



Morphometric indicators of changes in the kidneys of experimental rats under the conditions of administration of the venom of the viper *Vipera berus berus*

The aim of the study: to determine the morphometric changes in the kidneys of experimental rats under the conditions of administration of the venom of the viper *Vipera berus berus*.

Materials and Methods. The animals were divided into two groups: control and experimental (10 individuals each). Experimental rats received an intraperitoneal injection of a semi-lethal dose (LD_{50}) of the venom of *Vipera berus berus* in saline at a concentration of 1.576 µg/g. The control group was administered only saline. After 24 h, the rats were euthanised by decapitation after anaesthesia. A morphometric study of the area of the epithelium of the proximal and distal tubules was performed.

Results. In the experimental group, oedema and dystrophy of the epithelial cells of the proximal convoluted tubules, along with reduction or even disappearance of their lumen, led to a statistically significant increase in their area compared with the control group. An increase in the standard deviation by 2.4 times indicates uneven manifestations of edematous and other pathological processes in the proximal tubules of the kidneys of rats. If some tubules remained unchanged or had moderate signs of pathology, others, on the contrary, had edematous epithelium and narrowed lumens. As a result of the cumulative effect of pathological changes in the epithelial cells of the distal tubules themselves, which caused their flattening, and in the surrounding tissues, in particular in the circulatory component, which led to obturation and deformation of the lumens of the tubules, the area of the epithelium in this experimental group decreased statistically significantly compared to the control group.

Conclusions. Morphometric analysis of changes in the kidneys of experimental rats under the conditions of administration of the venom of the viper *Vipera berus berus* revealed a statistically significant increase in the area of the epithelium of the proximal convoluted tubules against the background of a sharp decrease in the area of epithelial cells of the distal tubules.

Key words: venom; vipers; kidneys; morphometry; inflammation; rats.

Problem Statement and Analysis of Recent Research. *Viper bites* can cause hemostasis disorders, leading to massive bleeding, myonecrosis, dermonecrosis, renal dysfunction, and other symptoms that can lead to disability or death [1–4]. *Viper* venom toxins cause inflammation and affect antioxidant levels [5–8]. Mitochondria in the renal tubular epithelium generate the energy needed to clear metabolic waste products from the blood and maintain fluid and electrolyte balance, and they can adapt to different metabolic states through signalling pathways such as mTOR and AMPK. These pathways activate transcriptional coactivators such as PGC1α and help regulate mitochondrial dynamics and energy production, ensuring cellular homeostasis. When mitochondria are damaged, ATP production is reduced, leading to impaired cellular function and changes in renal structure [9–12]. Mechanisms help preserve mitochondrial function during hypoxia. When oxygen levels are low, ATP production is reduced, leading to cell death. Under normoxic conditions, hypoxia-inducible factor 1α (HIF1α) is degraded in the presence of oxygen and α-ketoglutarate

(an intermediate of the tricarboxylic acid cycle). Under hypoxia, HIF1α associates with HIF1β to form a transcription factor that activates genes with a hypoxia response element (HRE), including genes for glycolytic enzymes and glucose transporters in kidney cells [13]. At the submicroscopic level, mitochondria swell and fragment, with decreased ATP production, increased reactive oxygen species, cytochrome C release, and damage to mitochondrial cristae. Numerous animal models of acute renal failure, particularly sepsis, demonstrate decreased ATP levels and mitochondrial dysfunction, leading to loss of mitochondrial respiratory chain proteins in the proximal tubules [14]. Renal ischemia and hypoxia induced by venomous snake bites impair the transport and oxidation of fatty acids in cells. This leads to fatty acid accumulation in the cytoplasm, decreased ATP production, and mitochondrial dysfunction due to impaired respiratory chain activity [15–17]. The kidney is particularly vulnerable to oxidative stress because its lipid membranes contain long-chain polyunsaturated fatty acids. Components of viper venom can cause direct nephrotoxicity as well as

indirect hemolytic and rhabdomyolytic effects. This process occurs in several phases and leads to irreversible kidney damage.

The aim of the study: to determine morphometric changes in the structure of the kidneys of experimental rats under the conditions of administration of the venom of the viper *Vipera berus berus*.

Materials and Methods. We conducted experimental studies on white non-linear male rats. After a 7-day acclimatisation period in the vivarium of the Taras Shevchenko National University of Kyiv, the animals were kept under controlled temperature and lighting conditions. They received a standard diet and water. All procedures complied with the NIH recommendations for the care of laboratory animals and the European Council Directive (86/609/EEC) of November 24, 1986. The study was approved by the Bioethics Commission of the National Scientific Centre "Institute of Biology and Medicine" at the Taras Shevchenko University (protocol No. 2 dated August 19, 2021) and the Bioethics Committee of the M. I. Pirogov National Medical University (protocol No. 4 dated April 1, 2024).

Viper venom (*Vipera berus berus*) was obtained from the V. N. Karazin Kharkiv National University. We stored the freeze-dried native venom at -20 °C and diluted it in saline immediately before the experiment.

We divided the animals into two groups: control and experimental (10 rats each). The experimental group was injected intraperitoneally with *Vipera berus berus* venom at a semi-lethal dose (LD_{50}) in saline, the concentration of which was 1.576 µg/g. The control group received saline only. After 24 h, the rats were anaesthetised and then euthanised by decapitation.

For a full morphometric analysis of the selected parameters, measurements were performed on digital images taken under a microscope of kidney tissue sections stained with eosin and hematoxylin using the classical method, as well as using a specific immunohistochemical technique to detect TNF-α in order to quantitatively assess the manifestations of the immune response in this organ by analyzing the localisation areas of this marker near the epithelial cells of the proximal renal tubules. The number of these expression foci was counted and, thus, the level of acute inflammation in the tubules was quantitatively assessed. To count cells expressing TNF-α, we used the "Cell Counter" plugin in Fiji (ImageJ), into which we loaded digital images of immunohistochemically stained kidney sections. We then statistically processed all data in Excel.

Results. For the morphometric study, we selected digital images of kidney preparations stained with eosin and hematoxylin according to the classical method. With such staining, it is possible to clearly

determine the type of tubules, distinguish their boundaries from the surrounding connective tissue interstitium and find the lumens in their centre, which allows measuring the total area of the tubule and separately the area of the lumen and, by finding the difference between these two indicators, obtain the area of the epithelium itself. For measurements, we selected the image obtained at ×100, since it showed enough complete tubules in the field of view. We measured four randomly selected proximal tubules in five different digital images, yielding a numerical series of 20 indicators sufficient for further statistical analysis.

When we plotted the distribution of our numerical series, it did not match a Gaussian curve, so we used the nonparametric Mann-Whitney test to determine the significance of differences between the experimental groups. According to this test, we found a statistically significant ($p \leq 0.01$) difference between the experimental groups and the control group (Fig. 1).

If, for the control group of animals, the average value of the epithelial area was 8271.15 (first quartile – 7639.25; third quartile – 9109.5), then for the group of animals exposed to the venom of the viper *Vipera berus berus*, it was 11349.95 (first quartile – 10028.5; third quartile – 13383.5). The statistically significant difference between these two groups can be explained by the negative effect of components of *Vipera berus berus* venom on the proximal tubules of nephrons, as confirmed by morphological studies. If, in the control group, the epithelial lining of the tubules showed a classic high cuboidal epithelium with typical eosinophilic cytoplasm, without signs of oedema or rounded nuclei, and the lumens were narrow and free of detritus, then under the influence of the venom this picture changed significantly.

The cells of the proximal convoluted tubules showed the most pronounced pathological changes. The statistically significant increase in their area compared with the control group was influenced, in particular, by oedema, manifested by cytoplasmic lumens and vacuoles that displaced the nuclei to the periphery. In addition, cell size increased due to the accumulation of small eosinophilic granules, characteristic of granular and hydropic dystrophy. The apical surface of the cells was also damaged, which was manifested in the fragmentation of the brush border and could lead to partial detachment of the surface zone of the cytoplasm into the lumen of the tubules, thus reducing them and causing an increase in the total area of the epithelium.

Oedema, the main factor in the increase in cell and component size, affected not only the cytoplasm but also the nuclei, which also had luminal areas.

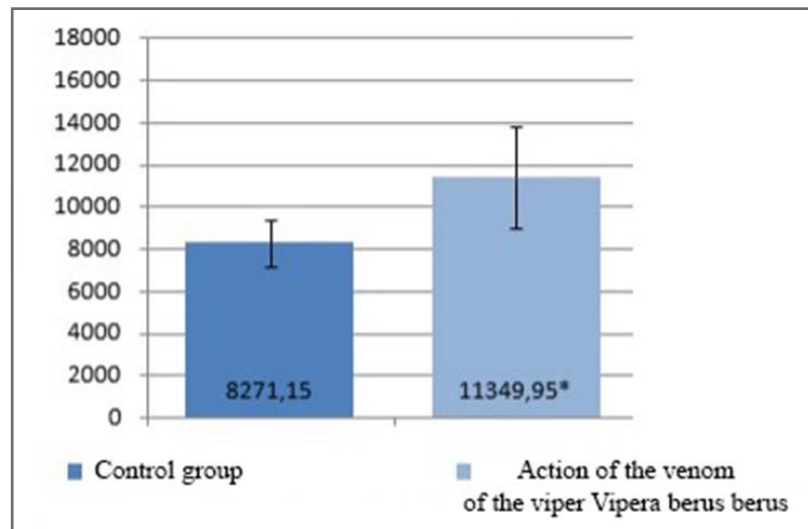


Fig. 1. The average value of the area of the proximal convoluted tubules of the kidney with standard deviation in the control and experimental groups with the introduction of the venom of the viper *Vipera berus berus*.

Note. * – the difference from the control group is significant at $p \leq 0.01$.

Epithelial cell swelling led to a decrease in the size of the tubular lumens, which also affected our calculations, because we calculated the epithelial area as the difference between the total area of the tubule and the area of its lumen. The standard deviation also differed between groups: 1088.3 in controls and 2402.28 in the experimental group. Such an increase indicated uneven manifestations of edematous and other pathological processes in the renal tubules of experimental animals (Fig. 2). If some tubules remained unchanged or had moderate signs of pathology, others, on the contrary, had edematous epithelium and narrowed lumens.

In contrast to the above results, morphometric analysis of the epithelial area of the distal renal tubules demonstrated that in renal tissues exposed to *Vipera berus berus* venom, the epithelial area was smaller than in intact animals (Fig. 3).

To understand such features of changes in the area of the tubular epithelium, it is necessary to consider each group separately and refer to the data of the morphological description of each of them. In the control group, the average area of the distal tubular epithelium was 18178.85 (first quartile – 14662; third quartile – 18439.25), while in the first experimental group – with the introduction of *Vipera berus berus*

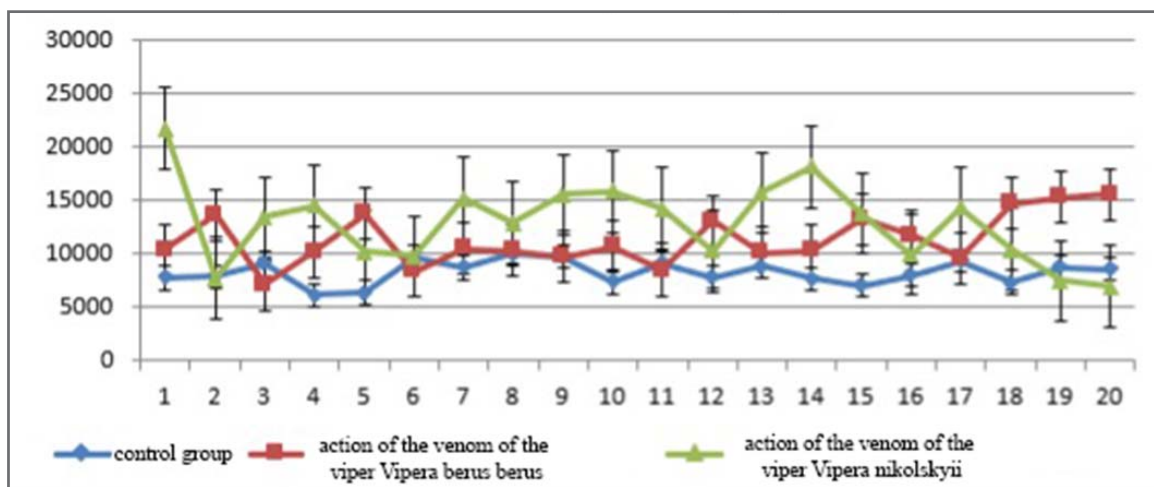


Fig. 2. Individual indicators of the area of the proximal convoluted tubules of the kidney in the control and experimental groups with the introduction of the venom of the viper *Vipera berus berus*.

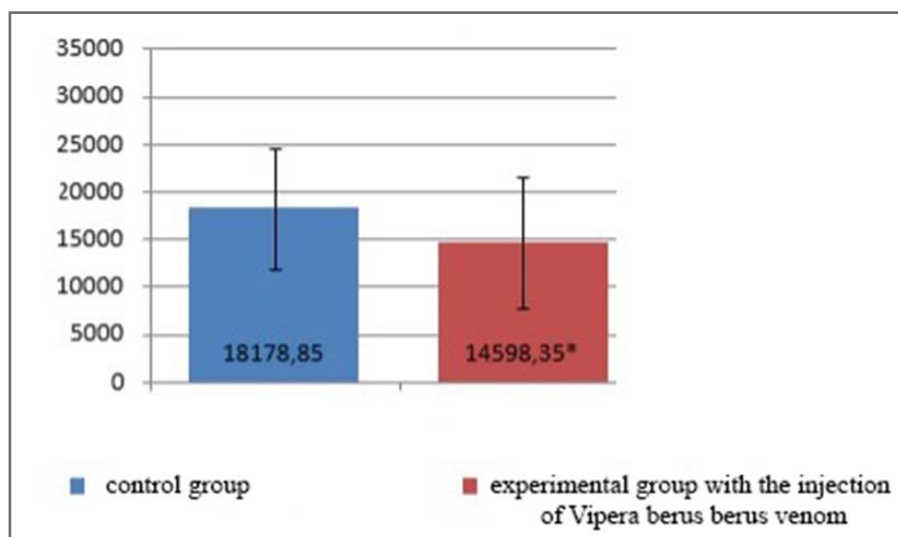


Fig. 3. The average value of the area of distal kidney tubules with standard deviation in the control and experimental groups with the injection of *Vipera berus berus* venom.

Note. * – the difference from the control group is significant at $p \leq 0.01$.

venom – this indicator was significantly lower and amounted to only 14598.35 (first quartile – 10052.75; third quartile – 15890.25). At the same time, the standard deviation was comparable in both groups, at 6402.72 and 6868.73, respectively.

At the same time, the morphological analysis of the distal tubules of the kidney in the control group confirmed the absence of deviations from the normal state: their boundaries were clear, and the lumen was wider than in the proximal ones, as expected. The cytoplasm of the epithelial cells themselves was uniformly eosinophilic in colour, and the nuclei were not shifted to the periphery, which indicated the absence of edematous or dystrophic processes that could affect the area of these cells.

A statistically significant decrease in the area of the distal tubules in the kidneys of animals under conditions of intoxication with the venom of the viper *Vipera berus* can be explained by a number of factors that were described during the morphological study of this organ. As we have already mentioned, our calculations were based on measuring the area of the entire tubule and its lumen. For this experimental group, both a decrease in the first parameter and an increase in the second were characteristic.

Epitheliocytes of the studied tubules became flattened from cubic, and their cytoplasm had no signs of oedema, but there were signs of fine-droplet vacuolization in it, which could not affect the increase in the area of these cells. In turn, the lumens of the distal tubules were often sharply dilated and filled

with hyaline and pigment cylinders. Obturation of the lumens could lead to overstretching if the accumulated mass of cellular detritus and protein cylinders was significant and pressed on the walls.

Thus, due to the combined effect of pathological changes in the epithelial cells themselves, which caused their flattening, and in the surrounding tissues, in particular in the circulatory component, which led to obturation and deformation of the lumens of the tubules, the area of the epithelium in this experimental group decreased statistically significantly compared to the control group.

Discussion. The results of our study generally support the idea of primary damage to the tubular apparatus and allow us to determine its characteristics at the level of individual nephron segments. In contrast to most clinical and pathomorphological studies, which mainly describe tubular changes as degeneration, necrosis, epithelial desquamation, and lumen expansion, we found statistically significant changes in the epithelial areas of the proximal and distal tubules. These results complement traditional descriptive methods by quantitatively assessing nephron structural reorganisation. The results regarding the proximal tubules deserve special attention. In our study, the effect of *Vipera berus berus* venom was associated with a significant increase in the area of the epithelium. We also observed epithelial oedema, cytoplasmic vacuolization, nuclear displacement, granular and hydropic dystrophy, and brush border damage. This morphological profile is

consistent with existing data on snake envenomation-induced tubular injury. The literature reports degeneration and necrosis of epithelial cells in various parts of the tubules; during the hemotoxic action of snake venom, haemoglobin granules can accumulate in proximal tubule cells, and haemoglobin casts can form in the lumen of damaged tubules [18]. The increase in the area of proximal tubular epithelium that we observed contrasts with the typical morphological features seen in clinical biopsies of snake envenomations. Acute tubular necrosis is usually characterised by dilation of the tubules, flattening of the epithelium, desquamation of necrotic cells, and formation of casts in the lumen. However, our experiment showed mainly early or less significant dystrophic-edematous changes, which probably caused the opposite reaction - an increase in the area of epithelial cells due to intracellular oedema. Therefore, the morphometric index may reflect a certain phase of the pathological process, rather than the final state of damage, such as atrophy or necrosis [19]. Interestingly, the morphometric data for the distal tubules are more consistent with descriptions of acute tubular injury in the literature. In animals exposed to *V. berus berus* venom, the area of the distal tubule epithelium was significantly reduced compared with the control group, with morphological features including flattening of the epithelium, expansion of the lumen containing hyaline and pigmented casts. Tubular dilation, flattening of the epithelium, and casts are typical indicators of acute tubular injury, as documented in studies of renal pathology after snakebite [20].

The similarity of our results to the morphological picture described in the literature is particularly important for interpreting changes in the distal tubules. A comprehensive review of the histopathology of the kidneys after snakebites showed that hyaline, granular, and pigmented casts often coexist with dilated tubules lined with flattened epithelium and necrotic, sloughed cells. Therefore, the observation of these features in our experimental group supports the idea that a decrease in the area of the distal tubule

epithelium is a morphometric sign of tubular disorganisation.

Our results are consistent with other studies on renal pathology after viper envenomation. For example, a clinicopathological study of patients with acute renal failure after viper bites revealed that renal biopsy most often reveals tubular necrosis, tubular degeneration, and glomerular changes. A study of 22 biopsies from patients with AKI caused by snakebite showed that 91% of cases had acute tubular necrosis, and almost half of these cases also had acute interstitial nephritis [21]. Thus, our results support the general picture of tubular involvement in renal injury demonstrated here in an experimental model and quantified by morphometric analysis.

Conclusions. Morphometric analysis of changes in the structure of the kidneys of experimental rats under the conditions of administration of the venom of the viper *Vipera berus berus* revealed a statistically significant increase in the area of the epithelium of the proximal convoluted tubules against the background of a sharp decrease in the area of the epithelial cells of the distal tubules. This is explained by oedema and dystrophy of the epithelium of the proximal convoluted tubules of the kidney and the reduction/disappearance of their lumen, on the one hand; and flattening of the epithelial cells, which leads to obturation and deformation of the lumens of the distal tubules, on the other.

Conflict of interest. The authors declare no conflict of interest.

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Authors' contribution. Ahafonov Kostiantyn – literature review, collection of material, analysis of the results obtained, preparation of the text of the article and design of figures.

Prospects for further research. Further studies of morphometric changes in the kidney tissue parameters of rats under the influence of the venom of the viper *Vipera berus berus* and *Vipera berus nikolskii* and the comparative characterisation of changes in these parameters.

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Морфометричні показники змін у структурі нирок експериментальних щурів за умов введення отрути гадюки *Vipera berus berus*

Мета роботи: визначити морфометричні зміни у структурі нирок експериментальних щурів за умов введення отрути гадюки *Vipera berus berus*.

Матеріали і методи. Тварин поділили на дві групи: контрольну та експериментальну – по 10 особин у кожній. Експериментальні щури отримали внутрішньочеревну ін'єкцію напівлетальної дози (LD₅₀) отрути *Vipera berus berus* у фізіологічному розчині в концентрації 1,576 мкг/г. Контрольній групі вводили лише фізіологічний розчин. Через 24 год щурам було проведено евтаназію шляхом декапітації після анестезії. Проводили морфометричне дослідження площі епітелію проксимальних та дистальних канальців.

Результати. Сукупність процесів набряку і дистрофії клітин епітелію проксимальних звивистих каналців нирки та зменшення чи навіть зникнення їх просвіту в експериментальній групі призводили до статистично достовірного зростання їх площі відносно групи контролю. Збільшення показника стандартного відхилення у 2,4 раза свідчить про нерівномірні прояви набрякових та інших патологічних процесів у проксимальних каналцях нирок щурів. Якщо деякі каналці лишались незмінними або мали помірні ознаки патології, інші, навпаки, мали набряклий епітелій та звужені просвіти. Внаслідок сукупного впливу патологічних змін у самих епітеліоцитах дистальних каналців, що викликали їх сплюснення та в навколишніх тканинах, зокрема в кровоносній складовій, які призвели до обтурації та деформації просвітів каналців, площа епітелію в даній експериментальній групі зменшилась статистично достовірно до групи контролю.

Висновки. При морфометричному аналізі змін нирок експериментальних щурів за умов введення отрути гадюки *Vipera berus berus* встановлено статистично достовірне зростання площі епітелію проксимальних звивистих каналців на тлі різкого зменшення площі епітеліоцитів дистальних каналців.

Ключові слова: отрута; гадюки; нирки; морфометрія; запалення; щури.

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