



TNF- α^+ expression in the kidneys of rats 1 and 3 hours after the injection of the venom of the scorpion *Leiurus macroctenus*

The aim of the study: to investigate the expression of the immunohistochemical marker TNF- α^+ in the kidneys of experimental rats after injection of venom from the scorpion *Leiurus macroctenus*.

Materials and Methods. The study used 15 white male laboratory rats weighing (200 $\pm$ 10) g. The animals were divided into three groups: a control group (5 rats), in which no venom injection was performed, and material was collected 1 hour after saline injection; and two experimental groups (5 rats each), in which histological material was collected 1 and 3 hours after venom injection, respectively. The venom of scorpions of the *Buthidae* family, genus *Leiurus*, species *Leiurus macroctenus* was administered to animals once intramuscularly (0.5 ml of venom solution previously dissolved in saline; 28.8 μ g/ml; LD₅₀=0.08 mg/kg). We euthanised rats by carbon dioxide inhalation. Kidneys were isolated at 4 °C immediately after euthanasia. To assess the regulation of the immune response and inflammation in kidney tissue, we used immunohistochemical staining with a panel of specific markers, particularly TNF- α^+ .

Results. Intoxication with the venom of the scorpion *Leiurus macroctenus* causes focal expression of TNF- α^+ , mainly in the proximal tubules of the kidney nephron 1 h after the start of the experiment as a local response to the initial phase of acute toxic stress. Three hours after the introduction of scorpion venom, immunohistochemical staining with antibodies to TNF- α^+ revealed a sharp increase in immunoprecipitation in kidney tissue: a high positive reaction was observed in renal corpuscles, and a moderate reaction to this pro-inflammatory cytokine in peritubular interstitial areas.

Conclusions. Within 1–3 hours after introducing scorpion venom, early signs of an immune response are detected in kidney tissues: focal, mainly weak or moderate TNF- α^+ expression is detected, indicating the initial initiation of the inflammatory process.

Key words: venom; scorpions; kidneys; immunohistochemistry; morphology; TNF- α^+ marker; inflammation; rats.

Problem Statement and Analysis of Recent Research. Currently, special attention is paid to studying the mechanisms of cellular homeostasis because, as established, cells are the first barrier against pathogenic factors. Membrane receptors can recognise changes in the extracellular matrix and signal cells about disruptions in homeostasis and the need to launch signalling cascades to normalise it [1]. Cellular responses to certain stimuli vary significantly and depend mainly on the duration of disturbances in the constancy of the organism's internal environment. Endothelial cells of the vascular walls play an important role in recognising and protecting the body from toxins of poisonous animals. Being the main point of contact with toxic components of natural poisons that have entered the bloodstream, endothelial cells perform the function of recognising them by expressing numerous PRRs, including Toll-like receptors (TLRs), TNF receptors, and IL-1, which cause the activation of pro-inflammatory genes and ultimately pathological changes in the vascular microenvironment. Endotheliocytes also express major histocompatibility complex (MHC) molecules of classes I and II and CD40 ligands, ensuring intravascular presentation of foreign agents, particularly

poison toxins, to immune effector cells [2]. Endothelial cells modulate immune function by influencing leukocyte transport (migration). Adhesion and extravasation are characteristic responses driven by the selective expression of cell adhesion molecule-1 (ICAM-1) and selectin on the apical surface of endothelial cells [3, 4]. Based on the above data on the participation of the endothelium of the vascular walls in the regulation of the immune response, it can be argued that its dysfunction, which develops as a result of the negative impact of poison toxins and is characterized by distortion of the morphology of endothelial cells, disruption of the organization of their cytoskeleton and viability, may lead to disorders of the body's immune reactivity in response to bites of predatory animals, the appearance of severe complications. Currently, immunohistochemical studies are a crucial tool for determining the degree of tissue damage caused by harmful factors. They also significantly improve our understanding of complication development. The TNF- α^+ marker may indicate an inflammatory reaction. Currently, few studies have examined TNF- α^+ expression in the kidneys of experimental animals exposed to *Leiurus macroctenus* venom. Therefore, assessing TNF- α^+ expression in

the kidneys of rats after exposure to scorpion venom can provide valuable information about the localisation, severity, and duration of the inflammatory reaction [5].

The aim of the work: to investigate the expression of the immunohistochemical marker TNF- α in the kidneys of experimental rats under conditions of administration of the venom of the scorpion *Leiurus macroctenus*.

Materials and Methods. The study used 15 white male laboratory rats, each weighing approximately (200 $\pm$ 10) g. They were raised in the vivarium of the Educational and Scientific Centre "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv. The rats received a standard diet and were kept in an accredited vivarium in accordance with the Standard Rules for the Arrangement, Equipment and Maintenance of Experimental Biological Clinics (Vivaria). All procedures complied with the current standards for working with experimental animals and the principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [6]. Also, all work with animals was carried out in accordance with 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 01/27/25).

Rats in the experiment were divided into three groups: a control group of 5 animals that did not receive poison; their samples were collected 1 and 3 hours after injection of saline; and two experimental groups of 5 rats each, in which tissues were collected 1 and 3 hours after injection of poison, respectively. The venom used was from the family *Buthidae*, genus *Leiurus*, species *Leiurus macroctenus*, administered intramuscularly as a single dose (0.5 mL venom solution in saline; 28.8 μ g/mL; LD₅₀ = 0.08 mg/kg) [7, 8]. Rats were euthanised by carbon dioxide inhalation, and their kidneys were immediately isolated at 4 °C after euthanasia.

Immunohistochemical staining with a panel of specific markers, including TNF- α , was used to assess immune response regulation and inflammation in kidney tissues.

Antigens were detected by temperature-induced retraction in EnVision FLEX Target Retrieval Solution with high pH buffer (pH 9.0) using a KOS histology processor (Milestone, Italy) at 98 °C for 20 min. Endogenous peroxidase activity was blocked with 3 % aqueous H₂O₂. The cells were then incubated for 60

min with specific primary antibodies: rabbit polyclonal antibodies Anti-TNF- α (cat. no. ab125212, Abcam, USA) to identify TNF- α cells [9, 10].

Immune complexes were visualised using the Mouse/Rabbit PolyVue™ HRP/DAB detection system (Diagnostic BioSystems, USA), which provides high sensitivity and specificity due to polymer-conjugated secondary antibodies with peroxidase activity and subsequent chromogenic development with diaminobenzidine (DAB). We counterstained cell nuclei with Mayer's hematoxylin according to standard procedures. Samples were examined under a MICROMED SEO SCAN light microscope, and photodocumentation was performed using a Vision CCD camera.

Results. Immunohistochemical studies of TNF- α as a marker that plays a key role in the regulation of the immune response and inflammation in the kidney tissue of the control group showed the appearance of a weak immunoprecipitation reaction (+/-) in the areas of the proximal renal tubules, while the reaction is negative in the glomerular apparatus (Fig. 1). These signs indicate the complete absence of inflammatory processes in the organ.

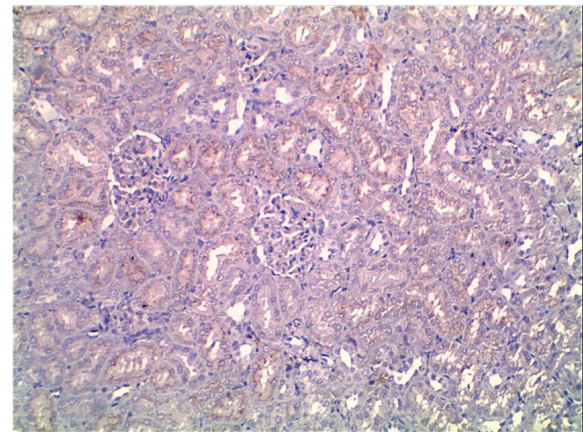


Fig. 1. Expression of TNF- α in the kidneys of rats from the control group. Presence of single TNF- α cells in the renal tubular apparatus of rats from the intact group. Immunohistochemical staining using an antibody to TNF- α , counterstaining with Mayer's hematoxylin. $\times 100$.

One hour after the administration of scorpion venom, focal expression of TNF- α was immunohistochemically detected, mainly in the proximal tubules of the nephron of the kidney, with a characteristic pale brown staining of the immunoprecipitate (Fig. 2). A weak reaction (+/-) of the TNF- α marker was observed, i.e. a local response to the initial phase of acute toxic stress.

After 3 hours of the experiment after the introduction of scorpion venom, immunohistochemical staining of kidney tissue with antibodies to TNF- α

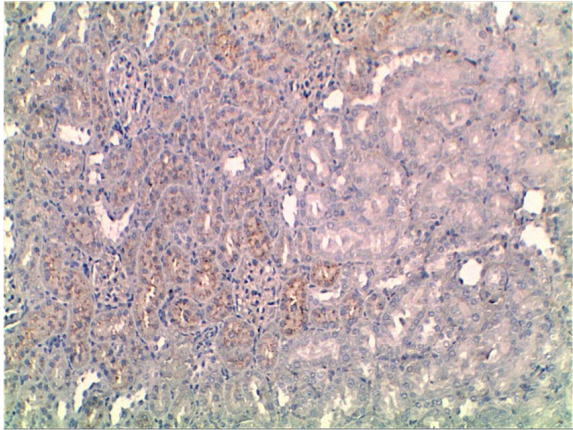


Fig. 2. TNF- α ⁺ expression in rat kidney 1 h after scorpion venom administration. TNF- α ⁺ positive cells are detected in the nephron's structural components. Immunohistochemical staining using a TNF- α ⁺ antibody, counterstained with Mayer's hematoxylin. $\times 100$.

revealed a sharp increase in immunoprecipitation (++-), and a high positive reaction (+++) was observed in renal corpuscles. Immunopositive staining of TNF- α ⁺ proximal tubules of the nephron is characterised by moderate or high intensity with the formation of brown immunoprecipitate in the cytoplasm. Areas of the peritubular interstitium with a moderate reaction (++-) to this pro-inflammatory cytokine are also detected (Fig. 3).

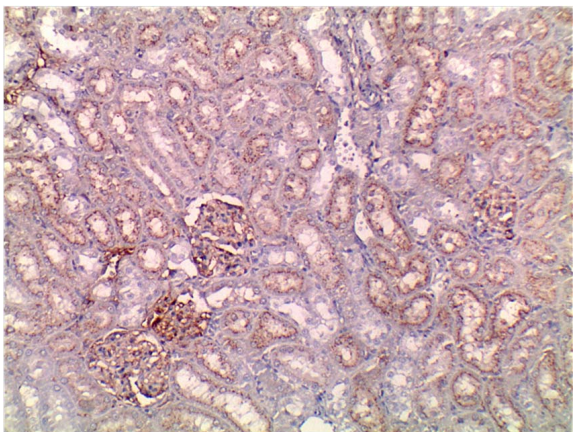


Fig. 3. TNF- α ⁺ expression in rat kidneys 3 h after scorpion venom administration. Presence of TNF- α ⁺ positive cells in the renal tubular and glomerular apparatus. Immunohistochemical staining using TNF- α ⁺ antibody, counterstaining with Mayer's hematoxylin. $\times 100$.

Discussion. Among the currently known pathological processes associated with scorpionism, according to experimental studies, kidney damage is considered one of the most critical complications, which is often fatal [11–13]. In particular, the venoms of *Hemiscorpius lepturus* and *Androctonus australis*

exhibit pronounced nephrotoxicity. Venom toxins can directly or indirectly affect kidney tissue, causing mesangiolysis, glomerulonephritis, vasculitis, interstitial nephritis, cortical and tubular necrosis, hypoxia, and renal infarction [14, 15]. Of these pathologies, tubular necrosis is most often observed in victims and is characterised by decreased reabsorption and increased secretion of Na⁺, K⁺, and Cl⁻. Venom inoculation affects Na⁺ channels in the kidneys. At the same time, catecholamine levels increase in victims' blood. In the cytosol of nephron tubule cells under conditions of poisoning with scorpion toxins, the concentration of Ca²⁺ ions increases.

When studying the effect of *Hemiscorpius lepturus* scorpion venom on kidney morphology, histological examination revealed widening of the lumens and thinning of the walls of the proximal nephron tubules, as well as the accumulation of fibrin in the glomerular apparatus. The glomerular capillaries contained clusters of erythrocytes; their endothelial walls were swollen 4 hours after venom administration in laboratory animals. In the proximal tubules, flattening of the epithelial cells of their inner membrane, loss of their brush border, and an atypical reaction of the nuclei of these cells were noted, which indicated the development of acute tubular necrosis [16].

The body's defence mechanisms contribute to increased renal parenchymal levels of pro-inflammatory cytokines, namely IL-1 β , TNF- α , NO, as well as platelet aggregation factor, PG, leukotrienes, kinins, angiotensin, and ET. 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 hemoglobinuria and proteinuria [17]. Macrophages have been shown to contribute to acute kidney injury in scorpion bites. Thus, MCP-1 levels increase in the victim's body, indicating the onset of an inflammatory process in the organ and signalling the risk of complications, since it directly affects macrophage migration and leukocyte proliferation and differentiation in the epithelium of human kidney tubules. MCP-1 stimulates IL-6 secretion and intracellular expression of ICAM-1. In addition, by binding to CC2 chemokine receptors on podocyte surfaces, 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 renal failure, disruption of the podocyte cytoskeleton, and disorders of the filtration process [18].

Conclusions. Intoxication with the venom of the scorpion *Leiurus macroctenus* causes focal expression

of TNF- α +, mainly in the proximal tubules of the kidney nephron 1 h after the start of the experiment, as a local response to the initial phase of acute toxic stress. After 3 h of the experiment, following the introduction of scorpion venom, immunohistochemistry shows a sharp increase in TNF- α expression in kidney tissue: the renal corpuscles show a strong positive reaction, and the peritubular interstitium shows a moderate reaction to this pro-inflammatory cytokine.

Thus, within 1–3 h after introduction of scorpion venom, early signs of an immune response are established in kidney tissues: focal, mainly weak or moderate TNF- α expression is detected, indicating the initiation of the inflammatory process.

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Prospects for further research. Further studies of the expression of other immunohistochemical markers of rat kidney tissue under conditions of exposure to the venom of the scorpion *Leiurus macroctenus* and comparative characterisation of changes in these indicators.

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Експресія $\text{TNF-}\alpha^+$ у нирках щурів через 1 та 3 години після введення отрути скорпіона *Leiurus macroctenus*

Мета роботи: дослідити експресію імуногістохімічного маркера $\text{TNF-}\alpha^+$ в нирках експериментальних щурів за умов введення отрути скорпіона *Leiurus macroctenus*.

Матеріали і методи. У дослідженні використали 15 білих лабораторних щурів-самців масою (200 ± 10) г. Тварин поділили на три групи: контрольна – 5 щурів, введення отрути не проводилося, забір матеріалу через годину після введення фізіологічного розчину; 2 експериментальні – по 5 щурів, відбір гістологічного матеріалу через 1 та 3 год відповідно після введення отрути. Отруту скорпіонів родини *Buthidae*, роду *Leiurus*, виду *Leiurus macroctenus* вводили тваринам одноразово внутрішньом'язово (0,5 мл розчину отрути, попередньо розчиненого у фізіологічному розчині; $28,8 \text{ мкг/мл}$; $\text{LD}_{50} = 0,08 \text{ мг/кг}$). Щурів піддавали евтаназії шляхом інгаляції вуглекислого газу. Відділяли нирки при 4°C відразу після евтаназії. Для встановлення ступеня регуляції імунної відповіді й запалення у тканині нирки застосовували метод імуногістохімічного фарбування із використанням панелі специфічних маркерів, зокрема $\text{TNF-}\alpha^+$.

Результати. Інтоксикація отрутою скорпіона *Leiurus macroctenus* зумовлює фокальну експресію $\text{TNF-}\alpha^+$, головним чином, у ділянках проксимальних каналців нефрона нирки через 1 год від початку експерименту як локальну відповідь на початкову фазу гострого токсичного стресу. Через 3 год після введення отрути скорпіона в тканині нирки імуногістохімічно після забарвлення антитілами до $\text{TNF-}\alpha^+$ виявляється різке підвищення імунопреципітації: у ниркових тільцях спостерігається висока позитивна реакція, а в ділянках перитубулярного інтерстицію – помірна реакція до цього прозапального цитокіну.

Висновки. У терміни 1–3 год після введення отрути скорпіона в тканинах нирки встановлено ранні ознаки імунної відповіді: виявляється фокальна, переважно слабка або помірна експресія $\text{TNF-}\alpha^+$, що свідчить про початкову ініціацію запального процесу.

Ключові слова: отрута; скорпіони; нирки; імуногістохімія; морфологія; маркер $\text{TNF-}\alpha^+$; запалення; щури.

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