

Complex Phytochemical Preparation (*Herba centaurii*, *Radix levistici* and *Folia rosmarini*) Protects Kidney during Glomerulonephritis

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Abstract.

Chronic kidney disease (CKD) is a global problem all over the world. Special attention is paid to the action of natural compounds. This research was aimed to investigate the effect of complex phytochemical preparation (*Herba centaurii*, *Radix levistici* and *Folia rosmarini*) on dynamics of morphological changes in kidney tissue in animals with glomerulonephritis. We found a positive dynamics in the structure of the nephron, which allows us to conclude that its nephroprotective effect is expressed.

Key words: phytochemical composition, kidney, cardiovascular disease, rat, biochemical parameters.

INTRODUCTION

Chronic kidney disease (CKD) is a global problem all over the world, presenting itself a high economic costs for the health system and associated with a high risk of developing cardiovascular diseases (CVD). Timely and early diagnosis at potentially reversible stages of the disease, maximum long-term preservation of kidney function, postponement of complications, improvement of patients' quality of life are the priority directions in the work of a nephrologist, cardiologist, and therapist^{1,2}.

At present, with the introduction of evidence-based medicine, we can predict the effect of the use of a number of nephroprotective drugs in complex therapy of the disease. Their choice for today is great, but interest in nephroprotective drugs of plant origin does not decrease³⁻⁹. The highest requirements for the production of phytopreparations at all stages and the rational approach to phytotherapy, modern scientific and technical achievements of the pharmaceutical industry have become part of the concept of phyto-engineering (phytoneering) - the priority direction of the work of a number of companies, including Bionorica (Germany). The company developed and produced phytochemical preparation Kanefron® (*Herba centaurii*), lovage (*Radix levistici*), rosmarin (*Folia rosmarini*), widely used in urology and nephrology. The high degree of safety, excellent drug tolerance, the possibility of prolonged use without side effects is confirmed by experimental and clinical studies^{10,11,12}. However, in the available scientific and patent literature, we did not find data on the morphological confirmation of the effectiveness of this phytopreparation in glomerular and tubulo-interstitial kidney damage. The existing methods of assessing the effectiveness of the drug are based only on clinical and laboratory criteria. No morphological studies have been conducted and, accordingly, there is no data on the dynamics of structural and ultrastructural changes in renal tissue in complex therapy of glomerulonephritis with the use of Kanefron®.

The purpose of our research work was to study the nephroprotective effect of the drug Kanefron® as part of complex therapy in the experiment, using an objective status assessment, laboratory data, comparative dynamics of morphological changes in kidney tissue in animals with glomerulonephritis.

MATERIALS AND METHODS

The experiment was performed on 33 animals - male and female outbred albino rats of four months of age contained in standard vivarium conditions: content of fine plastic cages with wood chips, no more than 10 per cell. Diet and drinking regime vivarium was a standard one. All experiments were carried out in accordance with the "Rules for carrying out work using experimental animals" (Appendix to the Order of the Ministry of Health of the USSR of 12.08.1977 N 755) and "European Convention for the Protection of Vertebrate Animals Used for Experiments or Other Scientific Purposes" from March 18, 1986. Immune-complex glomerulonephritis was reproduced according to the original patented procedure "Method of simulation of chronic glomerulonephritis in experiment" Russian patent #2383063 of November 19, 2008 registered in the State Register of Inventions of the Russian Federation in 27.02.2010 (by Kolomeets N.Y., Averyanov N.I. Kosarev P.V.).

At all stages of the experiment, the objective status of the animals was evaluated (phenotype, general motor activity, food and drink requirements, body weight was determined twice a week), as well as renal excretion by the number of spontaneous urination per day and proteinuria in a single urine sample, using a qualitative reaction with 20% sulfosalicylic acid. Blood sampling for biochemical analysis was performed by the method of intravital puncture of the cardiac bag, in the morning on an empty stomach. The total protein, urea, creatinine, cholesterol, alkaline phosphatase (PH), phosphorus, potassium, activity of alanine aminotransferase (ALT), aspartate aminotransferase (ACT) were determined on the StatFax

1904+ biochemical analyzer using a set of Spinreact (USA) reagents.

At the end of the experiment, the animals were removed from the experiment in compliance with the rules of euthanasia (euthanasia was performed under ether anesthesia in accordance with the requirements of the Geneva Convention "International Guiding Principles for Biomedical Research Involving Animals", 1990). Internal organs were collected for histological examination. The material was fixed in Lilly buffered formalin (pH 7.2), subjected to dehydration in alcohols of increasing strength, and placed into histamix. Sections were obtained on a rotary microtome (3-4 μm thick), stained with hematoxylin and eosin, pyrofuxin by Van Gieson, Mason, Foot silver nitrate staining, and a periodic acid Schiff (PAS) reaction were performed. Microscopy of the preparations in transmitted light was carried out using a microscope Micros MC 50 (Austria) with an increase of $\times 60$, $\times 150$, $\times 600$, $\times 1500$. The histological images were captured using a CAM V200 Micros microscope camera "Handelsgesellschaft m.b.H." (Austria).

Morphometric analysis of the obtained images was carried out using specialized software for medicine and biology BioVision, version 4.0 (Austria). To ensure the reliability of the data, a series of duplicate measurements was used to determine each parameter, in at least 10 fields of view of the microscope in each histological specimen. The following parameters were analyzed: the thickness of the parietal leaf of the Shumlyansky-Bowman capsule, the area of the glomerulus with the capsule, the urinary space on the sections passing through the maximum diameter of the renal corpuscle, the area of the capillary loops with the interstitial space. For electron microscopic examination, the tissue was subjected to double fixation in a buffered mixture of glutaraldehyde and paraformaldehyde according to Karnovsky, after being doped with 1% solution of osmium tetroxide, dehydrated and poured into epon-812 (Karnovsky, M.J., J. Cell Biol., 27, 137A (1965) Hayat, MA Principles & Tech., Of EM (Univ. Park Press, 68 (1981)). Semi-thin sections from epoxy blocks were stained with toluidine blue and basic fuchsine (Spurlock B.O. et al 1996), methylene blue and viewed in a light microscope. Ultrathin sections were contrasted with lead uranyl acetate and lead citrate (Reynolds J., 1967). The contrasted samples in the ranges of electron-optical magnifications 1250-20000X were viewed on a Zeiss Libra 120 EFTEM electron microscope equipped with a digital SSCCD camera Gatan UltraScan 1000. In the course of the experiment, 3 groups were formed to create a model of immune-complex glomerulonephritis: Group I - control (intact) animals, n = 7; Group II - animals of the same litter immunized with a suspension of the renal antigen obtained from the maternal kidney, n = 7; III group - the offspring of Group II animals immunized with nephrotoxic serum obtained from parents (group II), n = 19.

To produce nephrotoxic serum, a 250.4 g female white mongrel rat was taken. Immediately after decapitation, the kidneys were perfused *in situ* with a sterile cooled medium 199 until a uniform gray-brown color was obtained, cut off from the renal pedicle, and

transferred to a sterile tray. All subsequent manipulations were performed at a temperature of $+40^{\circ}\text{C}$. Separating from the capsule, the kidneys were cut sagittally, the cortical layer was isolated (the kidney mass was 1.780 mg, the mass of the cortical layer was 940 mg). The cortex was suspended in a sterile mortar, further crushed in an ultrasonic disintegrator "USDI-2T", at 44 MHz, for 10 minutes, then the suspension was transferred to a centrifugal cup, adding a cooled saline solution to 10 ml and a merthiolate 1 mg as an antiseptic. The resulting suspension was centrifuged cold 3 times during 3 minutes at 2000 rpm, each time removing the supernatant and bringing the suspension to 10 ml with a cooled saline solution. The suspension was then centrifuged at 5000 rpm for 10 minutes. The sediment was transferred to a sterile foil and weighed on a torsion balance. The weight of the suspension was 540 mg. The resulting precipitate consisted predominantly of glomeruli, which were well visualized by microscopy of unstained preparations. To prepare the antigen, a complete Freund's adjuvant (Disko-lab, USA) was added to the suspension pellet at a rate of 1.0 ml per 20 mg of precipitate. The resulting weighted antigenic suspension of the cortical layer of the kidney of the rat was stored at a temperature of 0°C to $+4^{\circ}\text{C}$. The antigen produced in this way was immunized with the progeny of group II rats (1st generation), based on 100 μl of the antigenic suspension obtained per 100 g of body weight of the animal, as follows: 3 times intraperitoneally once a day with an interval between immunizations of 48 hours. Repeated immunization was performed once, after 3 weeks from the time of the first administration of the antigenic suspension. After 30 days from the start of immunization, these animals were excised from the experiment by cutting the spinal cord under ether anesthesia in compliance with the rules of euthanasia. Serum was taken from the heart cavities immediately after the biological death of the animal, centrifuged at 3000 rpm for 10 minutes until a clear liquid was obtained. The resulting nephrotoxic serum was stored for a further 2 days at a temperature of $+4^{\circ}\text{C}$. Then the animals of the group III (II generation) were immunized, in order to form an immune-complex glomerulonephritis, according to the following scheme: 2 times intraperitoneally with an interval of 24 hours, at the rate of 100 μl of nephrotoxic serum per 100 g of body weight of the animal.

RESULTS AND DISCUSSION

Observation of the animals for 14 days (before the start of treatment) showed that all animals of the control (intact) group were marked with active behavior, had a stable weekly weight gain (for the entire period of the experiment the increase was 80.9 ± 0.56 g), exhibited interest to food and drink. They had shiny eyes, wool, visible mucous were moist and pink. The number of urinations per day averaged 5-6 times. There were no laboratory signs of the disease. In animals immunized with nephrotoxic serum, changes in behavior were observed at the end of the first week after immunization: a decrease in overall motor activity was noted in 40%, by the end of the second week adynamia was observed in all animals. The

wool of animals became dull, tousled, with traces of excrement, they did not take care of themselves. Visible mucous membranes were dry, pale; eyes were dull. The need for food was decreased (they leaved the volume of food after serving a portion), but the need for liquid was increased. The weight gain was 36.7 ± 0.42 g ($p < 0.001$). The total number of urination was reduced to 2-4 times a day, by 14 days from the moment of immunization in single urine samples, proteinuria was determined in all animals. In the blood serum, a significant increase in the level of creatinine was observed 189.90 ± 14.72 $\mu\text{mol/l}$, ($p < 0.06$), a decrease in the total protein content 53.63 ± 0.86 g/l ($p < 0.06$). For the purpose of morphological confirmation of the presence of immune-complex glomerulonephritis in animals, 3 animals of group III were withdrawn from the experiment by random sampling. Control was an autopsy material of the kidneys of 3 intact rats. Histologically examination of the kidneys of intact animals revealed a capsule, a cortical substance with renal corpuscles and convoluted tubules, as well as a brain substance. Brain rays, deepen into the cortical substance of the kidney, papilla and renal pelvis lined with a transitional epithelium were well visualized. The straight tubules in the brain substance and the collecting tubes were without special features. The vascular glomeruli were unchanged, the urinary spaces were free, the Shumlyansky-Bowman capsule was not thickened. Between the renal corpuscles were proximal and distal renal tubules with a typical structure. The walls of the vessels had a typical structure, not thickened, the endothelium is represented by a single layer of flat cells lying on the basal membrane. As shown by the results of our morphometric, electron microscopic studies, the kidneys of control (intact) white rats had a typical structure, no pathological changes were detected.

When studying the kidneys of immunized animals, the fullness of the peritubular capillaries, arched and interlobular vessels was revealed. More than 80% of the enlarged glomeruli showed diffuse mesangial proliferation with a significant decrease in the urinary space, an increase in the area of the capillary lumen, edema and thickening of the parietal leaf of the Shumlyansky-Bowman capsule by the appearance of adhesions between the capillary loops and the capsule, in some cases with the formation of cellular crescents, difference ($p < 0.05$) with an intact group of animals [Table 1].

When the kidney tissue was stained with picrofuchsin according to Van Gieson-Masson, we also noted the plethora of capillaries of the vascular glomeruli, the presence of capillary loop adhesions with the parietal leaf of the Shumlyansky-Bowman capsule, which caused the glomerularity of the glomeruli, the collagenization of the parietal leaflet of the glomerulus capsule, significant narrowing of the urinary space, and the proliferation of mesangial cells. When the kidney tissue was stained with silver nitrate according to Foot, the destruction and deformation of the capillary walls were observed in the part of the glomeruli with the formation of thrombi in the capillary lumens, spines on the glomerular basement membrane were detected, the thickening of the basal membrane of both the parietal and visceral sheets of the

Shumlyansky-Bowman capsule, the basal membrane Visceral leaf had a two-contour form. In the interstices and tubules of the kidneys there was edema of the tissues, foci of inflammation and sclerosis. Cortical matter in the proximal and distal tubules of the kidneys revealed granular and hydropic degeneration of epithelial cells, and desquamation into the lumen of the tubules was detected. In the brain, the peritubular capillaries are enlarged and full-blooded. Epithelium of tubular structure was without dystrophic changes.

Using electron microscopy of kidney preparations of animals with immune-complex glomerulonephritis, we found changes in the glomeruli; leukocytes and sub-endothelially located immune complexes were visualized in the capillary lumens [Fig. 1]. The lumens of the glomerular capillaries were of different sizes, full-blooded, and in a number of cases it were obliterated.

In the cytoplasm of the tubular epitheliocytes, numerous large, interconnected, electron-dense inclusions were present, which had a non-homogeneous structure when contrasting. When performing ESI-spectroscopy, it was revealed that inclusions did not contain Fe, therefore, they were not iron-containing hemoglobin derivatives. According to the peculiarities of the binding of contrast agents, the inclusion data corresponded to lipids to a greater degree. In the epithelial cells of the proximal tubules, deformation of the microvilli of the brush border on the apical surface was noted [Fig. 2]. In the interstitium, single plasma cells, tubular necrosis with initial interstitial fibrosis phenomena (newly formed collagen fibers among dead tubules) were detected [Fig. 3].

Thus, at the morphological level, the presence of immune-complex glomerulonephritis was confirmed, which allowed us to extrapolate the results obtained to the entire III group of animals immunized with nephrotoxic serum.

In order to study the effectiveness of Kanefron® H, 3 subgroups were formed from the 16 remaining animals in this group. Control was presented by 3 healthy rats, which during the whole experiment were kept in standard conditions of the vivarium; food ration and drinking regime were also standard. The I-st subgroup consisted of immune-complex glomerulonephritis, without treatment, $n = 5$; II-nd subgroup – immune-complex glomerulonephritis, treatment with prednisolone (Akrihin HFC JSC, Russia), $n = 5$; III-rd subgroup – immune-complex glomerulonephritis, treatment with prednisolone + Kanefron® (Bionoric AG, Germany), $n = 6$.

Experimental therapy was started 14 days from the last day of immunization with nephrotoxic serum and was carried out for 10 days. Prednisolone was administered at a dose of 2.88 mg / kg, Kanefron® H - 1 drop orally once a day (the conversion factor from the human dose to the dose of the experimental animal 0.018 (Volchegorsky IA, Dolgushin II, Kolesnikov O.L. , Tseylikman V.E. // Experimental modeling and laboratory evaluation of adaptive reactions of the organism. - Chelyabinsk, 2000.- 167 pp.)). In all animals that did not receive treatment, by the end of the experiment, a sharp adynamia was observed, there was no interest to food, only a small amount of

portioned food was eaten, the need for liquid was decreased. The animals did not arrive at all in bulk, they gave off an unpleasant smell, the hair was dull, with traces of excrement, visible mucous were pale and dry. The number of spontaneous urination decreased to 1-2 times a day, proteinuria was determined in all animals.

Animals with immune-complex glomerulonephritis who received both prednisolone and prednisolone with Kanefron® H had a more lively behavior at the end of the treatment: general motor activity increased, there was a constant interest to food, but the need for liquid was higher than usual in the group of animals that received only prednisolone. In contrast to the animals that received prednisolone and Kanefron®, for which the need for a liquid was within the normal drinking regime. The animal's fur remained dull, disheveled, but the rats began to take care of themselves. Visible mucous membranes were pale and moist. By the end of therapy, the number of spontaneous urination in animals treated with prednisolone was 5-6 times a day, proteinuria in single urine samples was determined in 3 animals, and in animals treated with prednisolone and Kanefron® 7-8 times a day and proteinuria were determined only in one animal. In the blood serum, the creatinine concentration still remained significantly high in both subgroups ($102.44 \pm 9.06 \mu\text{mol/L}$, $98.38 \pm 7.12 \mu\text{mol/L}$, $p < 0.06$), total protein content in serum of animals of both subgroups tended to increase and did not have significant differences from the indices of animals of the intact group ($68.52 \pm 0.31 \text{ g/l}$, 70.12 ± 2.5 , $p > 0.06$) (Table 2).

A light-optical study of the structure of the half-thin sections of the kidneys of animals treated with prednisolone showed that 60% of the glomeruli had practically restored their structure, while the others showed changes (preserved plethora of capillaries, pronounced lobularity, double-contour of the basal membrane of the visceral leaf of the Shumlyansky-Bowman capsule). The visual decrease of the urinary space and the distinct collagenization of the capsule of the glomeruli were not noted. In animals treated with prednisolone and Kanefron®, the structure was restored to almost 70% of the glomeruli.

In animals of both subgroups, the cortical substance was visualized with the remaining fullness of the peritubular capillaries; granular and hydropic degeneration of the epithelial cells of the proximal convoluted tubules was detected. The tubular structures of the medulla were not changed. In the interstitial tissue, inflammatory changes were absent. However, in most preparations of animals treated with prednisolone alone, the fullness of the arched and interlobular vessels was preserved, in contrast to the animals treated with prednisolone and Kanefron®, which showed little vascular adherence.

According to the morphometric analysis in the group of animals treated only with prednisolone, the

thickness of the parietal capsule ($1.709 \pm 0.045 \mu\text{m}$, $p < 0.05$), the cross-sectional area of the glomerulus with the Shumlyansky-Bowman capsule ($6260.09 \pm 302.5 \mu\text{m}^2$, $p < 0.05$), the lumen of the capillaries in the glomerulus ($4,961.4 \pm 98.11 \mu\text{m}^2$, $p < 0.05$) remained significantly higher than the indices of intact animals. The area of the urinary space in the renal corpuscle, its values had no significant differences ($1\ 631.01 \pm 250.7 \mu\text{m}^2$, $p > 0.05$) from the indices of the area of animals of the intact group.

In the group of animals treated with prednisolone and Kanefron®H, the thickness of the parietal leaf of the Shumlyansky-Bowman capsule ($1.522 \pm 0.048 \mu\text{m}$, $p > 0.05$), the cross-sectional area of the glomerulus with the Shumlyansky-Bowman capsule ($6,285.3 \pm 695, 2 \mu\text{m}^2$, $p > 0.05$) were higher and had a significant difference from the indices of the animals in the intact group. The luminal area of the capillaries in the glomerulus ($3,996.8 \pm 380.2 \mu\text{m}^2$, $p > 0.05$) and the urinary space in the renal corpuscle ($1\ 112.5 \pm 286.1 \mu\text{m}^2$, $p > 0.05$) had no significant differences from Indices of intact animals (Table 1).

In the electron microscopic study of ultrathin sections of the kidneys of animals treated with prednisolone, the lumens of the glomerular capillaries remained uneven, the capillaries showed open, sometimes greatly expanded, and with a narrowed or closed lumen, and in certain glomeruli subendothelial sites immune-complex deposits (Fig. 4). In animals treated with prednisolone and Kanefron®H, glomeruli with a normal structure prevailed, pathological inclusions and immune complexes were not visualized [Fig. 5].

In the animals of both subgroups, dystrophic changes in the epithelium of the proximal convoluted tubules persisted, thereby confirming the primary significant damage to the tubulo-interstitial tissue, which can be observed simultaneously with the glomerular and significantly affect the clinical course of the disease. In the epithelial cells of the tubules, expansion of intercellular spaces, an increase in the height of the microvilli of the brush border on the apical part and their destruction were noted. Inclusions of different electron density were visualized in the cytoplasm. The number of mitochondria in the apical zone was reduced. Necrotic masses were detected in the lumens of individual tubules [Fig. 6, 7].

Considering the precise mechanisms of the action of this phytochemical preparation, a few speculations may be raised. First, it is possible to suggest that the used plants - *Centáurium erythraea*, *Levisticum officinale* and *Rosmarinus officinalis* – are plants with increased content of silicon. The former may restore nephron at molecular level that were previously reported for some bacteria^{13,14}. Also, there is a possibility to act at very low doses like some insecticides¹⁵ that also should be clarified in future studies. Moreover, it is possible to suggest that these plants acting via modulation of neurotransmission to reduce inflammation¹⁶.

Table 1. Morphometric parameters of the renal corpuscle of the kidneys of laboratory animals (* $p < 0.05$ compared to control (intact) animals).

Structure	Parameter	Control, n=3 (M±SD)	Immuno-complex GN, n=3 (M±SD)	Treatment with prednisolone alone, n=5 (M±SD)	Treatment with prednisolone plus Kanefron®, n=6 (M±SD)
Renal body	thickness of the parietal leaf of the Shumlyansky-Bowman capsule, mcm	0,437 ±0,088	1,873±0,031*	1,709±0,045*	1,522±0,048*
	The area of the cross-section of the glomerulus with the Shumlyansky-Bowman capsule mcm ²	5 394,8±839,3	7 489,01±512,2*	6 260,09±302,5*	6 285,3±695,2*
	The area of the urinary space in the renal corpuscle, mcm ²	1 565,6±344,1	931,04±119,5*	1 631,01±250,7	1 112,5±286,1
	The area of the lumen of capillaries in the glomerulus, mcm ²	3 872,1±584	5 161,4±138,1*	4 961,4±98,11*	3 996,8±380,2

Table 2. Biochemical parameters of blood serum of laboratory animals on day 10 of the experimental therapy (* $p < 0.05$ compared to control (intact) animals).

Parameter	Control, (M±SD)	Immuno-complex GN, (M±SD)	Treatment with prednisolone alone, (M±SD)	Treatment with prednisolone plus Kanefron®, (M±SD)
Total protein, g/L	71,46±1,63	53,63±0,86*	68,52±0,31	70,12±2,5
Urea, mmol/L	4,67±0,14	4,78±0,51	4,31±0,34	4,88±0,11
Creatinine, mmol/L	59,93±2,32	189,90±14,72*	102,44±9,06*	98,38±7,12*
Cholesterol, mmol/L	2,04±0,09	2,01±0,28	3,51±0,1	2,9±0,23
Alkaline phosphatase, UI/L	290,83±32,29	363,66±31,81	346,31±21,1	321,34±34,9
Alanine aminotransferase, UI/L	91,65±12,06	175,0±5,51*	117,01±4,13	120,34±3,1
Aspartate aminotransferase, UI/L	142,83±7,53	314,5±11,7*	208,01±9,5*	214,67±5,3*
Phosphorus, mmol/L	2,53±0,15	2,35±0,15	2,98±0,3	2,34±1,2
Potassium, mmol/L	4,45±0,16	4,72±0,51	4,96±0,24	4,24±0,13

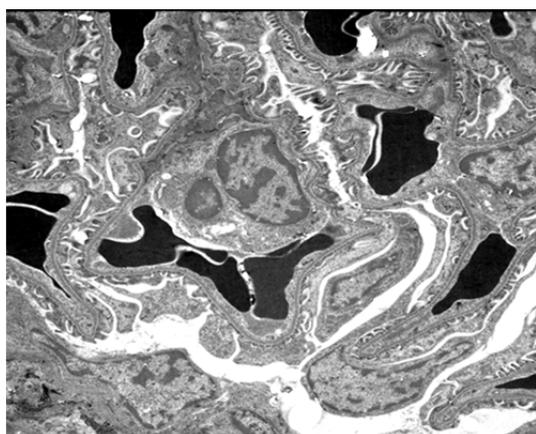


Fig. 1. Kidney of animal with immuno-complex GN: a fragment of a vascular glomerulus. In the lumen of the capillary is visualized located subendothelial immune complex (x1260).

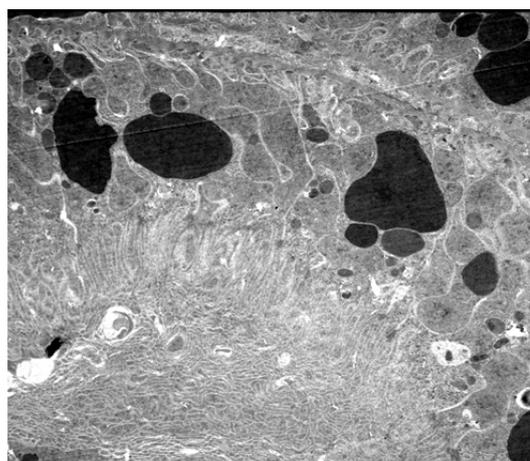


Fig. 2. Kidney of the animal with immuno-complex GN: proximal tubule with brush border. In the cytoplasm of epithelial cells there are large lipid inclusions (x1575).

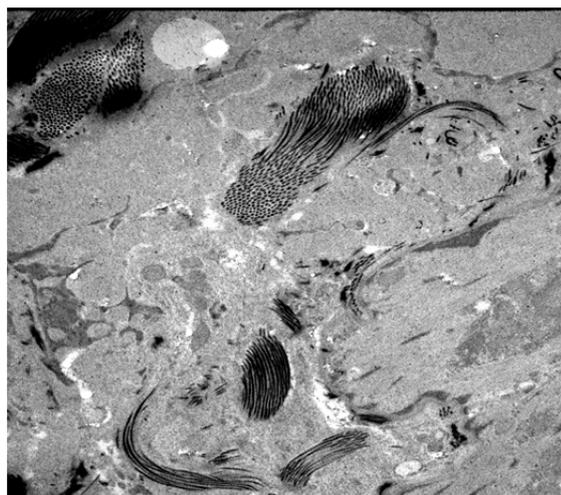


Fig. 3. Kidney of the animal with immuno-complex GN: tubular necrosis, newly formed collagen fibers in the interstices (x2520).

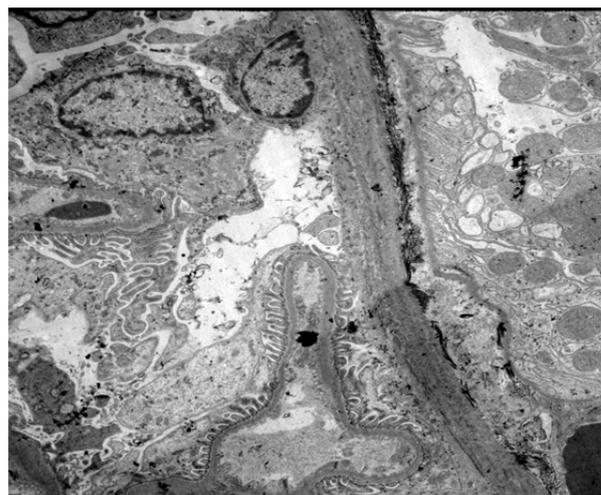


Fig. 6. Kidney of the animal with immuno-complex GN, treatment with prednisolone: a fragment of the glomerulus without changes. To the right side, there is visualized the basal part of epitheliocyte proximal tubule with degenerative changes in the cytoplasm (x1260).

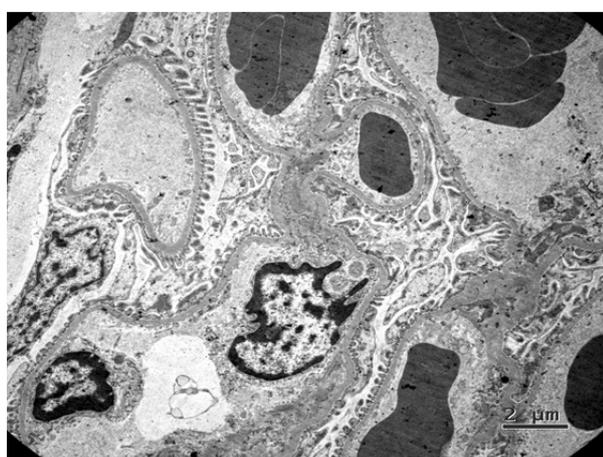


Fig.4 Kidney of the animal with immuno-complex GN, treatment with prednisolone: a fragment of the glomerulus. To the right side, there are rendered immune complex deposits (x2520).

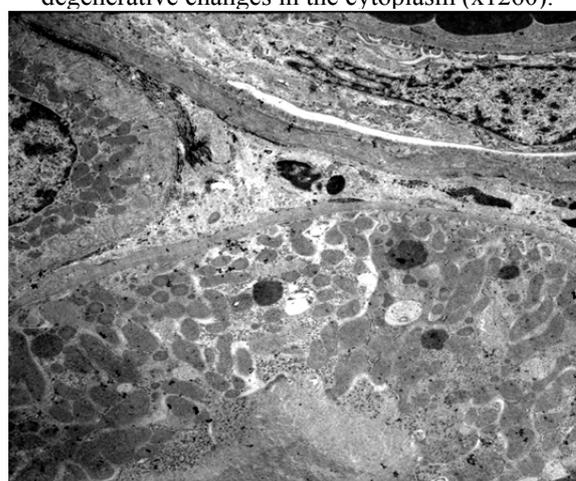


Fig. 7. Kidney of the animal with immuno-complex GN, treatment with prednisolone and Kanefron®H: epithelioid tubule with the destruction of the brush edges and a separate (small) lipid inclusions in the cytoplasm (x1260).

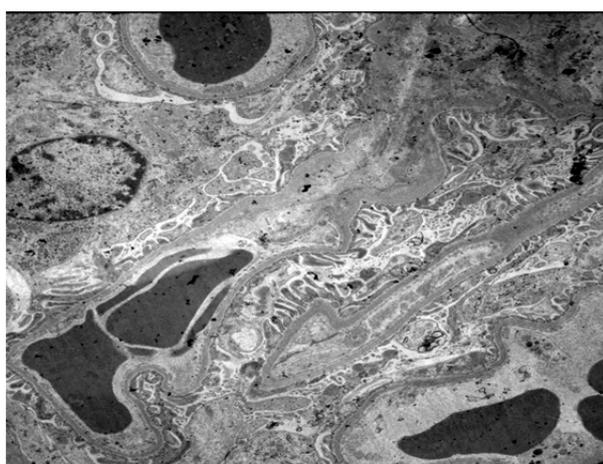


Fig. 5. Kidney of the animal with immuno-complex GN, treatment with prednisolone and Kanefron®H: a fragment of the glomerulus without changes (x1260).

CONCLUSION

Thus, on the basis of an experimental study in animals with immune-complex glomerulonephritis, modeled according to the patented procedure, treated only with prednisolone and prednisolone + Kanefron®, clinical and laboratory data were obtained, indicating a higher effectiveness of combined therapy. Morphological studies of autopsy medications in the kidneys of animals withdrawn from the experiment after the therapy confirm that the inclusion of the drug Kanefron® in the treatment leads to a positive dynamics in the structure of the nephron, which allows us to conclude that its nephroprotective effect is expressed. Undoubtedly, this preclinical study will serve as a determining factor for including this herbal preparation not only in complex therapy for patients with glomerular and tubulo-interstitial changes in the tissues of the kidneys, but also as a promising, delicate, preventive nephrotector for this group of patients.

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