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MORPHOLOGICAL EVALUATION OF THE EFFECTIVENESS OF ANTIOXIDANT THERAPY OF DIETARY SUPPLEMENTS "GEPAVIT" ON THE MODEL OF HEAVY METAL POISONING IN THE EXPERIMENTAL CONDITIONS

Makhmudov K.O.
State Institution "Republican Specialized Scientific and Practical Medical Center for Surgery named after Academician V. Vakhidov"

The aim is to study the use of antioxidants in heavy metal poisoning.

Introduction

As you know, all living things are always directly or indirectly affected by various physical, mechanical, chemical or biological factors. These factors can cause pathomorphological and pathophysiological changes in the macroorganism of an individual, depending on the area or method of exposure to the body [11, 13].

Most living units in the biological food chain have the characteristics of an open system. The maintenance of the life form of these units is provided on the basis of respiration and nutrition [4, 2, 10].

The fact that the basis of life is food and water supply is substantiated by known biological knowledge [2, 3]. For this reason, micro- and macroelements in the food chain form the basis of all life processes and determine its existence in nature [5].

At present, almost all elements of the periodic system of chemical elements are involved in the metabolism of organisms. Interestingly, the majority of them are metallic elements. These elements can enter the body only through food mixtures from the external environment [8, 9, 14]. For this reason, if the number of elements in any food mixture exceeds the norm, then poisoning with this element occurs in the body. The degree of poisoning is necessarily proportional to the number and duration of exposure to the elements. For this reason, various poisonings that occur for a long time are referred to as chronic poisonings and lead to various stages of changes in the body [7].

Based on the foregoing, treatment of such poisonings with substances capable of biochemically oxidizing these elements, removing them from the body or converting them into other forms that are easily excreted without any harm is now widespread. They are usually called antioxidants [1, 6].

Antioxidant forms differ from each other in types and methods of application [15].

Keywords: heavy metals, poisoning, morphology and antioxidants.

As a result of the analysis of the available literature, it became clear that the clinical, laboratory and pathomorphological changes that occur during poisoning with mixtures of various chemical elements, especially heavy metals, at the same time have not been fully proven. Also, the fact that the various organic changes that can occur due to these elements are not sufficiently studied in a certain systematic relationship determined the goal of our work on sheep. In a word, it differed from the assessment of pathomorphological changes that occur during chronic poisoning with

various heavy metal compounds, and the morphological assessment of changes that occur during treatment with a mixture of antioxidants.

Based on the foregoing, pathomorphological changes occurring in organs when heavy metals enter the body, and the results of their treatment with the help of the antioxidant mixture proposed by us, can be clearly demonstrated only by experimental comparative pathomorphological evaluation.

Material and methods

The experiment was carried out on 30 white male rats weighing 150-180 g, kept in normal microclimatic conditions of the vivarium, in accordance with the current ethical standards.

Our experiment consisted of two stages. In each of the stages, the animals were initially divided into groups in accordance with the goals of further research.

In the first series of experiments, the experimental animals were divided into 2 groups. The pathomorphological nature of the toxic effect of the studied metal compound was studied in the first group of 10 animals, the control group and 20 experimental animals with simulated toxic hepatitis.

In the second series, a 3-week course of antioxidant therapy with the antioxidant dietary supplement "GEPAVIT" was carried out in 10 rats simulating toxic hepatitis.

In the 1st series of experiments, conducted on 20 rats in the simulation of chronic poisoning with toxic hepatitis, a mixture of metals was intraperitoneally received, consisting of compounds of copper, manganese, molybdenum and chromium (Elbekyan K.S., 2006). The introduction of the mixture was carried out daily for 4 weeks. The ratio of the amount of metals in the charge approximately corresponds to the Almalyk mining and metallurgical plant, corresponded to the ratio in soils and was Cu:Mn:Mo:Cr=100:19:16:2 [12].

Liver, spleen, intestines and kidneys of animals were taken for pathomorphological examination.

At the same time, biomaterials obtained from experimental animals after surgery were examined on days 3, 7, and 10.

As mentioned above, although the treatment with the antioxidant dietary supplement GEPAVIT was carried out for 3 weeks, 10 days of the experiment were taken for a correct dynamic assessment and a correct comparative assessment of the results. We did not dwell on the remaining periods, since the dynamics of pathomorphological analyzes during these periods could fully reflect our conclusions.

The obtained biomaterials were fixed in 10% formalin solution in phosphate buffer. Paraffin sections were stained with hematoxylin and eosin.

Light-optical micrographs were taken on a DN-300M microscope coupled with a digital camera and a computer.

All microphotographs were processed and saved on a computer using Microsoft-"Windows 10 pro" application programs.

Results

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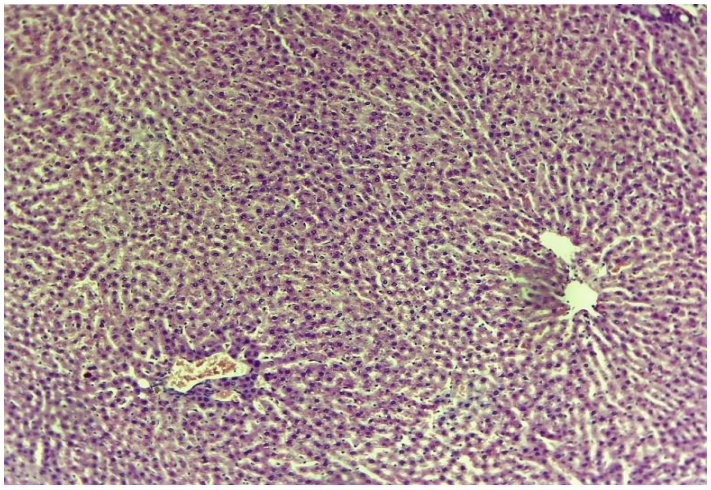
In this study, in the pathomorphological aspect, biomaterials obtained from a group with simulated toxic hepatitis and a group that received the antioxidant dietary supplement "GEPAVIT" were studied.

On the 3rd day of the experiment, in the group with simulated toxic hepatitis, in comparison with control animals, alterative-dystrophic changes in the inflammatory process were revealed. Violations of the architecture of common liver hepatocytes: hepatocytes and hepatocellular dystrophy, necrosis with various foci, edema of varying degrees. At the same time, despite obvious pathological changes, the phenomena of lymphoid-plasmatic infiltration and fibrosis were not revealed. In our opinion, such changes are the result of a toxic effect on the liver and differ from other types of inflammation (Fig. 1 and 2).

On the 7th day of the experiment, the above changes continued to develop in the liver, that is, hyperchromic nuclei, degeneration and necrosis of hepatocytes began to alternate with proliferative changes. In large foci of necrosis, fibroblast connective tissue began to form, microcirculation disorders were detected - stasis and sludge, a distinct polymorphism of hepatocytes, hyperchromia of the nuclei. In a word, predominantly proliferative-dystrophic processes of inflammation prevailed (Fig. 3).

By the 10th day of the experiment, septal fibrotic changes in the liver, lymphoid infiltrates in the portal tracts, and various stasis-like disorders in the microcirculatory vein were observed (Fig. 4).



Rice. 1. Normal architecture of the liver and hepatocytes. Control group. G-E. CM. 10x20.

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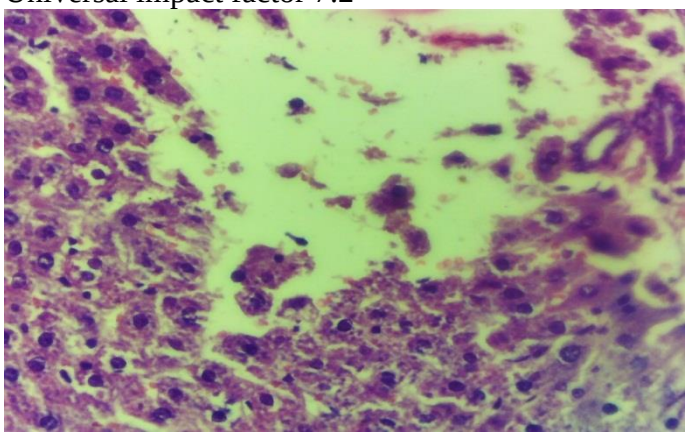
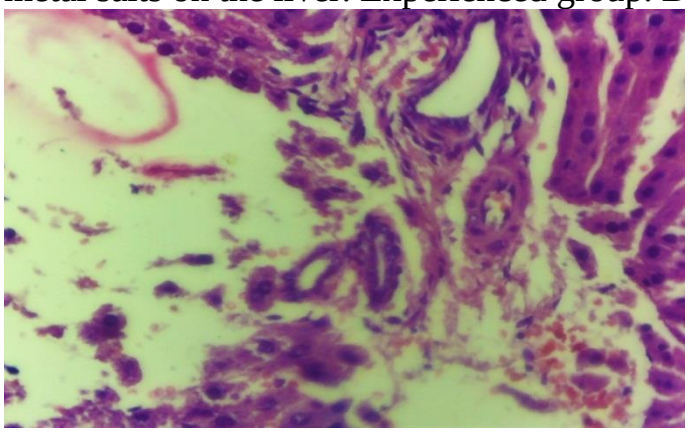
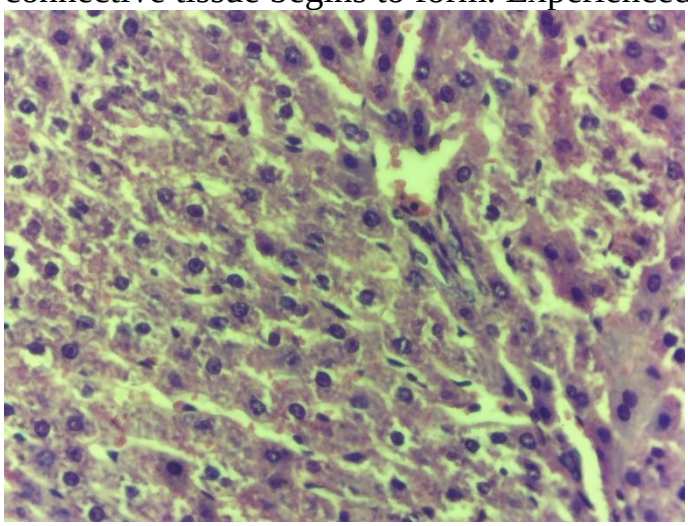


Rice. 2. Liver. Incorrect architecture of hepatocytes. Subcapsular area hepatocytic dystrophy, necrosis. The nucleus of hepatocytes is hyperchromic. Effect of heavy metal salts on the liver. Experienced group. Day 3 G-E. CM. 10x40.



Rice. 3. Macrofocal necrosis is visible, microcirculatory disorders - stasis and sludge, pronounced polymorphism of hepatocytes, nuclear hyperchromia. Fibroblast connective tissue begins to form. Experienced group. Day 7 G-E. CM. 10x40



Rice. 4. Liver tissue. Fibrous changes in the septum, lymphoid infiltrates in the portal tracts, microcirculatory disorders with various manifestations of stasis. Effect of heavy metal salts on the liver. Experienced group. 10 days. G-E. CM. 10x40.

Pathological examination of the kidney tissue revealed moderate dystrophic changes and moderate edema in nephrocytes compared with the control group (Fig. 5) on the

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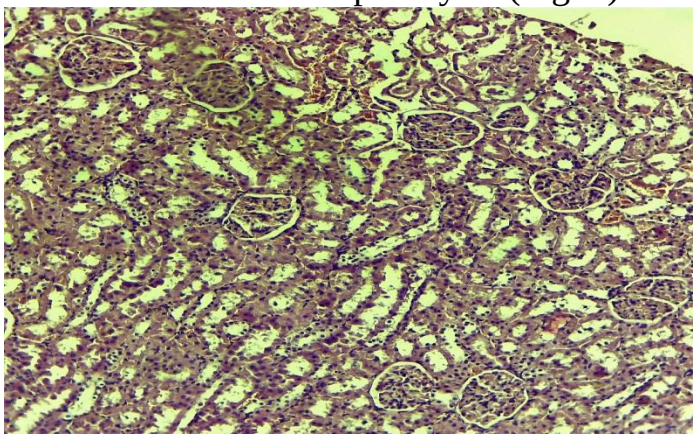
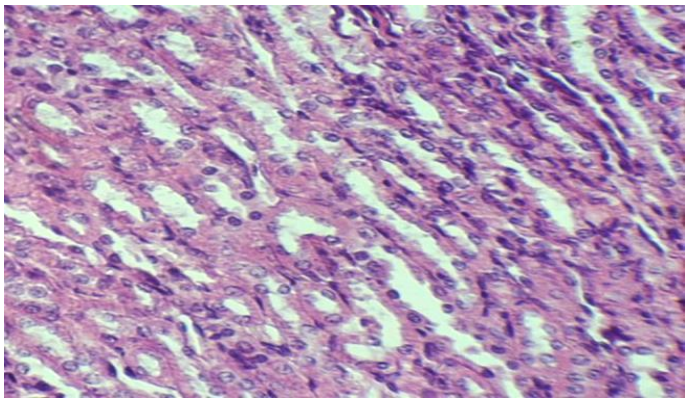
3rd day of the experiment. These changes are observed in all parts of the kidney parenchyma (Fig. 5 and 6).

On the 7th day of the experiment, additional proliferative processes joined the above changes, as we noted in our previous studies. Observed nephrocytic dystrophy, nuclear hyperchromia, Shumlyansky-Bowman's capsule and edema throughout the tissue. This, in our opinion, indicates that it is this area of the renal tissue that is the main damage in case of poisoning (Fig. 7).

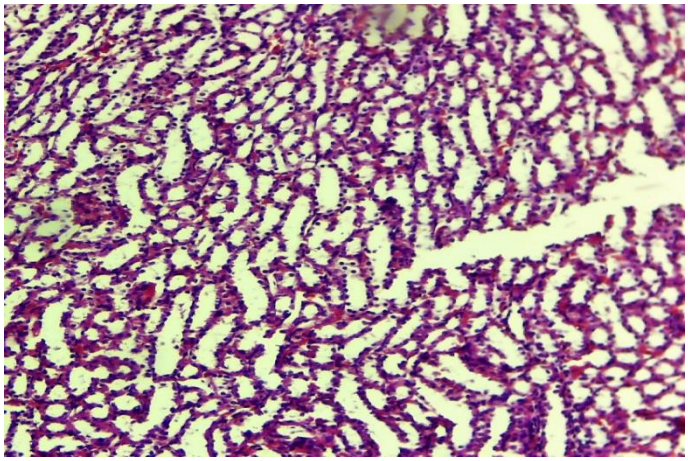
By the 10th day of the experiment, the process proceeded with the meeting of several foci of infiltration around the glomerular apparatus, expansion of the tubular space, and vacuolization of nephrocytes (Fig. 8).



A



B



C

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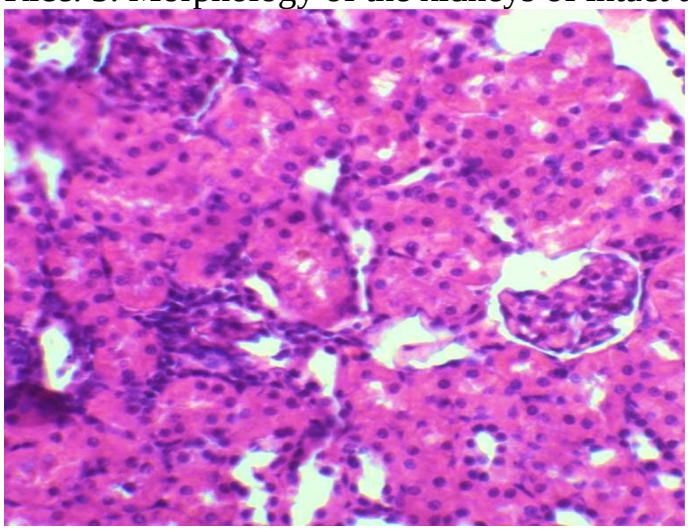
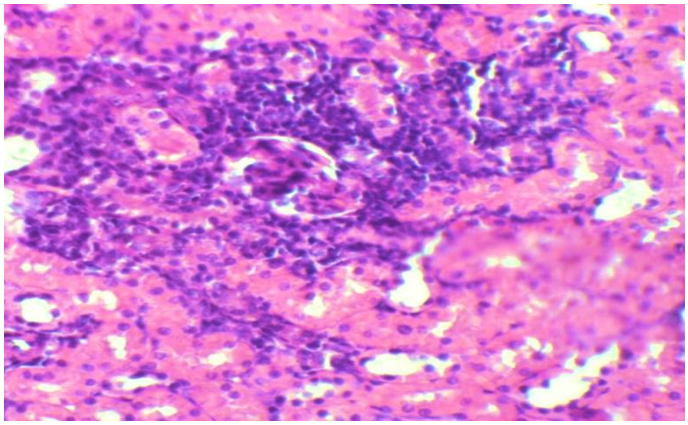
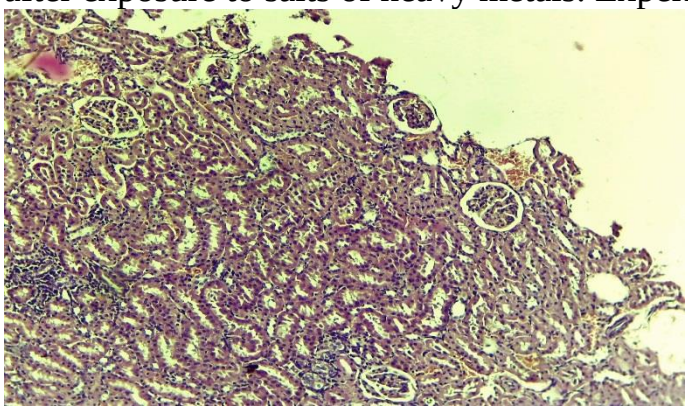
A. cortical layer. B and C - medulla.
Rice. 5. Morphology of the kidneys of intact animals. G-E. CM. 10x20.



Rice. 6. Degeneration of nephrocytes, hyperchromia of the nuclei, pronounced edema of the Shumlyansky-Baumen capsule. Morphology of rat kidneys after exposure to salts of heavy metals. Experienced group. Day 3 G-E. CM. 10x40..



Rice. 7. Degeneration of nephrocytes, infiltration, edema. Morphology of rat kidneys after exposure to salts of heavy metals. Experienced group. Day 7 G-E. CM. 10x40



A.

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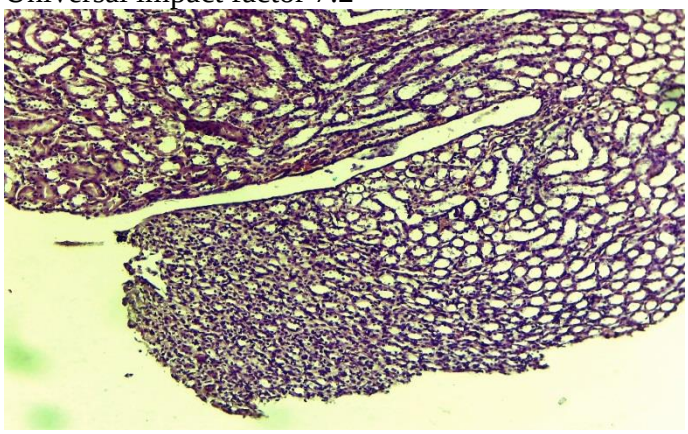


B

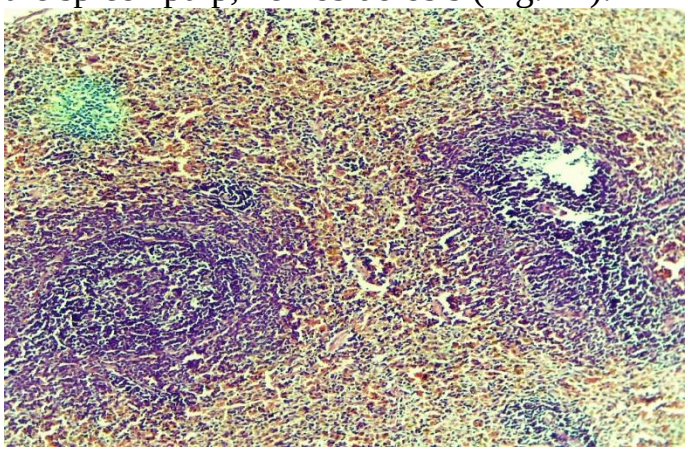
A. Expansion of the lumen of the tubules, hyperchromia of the nuclei. G-E. CM. 10x20.

B. Vacuolization of nephrocytes. G-E. CM. 10x20.

Rice. 8. Morphology of rat kidneys after exposure to salts of heavy metals. Experienced group. Day 10

On the 3rd day of the experiment, hyperplasia of the mantle layer of the follicle was observed in the spleen, venous plethora of the red pulp (Fig. 9) compared with animals of the control group (Fig. 10). By the 7th day of the experiment, these changes were supplemented by a diffuse distribution of heme-like elements (Fig. 11).

On the 10th day of the experiment, microcirculation disturbances are observed in the red pulp of the spleen: stasis and sludge, "depletion" of the cellular composition of the spleen pulp, hemosiderosis (Fig. 12).



Rice. 9. Follicle of the spleen. Morphology of the spleen of intact animals.
G-E. CM. 10x40.

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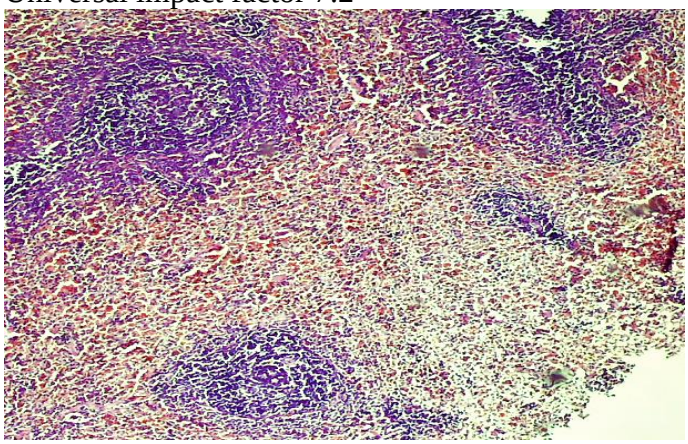
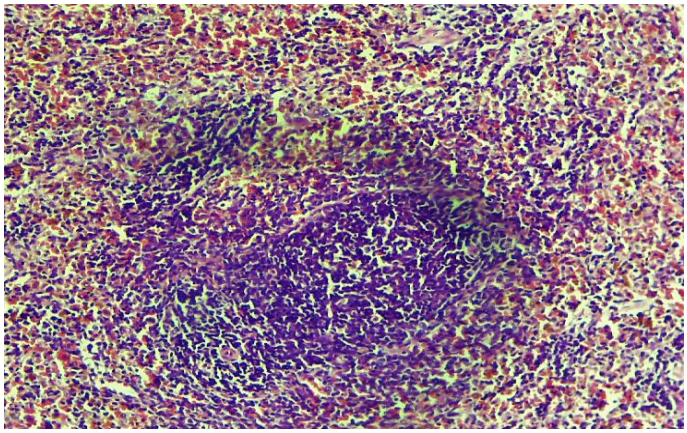
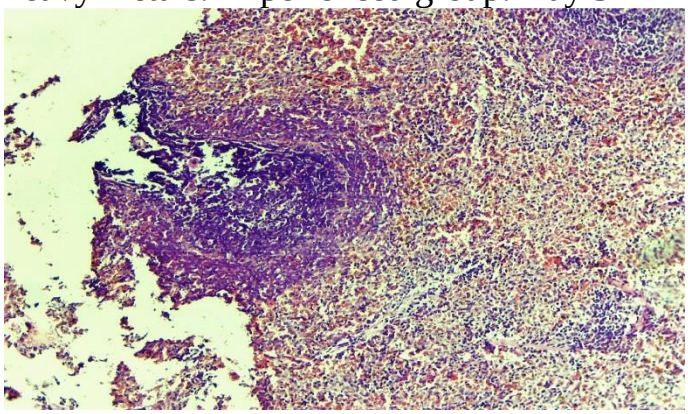


A.



B

A, B. Moderate hemosiderosis of the red pulp. G-E. CM. 10x20, 10x40. Rice. 10. Morphology of the spleen of rats after the development of poisoning with salts of heavy metals. Experienced group. Day 3



Rice. 11. Hyperplasia of the mantle layer of the follicle, venous plethora of the red pulp. Morphology of the spleen of rats after the development of poisoning with salts of heavy metals. Experienced group. Day 7 G-E. CM. 10x20.

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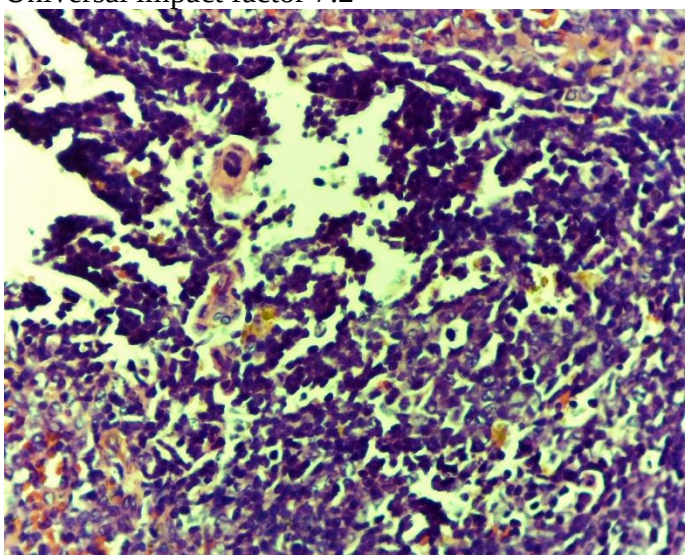
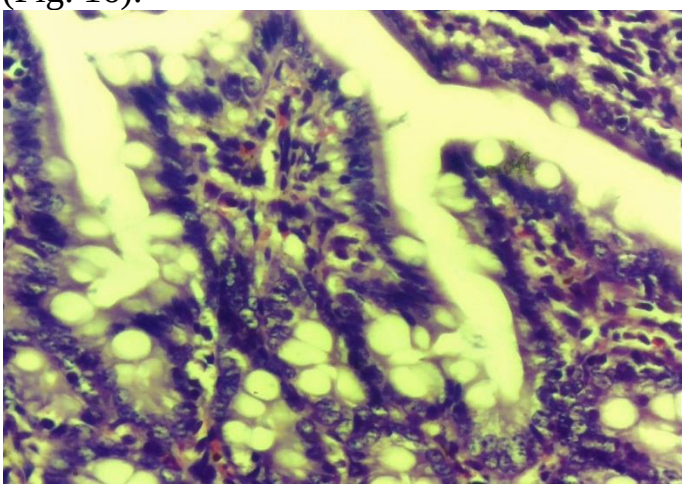


Rice. 12. Microcirculatory disorders in the red pulp of the spleen, "depletion" of its cellular composition, hemosiderosis. Morphology of the spleen of rats after the development of poisoning with salts of heavy metals. Experienced group. Day 10 G-E. CM. 10x40.

When studying the morphology of the small intestine, compared with the control group (Fig. 13), on the 3rd day of the experiment, small intestinal crypts were often enlarged, and secretory granules in Paneth cells were practically absent. In the apical branch of the mucous membrane, superficial dystrophic changes, edema, and a decrease in intraepithelial lymphocytes were found (Fig. 14). By the 7th day of the experiment, smoothing of the microvilli of enterocytes and an increase in the above signs were noted (Fig. 15).

By the 10th day of the experiment, it was found that the basal part of enterocytes had edema and thickening of the basement membrane. There were also pronounced hydropic disorders of mesotheliocytes of the serous membrane of the small intestine (Fig. 16).



A.

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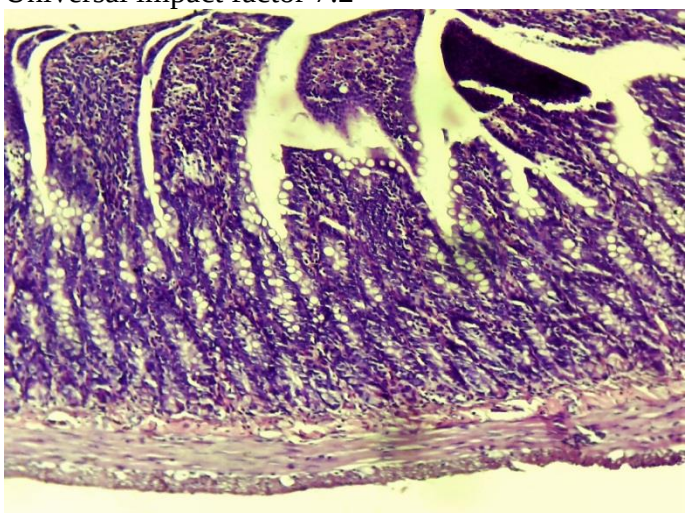
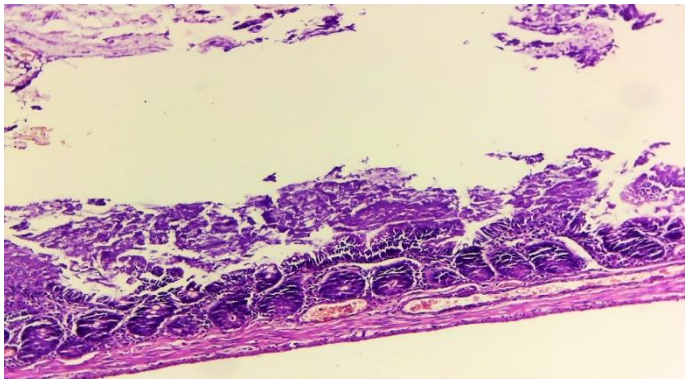


B.

A. The mucous membrane of the jejunum with straight, even villi,
B. Crypts of the jejunum with a moderate number of Paneth cells.
Fig.13. The mucous membrane of the jejunum of an intact rat. G-E. CM. 10x40, 10x20.



Rice. 14. Small intestine. The crypts smoothed out, the number of epitheliocytes decreased. Secretory granules are almost absent. Superficial dystrophic changes, edema and a decrease in intraepithelial lymphocytes in the apical branch of the mucosa. 3rd day of the experimental group with poisoning with salts of heavy metals. G-E. CM. 10x40.

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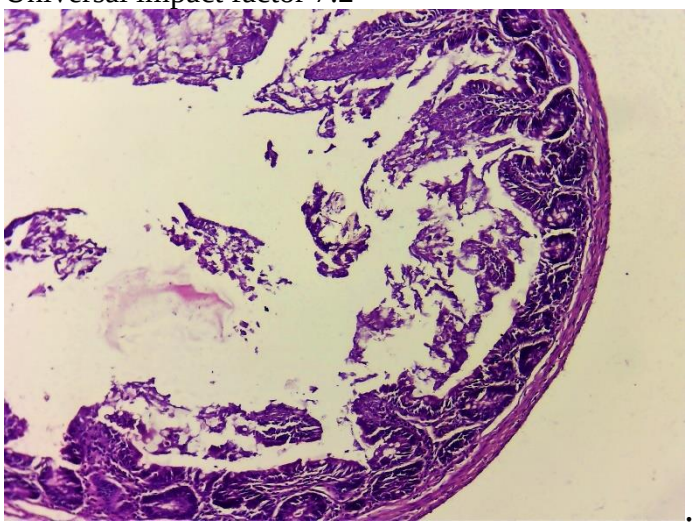

Rice. 15. Small intestine. Microvilli of enterocytes are smoothed. Superficial dystrophic changes, edema and a decrease in intraepithelial lymphocytes in the apical branch of the mucosa. 7th day of the experimental group with poisoning with salts of heavy metals. G-E. CM. 10x20.

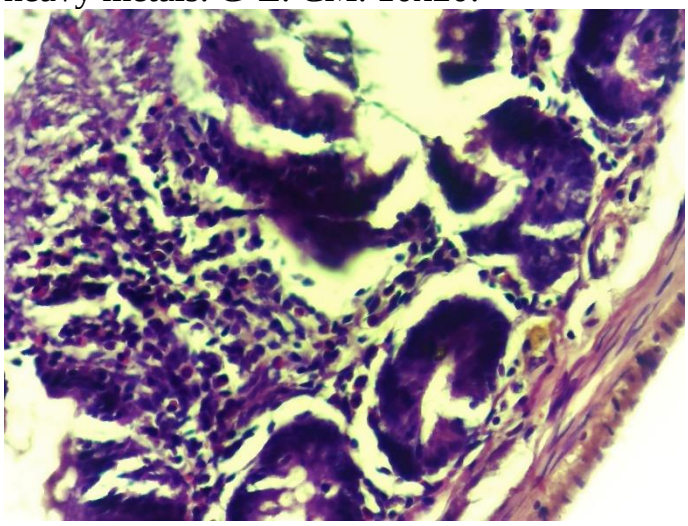

Fig.16. Small intestine. In the basal part of enterocytes, swelling and thickening of the basement membrane. Severe hydropic disorders of mesotheliocytes of the serous membrane of the small intestine were revealed. 10 days of the experimental group with poisoning with salts of heavy metals. G-E. CM. 10x40.

We found positive changes in the study of the morphology of the organs of experimental animals treated with the antioxidant dietary supplement GEPAVIT. On the 3rd day of the experiment, less dystrophic-necrobiotic phenomena were detected in the liver of animals treated with the antioxidant dietary supplement GEPAVIT. However, the phenomena of hepatocyte polymorphism and hyperchromia of their nuclei persisted. Microcirculation disorders were also observed: stasis and sludge, edema (Fig. 17).

These changes decreased accordingly during the 7 days of the experiment. A light-optical examination of the liver tissue revealed a decrease in lymphoid infiltration of the periportal tracts. At the same time, with the loss of glycogen granules in the

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cytoplasm of hepatocytes, their moderately pronounced vacuolar degeneration was observed (Fig. 18 and 19).

Normalization of the architecture of hepatocytes was observed on the 10th day of the experiment. Trabeculae and sinusoids around the central vein are determined. It has been established that the cytoplasm of hepatocytes is rich in glycogen granules (Fig. 20).

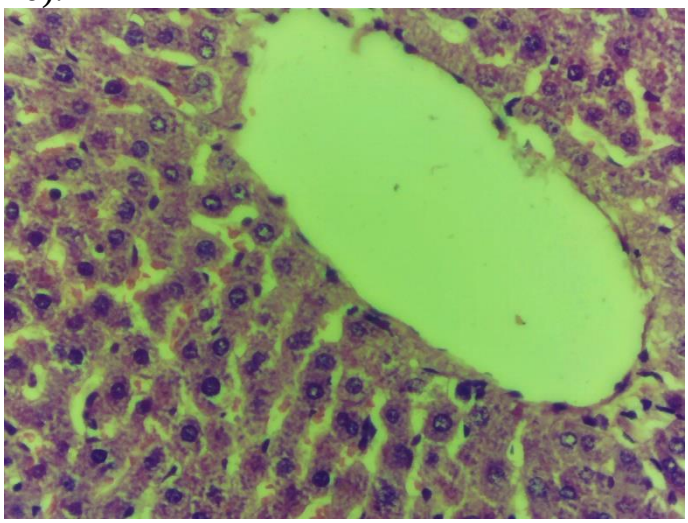

Fig.17. Dystrophy, hepatocyte polymorphism, nuclear hyperchromia. An experimental group that received the antioxidant dietary supplement GEPAVIT. 3 days. G-E. CM. 10x40.

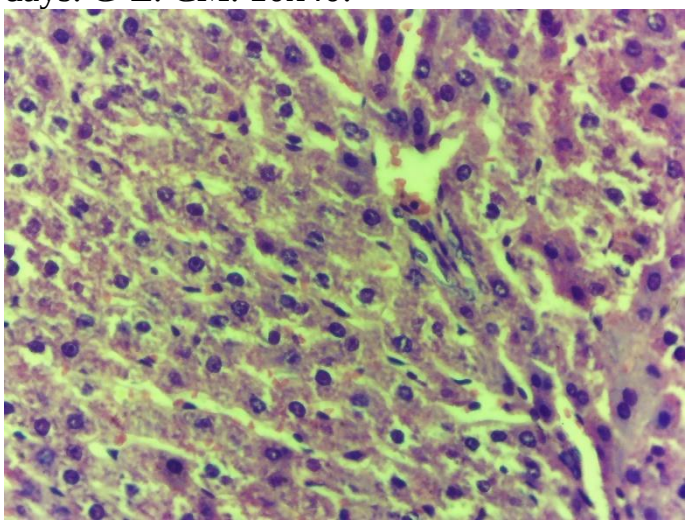

Fig.18. Dystrophy and small areas of hepatocyte necrosis, edema. An experimental group that received the antioxidant dietary supplement GEPAVIT. Day 7 G-E. CM. 10x40.

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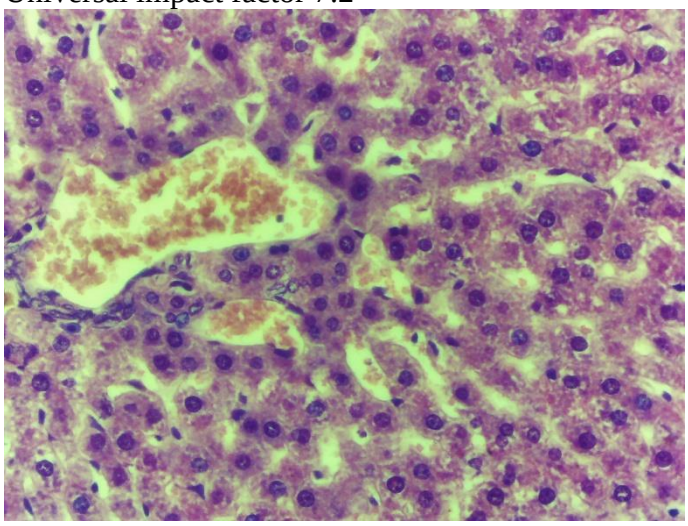
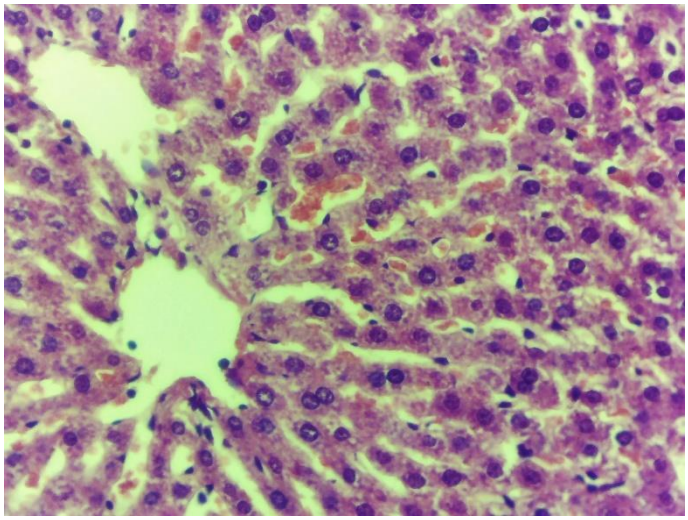


Rice. 19. Morphology of the liver of rats. Vacuolar degeneration of individual hepatocytes with the disappearance of glycogen granules in the cytoplasm. An experimental group that received the antioxidant dietary supplement GEPAVIT. Day 7 G-E. CM. 10x40.



Rice. 20. Rat liver lobule. Trabeculae, sinusoids around the central vein are visualized. The cytoplasm of hepatocytes is rich in glycogen granules. An experimental group that received the antioxidant dietary supplement GEPAVIT. Day 10 G-E. CM. 10x40.

On the 3rd day of the experiment, a morphological study of kidney tissue revealed a decrease in inflammatory infiltration both in the cortical layer and in the medulla in the experimental group, which received the antioxidant dietary supplement GEPAVIT. In areas in front of the renal glomeruli, hydropic dystrophy of the proximal and distal canals, covered with a single-layer epithelium, is revealed. In the parenchyma of the kidney, single elements of inflammation are determined (Fig. 21).

On the 7th day of the experiment, weak swelling and hyperchromia of nephrocytes prevailed in the prerenal glomerular areas. In this case, hydropic swelling of the channels is observed. Various infiltrates were also not observed (Fig. 22).

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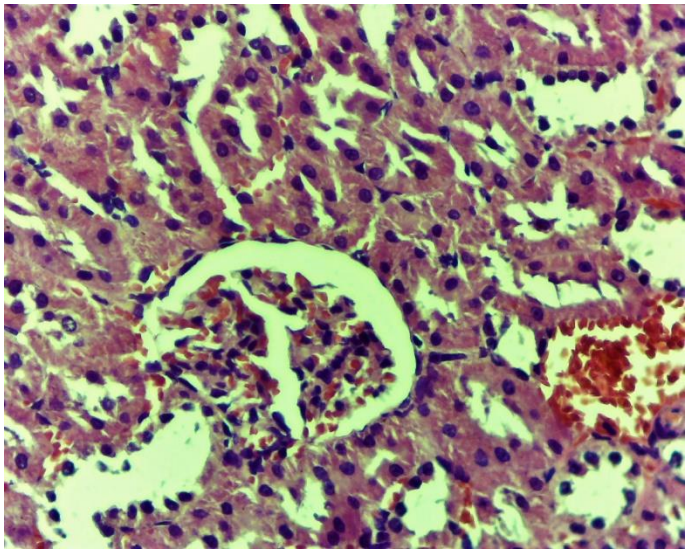
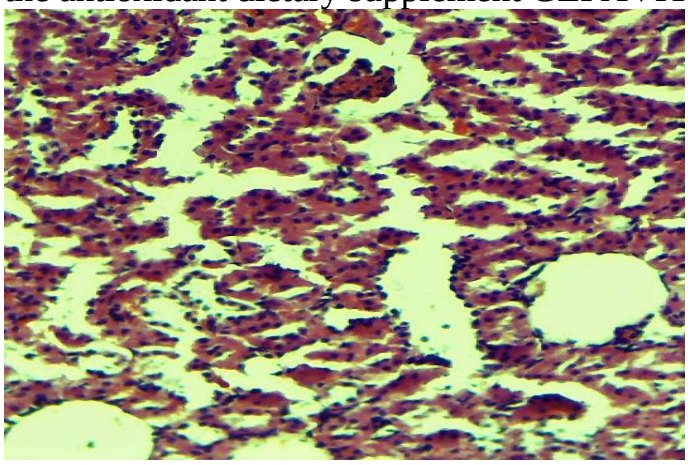
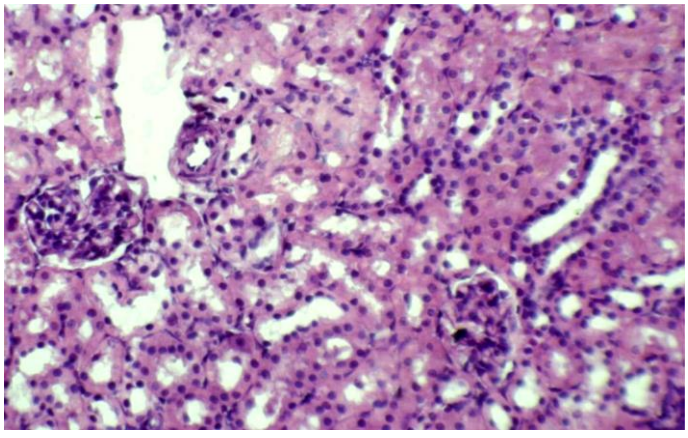
On the 10th day of the experiment, there is a significant decrease in edema in the kidney parenchyma, a moderate expansion of the tubule cavity (Fig. 23).



Rice. 21. Renal parenchyma of a rat with enlarged periglomerular zones with hydropic dystrophy of the tubular epithelium. An experimental group that received the antioxidant dietary supplement GEPAVIT. 3 days. G-E. CM. 10x40.



A.



B.

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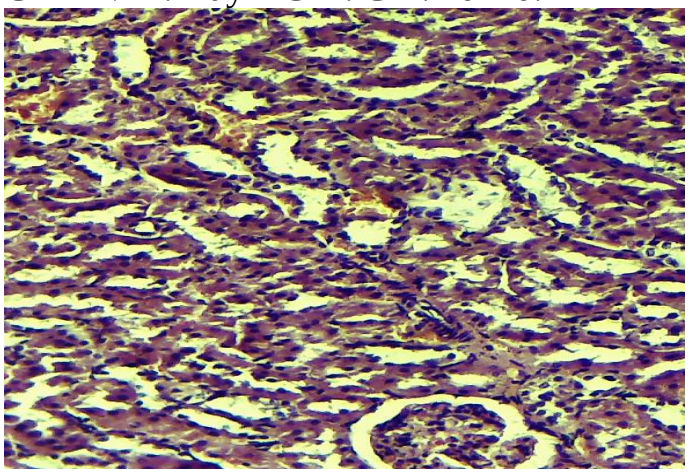
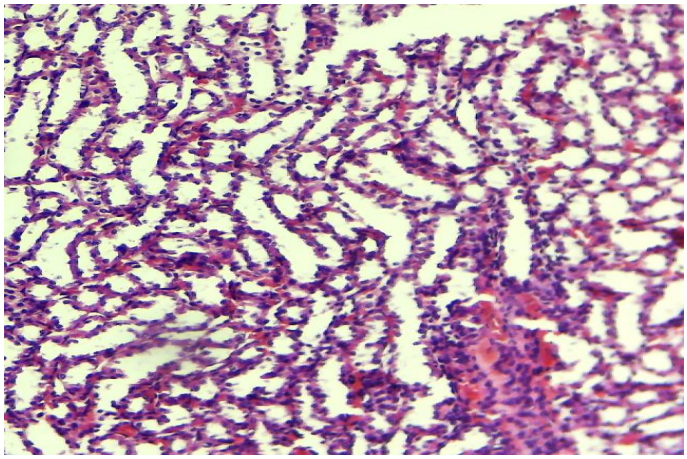
A. Moderately expressed dystrophy of nephrocytes.

B. Expansion of the lumen of the tubules, reduction of edema of the cavity of the Shumlyansky-Baumen capsule.

Fig.22. An experimental group that received the antioxidant dietary supplement GEPAVIT. Day 7 G-E. CM. 10x40.



A



B.

A. Reduced severity of edema.

B. Moderately pronounced expansion of the lumen of the tubules.

Rice. 23. Experimental group that received the antioxidant dietary supplement GEPAVIT. Day 10 G-E. CM. 10x40, 10x20

In the study of the spleen in the experimental group, which received the antioxidant dietary supplement GEPAVIT, on the 3rd day of the experiment, the intensity of the lymphoid reaction in the lymphoid tissue, microcirculatory disorders in the red pulp were observed (Fig. 24).

On the 7th day of the experiment, the above symptoms decreased, and reticular cells predominated in the follicle (Fig. 25).

On the 10th day of the experiment, these signs became more pronounced and the layers approached the norm. There were many division centers and histiocytes in the center of the spleen tissue (Figures 26 and 27).

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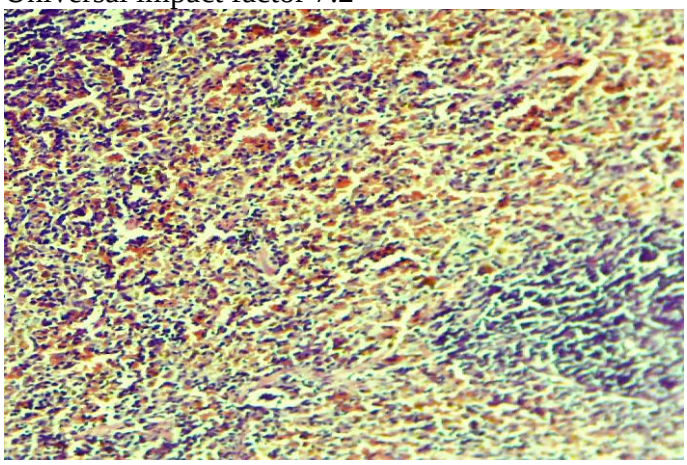
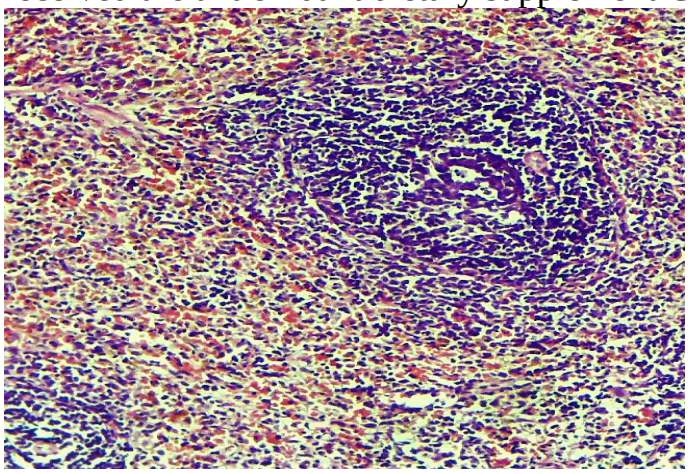


Fig.24. Reticular cells predominate in the follicle. An experimental group that received the antioxidant dietary supplement GEPAVIT. Day 3 G-E. CM. 10x20.



Rice. 25. Microcirculatory disturbances in the red pulp are slightly expressed. An experimental group that received the antioxidant dietary supplement GEPAVIT. Day 7 G-E. CM. 10x20.

Fig.26. Normalization of the layers of the spleen. Microcirculatory disturbances in the red pulp are expressed slightly. An experimental group that received the antioxidant dietary supplement GEPAVIT. Day 10 G-E. CM. 10x20.

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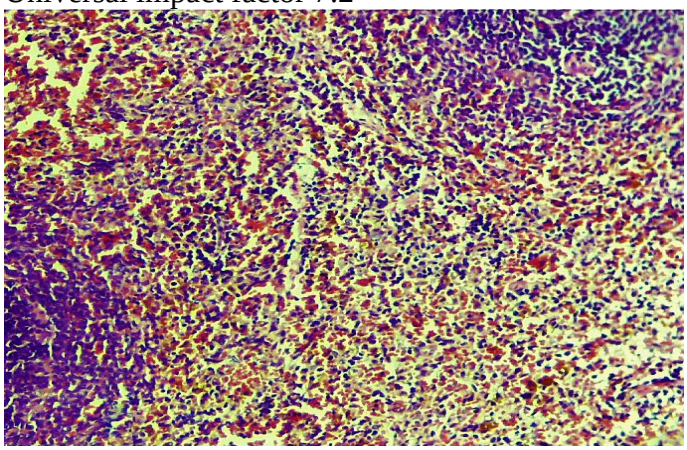
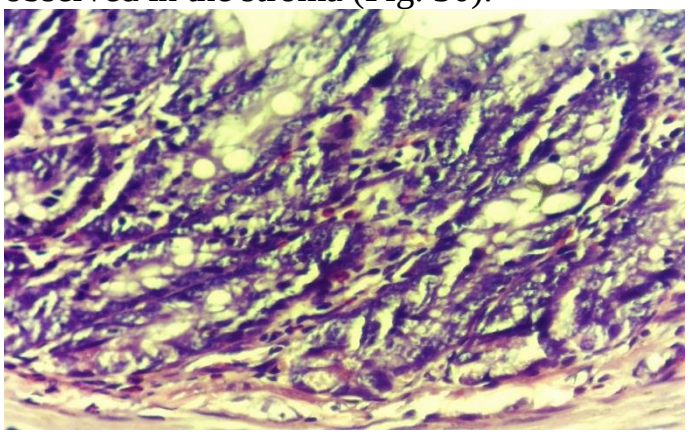


Rice. 27. Spleen tissue consists of many reproduction centers, histiocytes. An experimental group that received the antioxidant dietary supplement GEPAVIT. Day 10 G-E. CM. 10x40

During the pathomorphological assessment of the state of the small intestine in the experimental group, which received the antioxidant dietary supplement GEPAVIT, on the 3rd day of the experiment, the mucous membrane of the small intestine was straightened, moderate swelling was observed in the nipples. layers that have begun to form (Fig. 28).

On the 7th day of the experiment, normalization of the structures of the mucous membrane was noted (Fig. 29). By the 10th day, the mucous membrane of the small intestine is covered with enterocytes. A diffuse moderate lymphocytic infiltration is observed in the stroma (Fig. 30).



Rice. 28. The mucous membrane of the jejunum with straight, even villi, moderate edema. An experimental group that received the antioxidant dietary supplement GEPAVIT. Day 3 G-E. CM. 10x40.

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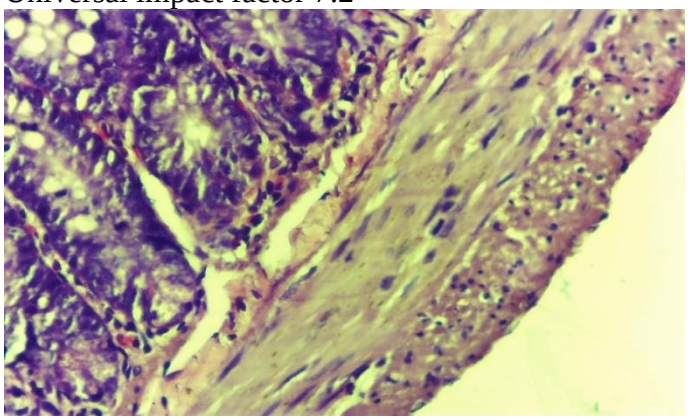
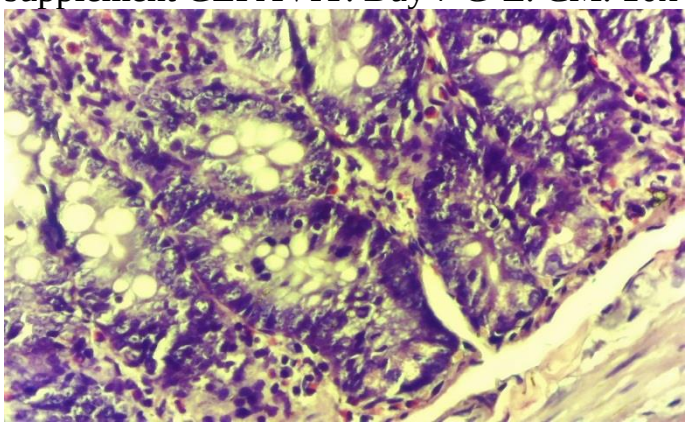


Rice. 29. Normalization of the structures of the mucous membrane of the small intestine of the rat. Hyperplasia of the crypts of the small intestine, a decrease in the height of the villi. An experimental group that received the antioxidant dietary supplement GEPAVIT. Day 7 G-E. CM. 10x40



Rice. 30. Small intestine with pronounced villi, lined with enterocytes, moderate lymphatic infiltration of the stroma. An experimental group that received the antioxidant dietary supplement GEPAVIT. Day 10 G-E. CM. 10x40.

Discussion

Summarizing the above morphological changes, we can come to the following conclusion: therapy with the antioxidant dietary supplement GEPAVIT has a pronounced antioxidant effect, thereby reducing the negative effect of free radicals on cell membranes. When analyzing liver histological preparations under a light microscope, it was found that the overall architecture and morphology of hepatocytes and their nuclei approached the morphological state observed in healthy animals. Similarly, this can be seen in another important feature, an increase in the amount of glycogen in the liver cells (Fig. 20).

Also, poisoning with salts of heavy metals causes distinct violations of the structures of the mucous membrane of the small intestine, which leads to a sharp violation of its physiological barrier function.

In the group that received the antioxidant dietary supplement "GEPAVIT", the results of morphological studies showed that after a course of enterosorption with the antioxidant dietary supplement "GEPAVIT", positive signs appear, the number of inflammatory elements in the wall of the small intestine of the experimental animals decreased, the number of goblet cells increased, and it is possible to assess the

location of cells and intestinal papillae. In our opinion, there is a tendency to improve the regenerative and reparative processes of the intestinal wall, which also indicates a decrease in the inflammatory process.

Morphological examination of histological preparations of kidney tissue revealed numerous glomeruli with intensely filled capillaries, mesangial cells. In the wide renal tubules, lined with a single-layer epithelium, edema and infiltration, expansion of the ulus decrease, and the filtration and absorption functions of the renal tissue improve.

Conclusion

Thus, the conducted histomorphological studies show that the treatment of dietary supplements "GEPAVIT" filled with antioxidants contributes to the normalization of the morphology of the studied organs: the liver, kidneys, spleen and intestinal mucosa, and to the improvement of its physiology.

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