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MORPHOMETRIC STUDY OF THE STRUCTURE OF RAT KIDNEYS DURING THE FIRST HOUR OF EXPOSURE TO LEIURUS MACROCTENUS SCORPION VENOM

Matkivska R.M. ✉ **Morphometric study of the structure of rat kidneys during the first hour of exposure to Leiurus macroctenus scorpion venom.**
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ABSTRACT. Background. Pathogens of external origin, entering the human body by various routes, can disrupt the typical structure of the kidneys and cause significant functional disorders. The degree of morphological changes depends on the strength, concentration and duration of exposure to the stimulus. Toxic compounds in animal venoms penetrate the victim's body and disrupt the stability of the internal environment. The primary mechanisms of pathological changes are linked to alterations in cellular structure, function, and the progression of biochemical reactions. During evolution, toxins have developed strategies for penetrating target organs, which leads to numerous pathological changes. **Objective.** Study of morphometric data of the structure of rat kidneys during the first hour of exposure to Leiurus macroctenus scorpion venom. **Methods.** The study used 10 white male laboratory rats weighing 200 g (±10 g), grown in the vivarium of the Educational and Scientific Centre "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv. The venom of scorpions of the 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). Morphometric studies were conducted using the SEO ImageLabBio, ImageJ, and STATISTICA 10.0 programs. The studies were conducted at the specified experimental periods in preparations stained with hematoxylin and eosin. Arithmetic means (M), errors of arithmetic means (m), coefficients of variation, and standard deviations were calculated. Changes were considered significant at $p \leq 0.05$. **Results and conclusion.** When a scorpion stings, after 1 hour, toxic substances cause the onset of substantial hemodynamic disorders and the initiation of inflammatory processes in the organ, acute renal failure as a result of acute damage to the glomerular apparatus, which is confirmed by a statistically significant increase in their parameters. Intracellular oedema and epithelial dystrophy lead to a substantial increase in the morphometric parameters of the organ's tubules.
Keywords: venom, scorpions, kidneys, morphometry, morphology, endothelium, glomerular apparatus, rats.

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Introduction

Pathogens of external origin that enter the human body through various means can disrupt the standard kidney structure and cause significant functional impairments. The extent of morphological changes depends on the stimulus's strength, concentration, and duration. Research shows that nearly all kidney structures are affected by such factors [1, 2, 3]. The kidneys' high vulnerability to harmful influences can lead to severe, destructive, and often irreversible damage. These conditions may result in disability or even death.

Toxic compounds in animal poisons penetrate the victim's body and disrupt the stability of the internal environment. The underlying mechanisms of pathological changes are related to alterations in cellular structure, functions, and the course of biochemical reactions. The first line of defence against toxin effects is a family of cells that help restore homeostasis or develop specific adaptations to new conditions [4, 5, 6, 7, 8]. Toxins from different poisonous animals can damage cell morpho-functional parameters by destroying membranes, forming pores, or disrupting ion channel activity [9, 10, 11]. Over the course of evolution, these toxins have developed strategies to penetrate target organs, resulting in numerous

pathological changes. The nervous, immune and endocrine systems play crucial roles in the body's response and adaptation to venom, activating signalling pathways. Their coordinated activity maintains vital functions, while dysfunction in these systems can cause severe complications or death from animal bites.

Research advances into the proteome, peptidome, and biological activity of scorpion venom components offer valuable insights into the specific features of morpho-functional changes in victims' bodies after bites [12, 13, 14, 15]. Nonetheless, the vast diversity of scorpion species complicates and slows down efforts to study all their venom components. As a result, the pathophysiological mechanisms behind some complications, such as kidney damage—an especially severe consequence of scorpionism—remain poorly understood. Current data on this issue are limited, highlighting the need for comprehensive and detailed research.

The study aimed to investigate the morphometric data of the rat kidney structure during the first hour of exposure to the venom of the scorpion Leiurus macroctenus.

Materials and methods

The study was carried out in accordance with the scientific research plans of the O. O. Bogomolets National Medical University and is a fragment of the scientific research works of the Department of Descriptive and Clinical Anatomy: "Morphological Features of Rat Organs under Experimental Exogenous Influence" (state registration number 0122U000491).

The venom of scorpions of the 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) [16, 17].

The study used 10 white male laboratory rats weighing 200 g (±10 g), raised in the vivarium of the Educational and Scientific Centre "Institute of Biology and Medicine" of the Taras Shevchenko National University of Kyiv (agreement on scientific and practical cooperation between the Taras Shevchenko National University of Kyiv, the M. I. Pirogov Vinnytsia National Medical University and the I. Ya. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, dated February 1, 2021. Rats were kept on 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 the current regulatory documents regulating 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" [18, 19]. 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". The rats selected for the experiment were divided into two groups: control - 5 rats, no poison was administered, material was collected one hour after the administration of saline; experimental - 5 rats, histological material was collected 3 hours after the administration of poison. Rats were euthanised by carbon dioxide inhalation. Kidneys were isolated from rats at 4 °C immediately after euthanasia.

Morphometric studies were performed using a visual analysis system for histological preparations. Images from histological preparations were displayed on a computer monitor using a MICROmed SEO SCAN microscope and a Vision CCD Camera. Morphometric studies were performed using SEO ImageLabBio, ImageJ, and STATISTICA 10.0 programs. The analyses were performed at specified times in hematoxylin and eosin-stained preparations.

The obtained digital material was processed by the variational statistics method using the Student's t-test. Arithmetic means (M), errors of arithmetic means (m), coefficients of variation, and standard deviations were calculated. Changes were considered significant at $p \leq 0.05$ [20].

Results and conclusion

Morphometric studies of the components of the renal corpuscle of intact white rats have established that the average value of the area of the renal corpuscle is (5468.3 ± 249.1) μm^2, and the average value of the area of the vascular glomerulus is (4325.6 ± 208.2) μm^2. In comparison, the average value of the location of the capsule lumen is (1142.7 ± 56.3) μm^2 (Table 1).

The conducted morphometric studies have shown that the diameter of the proximal convoluted tubules of the kidneys of the intact group of animals is (44.62 ± 2.13) μm, and the diameter of their lumen is (23.94 ± 1.17) μm. The cross-sectional area of the tubule is (1562.89 ± 75.14) μm^2, and the area of the lumen is (449.90 ± 20.49) μm^2. The height of epithelial cells is (12.35 ± 0.54) μm, and the width is (14.71 ± 0.62) μm. The area of the cells is (181.67 ± 8.21) μm^2. The area of the nuclei is (27.08 ± 1.26) μm^2. The nuclear-cytoplasmic ratio is 0.149 (Table 2). Morphometric studies of the distal convoluted tubules showed that their diameter is (42.11 ± 1.94) μm, and the area is (1392.00 ± 67.61) μm^2. The diameter of the lumen of the tubule is (20.73 ± 1.01) μm, while its cross-sectional area is (322.54 ± 14.34) μm^2. The values of the height and width of epithelial cells are (7.79 ± 0.29) μm and (13.26 ± 0.64) μm, respectively, and their area is (103.30 ± 5.14) μm^2. The area of the nuclei of these cells is (24.45 ± 1.18) μm^2. The nuclear-cytoplasmic ratio is 0.238 (Table 3).

The above data significantly change against the background of the effect of scorpion venom on the body of experimental animals. Morphometric studies of the cortical substance of the kidneys of white rats

after 1 hour of scorpion venom administration established that the average area of the renal corpuscles is (5868.7 ± 289.3) μm², which is 1.07 times greater than the normal value. At the same time, the average values of the area of the vascular glomerulus and the capsule cavity are equal to (4618.1±221.9) μm² and (1250.6±60.5) μm², respectively, which are 1.06 and 1.09 times greater than the similar parameters of the control group of animals (Table 1).

Table 1

Morphometric parameters of renal corpuscle components of rat kidneys 1 hour after scorpion venom administration, (M ± m)

	Control	1 hour
Renal corpuscle area, μm²	5468,3±249,1	5868,7±289,3
Glomerular area, μm²	4325,6±208,2	4618,1±221,9
Capsule cavity area, μm²	1142,7±56,3	1250,6±60,5

Morphometrically, it was established that the average diameter of the proximal renal tubules decreased insignificantly compared to the parameters of the control group of animals and amounted to (43.98 ± 1.96) μm, respectively, but the average area of the tubules decreased by 0.97 times ($p < 0.001$) and equalled (1518.38 ± 73.90) μm². The average values of the diameter and area of their lumen increased by 1.05 times and 1.10 times ($p < 0.001$) relative to the parameters of the animals of the control group, their values reaching (25.06 ± 1.21) μm, (492.98 ± 22.64) μm². The parameters of the epithelial cells of the proximal nephron underwent changes consisting of a partial increase in the height and width of these cells, and are (14.75 ± 0.63) μm ($p<0.05$) and (16.28 ± 0.74) μm. Taking into account the specified changes in the height and width of the cells, their average area increases by 1.32 times ($p<0.001$) relative to the norm and is (240.13 ± 11.05) μm². The average value of the area of the epithelial cell nucleus increases by 1.04 times relative to the parameter of the control group. It is 28.35 ± 1.39 μm², and the nuclear-cytoplasmic ratio decreases insignificantly relative to the similar indicator and is equal to 0.118 ± 0.003 (Table 2).

Table 2

Morphometric parameters of proximal convoluted tubules of rat nephrons one hour after administration of scorpion venom, (M ± m)

	Control	1 hour
Tubule diameter, μm	44,62±2,13	43,98±1,96
Tubule area, μm²	1562,89±75,14	1518,38±73,90*
Tubule lumen diameter, μm	23,94±1,17	25,06±1,21
Tubule lumen area, μm²	449,90±20,49	492,98±22,64*
Cell height, μm	12,35±0,54	14,75±0,63*
Cell width, μm	14,71±0,62	16,28±0,74
Cell area, μm²	181,67±8,21	240,13±11,05*
Nucleus area, μm²	27,08±1,26	28,35±1,39
Nuclear-cytoplasmic ratio	0,149±0,006	0,118±0,003

* – $p <0.05$ compared to the control group

Morphometric studies showed that the average values of the diameter of the distal tubules are insignificantly reduced and are equal to (41.43 ± 1.86) μm. Still, their area increased by 0.97 times ($p<0.001$) compared to the similar indicator of the control group and is (1347.41 ± 66.37) μm². The average value of the diameter of the lumen of the distal tubules increased by 1.10 times insignificantly compared to the control and is equal to (22.85 ± 1.08) μm. It was established that the average value of the area of the lumen of the distal tubules increased by 1.26 times ($p<0.001$) and is (409.51 ± 18.78) μm². In this period of the experiment, it was found that the average values of the height of epithelial cells of the convoluted distal tubules of the nephron increase and are respectively (8.35 ± 0.37) μm, however, the average value of the cell width decreases insignificantly relative to the parameters of the control group of animals and is equal to (12.90 ± 0.63) μm. The average value of the area of epithelial cells increases due to swelling of the cytoplasm and is equal to (107.72 ± 5.28) μm². The average value of the area of epithelial cell nuclei increases by 1.07 times relative to the similar parameter of the group of animals that were not injected with poison, and is (26.07 ± 1.25) μm. Violation of the structure of epithelial cells caused an increase in the nuclear-cytoplasmic ratio, which is 0.242 ± 1.25 (Table 3).

Table 3

Morphometric parameters of proximal convoluted tubules of rat kidneys 1 hour after administration of scorpion venom, (M ± m)

	Control	1 hour
Tubule diameter, μm	42,11±1,94	41,43±1,86
Tubule area, μm^2	1392,00±67,61	1347,41±66,37 *
Tubule lumen diameter, μm	20,73±1,01	22,85±1,08
Tubule lumen area, μm^2	322,54±14,34	409,51±18,78 *
Cell height, μm	7,79±0,29	8,35±0,37
Cell width, μm	13,26±0,64	12,90±0,63
Cell area, μm^2	103,30±5,14	107,72±5,28
Nucleus area, μm^2	24,45±1,18	26,07±1,25
Nuclear-cytoplasmic ratio	0,238±0,010	0,242±0,011

* – p <0.05 compared to the control group

Summary

When a scorpion stings, after 1 hour, toxic substances cause the onset of significant hemodynamic disorders and the initiation of inflammatory processes in the organ, acute renal failure as a result of acute damage to the glomerular apparatus, which is confirmed by a statistically significant increase in their parameters. Intracellular oedema and epithelial dystrophy lead to a substantial increase in the morphometric parameters of the organ's tubules.

Prospects for further development are related to studying morphometric 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. Морфометричне дослідження структури нирок щурів упродовж першої години впливу отрути скорпіонів *Leiurus macroctenus.*

РЕФЕРАТ. Актуальність. Патогени зовнішнього походження, що потрапляють в організм людини різними шляхами, можуть порушити нормальну структуру нирок і спричинити значні функціональні порушення. Ступінь морфологічних змін залежить від сили, концентрації та тривалості впливу подразника. Токсичні сполуки в отрутах тварин проникають в організм жертви та порушують стабільність внутрішнього середовища. Основні механізми патологічних змін пов'язані зі змінами в клітинній структурі, функціях та перебігу біохімічних реакцій. Протягом еволюції токсини виробили стратегії проникнення в органи-мішені, що призводить до численних патологічних змін. **Мета.** Вивчення морфометричних даних структури нирок щурів на протязі першої години 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 виду Leiurus macroctenus вводили щурам одноразово внутрішньом'язово (0.5 мл розчину отрути попередньо розчиненому у фізіологічному розчині; 28.8 мкг/мл; $ЛД_{50}$=0.08 мг/кг). Морфометричні дослідження проведені за допомогою програм SEO ImageLabBio, ImageJ та STATISTICA 10.0. Дослідження проводили у визначені терміни досліду в препаратах забарвлених гематоксиліном і еозином. Розраховували середні арифметичні величини (M), похибки середніх арифметичних (m), коефіцієнти варіації, а також середні квадратичні відхилення. Зміни вважали достовірними при $p \leq 0,05$. **Результати та підсумок.** При укусі скорпіона через 1 годину токсичні речовини спричиняють початок розвитку значних гемодинамічних розладів та запуску запальних процесів в органі, гострої ниркової недостатності як наслідок гострого ураження гломерулярного апарату, що підтверджується статистично значущим зростанням їх параметрів. Інтрацелюлярний набряк та дистрофія епітелію призводить до достовірного зростання морфометричних параметрів канальців органу.

Ключові слова: отрута, скорпіони, нирки, морфометрія, морфологія, ендотелій, гломерулярний апарат, щури.