

UDC: 615.275:616.61-008.64-072.74:612.465

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INFLUENCE OF ANTIOXIDANTS ON THE MORPHOLOGICAL CHANGES IN KIDNEYS AT EXPERIMENTAL ACUTE INJURY

Abstract. *Morphological changes in kidneys of white rats after the use of liposomal quercetin lipoflavon and lipin at the simulation of the myoglobinuric acute renal injury and gentamicin nephropathy have been studied. Expressed nephroprotective efficacy of lipoflavon both at one-time and repeated injection is revealed. At the same time lipin demonstrates its nephroprotective efficacy just in the case of its injection during 6 days.*

Key words: *myoglobinuric acute renal injury, gentamicin nephropathy, liposomal quercetin lipoflavon, lipin, morphological changes, rats.*

Introduction. There is a very wide range of diseases, which can be complicated by the occurrence of acute renal injury (ARI). Toxic effect of a number of substances on skeletal muscles [12] and their traumatic compression, thus developing rhabdomyolysis and myoglobinuria, is one of the leaders. Medicinal forms of ARI occur quite often due to nephrotoxic action of a number of drugs (about 60% of the cases are caused by the use of antibiotics, particularly aminoglycosides) [10].

It is well known, that renal form of ARI develops is a result of damage of the renal parenchyma, accompanied by a decrease in the intensity of tubular reabsorption, and renal hemodynamics, which occurs secondarily, leads to a drop in glomerular filtration rate [5]. According to the literature data, aminoglycoside antibiotic gentamicin, which accumulates in the cortical layer of the kidney, causes structural and functional disorders of the kidney tubules, mostly proximal, with partial preservation of functions of glomerular apparatus. Most researchers associate kidney injury with gentamicin with the formation of reactive oxygen species and the weakening of antioxidant protection [7].

It was shown that the introduction of antioxidants has a positive influence on the

development of experimental ARI [3]. This makes it possible to suggest that drugs and substances with antioxidant and membrane-stabilizing properties can be used to treat various forms of ARI [6, 11]. Particularly, it was proved experimentally, that antioxidants enhance the functional activity of the kidneys at the ARI, bring creatinine excretion to the control parameters and increase glomerular filtration rate significantly [1, 2].

Furthermore, it is still not quite clear how the improvement of renal function with antioxidants at ARI corresponds to morphological changes in the kidneys. Thus, the aim of our study is to study the morphological changes in the kidneys of rats with ARI at their correction with drugs with antioxidant action of lipoflavon and lipin.

Objective: to study of the morphological changes in the kidney tissues at the correction with lipoflavon and lipin of rhabdomyolic and gentamicin ARI.

Materials and methods. Study was performed with 24 white pubertal male outbred rats with the body weight of 120-180 g. Rhabdomyolic ARI was caused by the intramuscular injection of 50% glycerol solution at a dose of 10 mg/kg [8], gentamicin nephropathy was caused by the intramuscular injection of 80 mg/kg gentamicin

sulfate once a day for 6 days [9]. Lipoflavon (liposomal quercetin drug) was once injected intraperitoneally at a dose of 8 mg/kg [4] in 40 min after the injection of glycerol. Lipin, which is the basis for the lipoflavon, was injected similarly at a dose, which was equal to its content in lipoflavon (32 mg/kg) [4]. At the gentamicin nephropathy the drugs were injected similarly, but during all 6 days of the disease simulation.

Kidneys tissues for histological study were fixed in 10% solution of neutral buffered formalin for 48 hours, dehydrated in ascending alcohols, embedded in paraffin at 64 °C with following production of histological sections with thickness of 5 microns, stained with hematoxylin and eosin. For objectification and in order to improve the reproducibility of the results of quantitative research, computerized morphometry of objects in histological preparations was performed. Digital copies of optical images of areas of microscopic samples were obtained with digital camera Olympus (model C740UZ) and microscope LYUMAM-P8, bank of digital micrographs was created, and micrographs were further analyzed by the software "VideoTest - Size 5.0" (Ltd. "VideoTest", Russia).

Results and discussion. Morphological study of myoglobinuric simulated pathology confirms the displays of rhabdomyolysis, which leads not only to degenerative changes but also to coagulation necrosis in the kidneys.

Thus, in samples of animals with simulated pathology there are dilated vessels full of blood, sometimes there is perivascular hemorrhage, renal tubular epithelium is in a state of granular dystrophy ($94 \pm 1,0\%$), epithelial nuclei are not visible clearly, lumen is almost absent, and $2 \pm 0,1\%$ of epithelial cells are in state of coagulation necrosis, which is manifested as cytoplasm induration and karyopyknosis (Fig. 1, 2). There is an obstruction with myoglobin cylinders of $28 \pm 0,8\%$ of convoluted tubules of cortex and $61 \pm 1,2\%$ of excretory tubules of medulla. Myoglobin cylinders mostly expanded their lumens in their localization much.

Under the correction of ARI with lipoflavon the renal morphological state becomes better: obstruction of the lumen convoluted tubules with myoglobin cylinders is irregular and covers

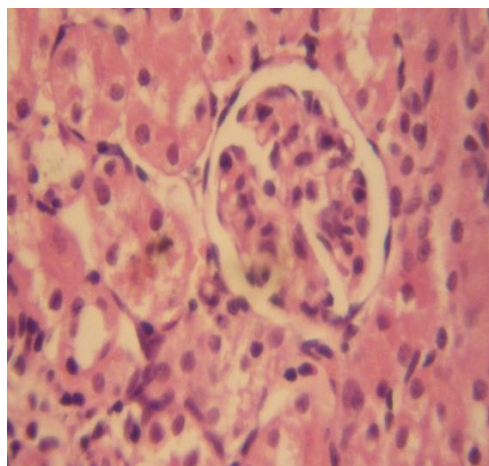


Fig. 1. Kidney cortex of an intact rat. Coloration with hematoxylin and eosin. Magnification: Objective $\times 40$, eyeglass $\times 10$.

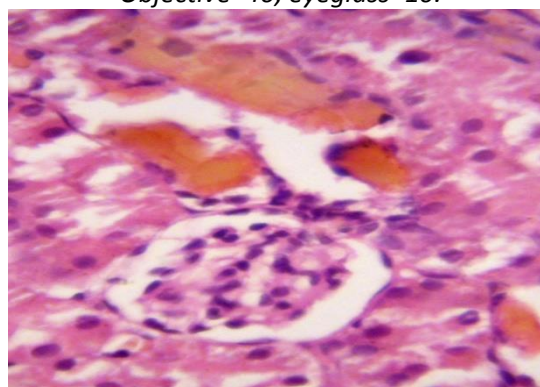


Fig. 2. Kidney cortex of a rat at the glycerol acute renal insufficiency on the 24th hour of the experiment

in average only $8 \pm 0,5\%$ of tubules (Figure 3). The cylinders are of various dimensions, but most of them are rather small. Degenerative processes cover $82 \pm 1,3\%$ of epithelial cells, and necrosis covers $1 \pm 0,1\%$ of them.

When using lipin the obstruction of the lumen of convoluted tubules with myoglobin cylinders is irregular and in average covers $31 \pm$

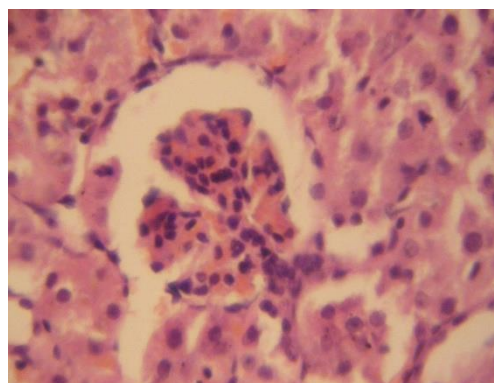


Fig. 3. Kidney cortex of a rat after the injection of lipoflavon. Coloration with hematoxylin and eosin. Magnification: Objective $\times 40$, eyeglass $\times 10$.

0,6% of tubules (Figure 4). The cylinders are small and obstruct the lumens slightly. Degenerative processes and necrotic effects are similar to ARI, particularly the dystrophy covers $93 \pm 0,9$ % of epithelial cells, and necrosis covers $2 \pm 0,3$ % of them.

In contrast to the intact animals (Fig. 5), in the kidneys of animals with gentamicin nephropathy

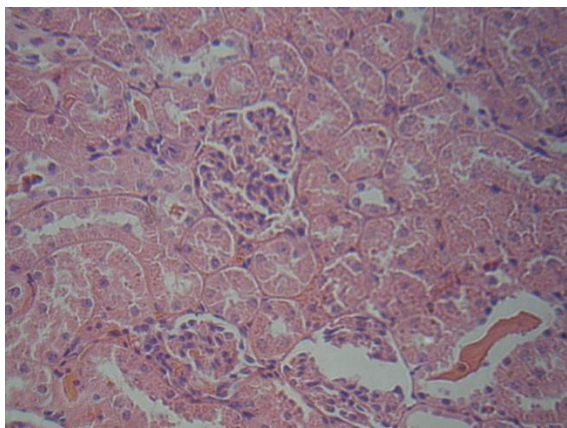


Fig. 4. Kidney cortex of a rat after the injection of lipin at the glycerol acute renal insufficiency.

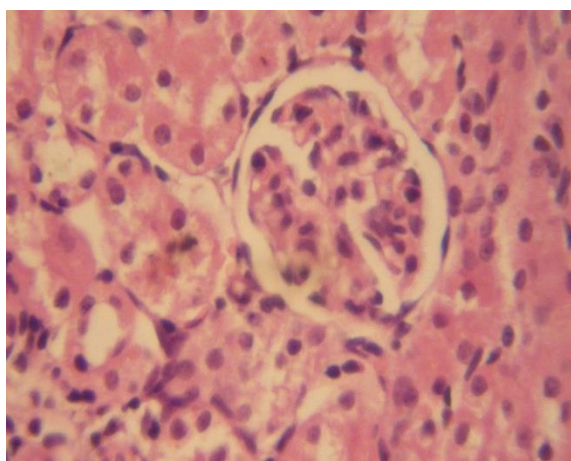


Fig. 5. Kidney cortex of an intact rat. Coloration with hematoxylin and eosin. Magnification: Objective \times 40, eyeglass \times 10.

(Fig. 6) granular dystrophy of epithelial tubules ($84 \pm 1,4$ %) is observed on the 6th day of the experiment, also small areas of necrosis of tubular epithelium are observed ($34 \pm 0,15$ %). In some cases, glomeruli seem to be atrophy. At the correction with lipoflavan (Fig. 7) and lipin (Fig. 8) the dystrophy of convoluted tubule epithelium decreases to $58 \pm 1,2$ % and $52 \pm 1,6$ % respectively. There are no atrophied glomeruli, the signs of necrosis are observed only in isolated epithelial cells mostly after application of lipin.

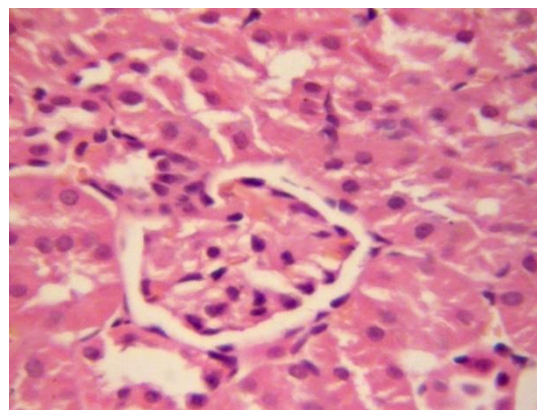


Fig. 6. Kidney cortex of a rat at the gentamicin nephropathy on the 6th day of the experiment. Coloration with hematoxylin and eosin. Magnification: Objective \times 40, eyeglass \times 10.

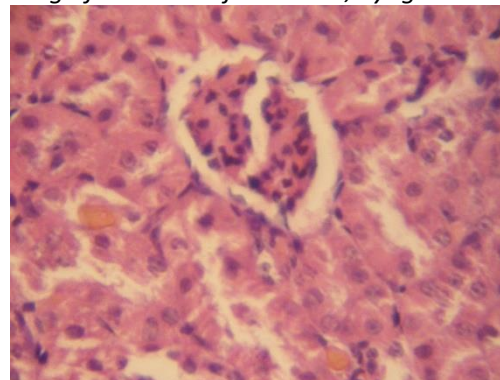


Fig. 7. Kidney cortex after injection of lipoflavan of a rat at the gentamicin nephropathy. Coloration with hematoxylin and eosin. Objective 40, eyeglass 10.

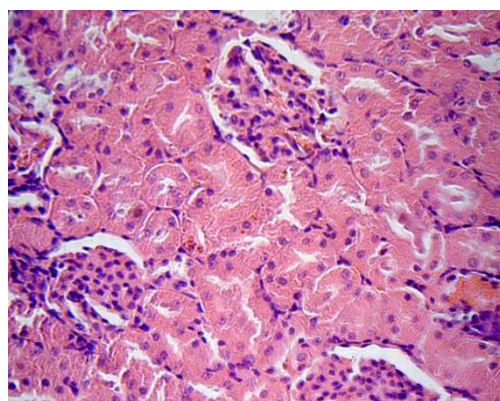


Fig. 8. Kidney cortex after injection of lipin of a rat at the gentamicin nephropathy. Coloration with hematoxylin and eosin. Objective 40, eyeglass 10.

Consequently, at the use of liposomal quercetin – lipoflavan – Its nephroprotective efficacy is confirmed both for myoglobinuric ARI and gentamicin nephropathy, accompanied by normalization of morphological state of kidney. However, the use of lipin causes almost no morphological changes in the kidney at the

myoglobinuric simulated pathology. At the same time, at the gentamicin nephropathy the nephroprotective efficacy of lipin is higher, perhaps because of the long-term (6 days) of the drug injection.

Conclusions. 1. According to the morphological study quercetin drug lipoflavon helps to protect kidney cells from the effects of pathogenic factors of renal damage, which is manifested in reducing of degenerative changes of nephrothelial.

2. Lipin almost does not change morphological state of kidneys in animals with acute renal injury after its one-time injection, but improves renal morphology at its long use.

Perspectives of further investigations. Obtained results give grounds to conclude that the investigated quercetin drug is effective nephroprotective agent at ARI, which allows recommending it for further clinical studies in order to implement it in clinical practice. The use of lipin may be one of the pharmacological approaches to the treatment of nephropathy.

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