. 6.** Microscopic changes in a white rat's lung under viper Vipera berus berus exposure. Alteration of the wall of the bronchus (1), voluminous inflammatory infiltrates in the wall of the bronchus (2), full-blooded vein (3), and respiratory department (4). Staining with hematoxylin and eosin. x100.

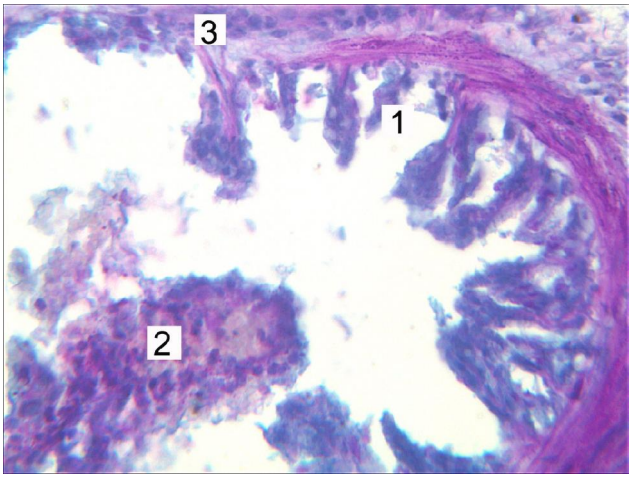


**Fig. 7.** Histochemical changes in a white rat's lung under exposure to the viper Vipera berus berus. Destruction of the epithelial plate of the wall (1), serous-mucous content in the lumen with moderately expressed PAS-positive properties (2), and alteration of the media (3). Staining according to the Mowry method. x200.

medium diameter. The ciliated epithelium of the mucous membrane is swollen, fragmented in many areas and desquamated in the lumen. Serous-mucous content with cellular detritus, which has bright PAS-positive properties, is determined in the lumen of the bronchi (Fig. 7).

Destructive damage to the respiratory epithelium is detected in most fields of observation; however, restoration of the epithelial plate due to interstitial cells is determined in some areas.

## Discussion

Thus, histological studies of the lungs of experimental animals after being bitten by the viper Vipera berus berus showed the development of significant destructive and inflammatory changes in all constituent components. The toxic effect of Vipera berus berus venom provoked coagulopathy. Blood circulation disorders were accompanied by the accumulation of fibrin intra- and extravascularly, which confirmed the development of the syndrome of disseminated intravascular blood coagulation. In the areas of haemorrhages, the presence of macrophages with brightly "Hale"-positive lumps of hemosiderin was histochemically revealed, which is the result of pathological accumulation and breakdown of haemoglobin in erythrocytes. Morphometrically, a reliable increase in the average value of the vascular area by 1.31 times ($p<0.001$) relative to the value of the intact group was established. The average values of the bronchi and lymphoid tissue area also increased progressively by 2.24 and 2.23 times ($p<0.001$) relative to the average values of the intact group. It was morphometrically investigated that under the conditions of exposure to Vipera berus berus viper toxins in the lungs of experimental animals, the relative proportions of dys- and atelectasis, emphysematously changed lung tissue increased reliably by 6.03, 7.15, and 2.00 times ($p<0.001$) relative to the parameters of the intact group.

The results of separate in-depth studies demonstrate the characteristics of broncho-pulmonary complications after bites of various types of snakes.

A. L. Ferrara and co-authors [11], in an experimental study, established that the secretory PLA2 poison of Naja mossambica mossambica leads to the development of an inflammatory process in the lungs associated with the activation of macrophages. It is known that PLA2 can hydrolyse the fatty acids of phospholipids of cell membranes. Among all species, their secretory PLA2 (sPLA2) represents the most prominent family, a low molecular weight $Ca^{2+}$-dependent enzyme. During the activation of the inflammatory cascade, sPLA2 is released into biological fluids and stimulates the activity of cells of the immune system, in particular, macrophages. In this case, under the influence of the enzyme, the authors observed increased production of cytokines by alveolar macrophages. They also demonstrated that sPLA2 of groups IIA and X stimulates the production of vascular endothelial growth factors A and C (VEGF-A and VEGF-C) by lung macrophages through a receptor-mediated mechanism. In addition, this enzyme from snake venom enhanced the activity of neutrophils. It induced the activity of its own cytosolic PLA2, leading to the production of arachidonic acid metabolites. It stimulated the synthesis of cytokines - TNF-α, IL-6 and IL-10, leading to a robust inflammatory process in lung tissue.

A rare complication in patients after a hump-nosed viper bite (Hypnale hypnale or Hump-nosed viper) was found by Srirangan A. et al. [34]. Pulmonary bleeding under conditions of moderate changes in the coagulogram was recorded in the early stages after venom inoculation. In addition, signs of severe blood stagnation and swelling of the lung tissue were observed. The authors suggest that the cause of the mentioned symptoms is the presence in the venom of a significant number of SVMPs, which are key participants in the development of coagulopathies.

Many authors in recent studies have described marked histological changes in the respiratory system due to the bite of Crotalus durissus terrificus. Examination of lung tissue samples 2, 6, and 12 hours after subcutaneous administration of crotoxin to mice revealed such changes as a decrease in the area of the alveolar sacs and an increase in the thickness of the alveolar walls. Stagnant phenomena, haemorrhages, infiltration of lung tissue by polymorphonuclear leukocytes, presence of foamy macrophages, and increased permeability of lung vessels were recorded. The growth of myeloperoxidase activity was determined in the homogenates. It has also been established that crotoxin from the venom of this viper species has a neurotoxic effect. It is a β-neurotoxin that induces the blockade of impulses in neuromuscular synapses, suppressing the release of acetylcholine from presynaptic membranes and postsynaptic desensitisation of nicotinic receptors, causing flaccid paralysis of the respiratory muscles. In addition, crotoxin PLA2 leads to the degradation of cell membrane phospholipids, the initiation of the arachidonic acid cascade and the production of prostaglandin E2 (PGE2), which is associated with myo- and neurotoxicity and the activation of the immune response [30].

According to the literature, the venom of the viper Crotalus durissus cascavella causes changes in the histological architecture of the bronchial tree, bronchioles and alveoli due to the development of emphysema and atelectasis. The specified structural organisation violations are accompanied by moderate swelling of the lung tissue, which is associated with the direct toxic effect of the poison. Also, a characteristic feature of the toxin is the induction of acute damage to the respiratory system, which is morphologically manifested by inflammatory infiltration of the lungs by lymphocytes and plasma cells [3].

## Conclusions

The toxic effect of Vipera berus berus venom caused significant changes in the vascular, stromal and

parenchymal components, which was confirmed by histological, histochemical and morphometric data. In the lungs of experimental animals, the priority is the remodelling of the vascular bed, with the formation of coagulopathies and thrombosis. The development of DIC syndrome and haemorrhages accompanies an increase in the permeability of the vessel wall. Inflammatory phenomena are found in the bronchi and respiratory departments' walls. There is a remodelling of the respiratory department components, with the formation of significant areas of dis- and atelectasis and zones of emphysema, which is confirmed morphometrically.

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**МОРФОЛОГІЧНИЙ СТАН ЛЕГЕНЬ ЩУРІВ ЗА УМОВ ВПЛИВУ ОТРУТИ ГАДЮК VIPERA BERUS BERUS**

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*В усьому світі щороку реєструють близько 5,4 млн випадків укусів змій. Приблизно половина з них стає причиною 81 000-138 000 смертей або призводить до інвалідності 400 000 людей. Не дивлячись на це, дана проблема є занедбаною в багатьох країнах Азії, Африки та Латинської Америки. Вважають, що смерть від укусів змій асоційована з бідністю, оскільки відсутність належного доступу до медичних закладів у таких прошарків населення зумовлює розвиток летальних ускладнень. Метою дослідження є вивчення морфологічного стану легень щурів за умов впливу отрути гадюк Vipera berus berus. Експериментальні дослідження проводили на білих нелінійних щурах самцях. Тварин умовно розподіляли на дві групи - контрольну і дослідну по 10 особин в кожній. Дослідним щурам внутрішньоочеревинно вводили напівлетальну дозу ($LD^{50}$) (1,576 мг/г$^{-1}$) отрути Vipera berus berus на фізіологічному розчині. Тваринам контрольної групи внутрішньоочеревинно вводили лише фізіологічний розчин. Виводили щурів з експерименту через 24 години після впливу отрути під анестезією, знеживлюючи шляхом цервікальної дислокації. Для мікроскопічного дослідження вилучали зразки тканини легень. Фіксацію матеріалу та приготування парафінових блоків проводили за загальноприйнятими методиками. Забарвлення гістологічних препаратів легень здійснювали гематоксиліном та еозином, за Массоном, проводили ШИК+"Хейл" реакцію за методом Моурі. Гістологічні препарати вивчали за допомогою світлового мікроскопа SEO SCAN. Токсичний вплив отрути гадюки Vipera berus berus спричинив значні зміни судинного, стромального та перенхіматозного компонентів, що підтверджується гістологічними, гістохімічними та морфометричними даними. В легенях дослідних тварин першочергово відбувається перебудова судинного русла, з формуванням коагулопатій, тромбозів. Підвищення проникності стінки судин супроводжується розвитком ДВЗ-синдрому та крововиливами. В стінці бронхів та респіраторному відділі виявляються запальні явища. Відбувається ремоделювання компонентів респіраторного відділу з формуванням значних площ дис- та ателектазів, зон емфіземи, що підтверджується морфометрично.*

**Ключові слова:** *гадюки, легені, отрута, запалення, щури.*

**Author's contribution**

*Lasavutz V. S.* - research, writing of the original draft.
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