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### **MORPHOLOGICAL CHANGES IN THE ADRENAL GLANDS OF RATS THREE HOURS AFTER EXPOSURE TO THE VENOM OF LEIURUS MACROCTENUS SCORPION**

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Morphological examination of the adrenal glands of rats three hours after administration of *Leiurus macroctenus* scorpion venom revealed a marked progression of pathological alterations compared with earlier experimental time points. In the zona glomerulosa of the cortex, nuclear deformation, loss of the orderly arrangement of cellular clusters, and the appearance of intracellular edema were observed. The cytoplasm of endocrine cells lost its homogeneity, became granular, and exhibited perinuclear clear zones indicative of endoplasmic reticulum destabilization. In the zona fasciculata, a combination of physiological and pathological cellular hypertrophy was detected, accompanied by increasing intra- and extracellular edema. The nuclei of some cells were in stages of karyopyknosis and karyorrhexis, indicating the development of necrobiotic processes. In the zona reticularis, moderate hypertrophy, cytoplasmic basophilia, focal areas of edema, and infiltration by immunocytes were recorded. In the medullary substance, early manifestations of destruction were noted, including swelling of chromaffin cells, loss of distinct cellular boundaries, and nuclear deformation. The vessels of both the cortex and medulla exhibited signs of congestion, with aggregates of erythrocytes in their lumina. The capsule of the gland was characterized by moderate loosening of collagen fibers and morphological alterations in fibroblasts.

**Key words:** anatomy, histology, adrenal glands, laboratory rats, venom of the scorpion *Leiurus macroctenus*.

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### **МОРФОЛОГІЧНІ ЗМІНИ В НАДНИРНИКАХ ЩУРІВ ЧЕРЕЗ ТРИ ГОДИНИ ПІСЛЯ ВПЛИВУ ОТРУТИ СКОРПІОНІВ LEIURUS MACROCTENUS**

Морфологічне дослідження наднирників щурів через три години після введення отрути скорпіонів *Leiurus macroctenus* показало виражене поглиблення патологічних змін у порівнянні з попередніми термінами експерименту. У клубочковій зоні кори відзначено деформацію ядер, втрату впорядкованості клітинних кластерів і появу внутрішньоклітинного набряку. Цитоплазма ендокриноцитів втрачала гомогенність, ставала зернистою, із перинуклеарними просвітленнями, що вказує на дестабілізацію ендоплазматичного ретикулюму. У пучковій зоні виявлено поєднання фізіологічної та патологічної гіпертрофії клітин із наростанням внутрішньо- та позаклітинного набряку. Ядра частини клітин перебували в стадіях каріопікнозу й каріорексису, що свідчить про розвиток некробіотичних процесів. У сітчастій зоні зафіксовано помірну гіпертрофію, базofilію цитоплазми, поодинокі ділянки набряку та інфільтрацію імуніцитами. У мозковій речовині спостерігались ранні прояви деструкції – набряк хромафінних клітин, втрата чітких контурів і деформація ядер. Судини кори та медулярної речовини мали ознаки повнокрів'я з агрегатами еритроцитів у просвітах. Капсула залози характеризувалася помірним розпушенням колагенових волокон і змінами фібробластів.

**Ключові слова:** анатомія, гістологія, наднирникові залози, лабораторні щури, отрута скорпіона *Leiurus macroctenus*.

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Bites and stings inflicted by venomous animals remain one of the pressing and important yet insufficiently studied problems of modern medicine. They represent a significant threat to human life and health, particularly in regions with tropical and subtropical climates. According to estimates by the World Health Organization (WHO), approximately one million cases of snake, scorpion, and other venomous arthropod bites occur annually worldwide, resulting in hundreds of thousands of deaths or severe complications leading to disability. However, even in developed European countries, this problem remains underestimated: bites from venomous vertebrates and invertebrates are often not recognized in a timely manner, and specific medical care is provided with delay. Consequently, current clinical observations and epidemiological studies emphasize the need to increase healthcare workers' vigilance regarding such cases and to improve diagnostic and therapeutic methods [13].

Particular attention is drawn to the situation in the Middle East and North Africa, where bites from venomous animals, such as scorpions, are highly prevalent and can acquire the character of regional epidemics. For instance, in Lebanon, envenomation following scorpion and snake bites remains a significant public health concern, especially in rural areas where access to specialized medical care is limited. The absence of standardized treatment protocols, restricted access to antitoxins, and a low level of

public awareness create serious challenges in both patient management and preventive strategies. Authors of high-quality studies emphasize that even when medical resources are available, the effectiveness of therapy is often reduced due to delayed presentation of victims and misinterpretation of clinical manifestations, which in turn decreases the likelihood of favorable outcomes without severe health consequences [8].

Epidemiological characteristics of scorpion stings in southern Iran indicate that this region belongs to a high-risk zone. Children and young adults constitute the majority of affected individuals. The most common clinical manifestations include localized pain, edema, tachycardia, arterial hypertension, hyperglycemia, and signs of systemic intoxication. Studies also demonstrate seasonal variations in sting frequency, peaking during the summer months, which correlates with increased scorpion activity and rising environmental temperatures. Such data highlight the importance of continuous monitoring, statistical analysis, and the development of region-specific preventive programs for venomous animal bites [7].

The problem of venomous animal bites is not limited to the Middle East. In Africa, particularly in rural areas of Tanzania, snake and scorpion bites are a frequent cause of hospitalization. Retrospective studies reveal that most victims do not receive timely medical care due to the remoteness of hospitals, low public awareness, and the absence of antidotes. This situation results in high mortality and disability rates, especially among children. Moreover, toxicological monitoring in such regions is hindered by underreporting and limited laboratory capacity. These factors underscore the need to study the mechanisms of venom action and the morphological changes occurring in target organs following envenomation [9].

In this context, research into morphofunctional changes induced by scorpion venom, particularly in the adrenal glands—which play a crucial role in the body’s stress response—acquires special importance. Representatives of the genus *Leiurus*, especially *Leiurus macroctenus*, belong to the most dangerous scorpion species in southwestern Asia. They are distributed in arid and semi-desert regions of Iran, Saudi Arabia, Syria, and Jordan, and their venom exhibits pronounced neurotoxic and cardiotoxic effects. According to recent reviews, more than 30 scorpion species have been recorded in Western Asia and the Middle East, at least 10 of which pose a direct threat to humans. Investigating their biology, behavior, and geographical distribution is an essential prerequisite for the effective prevention and treatment of envenomation outcomes [4].

Thus, the epidemiological situation, global prevalence of scorpion stings, and toxicological complexity of their venom necessitate experimental studies on morphological alterations in vital organs. The adrenal glands, in particular, represent a key target for investigation, as they reflect the systemic response of the organism to stress and intoxication. Analysis of structural rearrangements in the adrenal glands following short-term exposure to *Leiurus macroctenus* venom allows for a deeper understanding of the pathogenetic mechanisms of damage and potential directions for therapeutic correction.

**The purpose** of the study was to determine the morphological changes in the adrenal glands of male laboratory rats three hours after the administration of *Leiurus macroctenus* scorpion venom.

**Materials and methods.** The study involved 10 male white laboratory rats Wistar’s line weighing  $200 \pm 10$  g, bred in the vivarium of the Educational and Scientific Center “Institute of Biology and Medicine” of Taras Shevchenko National University of Kyiv. Experiments were carried out on the basis of the cooperation agreement between Taras Shevchenko National University of Kyiv, National Pirogov Memorial Medical University of Vinnytsya and I. Horbachevsky Ternopil National Medical University (2021). The housing conditions of the rats complied with the “Standard rules for the arrangement, equipment, and maintenance of experimental biological clinics (vivariums).” All procedures were performed in accordance with the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” [20] and the Law of Ukraine “On the Protection of Animals from Cruelty” (No. 3447-IV of February 21, 2006) (Bioethics of National Pirogov Memorial Medical University, Vinnytsya, protocol No. 11 from 12.11.2024).

Laboratory rats were divided into two groups: a control group (five rats intramuscularly injected with 0.5 mL of physiological saline) and an experimental group (five rats given a single intramuscular injection of 0.5 mL of *Leiurus macroctenus* venom solution at a concentration of  $28.8 \mu\text{g/mL}$ ;  $\text{LD}_{50} = 0.08$  mg/kg [6]). After 3 hours, euthanasia was performed by  $\text{CO}_2$  inhalation, and the adrenal glands were immediately excised at  $4^\circ\text{C}$ . All procedures complied with the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” and the Law of Ukraine “On the Protection of Animals from Cruelty” (No. 3447-IV of 21.02.2006) (Bioethics Committee of the National Pirogov Memorial Medical University, Vinnytsya; protocol No. 11 of 12.11.2024).

Tissue samples were fixed in 10 % neutral formalin, dehydrated through a graded ethanol series, and compacted in a Logos ONE histoprocessor (MILESTONE, Italy). The material was embedded in

paraffin blocks using an automated TEC 2800 station (HESTION, Australia). Histological sections 4–5  $\mu\text{m}$  thick were prepared with a rotary microtome AMR-400 (Amos Scientific Pty, Australia) and stained with hematoxylin-eosin and by the Azan Trichrome method.

For semithin sections (1–2  $\mu\text{m}$ ), the tissue was fixed in 2.5 % glutaraldehyde (pH 7.3–7.4) followed by postfixation in 1 % osmium tetroxide. After dehydration through graded alcohols, the material was embedded in a mixture of epoxy resins with subsequent polymerization. Sections were cut on an ultramicrotome Ultratome LKB 4801 A (Bromma, Sweden) and stained with methylene blue.

Microscopic examination of the adrenal gland was performed using an OLIMPUS BX 41 light microscope at magnifications of  $\times 100$ ,  $\times 200$ , and  $\times 400$ . Digital imaging and morphometric studies were conducted with QuickPhoto Micro 2.3 software. The description of histological preparations followed generally accepted pathomorphological criteria.

**Results of the study and their discussion.** Morphological examination of the adrenal gland tissues of rats at the third hour after scorpion venom administration revealed further intensification of the pathological alterations observed during the first hour [10]. This period was characterized by additional deformation of the nuclei of endocrine cells in the zona glomerulosa, which became intensely basophilic and somewhat disoriented, indirectly indicating disruption of the normal architecture of the round cellular clusters typical of this zone (Fig. 1).

The cytoplasm of the cells, apart from white droplets of steroid nature, also exhibited pale eosinophilic areas without distinct borders or shape, often perinuclear, presumably corresponding to intracellular edema. At early stages this swelling is largely due to the dilation of endoplasmic reticulum cisternae, an organelle essential for cellular detoxification. This organelle occupies a perinuclear position in the cytosol. The cytoplasm overall lost homogeneity and displayed granularity, often indicating disturbance of protein metabolism, most likely associated with the protein-toxic components of the scorpion venom. In many cells, the nuclei were eccentrically or even peripherally displaced toward the plasmalemma as a consequence of swelling. In certain regions, cellular boundaries became indistinct, and the nuclei of adjacent cells lay closely together due to their peripheral positioning, forming aggregates.

In the zona fasciculata, the cells demonstrated signs of hypertrophy (see Fig. 1, Fig. 2). In this case, both physiological and pathological hypertrophy could be observed. On the one hand, as the terminal component of the hypothalamic-pituitary-adrenal axis, the adrenal cortex – particularly its inner zones – increases corticosterone secretion intensity in response to pituitary release of adrenocorticotropic hormone (ACTH) during acute stress such as venom exposure. The cells synthesize on smooth endoplasmic reticulum and accumulate lipid droplets in the cytosol as secretory inclusions. Therefore, due to proliferation of the smooth endoplasmic reticulum and deposition of inclusions awaiting secretion, the endocrine cells physiologically enlarge.

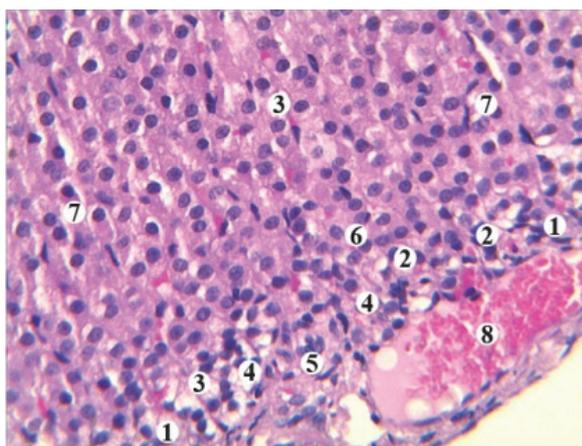


Fig. 1. Morphological structure of the zona glomerulosa and zona fasciculata of the rat adrenal cortex three hours after venom administration. Disorganization of endocrine cells into round clusters (1); cytoplasmic edema (2); cytoplasmic granularity (3); peripheral nuclear positioning (4); nuclear aggregation (5); indistinct cell boundaries (6); hypertrophy of zona fasciculata endocrine cells (7); congested vessel with erythrocyte aggregate in lumen (8). Hematoxylin-eosin staining.  $\times 200$ .

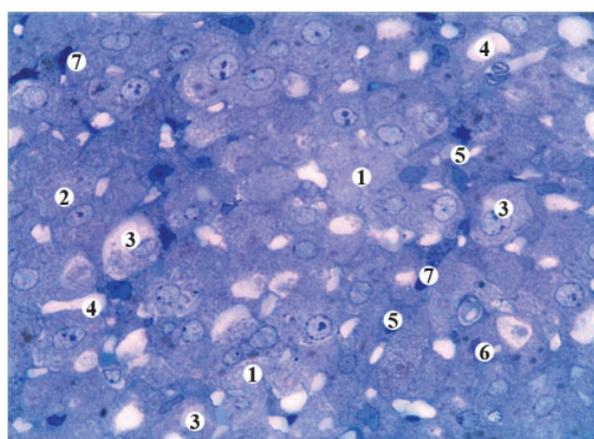


Fig. 2. Morphological structure of the zona fasciculata of the rat adrenal gland three hours after venom administration. Indistinct cell boundaries (1); cell hypertrophy (2); intracellular edema (3); intercellular edema (4); karyopyknosis (5); karyorrhexis (6); fibroblast nuclear deformation (7). Semithin section. Methylene blue staining.  $\times 400$ .

On the other hand, morphological analysis of adrenal sections from this group showed signs of edema – irregularly shaped pale areas in the cytoplasm, large elongated perinuclear vacuoles, and blurred

cellular borders (see Fig. 1, Fig. 2). These findings indicate pathological processes within the cells, with progression of intracellular swelling already noted at the first hour after venom administration [9], which further contributes to cellular enlargement.

Notably, morphological features of extracellular edema – previously unobserved – became evident, best seen in semithin sections (see Fig. 2). The zona fasciculata cells were separated by large round clear spaces, suggesting swelling within the extracellular matrix.

The nuclei of some endocrine cells in the zona fasciculata exhibited various stages of karyopyknosis and karyorrhexis (see Fig. 2). Some nuclei were dark, with indistinct nucleoli and condensed chromatin. Others were shrunken, triangular or stellate in shape, with surrounding cytoplasmic condensation – findings indicative of necrobiotic processes in endocrine cells caused by the cytotoxic effect of the venom.

Cells of the zona reticularis displayed signs of moderate hypertrophy. Their cytoplasm showed basophilia and uneven granularity, with areas of edema appearing lighter and poorly delineated (Fig. 3). The nuclei had somewhat indistinct contours but retained regular shapes with distinct nucleoli. Occasional endocrine cells exhibited perinuclear swelling. Cellular boundaries were blurred, and leukocytes were present between them. Such immunocytic infiltration in this cortical zone may be explained by the abundance of venous vessels in this region; their walls are highly permeable and represent typical sites of basophil and monocyte diapedesis into tissue.

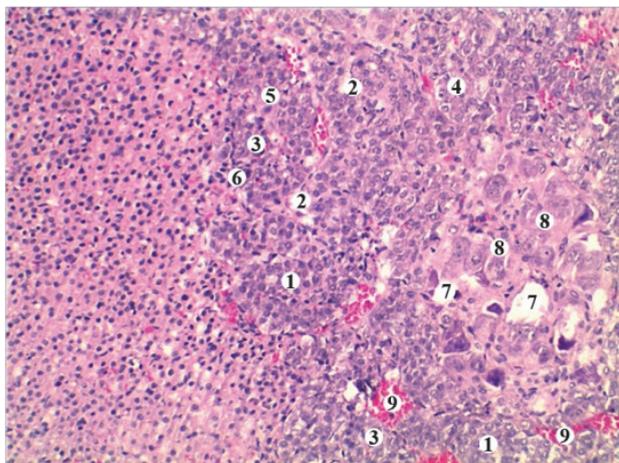


Fig. 3. Morphological structure of the zona reticularis and medulla of the rat adrenal gland three hours after venom administration. Cytoplasmic basophilia (1); cytoplasmic edema (2); cytoplasmic granularity (3); blurred nuclear contours (4); indistinct cell borders (5); immunocyte infiltration (6); edematous regions in medulla (7); indistinct borders of chromaffin cells (8); vascular congestion (9). Hematoxylin-eosin staining.  $\times 100$ .

While one hour after venom administration the medullary cells exhibited no pathological changes [10], by the third hour moderate degenerative alterations became apparent. Chromaffin cells showed indistinct borders, and edematous areas appeared within and between cells (see Fig. 3). Nuclei displayed indistinct outlines – some pale, typical of intracellular swelling; others dark and misshapen. Foci of connective-tissue infiltration by immune cells were also observed, likely originating from medullary veins via diapedesis. Both medullary and reticular zone veins exhibited congestion, irregular luminal shapes, and aggregates of red blood cells.

Examination of the stromal component of the adrenal glands began with the capsule. The dense connective tissue fibers were somewhat loosened, and fibroblast nuclei appeared blurred. Nevertheless, no striking differences were noted

compared with the earlier post-injection interval. Subcapsular vessels were dilated and contained large erythrocyte aggregates (see Fig. 1).

Regarding vascular conditions in other adrenal regions – previously described in context with corresponding zones – the interstitial morphology at this stage showed slight fibroblast nuclear deformation and chromatin condensation on semithin sections (see Fig. 2).

The data we get here partially shows that the adrenal glands are one of the leading targets for action in acute scorpion envenomation and present stressful both to three hours following causal morphological changes. This agrees with the most recent recognition of the involvement of endocrine system, and hypothalamic-pituitary-adrenal axis, in the pathogenesis of envenomation and that hormonal-metabolic dysregulation is a relevant player for the clinical manifestations associated to snake bite and scorpion sting [3].

Clinical experience, especially in young patients, provides robust evidence that envenomation by scorpions is not merely neurotoxic and cardiovascular phenomena but impose significant metabolic perturbations such as blood glucose fluctuations, dysregulation of the water–electrolyte balance and symptoms of acute stress [2].

The association of acute poisoning and subsequent occurrence of adrenal insufficiency is a matter that deserves special consideration. One of articles demonstrates a case of hyponatremia with adrenal insufficiency caused by the Mamushi bite, which suggests that exposure to just one type of snake venom can cause a series of endocrine tumors with severe electrolyte disorder [15].

Recent studies of hormone changes accompanying administration of scorpion venom demonstrate that altered endocrine function is not an accidental concomitant but a predictable feature of intoxication. Indeed, it has already been demonstrated that the venom of *Scorpio maurus palmatus* induces very significant variations in major hormones such as cortisol (predominance and very deep sensitization of response are highlights), clearly reflecting a systemic remodeling of stress-axis regulation [1].

It is worthy to say that animal models exposed to *Hemiscorpius lepturus* venom showed glucose, insulin, glucagon and cortisol alterations suggesting an exquisite imbalance in both arms of homeostasis (i.e., metabolism and glucocorticoid) [11]. Our morphological findings decreased lipid inclusions in cortical cells, evidence of increased synthetic load and early dystrophic events in mitochondria are also indicative that the reported hormonal shifts are directly deputed to modulation of cellular activity: the activated cortico-secreting apparatus fulfills its maximum adaptative capacity which is eventually reverberated by suprastructural alterations in adrenal tissue.

Similarities with snake venom also reinforce the interpretation of our observations. Viper venoms from the viperidae family can cause a marked ultrastructural alteration in the adrenal cortical mitochondria such as swelling, disruption of cristae, vacuolization with a loss from matrix [5]. 3 hours after the injection of *L. macroctenus* venom also corresponds to what we noticed, and this temporo-spectral combination suggests a rapid and direct or indirect (systemic hemodynamic and neurohumoral) toxic effect on the energy metabolism of the neurons of the cortex. These findings suggest that mitochondrial insufficiency represents an integral link between morphological damage and functional decompensation of hormonogenesis.

Morphometrical and histological research on the *Vipera berus berus* venom action also disclosed changes in microscopic structure of adrenal glands: disorganization of cortical zones, disturbance in blood flow and necrobiosis revealed by signs for the cells of cortex [12]. Comparison of our data with those would justify the use of a certain “common morphological phenotype” of adrenal involvement in two types of venom exposure – snake and scorpion. In the present model, we observed acute dystrophic and vascular changes without severe necrosis as it has been interpreted to be early stage of injury that was reversible but could lead to irreversible morphological defect if toxic influence continued.

Curious parallels are also observed in some studies of the effect of components isolated from the venom (especially crotonamine-like fractions) on adrenal glands. It is demonstrated that these toxins can cause significant ultrastructural changes in the adrenal cortex of mice, such as mitochondrial swelling, chromatin condensation and formation of apoptotic bodies [14]. Characteristics of the changes after *L. macroctenus* on adrenocorticocytes in rats, such as hyperchromatic nuclei in some cells and signs of karyopyknosis and karyorrhexis in others, fit into a similar pathomorphologic scheme. This leads us to speculate that certain fractions of scorpion venom could have a selective cytotoxic action on the cortical cells like the action of specific peptides in snake venom.

Therefore, the alterations that we reported – ranging from vascular engorgement, edema and dystrophy of cortical cells to mitochondrial disarray and signs of apoptosis – may be considered as the morphological counterpart to the systemic hormonal and metabolic derangements in scorpion- and snake-venomation.

There are no publications (except for the author) in the world scientific literature on morphological changes in the adrenal glands of laboratory animals after administration of the venom of the scorpion *Leiurus macroctenus*. There are only publications on morphological changes in other organs of male rats after administration of the venom of the scorpion *Leiurus macroctenus*, performed within the framework of the combined experiment given in the Materials and Methods.

## Conclusion

The venom of *Leiurus macroctenus* scorpions induces pronounced morphological alterations in the adrenal glands of laboratory rats as early as three hours after administration. The observed features combine destructive and adaptive-compensatory responses, indicating an acute functional rearrangement of the gland under conditions of toxic stress. The predominance of intra- and extracellular edema, disorganization of cellular structures, hypertrophy of endocrine cells, and the emergence of necrobiotic processes reflect intensive activation of the hypothalamic-pituitary-adrenal axis and depletion of steroidogenic reserves. Changes in the adrenal medulla and vascular network indicate the systemic nature of intoxication and the involvement of the sympathoadrenal system in the pathogenesis. The obtained results confirm the role of the adrenal glands as a key target organ in scorpion envenomation and provide a foundation for further investigations into the ultrastructural mechanisms of injury and the development of pathogenetically based corrective therapies.

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