ORIGINAL ARTICLE

CONTENTS

Comparative characteristics of structural changes in the kidneys of experimental rats 1 and 3 hours after exposure to the *Leiurus macroctenus* scorpion venom

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ABSTRACT

Aim: To compare structural changes in the kidneys of experimental rats 1 and 3 hours after exposure to the Leiurus macroctenus scorpion venom.

Materials and Methods: The venom of scorpions from the Buthidae family, genus Leiurus, species *Leiurus macroctenus*, was administered to rats (0.5 ml of venom solution; 28.8 μg/ml; LD_{50}=0.08 mg/kg). The study utilised 20 white male laboratory rats weighing 200 g (±10 g). The selected rats were divided into two groups: a control group – 10 rats, to which no venom was administered, and biological material was collected 1 and 3 hours after the administration of saline solution experimental group of 10 rats, from which histological material was collected 1 and 3 hours after the administration of the scorpion venom. For microscopic examination, kidney samples were taken from all groups. The specimens were fixed in a 10% formalin solution for 1 day. Histological samples of rat kidneys were stained with haematoxylin and eosin. Histological slides were examined using an SEO SCAN light microscope.

Results: One hour after the administration of *Leiurus macroctenus* scorpion venom to rats, significant hemodynamic disorders and the onset of inflammatory processes in the organ were noted, along with acute renal failure due to severe damage to the glomerular apparatus. Disruptions in the cytoskeleton of podocytes and the tubular apparatus of nephrons, along with manifestations of desquamation, hydropic, and hyaline-droplet dystrophies of the tubular epithelium, were established. The introduction of *Leiurus macroctenus* scorpion venom to rats was accompanied by markedly pronounced dystrophic changes in the structural components of the kidneys three hours after the beginning of the experimental study, in contrast to the earlier time point of the study. The emergence of inflammatory processes in the organ interstitium, characterised by plasmacytic, lymphocytic, and macrophage infiltration, was observed. Vascular and hemodynamic disorders were also detected, manifesting as desquamation of the endothelial lining of the vascular wall and increased permeability. The development of tubular necrosis characterised the renal tubular apparatus.

Conclusions: More pronounced histological changes in the structural organisation of rat kidneys were found 3 hours after exposure to *Leiurus macroctenus* scorpion venom than after 1 hour of study.

KEYWORDS: scorpions, kidneys, nephron, endothelium, rats

Wiad Lek. 2026;79(7):1563-1571. doi: 10.36740/WLek/222245 DOI

INTRODUCTION

According to the WHO, approximately 1.5 million cases of poisoning due to scorpion bites are reported annually worldwide, resulting in 2,000 to 3,000 deaths. Scorpionism is prevalent in the United States, Canada, Europe, and Australia. However, this issue is most widespread in Africa, India, Mexico, Brazil, Iran, Saudi Arabia, and Venezuela, where over 2 billion people face a constant threat. Climate change and the rapid urban development near the typical habitats of scorpions significantly heighten the likelihood of encounters with them. Specifically, in Brazil alone, the number of scorpion bite cases has increased from 64,000 to 124,000 per year since 2012 [1-8].

To date, data exists on approximately 2,000 species of scorpions. Most of these are dangerous to humans, particularly those from the families Buthidae, Scorpionidae, and Hemiscorpionidae. The clinical manifestations of poisoning from scorpion toxins vary significantly, depending on the species and the amount of venom that has entered the victim's body [9-12]. Researchers note that the composition of their venom is highly complex and heterogeneous. The most well-studied components are currently small

peptides, mainly due to their diversity and extensive pharmacological properties. Small peptides are classified into three major superfamilies based on their structure: peptides containing cysteine-stabilised (CS) α/β motifs with disulfide bridges, calcins, and peptides that lack disulfide bridges. However, it has been established that among the toxic components of scorpion venom, enzymes, mixtures of inorganic salts, free amino acids, nucleotides, and lipids are also recognised. [13-19].

According to scientific literature, the toxic components of scorpion venom adversely affect the nervous, cardiovascular, respiratory, and blood coagulation systems. In recent years, their role in the development of lesions in the excretory system has been demonstrated.

A thorough analysis of the scientific literature has shown that to date, studies on the effects of scorpion venom on structural and functional parameters of mammalian kidneys are extremely limited. In addition, the composition of the Leiurus macroctenus scorpion venom is not yet sufficiently known, and therefore the features of its influence on morphological changes in internal organs, including kidneys, have not been established. It is worth noting that the study of microscopic changes in the structure of the kidneys under the influence of scorpion venom at different time intervals is important for understanding the main pathogenetic links in the development of complications of scorpion bites. Thus, our study is timely and relevant.

AIM

The study aims to compare the structural changes in the kidneys of experimental rats one and three hours after exposure to Leiurus macroctenus scorpion venom.

MATERIALS AND METHODS

The venom from scorpions of the Buthidae family, genus Leiurus, species Leiurus macroctenus, was administered intramuscularly to rats (0.5 ml of venom solution previously dissolved in saline; 28.8 μg/ml; LD_{50}=0.08 mg/kg) [20].

The study utilised 20 white male laboratory rats, each 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 (Agreement on Scientific and Practical Cooperation between Taras Shevchenko National University of Kyiv, National Pirogov Memorial Medical University, Vinnytsia, and I. Horbachevsky Ternopil National Medical University, dated 1 February 2021). The rats were provided with a standard diet in an accredited vivarium, in accordance with the «Standard Rules for the Arrangement, Equipment and Maintenance of Experimental Biological Clinics (Vivaria)." The experiments were conducted in accordance with current regulatory documents governing the organisation of work involving experimental animals and adhering to the principles of the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» [21]. Furthermore, all interventions involving the animals complied with the Law of Ukraine No. 3447-IV of 21 February 2006, «On the Protection of Animals from Cruelty and Ethical Norms and Rules for Working with Laboratory Animals.»

The rats chosen for the experiment were divided into two groups: a control group consisting of 10 rats, which received no venom, with biological material collected 1 and 3 hours after saline solution administration; and an experimental group of 10 rats, for which histological material was collected 1 and 3 hours after administration of scorpion venom. The rats were euthanised using carbon dioxide inhalation. Their kidneys were isolated at 4 °C immediately following euthanasia.

For microscopic examination, kidney samples were collected from all animal groups. The samples were fixed in a 10% formalin solution for one day. Subsequently, the samples were dehydrated in increasingly concentrated alcohols and embedded in paraffin blocks. Histological samples of rat kidneys were stained with hematoxylin and eosin [22]. Histological slides were examined using an SEO SCAN light microscope and photodocumented with a Vision CCD Camera, equipped with an image output system for histological preparations.

AI STATEMENT

The author states that no AI tools were used in writing this article.

RESULTS

Studies of histological samples from the kidney, taken one hour after the introduction of scorpion venom into the experimental animals, revealed the onset of acute renal failure due to significant damage to the glomerular apparatus of the nephrons, the emergence of considerable haemodynamic disturbances, and the onset of inflammatory processes within the organ.

The fibrous capsule is oedematous and easily detached from the organ. The venous vessels dilate, and the arterial vessels ischaemiate. In the

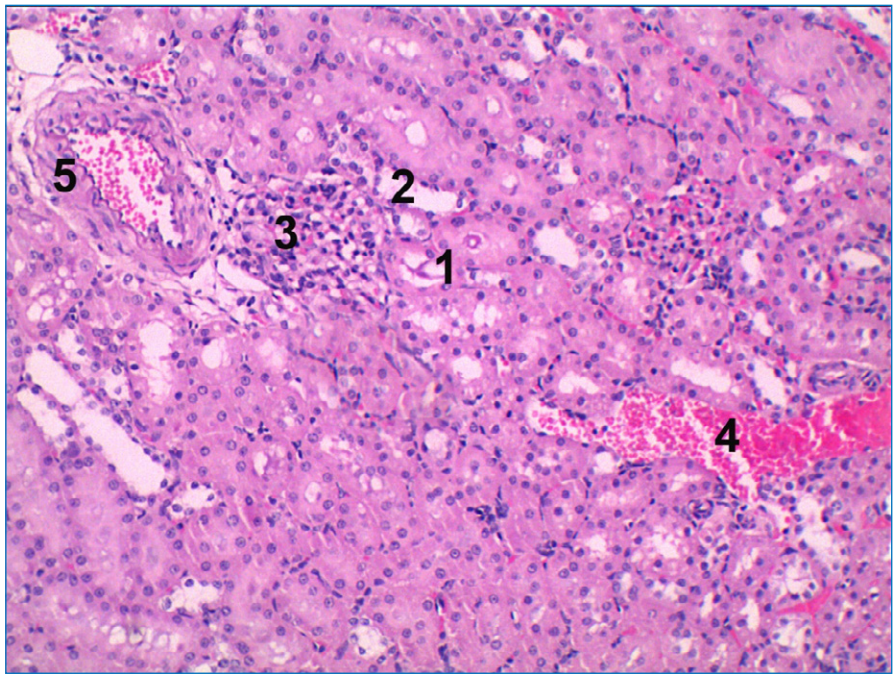

Fig. 1. Microscopic structure of the kidney of white rats 1 hour after the introduction of scorpion venom. 1 - dystrophy of proximal tubules with accumulation of cellular detritus in the lumen, 2 - destruction of distal tubules, 3 - hypertrophied renal corpuscle, 4 - area of haemorrhage, 5 - destructured artery. Staining with hematoxylin and eosin. Magnification. x 200
Authors' own work

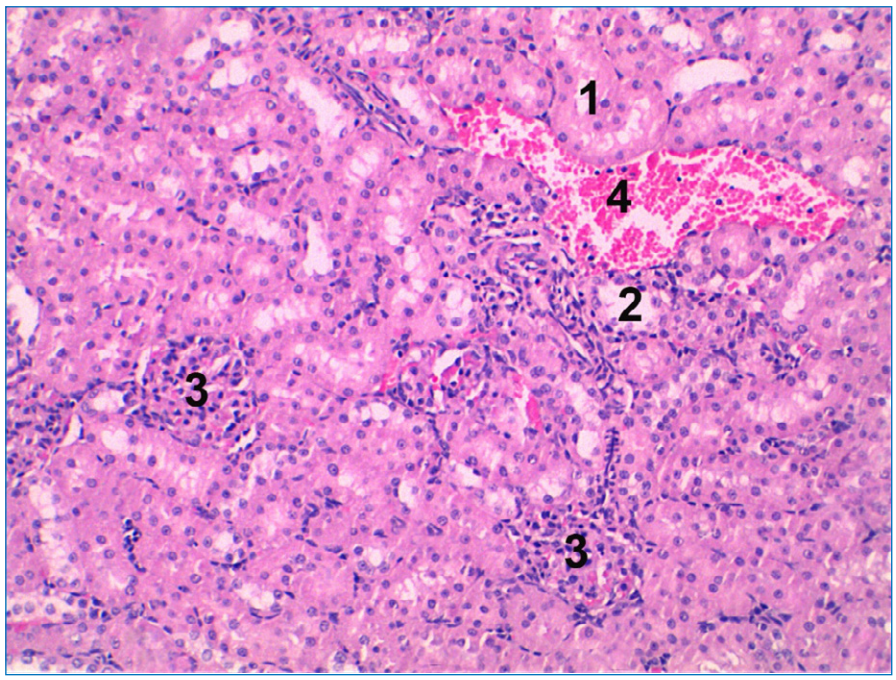

Fig. 2. Microscopic structure of the kidney of white rats 1 hour after the introduction of scorpion venom. 1 - dystrophy of proximal tubules, 2 - destruction of distal tubules, 3 - renal corpuscles with no urinary space, 4 - haemorrhage area. Staining with hematoxylin and eosin. Magnification x 200
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interstitium of the organ surrounding the vessels of the microcirculatory bed, perivascular oedema with infiltration by lymphocytes, macrophages, and histiocytes occurs.

In the cortical layer adjacent to the renal corpuscles, which retain their typical histological structure, hypertrophied renal corpuscles are observed where the lumen of the afferent and efferent arterioles is narrowed. Erythrocyte aggregation is enhanced within their lumens. The walls of the arteries and arterioles are thickened due to hypertrophy and slight leukocyte infiltration of the tunica media, while smooth myocytes, losing their spindle shape, become rounded. Their nuclei are hyperchromic, and the cytoplasm appears brightly oxyphilic. There is mild oedema and lympho-histiocytic infiltration in the subendothelial layer of the tunica intima and tunica adventitia. The endothelial cells of the tunica intima exhibit a cubic shape, and slight oedema can be seen in the cytoplasm. The nuclei are hyperchromic, and their plasmalemma is indistinct, showing significant protrusions into the lumen and invaginations. The basement membrane is continuous, although in some areas, signs of endothelial desquamation are present (Fig. 1).

In the glomeruli, stasis is present. In some areas, their lumens have collapsed due to spasms of the afferent

arteriole. The urinary spaces are sharply dilated. In the walls of the glomerular capillaries, initial signs of focal endothelial dystrophy are evident. The cytoplasm of the endothelial cells is swollen; the nuclei are hypertrophied and hyperchromatic, significantly protruding into the vessel's lumen. The basement membrane has thickened and appears swollen. Due to increased proliferative activity of mesangiocytes, there is an expansion of the mesangium and a rise in the amount of matrix. Mesangial hyperplasia has caused a sharp narrowing of the urinary space of the renal corpuscles, which, in most fields of view, appears slit-like or is not visualised at all (Fig. 2). The cells of the outer layer of Bowman's capsule are sharply flattened, their nuclei are hyperchromatic with invaginations of the karyolemma, and the basement membrane is thickened. The growth of the mesangium disrupts the cytoskeleton of the podocytes in the inner layer of the capsule. There is disorganisation of the cytopodia and cytotrabeculae of podocytes, resulting in the onset of filtration disorders.

In the proximal and distal renal tubules, the lumens are unevenly expanded with thinning of the walls. In the proximal and distal tubules, the epithelium is damaged. The brush border and basal striation are especially noticeable in dystrophic changes. In the foci, the cells almost wholly lose them, and desquamation of the epithelium into the lumen of the tubule is observed. The cells exhibit manifestations of hydropic and hyaline-droplet dystrophies, characterised by the appearance of vacuoles and single acidophilic granules in the weakly oxyphilic cytoplasm, indicating cytoplasmic swelling. The nuclei are hyperchromic and compacted, with indistinct contours. Fibrin accumulations were detected in the lumens of the tubules. In the thin tubules, hyperplasia of epithelial cells is due to the swelling of their cytoplasm. On the cross-section, thin tubules with slit-like lumens. When tubules with moderately dilated lumens are collected, epithelial cells almost completely lose microvilli and basal striation. Their cytoplasm is edematous, with single vacuoles in a weakly oxyphilic cytoplasm. The nuclei are hyperchromic and compacted. Along the course of the vessels of the myocirculatory bed of the interstitium, both in the cortex and medulla, there are locally single haemorrhages (Fig. 2).

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

Due to the oedema, the fibrous capsule is thickened. The organ shows dilation of the venous vessels and spasm of the arteries. The vessel wall thickens due to hypertrophy of the tunica media, accompanied by perivascular oedema and lymphohistiocytic infiltration. In their lumens, thrombi consisting of erythrocytes and solitary leukocytes are present, without clearly visualising the boundaries between them.

Within the interstitium of the organ surrounding the vessels of the myocirculatory bed, there is notable perivascular oedema and infiltration by lymphocytes, plasma cells, macrophages, tissue basophils, and eosinophils. In the lumens of the vessels, there is stasis, thrombi, and erythrocytes lacking clear boundaries. The capillary wall features an endothelium with disrupted intercellular contacts, and the basement membrane is significantly thinned and discontinuous. Focal areas of endothelial desquamation are present. The nuclei of endothelial cells are hyperchromatic and compacted. They protrude markedly into the lumen—increased vascular-tissue permeability of the interstitium results in pronounced hydration of the ground substance in loose connective tissue. Collagen fibres in areas with significant oedema appear brightly oxyphilic, creating spaces while retaining their fascicular nature, but are defibrotic. Disruptions in blood clotting processes and the integrity of the microcirculatory vessels within the organ lead to the emergence of small, multiple haemorrhages with pronounced erythrocyte hemolysis and substantial lymphohistiocytic infiltration at their periphery.

In comparison to the previous term of the experiment, there is an intensification of destructive-degenerative changes in the glomerular and tubular apparatus of nephrons within the cortical layer, alongside stromal-vascular disorders. The renal corpuscles of the nephrons exhibit varying sizes, predominantly being hypertrophied and deformed. Afferent and efferent arterioles remain in a spasmodic state, with thickened walls resulting from pronounced mucoid swelling, and stasis occurs within their lumens, characterised by significant hemolysis of erythrocytes. Lymphocytes, macrophages-histiocytes, and eosinophils significantly infiltrate the walls of the arterioles. Smooth myocytes of the tunica media appear shortened, displaying hyperchromic nuclei and markedly eosinophilic cytoplasm. The brightly eosinophilic collagen fibres swell in the subendothelial layer of both the tunica intima and tunica adventitia, maintaining their fascicular nature while demonstrating defibrotic characteristics. There is significant dehydration of the ground substance, which shows an increased presence of weakly basophilic glycosaminoglycans.

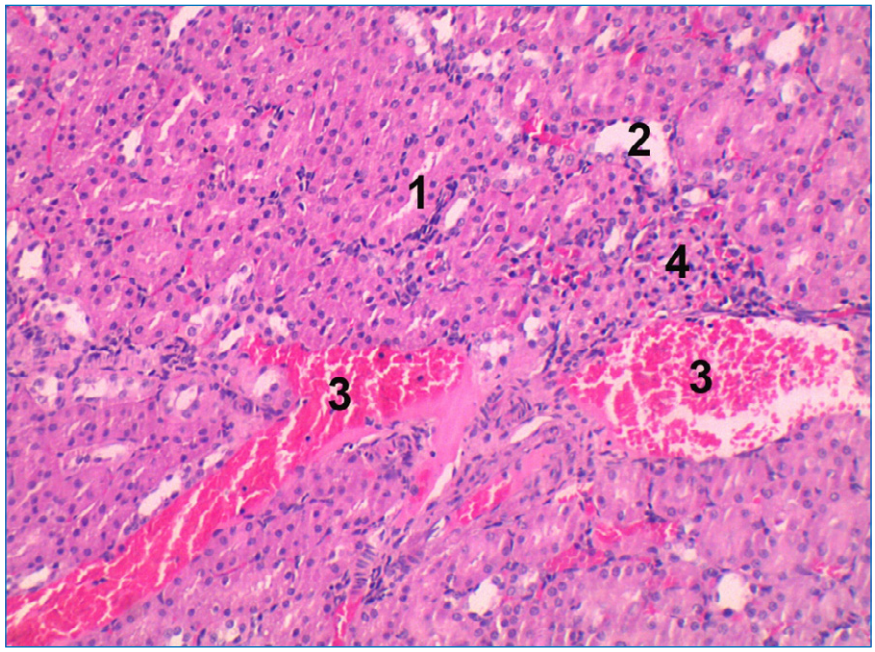

Fig. 3. Microscopic changes in the kidney of white rats 3 hours after the introduction of scorpion venom. 1 - proximal tubules with dystrophically altered epithelial cells, 2 - destruction of the distal tubule, 3 - areas of haemorrhage, 4 - deformed renal corpuscle with absent urinary space. Staining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

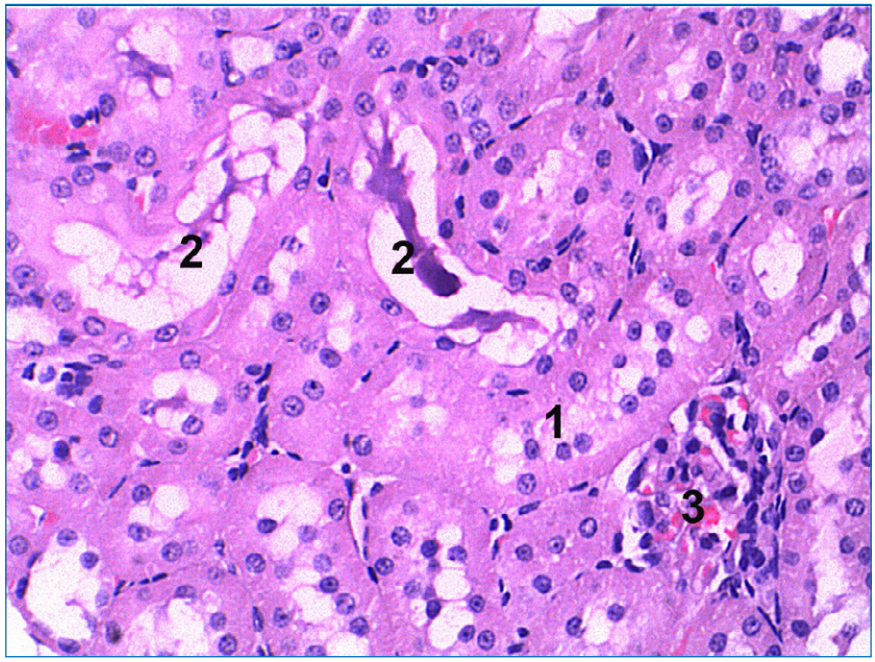

Fig. 4. Microscopic changes in white rats 3 hours after introducing scorpion venom. 1 - proximal tubules, 2 - cellular detritus in the lumen of the tubules, 3 - deformed renal corpuscle. Staining with hematoxylin and eosin. Magnification x 200
Authors' own work

Endotheliocytes display a swollen tunica intima and contain pyknotic, hyperchromic nuclei. The basement membrane is discontinuous and appears thinned, with focal areas of complete endothelial desquamation.

In the wall of glomerular capillaries, destructive degenerative disorders resemble those in the afferent arterioles. The expansion of the urinary spaces is preserved. The cells of the outer layer of the capsule are flattened; the cytoplasm is oxyphilic, and their nuclei are compacted and basophilic, while the basement membrane is thickened. The mesangium increases due to an augmentation in the matrix and active proliferation of mesangiocytes, resulting in significant oedema and destruction of the cytopodia and cytotrabeculae of podocytes. The urinary space was not visualised in such renal corpuscles (Fig. 3).

The proximal and distal renal tubules exhibit dilated lumens. Some tubules are characterised by acute necrotic damage to the epithelial cells and complete desquamation of the epithelium. The epithelium is rounded and loses its complete brush and striated borders; its cytoplasm is brightly oxyphilic. The nuclei are pyknotic, compacted, and intensely basophilic. There are individual cells with nuclei that possess an indistinct karyolemma. This indicates that the nuclei are undergoing lysis and the onset of acute necrosis. In the lumens of such tubules, there is an accumulation of cellular detritus. Simultaneously, a significant number of

tubules display manifestations of hydropic and hyaline-droplet dystrophy of the epithelium, complete loss of brush and basal striation, and desquamation into the tubule lumen. In their lumens, accumulations of protein masses and cellular detritus were observed (Fig. 4).

The above changes were fully confirmed during the morphometric study. Morphometric studies of the cortical substance of the kidneys of white rats after 1 hour of scorpion venom administration established that the average value of the area of the renal corpuscles is (5868,7±289,3) μm^2. In contrast, the average values of the area of the vascular glomerulus and the capsule cavity are (4618,1±221,9) μm^2 and (1250,6±60,5) μm^2, respectively. The average value of the diameter of the proximal renal tubules is (43,98 ±1,96) μm, respectively, but the average area of the tubules is (1518,38 ±73,90) μm^2. The average values of the diameter and area of their lumen are (25,06 ± 1,21) μm and (492,98 ± 22,64) μm^2, respectively. The parameters of the epithelial cells of the proximal nephron changed, consisting of a partial increase in height and width, with values of (14,75 ± 0,63) μm ($p < 0,05$) and (16,28 ± 0,74) μm, respectively. Taking into account the specified changes in the height and width of the cells, their average area is (240,13 ± 11,05) μm^2. The average value of the area of the epithelial cell nucleus is (28,35 ± 1,39) μm^2, and the nuclear-cytoplasmic ratio is 0,118 ± 0,003. The average value of the diameter of the distal tubules is insignificantly equal to (41,43 ± 1,86) μm, but their area has increased and is (1347,41 ± 66,37) μm^2. The average diameter of the lumen of the distal tubules is (22,85 ± 1,08) μm. It was found that the average value of the lumen area of the distal tubules is (409,51 ± 18,78) μm^2. The average height of the epithelial cells of the convoluted distal tubules of the nephron is (8,35 ± 0,37) μm, respectively, but the average value of the cell width decreases and is (12,90 ± 0,63) μm. The average area of the epithelial cells increases due to cytoplasmic swelling and is (107,72 ± 5,28) μm^2. The average value of the area of the epithelial cells' nuclei is (26,07 ± 1,25) μm. Violation of the structure of epithelial cells increased the nuclear-cytoplasmic ratio, which was 0,242 ± 1,25.

In contrast to these data, a significant change in the above parameters is observed in rats 3 hours after the introduction of scorpion venom into the experimental animals: the average value of the renal corpuscle area is (6646,4 ± 313,5) μm^2. The average values of the area of the vascular glomerulus and the capsule cavity are (5273,8 ± 254,1) μm^2 and (1372,6 ± 60,3) μm^2, respectively. The average diameter of the proximal renal tubules has decreased to (42,71 ± 1,94) μm. At the same time, the average area of the tubules is significantly reduced, measuring (1431,95 ± 69,75) μm^2. The average value of the diameter of the tubular lumen is (26,85±1,32) μm, and the average value of the tubular lumen area significantly ($p<0,001$) increases and reaches the value of (565,92±26,05) μm^2. It was established that the average value of the height of epithelial cells is (13,08±0,59) μm, and the value of the average width of cells significantly ($p<0,05$) increases and equals (17,35±0,81) μm. Accordingly, the average value of the area of epithelial cells is (226,94 ± 10,47) μm^2. The average value of the area of the epithelial cell nucleus decreases and is (25,19±1,20) μm^2. An insignificant decrease in the nuclear-cytoplasmic ratio to the value of 0,111±0,001 was established.

The average diameter of the distal tubules decreases to a value of (40,02 ± 1,78) μm, while the average area of the tubules significantly decreases ($p < 0,001$) to (1257,26 ± 61,18) μm^2. The average indicator of the diameter of the lumen of the distal tubules is (23,51 ± 1,11) μm. There is a significant ($p<0,001$) increase in the average value of the lumen area of the distal tubules, which is (433,89 ± 20,39) μm^2. The average value of the height of the epithelial cells of the convoluted distal tubules of the nephron is (9,28 ± 0,41) μm, and their width is (11,35 ± 0,55) μm. In contrast, the average area of the epithelial cells is (105,33 ± 5,23) μm^2. The average value of the area of epithelial cell nuclei decreases to (23,76±1,16) μm^2, and the nuclear-cytoplasmic ratio is 0,226±0,009.

DISCUSSION

Among the currently known pathological processes associated with scorpionism, kidney damage is regarded as one of the most critical complications, often resulting in fatality [23, 24, 25]. Notably, Hemiscorpius lepturus and Androctonus australis are observed to exhibit a pronounced nephrotoxic effect. The toxic compounds in their venom can directly or indirectly impact kidney tissue, leading to conditions such as mesangiolysis, glomerulonephritis, vasculitis, interstitial nephritis, cortical and tubular necrosis, as well as hypoxia and renal infarction [26, 27]. Among these conditions, tubular necrosis is most frequently reported in victims, characterised by reduced reabsorption and increased secretion processes of Na^+, K^+, and Cl^-. Studies have shown that venom inoculation affects Na^+ channels within the kidneys. Concurrently, there is an elevation in the blood levels of catecholamines among the victims. In the cytosol of nephron tubular cells, during scorpion toxin-induced poisoning, the concentration of Ca^{2+} ions rises. The body's protective mechanisms lead to an increase in pro-inflammatory cytokines within the renal parenchyma, including IL, TNF-α, NO, platelet aggregation factor, PG, leukotrienes, kinins, and angiotensin.

Clinical cases of bites accompanied by rhabdomyolysis, haemolysis, and disseminated intravascular coagulation have been documented to date. These processes have contributed to the development of acute renal failure through damage to the glomerular apparatus and have been associated with haemoglobinuria and proteinuria. The action of venom PLA2 can also lead to acute kidney injury. The enzymes in these conditions exhibit haemolytic and cytotoxic effects on the organ. Additionally, it promotes the generation of arachidonic acid and, consequently, the synthesis of eicosanoids. There is also evidence of increased activity of the hypothalamic-pituitary-adrenal system, which stimulates corticosteroid production. Simultaneously, an increase in the levels of acute phase proteins and significant haemodynamic disturbances has been recorded. It is known that the accumulation of a substantial number of haemoproteins accompanies haemolytic phenomena. Under these conditions, heme groups enhance the production of free radicals that accumulate in the renal cortex, exerting a toxic effect. An increase in NADPH oxidase activity facilitates the production of reactive oxygen species (ROS). In this context, apoptosis or necrosis of kidney cells is a frequent consequence [28].

Movahed A. et al. [29] studied the effect of the scorpion Hemiscorpius lepturus venom on the morphology of the kidneys. During the histological examination, they observed an expansion of the lumen and thinning of the walls of the proximal tubules of the nephron, as well as the accumulation of fibrin in the glomerular apparatus. The glomerular capillaries contained clusters of erythrocytes, and the endothelium of their walls was swollen four hours after the introduction of the venom to laboratory animals. In the proximal tubules, flattening of the epithelial cells of their inner membrane, loss of their brush border, and an atypical response of the nuclei of these cells were noted, indicating the development of acute tubular necrosis.

A thorough analysis of the scientific literature has demonstrated the involvement of macrophages in the development of acute kidney injury due to scorpion stings. Consequently, the level of MCP increases in the victim's body, indicating the initiation of an inflammatory process in the organ and signalling the risk of complications, as it directly affects the migration of macrophages and the 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. Additionally, binding to the chemokine receptor CC2 on the surface of podocytes can reduce the expression of both microRNA and nephrin protein. The latter is involved in forming filtration gaps; therefore, defects in this protein lead to renal failure, disruption of the podocyte cytoskeleton, and disorders of the filtration process [30].

CONCLUSIONS

1. One hour after administering *Leiurus macroctenus* scorpion venom to rats, we observed the development of significant haemodynamic disorders and the initiation of inflammatory processes in the organs, as well as acute renal failure resulting from damage to the glomerular apparatus. Disturbances in the cytoskeleton of podocytes and the tubular apparatus of nephrons, accompanied by desquamation, hydropic changes, and hyaline-droplet dystrophies of the tubular epithelium, were established.
2. The introduction of *Leiurus macroctenus* scorpion venom to rats resulted in significantly pronounced dystrophic changes in the structural components of the kidneys three hours after the commencement of the experimental study, in contrast to the earlier phase of the investigation. Inflammatory processes in the organ interstitium were observed, featuring plasmacytic, lymphocytic, and macrophage infiltration. Vascular and haemodynamic disorders were also detected, as evidenced by the desquamation of the endothelial lining of the vascular wall and increased permeability. The development of tubular necrosis characterised the renal tubular apparatus.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 19.03.2025
ACCEPTED: 20.05.2026