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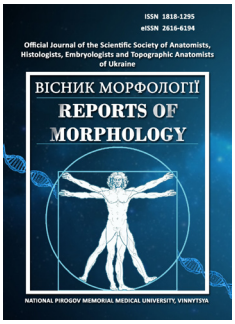

Histological changes in structural components of rat kidneys 1 day after exposure to Leiurus macroctenus scorpion venom

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Numerous cases of poisoning resulting from scorpion bites are reported annually in tropical and subtropical countries. Lethality from the effects of scorpion venom components is mainly associated with the ability of their neurotoxins to change the functional properties of ion channels. The kidneys are among the first to be affected by scorpion venoms, and among the main morphological manifestations of organ damage under these conditions are oedema, necrosis, haemorrhages, and lymphocytic infiltration. The study aims to examine the histological changes in rat kidney tissue 1 day after exposure to the venom of the scorpion Leiurus macroctenus. The study utilised 10 white male laboratory rats weighing 200 g (±10 g), bred in the vivarium of the Educational and Scientific Centre "Institute of Biology and Medicine" at the Taras Shevchenko National University of Kyiv. The venom of scorpions of the Buthidae family, genus Leiurus, species Leiurus macroctenus, was administered to rats once intramuscularly (0.5 ml of venom solution previously dissolved in saline; 28.8 μg/ml; LD50=0.08 mg/kg). Kidney samples of animals of all groups were taken for microscopic examination. Histological preparations of the kidneys were stained with hematoxylin and eosin. The toxic effect of scorpion venom on the first day of the experiment caused profound dystrophic-degenerative changes in the parenchymal components of the kidney, against the background of remodelling of the vascular bed of the organ, which is accompanied by the formation of stasis, thrombi, and volumetric haemorrhages. Significant alternative changes in the renal corpuscles are manifested by their deformation, atrophy, hyperemia and multiple haemorrhages. In the tubular system, pronounced hydropic, protein, and droplet-protein dystrophy were detected, and in their lumens, voluminous accumulations of cellular detritus were observed. Thus, in acute poisoning of rats with Leiurus macroctenus venom, stasis, thrombus formation, and massive haemorrhages in the cortical and medullary parts of the kidneys are observed within a day, as well as dystrophic changes in the tubular part of the nephrons (distal, proximal tubules, and collecting tubules). The indirect effect of the venom led to the formation of voluminous, inflammatory loci of leukocyte infiltrates in the interstitium and directly in the tubules and renal corpuscles.

Keywords: *poison, scorpions, kidneys, destructive changes, thrombosis, rats.*

Introduction

Numerous cases of poisoning due to scorpion stings are recorded annually in tropical and subtropical countries [7, 13]. The lethality of scorpion venom components is primarily attributed to the ability of their neurotoxins to alter the functional properties of ion channels in excitable tissues. However, the release of a significant amount of neurotransmitters leads to the appearance of various symptoms, such as pain, sweating, fever, and arterial hypertension. In more severe cases, these symptoms are associated with heart failure, pulmonary oedema and circulatory shock [1, 28].

Several mediators involved in immunoinflammatory

processes have been identified as playing a key role in kidney tissue damage under the influence of scorpion venom toxins [5, 9, 12]. The mechanisms of the inflammatory response are multifactorial and involve the generation of several bioactive molecules, such as cytokines, histamine, kinins, and lipid mediators derived from arachidonic acid. Prostaglandins are among the bioactive lipids that play crucial roles in various biological functions, including cell division and immune responses [2, 6, 29]. Cyclooxygenase (COX) is a key enzyme in the synthesis of prostaglandins from arachidonic acid, which is released by the action of phospholipase A_2 (PLA_2) on cell membrane phospholipids [27, 30]. COX converts arachidonic acid to PGH2, a common precursor of all prostanoids. Two isoforms of COX have been described: cyclooxygenase 1 (COX-1) and 2 (COX-2). COX-1 is constitutively expressed, whereas proinflammatory stimuli induce COX-2 and generate excessive prostaglandin production. The induction of COX-2 in immune cells, as well as in other cell types, such as fibroblasts and endothelial cells, is considered crucial for the production of prostaglandins during inflammatory processes. These mediators are involved in vasodilation and local recruitment, as well as the activation of inflammatory cells in the early stages of the immune response. According to the scientific literature, the kidneys are among the first to be affected by scorpion venoms, and among the main morphological manifestations of organ damage under these conditions are oedema, necrosis, haemorrhages and lymphocytic infiltration [3, 8, 31]. The body's defense mechanisms contribute to the increase in the renal parenchyma of pro-inflammatory cytokines, namely IL, TNF-α, NO, as well as platelet aggregation factor, prostaglandins, leukotrienes, kinins, angiotensin and endothelin.

To date, clinical cases of bites accompanied by rhabdomyolysis, hemolysis and disseminated intravascular coagulation are known. These processes contributed to the development of acute renal failure due to damage to the glomerular apparatus and were associated with haemoglobin and proteinuria. The action of PLA_2 venom can also cause acute kidney injury. The enzyme in these conditions exhibits hemolytic and cytotoxic effects on the organ. Additionally, it contributes to the increase in arachidonic acid, and accordingly, the synthesis of eicosanoids [4, 10, 11].

The aim of the study is to study histological changes in the kidneys of rats 1 day after exposure to the venom of the scorpion Leiurus macroctenus.

Materials and methods

The study utilised 10 white male laboratory rats weighing 200 g (±10 g), which were raised in the vivarium of the Educational and Scientific Centre "Institute of Biology and Medicine" at Taras Shevchenko National University of Kyiv. The rats were housed on a standard diet in an accredited vivarium, according to the "Standard Rules for the Arrangement, Equipment, and Maintenance of Experimental Biological Clinics (Vivaria)". The experiments were conducted by current regulatory documents governing the organisation of work with experimental animals and compliance with the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [16]. Also, all work with animals was carried out by the Law of Ukraine dated February 21, 2006 No. 3447-IV "On the Protection of Animals from Cruelty and Ethical Norms and Rules for Working with Laboratory Animals" (approved by the Bioethics Committee of the O. O. Bogomolets National Medical University, protocol No. 191 dated 27.01.2025).

The rats selected for the experiment were divided into two groups: control (5 rats), where no poison was administered, and material was collected one hour after the administration of saline; experimental (5 rats), where histological material was collected 1 day after the administration of poison. The venom of scorpions of the Buthidae family, genus Leiurus, species Leiurus macroctenus, was administered to rats once intramuscularly (0.5 ml of venom solution previously dissolved in saline; 28.8 μg/ml; LD_{50}=0.08 mg/kg) [18, 25]. Rats were euthanised by carbon dioxide inhalation. Rat kidneys were isolated at 4 °C immediately after euthanasia.

Kidney samples from all animal groups were taken for microscopic examination. The pieces were fixed in 10 % formalin solution for 1 day. Then the pieces were dehydrated in alcohols of increasing concentration and embedded in paraffin blocks. Histological preparations of rat kidneys were stained with hematoxylin and eosin [23]. Histological preparations were examined using an SEO SCAN light microscope and photographed using a Vision CCD Camera with an image output system.

Results

Histological examination of the kidney of white rats 1 day after the introduction of scorpion venom revealed the presence of pronounced destructive and degenerative changes in the structural components of the organ. The predominance of alteration processes in the stromal and parenchymal components of the kidney, remodelling of the blood vessel bed, impaired permeability of the vessel walls, and their altered morphology, against the background of the toxic effects of the venom components, are noted.

In the fibrous capsule, areas with defibering of collagen fibres in the composition of dense fibrous connective tissue and oedema of the amorphous component of the layers of loose connective tissue were observed. Congestive phenomena were detected in vessels of various calibres. In the lumens of the vessels, a significant number of aggregated erythrocytes that form thrombi are noted.

At this stage of the experiment, significant destructive changes were detected in the globular and tubular apparatus of the nephrons within the cortical substance of the kidney. Hypertrophied and single atrophied renal corpuscles were detected, which were deformed and had different volumes. Anaemia was observed in individual vascular glomeruli, but in most cases, congestive phenomena were determined. The wall of glomerular capillaries is

destructively changed, and oedema is detected in endothelial cells; their cytoplasm is weakly oxyphilic. Pictotic, altered-shaped nuclei are intensely basophilic, hyperchromic. The basement membrane is locally thinned, and areas with partial or complete desquamation of the endothelium into the lumen of the vessel are detected. The urinary spaces in most renal corpuscles were narrowed due to significant proliferation of mesangiocytes and oedema of the interstitial mesangial tissue and hyperemia of the hemocapillaries of the vascular glomerulus. Fusion of podocytes of the inner leaf with the squamous epithelium of the outer leaf is detected. The single-layered squamous epithelium of the outer leaf of the Bowman's capsule is thinned, its cells are flattened with oxyphilic cytoplasm and basophilic nuclei of various shapes. In the podocytes of the inner leaf of the capsule, significant alteration of cytotrabeculae and cytopodia was observed. Renal corpuscles with moderate or dilated urinary spaces were also detected. These changes in the structural components of the capsule cause significant damage to the filtration barrier, resulting in a violation of the first phase of urine formation, namely filtration. In the cortical substance, voluminous inflammatory infiltrates are detected, including lymphocytes, macrophages-histiocytes, neutrophils and eosinophils (Fig. 1).

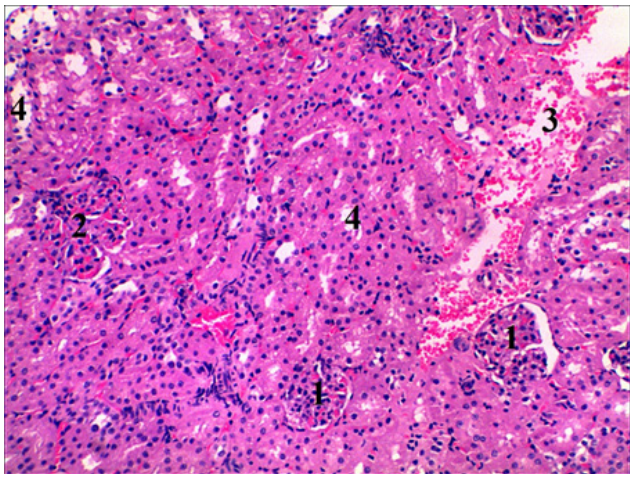

Fig. 1. Microscopic changes in the kidney of white rats 1 day after the administration of scorpion venom. Renal corpuscles with slit-like urinary lumens (1), hypertrophied renal corpuscle (2), haemorrhage (3), alteration of renal tubules (4). Staining with hematoxylin and eosin. ×100.

In the arteries, significant perivascular oedema of the adventitia was observed, characterised by increased hydration of the ground substance of loose fibrous connective tissue, local defiberization of oxyphilic collagen fibres, and in some areas, the formation of reticular-cellular structures. The basophilic nuclei of fibroblasts are deformed and pyknotic. Smooth myocytes in the media are disorganised, the internal elastic membrane is destructively changed and loses its tortuous course. Dexvamation of endothelial cells into the lumen is noted, their nuclei are pyknotic, intensely basophilic, weakly oxyphilic cytoplasm is swollen. Interstitial oedema is detected, as well as a violation of the vessel wall structure, which leads to the formation of haemorrhages with a predominance of erythrocytes (Fig. 2).

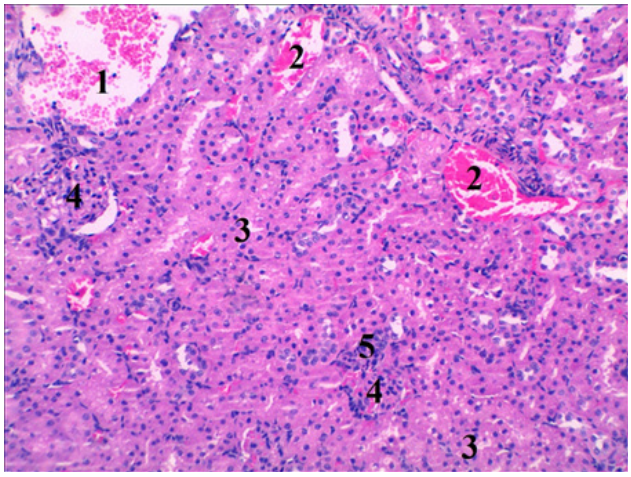

Fig. 2. Histological changes in the kidney of white rats 1 day after the administration of scorpion venom. Area of haemorrhage (1), blood-filled vessels (2), fissured lumens of proximal tubules (3), destructively altered renal corpuscles (4), histoleukocyte infiltration (5). Staining with hematoxylin and eosin. ×100.

The toxic effect of the poison led to significant alternative changes in the renal tubular apparatus. Locally, proximal and distal tubules with significantly thinned epithelium of their walls and desquamation of epithelial cells are detected. The shape of the epithelial cell changes, approaching a rounded one, with a loss of the brush border in its apical part and the absence of basal striation in the basal part of the cells. The cytoplasm of epithelial cells is intensely oxyphilic, with basophilic nuclei and signs of pyknosis. Weakly basophilic nuclei are detected, their karyolemma is unclearly contoured (Fig. 3). A significant manifestation of hydropic dystrophy of the epithelium characterises individual tubules. Cellular detritus is noted in the lumens of the tubules.

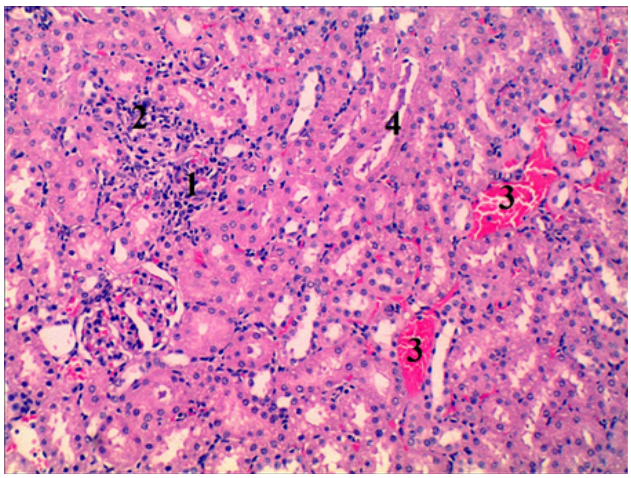

Fig. 3. Microscopic changes in the cortical substance of the kidney of white rats 1 day after the administration of scorpion venom. Deformed renal corpuscle (1), histoleukocyte infiltrate (2), blood-filled vessels (3), cellular detritus in the lumen of the distal tubules (4). Staining with hematoxylin and eosin. ×100.

Discussion

Scorpions of the genus Leiurus, known in the English-language scientific literature as “deathstalkers”, are among the most dangerous arthropods. Currently, 22 species of this genus have been identified. However, studies on the composition of the venom and the mechanisms of tissue damage have focused primarily on Leiurus quinquestriatus, Leiurus hebraeus, and Leiurus abdullahbayrami. According to recent data, Leiurus macroctenus boasts one of the highest LD_{50} values not only among scorpions but also among all arthropods worldwide. Although the species Leiurus macroctenus is considered particularly dangerous, the potential of its venom to cause damage to various organs remains unexplored [21]. Data from studies on lung damage after exposure to Leiurus macroctenus venom indicate that the venom affects both the cytokine profile and the levels of their regulators in the lungs of rats, reflecting specific changes in the innate immune response caused by the studied venom components [20]. The venom exposure results in a significant decrease in overall proteolytic activity, indicating the importance of metalloproteinases in this process. At the same time, the increase in the levels of metalloproteinases and tissue inhibitor of metalloproteinases-1 in all organs indicates that Leiurus macroctenus poisoning leads to systemic effects, potentially causing multiorgan dysfunction, mainly due to uncontrolled metalloproteinase activity [19]. However, the mechanisms underlying these changes require further investigation.

Hemiscorpius lepturus and Androctonus australis exhibit pronounced nephrotoxic effects. Toxic compounds in their venom can directly or indirectly affect renal tissue, causing mesangiolysis, the development of glomerulonephritis, vasculitis, interstitial nephritis, cortical and tubular necrosis, as well as hypoxia and renal infarction [15, 26]. Of the above pathologies, tubular necrosis is most often observed in victims, which is characterised by a decrease in reabsorption and an increase in the processes of secretion of Na+, K+, and Cl-. It has been demonstrated that inoculation of the poison affects the Na+ channels in the kidneys. At the same time, there is an increase in the blood levels of catecholamines in the victims. In the cytosol of nephron tubule cells under conditions of poisoning with scorpion toxins, the concentration of Ca^{2+} ions increases. There is also evidence of increased activity of the hypothalamic-pituitary-adrenal system, which stimulates the production of corticosteroids. At the same time, an increase in the levels of acute phase proteins and significant hemodynamic disorders are recorded. It is known that the accumulation of a substantial amount of hemoproteins accompanies hemolysis phenomena. Under these conditions, the presence of heme groups increases the production of free radicals, which accumulate in the renal cortex, having a toxic effect. The production of ROS is facilitated by increased NADPH oxidase activity. In this case, apoptosis or necrosis of kidney cells is a frequent consequence [14].

There is evidence of the involvement of macrophages in the development of acute kidney injury in scorpion bites. Thus, the level of MCP increases in the victim’s body, indicating the initiation of an inflammatory process in the organ and signaling the risk of complications, as it directly affects the migration of macrophages, proliferation, and differentiation of leukocytes in the epithelium of the human kidney tubules. MCP-1 stimulates the secretion of IL-6 and the intercellular expression of ICAM-1. In addition, by binding to chemokine receptors CC2 on the surface of podocytes, it can reduce the expression of both microRNA and nephrin protein. The latter is involved in the formation of filtration gaps, so defects in this protein lead to the development of renal failure, disruption of the podocyte cytoskeleton, and disorders of the filtration process [17].

Histological studies of the kidneys exposed to the venom of the scorpion Hemiscorpius lepturus revealed an expansion of the lumen and thinning of the walls of the proximal tubules of the nephron, and the accumulation of fibrin in the glomerular apparatus. The glomerular capillaries contained clusters of erythrocytes, and the endothelium of their walls was swollen 4 hours after the introduction of the venom to laboratory animals. In the proximal tubules, flattening of the epithelial cells on their inner membrane, loss of their brush border, and an atypical reaction of the nuclei of these cells were noted, indicating the development of acute tubular necrosis [24].

J. D. Galíndez-Cerón et al. [17] found that when rats were injected with the venom of the scorpion Centruroides margaritatus, the perfusion pressure and renal vascular resistance in the kidneys increased. However, glomerular filtration and perfusion rates, as well as the transport of sodium, potassium, and chloride in the tubular apparatus, showed a tendency to slow down. Centruroides margaritatus venom did not exhibit toxic effects on the nephron tubular epithelial cell line; however, it reduced the viability of proximal renal tubular cells at concentrations ranging from 6.25 to 200 μg/mL. Histological studies revealed hydropic degeneration, oedema, and protein accumulation in the renal tubules. Flow cytometry showed that nephron cell death primarily occurred through necrosis.

Thus, the data we obtained coincide with the results of other studies on the effects of scorpion venoms on the mammalian body, but significantly deepen and expand the understanding of the development of changes in the cytohistoarchitectonics of the kidneys during these processes, and also fill the gaps in information regarding the specific effects of the venom of the scorpion species Leiurus macroctenus on this organ.

Conclusions

1. The toxic effect of scorpion venom on the 1st day of the experiment caused profound dystrophic-degenerative changes in the parenchymal components of the kidney against the background of remodelling of the vascular bed of the organ, which is accompanied by the formation of stasis, thrombi and volumetric haemorrhages.

2. Significant alternative changes in the renal corpuscles are manifested by their deformation, atrophy, hyperemia and multiple haemorrhages. In the tubular system, pronounced hydropic protein and droplet-protein dystrophy were detected, and in their lumens, volumetric accumulations of cellular detritus were observed.

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ГІСТОЛОГІЧНІ ЗМІНИ СТРУКТУРНИХ КОМПОНЕНТІВ НИРОК ЩУРІВ ЧЕРЕЗ 1 ДОБУ ПІСЛЯ ВПЛИВУ ОТРУТИ СКОРПІОНІВ LEIURUS MACROCTENUS

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Щорічно в тропічних і субтропічних країнах реєструють чисельні випадки отруєння внаслідок укусів скорпіонів. Летальність від впливу компонентів отрути скорпіонів, головним чином, пов'язана зі здатністю їхніх нейротоксинів змінювати функціональні властивості іонних каналів. Нирки одними з перших піддаються ураженню отрутами скорпіонів, причому серед основних морфологічних проявів пошкодження органу за даних умов є набряк, некроз, крововиливи та лімфоцитарна інфільтрація. Мета дослідження – вивчення гістологічних змін тканини нирок щурів через 1 добу після впливу отрути скорпіонів Leiurus macroctenus. 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 вилучали зразки нирок тварин всіх груп. Забарвлення гістологічних препаратів нирок здійснювали гематоксиліном та еозином. Токсичний вплив отрути скорпіона на 1 добу експерименту спричинив глибокі дистрофічно-дегенеративні зміни паренхіматозних компонентів нирки на тлі ремоделювання судинного русла органу, що супроводжується формуванням стазів, тромбів та об'ємних геморагій. Значні альтеративні зміни ниркових тілець проявляються їх деформацією, атрофією, гіперемією та множинними геморагіями. В канальцевій системі встановлено виражену гідропічну, білкову та крапельно-білкову дистрофії, а в їх просвітах – об'ємні скупчення клітинного детриту. Таким чином, при гострому отруєнні щурів отрутою Leiurus macroctenus через добу спостерігаються стази, тромбоутворення та масовані крововиливи в кірковій та мозковій речовині нирок, а також дистрофічні зміни тубулярної частини нефронів (дистальних, проксимальних канальців та збірних трубочок). Опосередкована дія отрути призвела до утворення об'ємних, запальних локусів лейкоцитарних інфільтратів інтерстицію та безпосередньо канальців і ниркових тілець.

Ключові слова: *отрута, скорпіони, нирки, деструктивні зміни, тромбоз, щури.*

Author's contribution

Matkivska R. M. – conceptualisation, research, writing of the original draft.
Kucherenko O. S. – project administration, resources.
Karliychuk M. A. – methodology, formal analysis.
Halahan Y. V. – validation.
Samborska I. A. – software.