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STRUCTURAL CHANGES IN THE KIDNEYS OF EXPERIMENTAL RATS UNDER CONDITIONS OF ADMINISTRATION OF VIPERA BERUS BERUS VENOM

Ahafonov K.M. ✉ **Structural changes in the kidneys of experimental rats under conditions of administration of Vipera berus berus venom.**
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ABSTRACT. Background. Violation of the hemostasis system, the development of massive bleeding, myonecrosis, dermonecrosis, kidney dysfunction and other manifestations become the causes of disability or lethal consequences of viper bites. The kidneys are organs whose cells require a significant number of mitochondria to eliminate metabolic products from the blood and regulate fluid and electrolyte balance. Direct nephrotoxic or indirect hemolytic and rhabdomyolytic effects of viper venom components, activation of the OS phenomenon, which unfolds in several phases, cause irreversible kidney damage. **Objective.** Study of structural changes in the kidneys of experimental rats under conditions of administration of Vipera berus berus venom. **Methods.** Experimental studies were conducted on 20 male rats, which were intraperitoneally injected with a semi-lethal dose (LD_{50}) (1.576 mg· g^{-1}) of Vipera berus berus venom in saline. Kidney samples from animals of all groups were taken for microscopic examination. Histological preparations of the heart were stained with hematoxylin and eosin, and azan trichrome. **Results and conclusion.** Intoxication with the venom of the viper Vipera berus berus causes the developm ent of acute necrotic nephrosis, which is characterised by a combination of deep parenchymal dystrophy, destruction of the glomerular apparatus and massive hemorrhagic syndrome. The destruction of the histohematological barrier of the kidneys is noted. The venom causes enzymatic lysis of the basement membranes of the glomerular capillaries and Bowman's capsule, and hydropic and granular dystrophy of the epithelium is observed. The vasotoxic effect of the venom manifests as multiple extravasations.

Key words: venom, vipers, kidneys, morphology, glomerular apparatus, rats.

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Introduction

Violations of the hemostasis system, the development of massive bleeding, myonecrosis, dermonecrosis, kidney dysfunction and other manifestations become causes of disability or lethal consequences of viper bites [1-4]. Currently, the development of inflammation and an imbalance in the antioxidant status system under the influence of viper venom toxins has been proven and is actively undergoing comprehensive research [5-8].

The kidneys are among the organs whose cells require a significant number of mitochondria to eliminate metabolic products from the blood and regulate fluid and electrolyte balance. Mitochondria provide energy to perform these important functions and can adapt to different metabolic conditions through a number of signalling pathways (e.g., the mechanistic

target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) pathways, which induce the transcriptional coactivators peroxisome proliferator-activated receptor γ and coactivator 1α (PGC1α), and by balancing mitochondrial dynamics and bioenergetics to maintain homeostasis. Mitochondrial dysfunction leads to reduced ATP synthesis, altered cellular function, and renal structural changes [9-12]. There are mechanisms to maintain mitochondrial function under hypoxia. Oxygen deprivation under hypoxia reduces ATP production and induces cell death. Under normoxic conditions, hypoxia-inducible factor 1α (HIF1α) is degraded in the presence of oxygen and α-ketoglutarate (an intermediate of the glutamate cycle). tricarboxylic acids). However, under hypoxic conditions, HIF1α heterodimerizes with HIF1β to form a transcription factor that binds to the hypoxia response element (HRE) present in genes encoding glycolytic enzymes and glucose transporters in the kidney [13]. At the submicroscopic level, mitochondrial swelling and fragmentation occur, accompanied by decreased ATP synthesis, increased ROS production, cytochrome c release, and destruction of mitochondrial cristae. Decreased ATP levels and mitochondrial dysfunction have been demonstrated in many animal models of ARF, including sepsis, and result in loss of mitochondrial respiratory chain proteins in the proximal tubules [14]. The development of ischemia and hypoxia in the kidneys during bites by venomous snakes and vipers also leads to impaired transport and oxidation of fatty acids in cells of the organ, causing the accumulation of the latter in the cytoplasm, a decrease in ATP production, and mitochondrial dysfunction, due to changes in the functioning of the respiratory chain [15-17].

The kidneys are very sensitive to OS because they contain long chains of polyunsaturated fatty acids in lipid molecules. Direct nephrotoxic or indirect hemolytic, rhabdomyolytic effects of viper venom components, activation of the OS phenomenon, which unfolds in several phases, cause irreversible kidney damage.

The study aims to investigate structural changes in the kidneys of experimental rats under conditions of Vipera berus berus venom administration.

Materials and methods

Experimental studies were conducted on white non-linear male rats. For preliminary acclimatisation, the animals were kept in the animal facility of Taras Shevchenko National University of Kyiv for 7 days, and then in laboratory conditions under controlled temperature and light regimes. Rats received standard food and water. All experiments were conducted in accordance with the Recommendations of the National Institute of Health for the Care and Use of Laboratory Animals and the European Council Directive of November 24, 1986, for the Care and Use of Laboratory Animals (86/609/EEC). The studies were approved and confirmed by the Bioethics Commission of the National Scientific Centre "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv (protocol No. 2 dated 08/19/2021) and the Bioethics Committee of the National Pirogov Memorial Medical University (protocol No. 4 dated April 1, 2024).

The venom of the viper Vipera berus berus was obtained from the V. N. Karazin Kharkiv National University. Lyophilised native venom was stored at -20°C, and then dissolved in saline immediately before the experiment.

The animals were conditionally divided into two groups - control and experimental, 10 individuals each. Experimental rats were intraperitoneally injected with a semi-lethal dose (LD_{50}) (1.576 mg· g^{-1}) of Vipera berus berus venom in saline. Animals of the control group were intraperitoneally injected with only saline. Rats were removed from the experiment 24 hours after exposure to the venom and anaesthetised by decapitation.

For microscopic examination, kidney samples from animals in all groups were collected. 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 the liver were stained with hematoxylin and eosin. For the purpose of morphological assessment of fibrous changes and the structure of the stroma of the studied organ, paraffin sections were stained using the Azan trichrome method. At the initial stage, the sections were stained with azocarmine G, followed by differentiation in aniline-alcohol solution to achieve optimal contrast and selective staining of tissue components. After treatment with phosphomolybdic acid, a final counterstaining with aniline blue was performed to visualise collagen fibres.

Histological preparations were studied using an SEO SCAN light microscope and photodocumented using a Vision CCD Camera with an image output system.

Results and discussion

Histological examination of rat kidneys under the conditions of administration of Vipera berus berus venom revealed marked disorganisation of renal corpuscles. The vascular glomeruli were full-blooded, the capillary loops were dilated, and overflowed with erythrocytes. Some glomeruli showed signs of mesangiolysis (destruction of mesangial structures), making them appear fragmented. The lumen between the inner and outer leaves of the Shumlyansky-Bowman capsule was unevenly dilated. Accumulations of a weakly oxyphilic granular substrate — protein exudate — were visualised in it, indicating impaired permeability of the glomerular filter. Epitheliocytes of the outer leaf of the capsule were characterised by signs of oedema and desquamation. In some fields of view, shrinkage of the vascular glomeruli and a sharp expansion of the urinary spaces were noted.

The most pronounced changes were found in the cells of the proximal convoluted tubules, which is due to their high metabolic activity. Their lumens are lined with a single-layer cuboidal epithelium.

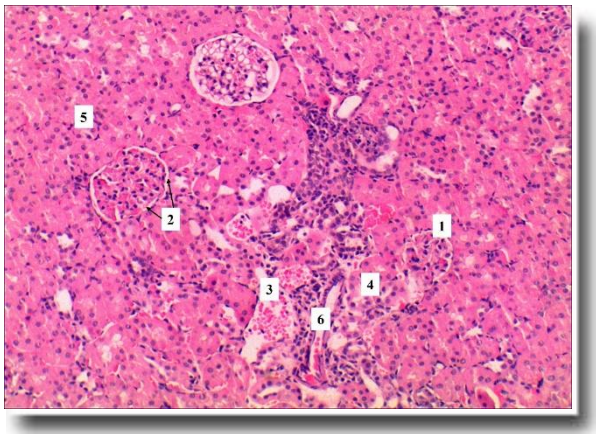

Fig. 1. Morphological changes in rat kidneys under conditions of intoxication with the venom of the viper Vipera berus berus. Shrinkage of the renal corpuscle (1), uneven expansion of the urinary space (2), haemorrhages (3), distal nephron tubules (4), proximal nephron tubules (5), vessel lumen with erythrocyte aggregation (6). Staining with hematoxylin and eosin. ×100.

The cytoplasm of these cells is clear, filled with small eosinophilic granules, which are manifestations of hydropic and granular dystrophy. In many epithelial cells, vacuoles of various sizes are visualised, which shift the nucleus to the periphery. The brush border is either not differentiated or appears fragmented, indicating serious damage to the apical surface of the cells. Mosaic changes were observed in the nuclei of the cells, from hyperchromatosis to karyopycnosis. Anucleate cells are found in the foci of the most intense effect of the poison. Heterochromatin with a marginal position under the karyolemma prevailed in most nuclei; the karyoplasm was clear and swollen. The lumen of the proximal tubules of the nephron was significantly narrowed due to epithelial cell oedema; inside, it contained desquamated cells and single erythrocytes. The interstitial tissue between the tubules was loose and swollen, which caused compression of the peritubular capillaries. The vessels of the cortex and medulla were significantly dilated, filled with erythrocyte aggregates. Single diapedetic haemorrhages in the interstitium were detected. The distal tubules of the nephrons under conditions of intoxication with the venom of the viper Vipera berus berus demonstrated the presence of pronounced pathological changes. The epithelial cells of the distal tubules lost their cubic shape, becoming more flattened. They accumulated a homogeneous eosinophilic substrate - hyaline and pigmented cylinders. The latter had a brown tint, indicating the presence of haemoglobin breakdown products from haemolysis, which led to tubular obstruction and tubular dilatation. The intertubular connective tissue was sharply swollen, which visually pushes the nephron structures apart. In the places of the most intense haemorrhages, focal infiltration by single leukocytes is observed, indicating the beginning of a reactive inflammatory response to necrobiotic changes in the parenchyma.

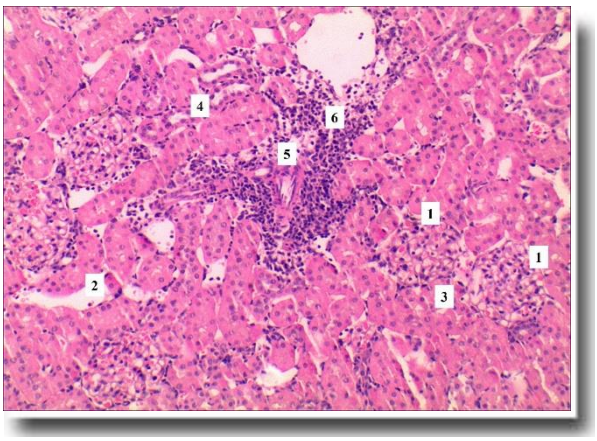

Fig. 2. Microscopic changes in rat kidneys under conditions of intoxication with the venom of the viper Vipera berus berus. Renal corpuscles (1), oedema of the interstitial connective tissue (2), proximal nephron tubules (3), distal nephron tubules (4), vascular lumen (5), lymphoplasmacytic infiltration of interstitial connective tissue components (6). Staining with hematoxylin and eosin. ×100.

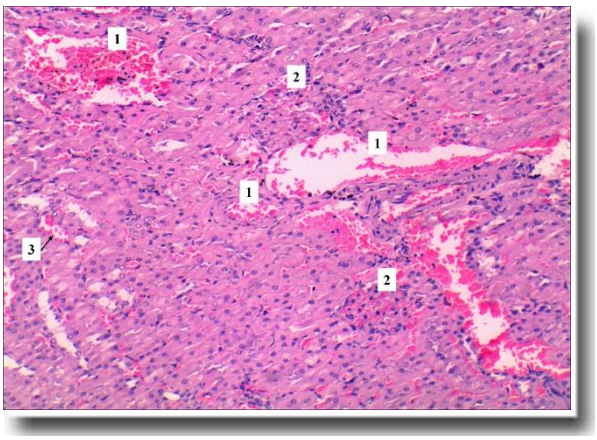

Fig. 3. Histological organisation of rat kidneys under conditions of intoxication with the venom of the viper Vipera berus berus. Blood vessel lumens with full blood and erythrocyte sludge (1), renal corpuscles (2), haemorrhages (3). Staining with hematoxylin and eosin. ×100.

The vessels of the kidneys' microcirculatory beds in experimental rats also underwent profound destructive changes involving all layers of the vascular wall. The endothelial cells of their tunica intima showed signs of oedema; their nuclei were enlarged and hyperchromic, and, in some areas, detachment from the basement membrane (endothelial desquamation) was observed. The walls of the vessels, upon microscopic examination, were homogenised and intensely oxyphilic. Their lumens were unevenly dilated, filled with erythrocyte aggregates, resulting in a sludge phenomenon. A characteristic feature of the action of the Vipera berus berus venom is the pronounced destruction of the capillary network, which leads to the appearance of numerous haemorrhages. Large foci of extravasates are visualised in the interstitium of the cortex and medulla of the kidneys. The most severe haemorrhages are localised around the renal corpuscles and between the convoluted tubules, increasing ischaemia and parenchymal compression.

Staining of rat kidney preparations with azan is critically important for assessing the nephrotoxicity of the venom of the viper Vipera berus berus, as it allows for a detailed study of the condition of the basement membranes and vascular framework of the glomeruli, which are usually the first to undergo destruction under the action of metalloproteinases and phospholipases of the venom. When stained with azan, the basement membranes of the glomerular capillaries, which normally have the appearance of clear blue contours, appear deformed. Uneven thickening or, conversely, ruptures of the basement membranes of the capillary loops were observed.

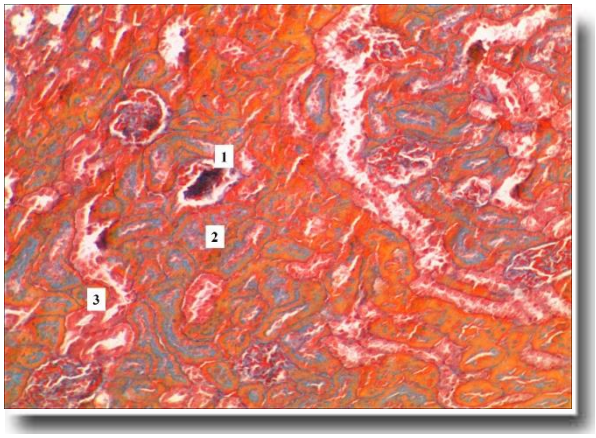

Fig. 4. Morphological features of rat kidneys under conditions of intoxication with the venom of the viper Vipera berus berus. Shrinkage of the renal corpuscle (1), proximal nephron tubules (2), and distal nephron tubules. Staining with azan. ×100.

Blue reticular and collagen fibres are clearly visualised between the nephron tubules, which are separated by edematous fluid. 1 day after intoxication, these fibres appear thin and stretched.

The blue colour of the membranes becomes blurred, indicating disorganisation and increased permeability. The outer leaf of Bowman's capsule was fibrous, in places discontinuous. Fine-grained protein masses stained with azan in a pale blue or greyish colour were found in the urinary space. The most diagnostically significant changes were noted in the nephron tubule system. The contours of the basement membranes of the proximal tubules were blurred, not clear. In areas of pronounced epithelial cell dystrophy, cell detachment from the basement membrane was observed, resulting in subepithelial voids. In the lumen of the distal parts of the nephron tubules, haemoglobin cylinders were noted (a consequence of hemolysis caused by viper venom), which were stained in a rich red or orange colour, contrasting sharply with the blue basement membranes.

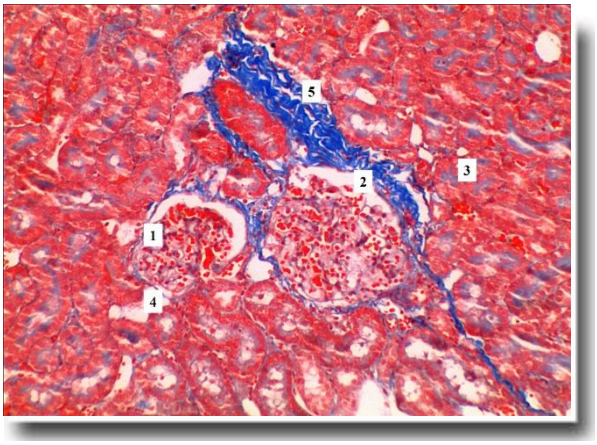

Fig. 5. Microscopic changes in rat kidneys under conditions of intoxication with the venom of the viper Vipera berus berus. Renal corpuscle (1), urinary space (2), proximal nephron tubules (3), distal nephron tubules (4), interstitial connective tissue fibres (5). Azan staining. ×100.

Conclusion

Thus, it was established that intoxication with the venom of the viper Vipera berus berus causes the development of acute necrotic nephrosis, which is characterised by a combination of deep parenchymal dystrophy, destruction of the glomerular apparatus and massive hemorrhagic syndrome. The destruction of the histohematological barrier of the kidneys is noted. The venom causes enzymatic lysis of the basement membranes of the glomerular capillaries and Bowman's capsule, and hydropic and granular dystrophy of the epithelium is observed. The vasotoxic effect of the venom manifests as multiple extravasations.

Prospects for further development are related to the study of histological changes in the kidneys of rats exposed to the venom of another species of viper – Vipera berus nikolskii, and to the comparative characterisation of the effects of these venoms.

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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References

1. Chaiyabutr N, Chanhome L, Vasaruchapong T, Laoungbua P, Khow O, Rungsipipat A, Sitprija V. The pathophysiological effects of Russell's viper (*Daboia siamensis*) venom and its fractions in the isolated perfused rabbit kidney model: A potential role for platelet activating factor. Toxicon X. 2020;7:100046. doi: 10.1016/j.toxcx.2020.100046.

2. Larréché S, Chippaux JP, Chevillard L, Mathé S, Résière D, Siguret V, Mégarbane B. Bleeding and

Thrombosis: Insights into Pathophysiology of *Bothrops* Venom-Related Hemostasis Disorders. Int J Mol Sci. 2021;22(17):9643. doi: 10.3390/ijms22179643.

3. Mora-Obando D, Salazar-Valenzuela D, Pla D, Lomonte B, Guerrero-Vargas JA, Ayerbe S, Calvete JJ. Venom variation in Bothrops asper lineages from North-Western South America. J Proteomics. 2020;229:103945. doi: 10.1016/j.jprot.2020.103945.

4. Park EJ, Choi S, Kim HH, Jung YS. Novel Treatment Strategy for Patients with Venom-Induced Consumptive Coagulopathy from a Pit Viper Bite. Toxins (Basel). 2020;12(5):295. doi: 10.3390/toxins12050295.

5. Adisakwattana P, Chanhome L, Chaiyabutr N, Phuphisut O, Onrapak R, Venom-gland transcriptomics of the Malayan pit viper (*Calloselasma rhodostoma*) for identification, classification, and characterization of venom proteins. Heliyon. 2023;9(5): e15476. doi: 10.1016/j.heliyon.2023.e15476.

6. AlShammari AK, Abd El-Aziz TM, Al-Sabi A. Snake Venom: A Promising Source of Neurotoxins Targeting Voltage-Gated Potassium Channels. Toxins (Basel). 2023;16(1):12. doi: 10.3390/toxins16010012.

7. Frangieh J, Rima M, Fajloun Z, Henrion D, Sabatier JM, Legros C, Mattei C. Snake Venom Components: Tools and Cures to Target Cardiovascular Diseases. Molecules. 2021;26(8):2223. doi: 10.3390/molecules26082223.

8. Siigur J, Siigur E. Biochemistry and toxicology of proteins and peptides purified from the venom of Vipera berus berus. Toxicon X. 2022;15:100131. doi: 10.1016/j.toxcx.2022.100131.

9. Albert V, Hall MN. mTOR signaling in cellular and organismal energetics. Curr Opin Cell Biol. 2015;33:55-66. doi: 10.1016/j.ceb.2014.12.001.

10. Chun Y, Kim J. AMPK-mTOR Signaling and Cellular Adaptations in Hypoxia. Int J Mol Sci. 2021;22(18):9765. doi: 10.3390/ijms22189765.

11. Fantus D, Rogers NM, Grahammer F, Huber TB, Thomson AW. Roles of mTOR complexes in the kidney: implications for renal disease and transplantation. Nat Rev Nephrol. 2016;12(10):587-609. doi: 10.1038/nrneph.2016.108.

12. Toyama EQ, Herzig S, Courchet J, Lewis TL Jr, Losón OC, Hellberg K, Shaw RJ. AMP-activated protein kinase mediates mitochondrial fission in response to energy stress. Science. 2016;351(6270): 275-81. doi: 10.1126/science.aab4138.

13. Zhang H, Xu R, Wang Z. Contribution of Oxidative Stress to HIF-1-Mediated Profibrotic Changes during the Kidney Damage. Oxid Med Cell Longev. 2021;2021:6114132. doi: 10.1155/2021/6114132.

14. Palikaras K, Tavernarakis N. Mitochondrial homeostasis: the interplay between mitophagy and mitochondrial biogenesis. Exp Gerontol. 2014;56:182-8. doi: 10.1016/j.exger.2014.01.021.

15. Singh JP, Singh AP, Bhatti R. Explicit role of peroxisome proliferator-activated receptor gamma in gallic acid-mediated protection against ischemia-reperfusion-induced acute kidney injury in rats. J Surg Res. 2014;187(2):631-9. doi: 10.1016/j.jss.2013.11.1088.

16. Whitaker RM, Corum D, Beeson CC, Schnellmann RG. Mitochondrial Biogenesis as a Pharmacological Target: A New Approach to Acute and Chronic Diseases. Annu Rev Pharmacol Toxicol. 2016;56:229-49. doi: 10.1146/annurev-pharmtox-010715-103155.

17. Zhang J, Pan W, Zhang Y, Tan M, Yin Y, Li Y, Li H. Comprehensive overview of Nrf2-related epigenetic regulations involved in ischemia-reperfusion injury. Theranostics. 2022;12(15):6626-45. doi: 10.7150/thno.77243.

Агафонов К.М. Структурні зміни нирок експериментальних щурів за умов введення отрути гадюк *Vipera berus berus.*

Вінницький національний медичний університет імені М.І. Пирогова, Вінниця, Україна.

РЕФЕРАТ. Актуальність. Порушення системи гемостазу, розвиток масивних кровотеч, міонекроз, дермонекроз, розлади функціонування нирок та інші прояви стають причинами інвалідності чи летальних наслідків укусів гадюк. Нирки належать до органів, клітини яких потребують значної кількості мітохондрій для елімінації продуктів обміну із крові та регулювання балансу рідини і електролітів. Пряма нефротоксична чи непряма гемолітична, рабдоміолітична дія компонентів отрути гадюк, активація феномену ОС, який розгортається в декілька фаз, зумовлюють незворотне пошкодження нирок. **Мета.** Вивчення структурних змін нирок експериментальних щурів за умов введення отрути гадюк Vipera berus berus. **Методи.** Експериментальні дослідження проводили на 20 щурах-самцях, яким внутрішньоочеревинно вводили напівлетальну дозу (LD50) (1.576 мг· г-1) отрути Vipera berus berus на фізіологічному розчині. Для мікроскопічного дослідження забирали зразки нирок тварин всіх груп. Фарбування гістологічних препаратів серця здійснювали гематоксиліном та еозином, азан трихром. **Результати та підсумок.** Інтоксикація отрутою гадюки Vipera berus berus зумовлює розвиток гострого некротичного нефрозу, який характеризується поєднанням глибокої паренхіматозної дистрофії, деструкції гломерулярного апарату та масивного геморагічного синдрому. Відмічається руйнування гістогематичного бар'єру нирок. Отрута викликає ферментативний лізис базальних мембран капілярів клубочків та капсули Боумена, спостерігається гідропічна та зерниста дистрофія епітелію. Вазотоксична дія отрути проявляється множинними екстравазатами.

Ключові слова: отрута, гадюки, нирки, морфологія, гломерулярний апарат, щури.