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HISTOLOGICAL CHANGES IN THE KIDNEYS OF EXPERIMENTAL RATS 3 HOURS AFTER EXPOSURE TO THE VENOM OF THE SCORPION LEIURUS MACROCTENUS

Matkivska R.M. ✉ **Histological changes in the kidneys of experimental rats 3 hours after exposure to the venom of the scorpion Leiurus macroctenus.**
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ABSTRACT. Background. Throughout life, the human body interacts closely with environmental factors that can have either a positive or negative impact. The dynamic equilibrium between the organism and its environment results from the influence of natural, anthropogenic, and social factors. The action of any exogenous factors leads to the development of adaptive changes. **Objective.** To study the histological changes in the kidneys of rats three hours after exposure to the venom of the scorpion Leiurus macroctenus. **Methods.** The study involved 10 white male laboratory rats weighing 200 g (±10 g), reared in the vivarium of the Educational and Scientific Centre "Institute of Biology and Medicine" at Taras Shevchenko National University of Kyiv. The venom of scorpions in the Buthidae family, genus Leiurus, species Leiurus macroctenus was administered to the rats once intramuscularly (0.5 ml of venom solution previously dissolved in saline; 28.8 μg/ml; LD_{50}=0.08 mg/kg). For microscopic examination, kidney samples were collected from animals across all groups. Histological preparations of the kidneys were stained with hematoxylin and eosin. **Results and conclusion.** The administration of the venom from the scorpion Leiurus macroctenus was associated with pronounced dystrophic changes in the structural components of the kidneys three hours after the commencement of the experimental study. The appearance of inflammatory processes within the organ interstitium, with infiltration by plasmacytes, lymphocytes, and macrophages, was observed. Vascular-hemodynamic disorders were also identified, manifesting as desquamation of the endothelial lining of the vascular wall and increased permeability of the vascular wall. The renal tubular apparatus exhibited features of tubular necrosis.
Key words: venom, scorpions, kidneys, endothelium, tubular necrosis, rats.

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Introduction

Throughout life, the human body interacts closely with environmental factors that can have both positive and negative effects. The dynamic balance between the organism and its environment is influenced by natural, anthropogenic, and social factors. The actions of these external influences dictate the development of adaptive changes. Nearly all organs and systems contribute to the formation of these adaptive mechanisms, and their coordinated activities ensure the maintenance of a relatively constant internal environment known as homeostasis. Research indicates that homeostasis is upheld by the normal functioning of the immune defence system, alongside the nervous and endocrine systems. Together, these systems constitute the neurohumoral regulation of the body's functions. It is essential to consider the maintenance of internal environmental constancy not only at the tissue, organ, or systemic levels but also at the molecular and cellular levels, where the primary response to foreign agents begins. According to the literature, achieving dynamic equilibrium in the organism's activity is possible only with short-term impacts from damaging factors and depends on the strength of the irritating stimulus. In this context, the organism can exhibit properties of self-regulation, reactivity, and stability. However, any damage to the structural and functional parameters of the systems responsible for maintaining homeostasis can disrupt coordinated activity and lead to the emergence of pathological reactions.

A careful analysis of the scientific literature demonstrates that the action of toxins from various sources, including components of animal venom, can lead to significant disruptions in normal functioning and structural rearrangements of organs. This underscores the growing importance of the issue, as the

considerable variety of predatory animals with insufficiently studied proteomes, peptidomes, and the biological activity of their venoms drives an increasing number of experimental studies in this area [7, 8, 9].

The study aimed to investigate the histological changes in the kidneys of rats three hours after exposure to the venom of the scorpion Leiurus macroctenus.

Materials and methods

The venom of scorpions from the Buthidae family, genus Leiurus, and species Leiurus macroctenus, was administered to rats via intramuscular injection (0.5 ml of venom solution in saline; 28.8 μg/ml; LD_{50}=0.08 mg/kg) [10, 11].

The study involved 10 white male laboratory rats weighing 200 g (±10 g), reared in the vivarium of the Educational and Scientific Centre, "Institute of Biology and Medicine" at Taras Shevchenko National University of Kyiv (in accordance with the agreement on scientific and practical cooperation between Taras Shevchenko National University of Kyiv, Vinnytsia National Medical University named after M. I. Pirogov, and Ternopil National Medical University named after I. Ya. Horbachevsky of the Ministry of Health of Ukraine, dated 1st February 2021). The rats were maintained on a standard diet in an accredited vivarium, adhering to the "Standard Rules for the Arrangement, Equipment and Maintenance of Experimental Biological Clinics (Vivaria)". The experiments complied with current regulatory documents governing the organisation of work with experimental animals, ensuring alignment with the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes". Furthermore, all work with the animals was conducted in accordance with the Law of Ukraine dated 21st February 2006, No. 3447-IV, "On the Protection of Animals from Cruelty and Ethical Norms and Rules for Working with Laboratory Animals". The rats selected for the experiment were divided into two groups: the control group, consisting of 5 rats that were not administered any poison and from which samples were collected one hour after saline administration; and the experimental group, consisting of 5 rats, from which histological material was collected 3 hours after the administration of poison. The rats were euthanised via carbon dioxide inhalation, and the kidneys were isolated at 4 °C immediately after euthanasia.

Kidney samples from animals of all groups were collected for microscopic examination. The pieces were fixed in a 10% formalin solution for 1 day. They were then dehydrated in alcohols of increasing concentration and embedded in paraffin blocks. Histological preparations of rat kidneys were stained with hematoxylin and eosin. Histological preparations were examined using an SEO SCAN light microscope and photographed using a Vision CCD Camera with a system for displaying images from histological preparations.

Results and discussion

Microscopic examination of the kidneys of white rats three hours after the introduction of scorpion venom into the experimental animals revealed a significant degree of dystrophic changes in the organ's structural components. Local inflammatory infiltrates were detected in the organ against a background of substantial vascular changes, coagulopathy, acute renal failure, and tubular necrosis.

Due to the oedema, the fibrous capsule is thickened. The organ displays dilation and engorgement of the venous vessels, alongside spasms of the arteries. The vessel wall is thickened as a result of the hypertrophy of the media, accompanied by perivascular oedema and lymphohistiocytic infiltration. In their lumens, thrombi consisting of erythrocytes and single leukocytes are present, with the boundaries between them not clearly visualised.

In the interstitium of the organ surrounding the vessels of the myocirculatory bed, notable perivascular oedema and infiltration by lymphocytes, plasma cells, macrophages, tissue basophils, and eosinophils are present. Within the lumens of the vessels, stasis, thrombi, and erythrocytes without clear boundaries can be observed. The capillary wall features an endothelium with disrupted intercellular contacts, while its basement membrane is significantly thinned and discontinuous. Focal areas of endothelial desquamation and oedema are observed in the cytoplasm of the endothelium. The nuclei appear hyperchromic, compacted, and protrude significantly into the lumen. Increased vascular-tissue permeability in the interstitium results in pronounced hydration of the ground substance of loose fibrous connective tissue. In areas of marked oedema, collagen fibres exhibit bright oxyphilia, retain their fascicular nature, and form cells, although they are defibrotic. Disruption of blood coagulation processes and the integrity of the walls of the vessels in the microcirculatory bed of the organ interstitium leads to the emergence of small, multiple haemorrhages with pronounced haemolysis of erythrocytes and significant lymphohistiocytic infiltration along their periphery.

In the cortical substance, alongside stromal-vascular disorders, destructive-degenerative changes in the globular and tubular apparatus of nephrons are also intensified. The renal corpuscles of the nephrons vary in size, predominantly appearing hypertrophied and deformed. Afferent and efferent arterioles remain spasmodic, with their walls thickened due to pronounced mucoid swelling, and their lumens exhibit stasis accompanied by marked haemolysis of erythrocytes—the walls of the arterioles show significant infiltration of lymphocytes, macrophages-histiocytes, and eosinophils. Smooth myocytes in the media are shortened, exhibiting hyperchromic nuclei and brightly eosinophilic cytoplasm. Brightly eosinophilic collagen fibres in the subendothelial layer of the intima and adventitia swell but retain their bundled nature and are depopulated. Notable dehydration occurs in the ground substance, wherein the count of weakly basophilic glycosaminoglycans increases.

The endotheliocytes of the intima are swollen and exhibit pyknotic, hyperchromic nuclei. The basement membrane is discontinuous, thinned, or presents focal areas, with complete desquamation of the endothelium—glomeruli exhibit marked anaemia. In the walls of glomerular capillaries, destructive-degenerative disorders akin to those in the afferent arterioles manifest in endothelial cells. Expansion of the urinary spaces remains intact. The cells of the outer leaf of the capsule are flattened; their cytoplasm is oxyphilic while their nuclei are compact, basophilic, and the basement membrane is thickened. The mesangium expands due to an increase in the matrix and active proliferation of mesangiocytes, resulting in considerable oedema and destruction of the cytopodia and cytotrabeculae of podocytes. The urinary space is not visualised in such renal corpuscles (Fig. 1).

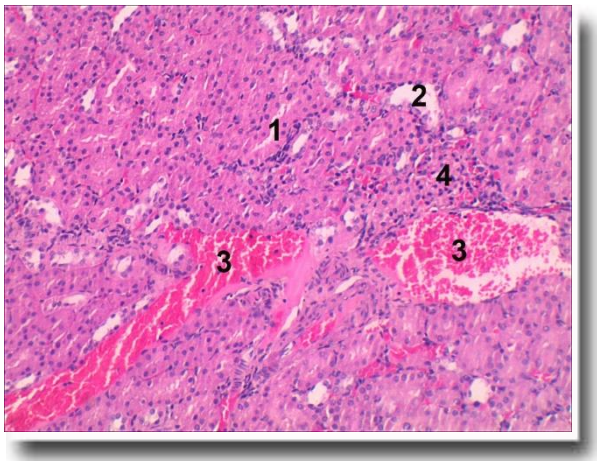

Fig. 1. Microscopic changes in the kidney of white rats 3 hours after the introduction of scorpion venom. 1 - proximal tubules with dystrophically altered epithelial cells, 2 - destruction of the distal tubule, 3 - areas of hemorrhage, 4 - deformed renal corpuscle with absent urinary space. Staining with hematoxylin-eosin. ×200.

Proximal and distal renal tubules exhibited dilated lumens, with focal tubules showing acute necrotic damage to epithelial cells and complete desquamation of the epithelium. The epithelium appears rounded, entirely losing its brush and striated borders, and the cytoplasm is vividly oxyphilic. The nuclei are pyknotic, compact, and intensely basophilic. Some individual cells display nuclei with an indistinct karyolemma, indicating nuclear lysis and the onset of acute necrosis. In the lumens of these tubules, there is an accumulation of cellular detritus. Simultaneously, many tubules show signs of hydropic and hyaline-droplet dystrophies of the epithelium, along with a complete loss of brush and basal striation, accompanied by desquamation into the lumens of the tubules. Within these lumens, accumulations of protein masses and cellular detritus were detected (Fig. 2).

Epithelial cell hyperplasia, accompanied by signs of dystrophy, is observed in the thin tubules. The collecting tubules display dilated lumens filled with dense oxyphilic masses. Epithelial cells lack microfornix and basal striation. The nuclei appear compacted and pyknotic, exhibiting uneven karyolemma. Numerous vacuoles are evident in the oxyphilic cytoplasm, indicating hydropic dystrophy. Localised haemorrhages and large inflammatory foci of histoleukocyte infiltrates are noted in the cortical substance (Fig. 3).

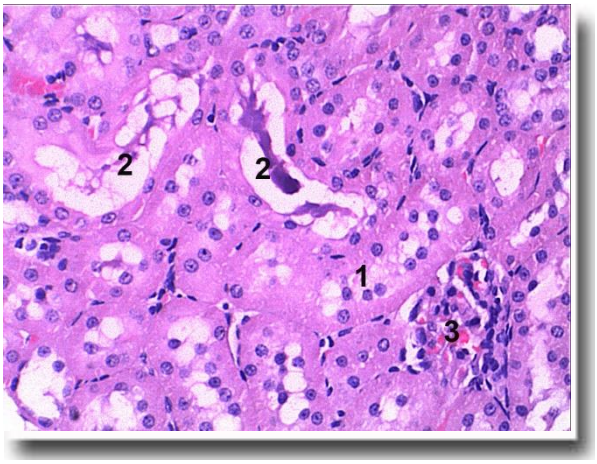

Fig. 2. Microscopic changes in white rats 3 hours after introducing scorpion venom. 1 - proximal tubules, 2 - cellular detritus in the lumen of the tubules, 3 - deformed renal corpuscle. Staining with hematoxylin and eosin. ×400.

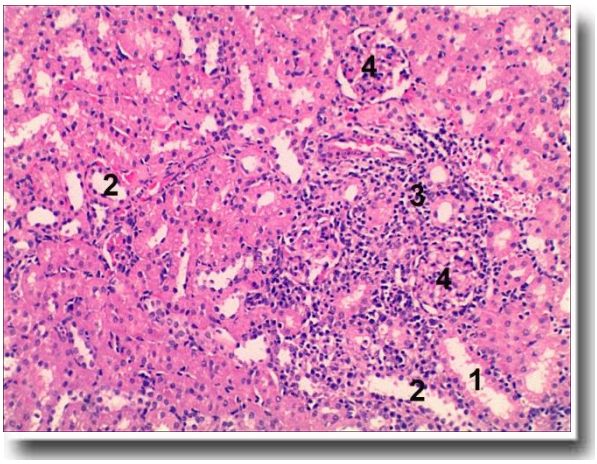

Fig. 3. Microscopic changes in the kidney of white rats 3 hours after the introduction of scorpion venom. 1 - proximal tubules with dystrophically altered epithelial cells, 2 - dystrophically altered distal tubule, 3 - voluminous histoleukocyte infiltrate, 4 - deformed renal corpuscles. Staining with hematoxylin-eosin. ×200.

Conclusion

The introduction of scorpion venom from Leiurus macroctenus to rats resulted in significant dystrophic changes in the structural components of the kidneys three hours after the experimental study commenced. Inflammatory processes in the organ's interstitium, characterised by plasmacytic, lymphocytic, and macrophage infiltration, were observed. Vascular and haemodynamic disorders were also identified, presenting as desquamation of the endothelial lining of the vascular wall and increased permeability. The occurrence of tubular necrosis characterised the renal

tubular apparatus.

Prospects for further development are related to studying histological changes in rat kidneys under the influence of Leiurus macroctenus scorpion venom at later stages of the experiment.

Information on conflict of interest

There are no potential or apparent conflicts of interest related to this manuscript at the time of publication and are not anticipated.

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Матківська Р.М. Гістологічні зміни в нирках експериментальних щурів через 3 години після впливу отрути скорпіонів Leiurus macroctenus.

РЕФЕРАТ. Актуальність. Протягом життя організм людини перебуває в тісному взаємозв'язу з факторами оточуючого середовища, які здійснюють на нього позитивний чи негативний вплив. Динамічна рівновага між організмом і середовищем його існування є результатом впливу природних, антропогенних та соціальних факторів. Дія будь-яких чинників екзогенного походження зумовлює розвиток пристосувальних та адаптаційних змін. **Мета.** Вивчення гістологічних змін нирок щурів через 3 години після впливу отрути скорпіонів *Leiurus macroctenus*. **Методи.** У дослідженні використано 10 білих лабораторних щурів-самців масою 200 г (±10 г), вирощених у віварії Навчально-наукового центру "Інститут біології і медицини" Київського національного університету імені Тараса Шевченка. Отруту скорпіонів родини Buthidae роду Leiurus виду Leiurus macroctenus вводили щурам одноразово внутрішньом'язово (0.5 мл розчину отрути попередньо розчиненому у фізіологічному розчині; 28.8 мкг/мл; ЛД50=0.08 мг/кг). Для мікроскопічного дослідження забирали зразки нирок тварин всіх груп. Фарбування гістологічних препаратів серця здійснювали гематоксиліном та еозином. **Результати та підсумок.** Введення отрути скорпіона *Leiurus macroctenus* супроводжувалось розвитком виражених дистрофічних змін структурних компонентів нирок через 3 години від початку експериментального дослідження. Спостерігали появу запальних процесів інтерстицію органу з плазмоцитарною, лімфоцитарною та макрофагальною інфільтрацією. Також виявлено судинно-гемодинамічні розлади, що проявлялись у вигляді десквамації ендотеліального вистелення судинної стінки та підвищення проникності судинної стінки. Канальцевий апарат нирок характеризувався розвитком тубулярного некрозу.

Ключові слова: отрута, скорпіони, нирки, ендотелій, тубулярний некроз, щури.