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MICROSCOPIC CHANGES IN THE INTERNAL ORGANS OF WHITE MICE DURING EXPERIMENTAL IRON (IV) CLATHROCHELATE TOXICOSISS

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Abstract. *Iron (IV) clathrochelate based on a macrobicyclic ligand of the hexahydrazide type is a unique compound that contains iron in a rare high valence IV. Preclinical and clinical studies of this complex, which were started for the first time in Ukraine, have an important theoretical and practical consequence as this complex can be recommended as an active substance in iron-containing drugs with antianemic action.*

In conducting preclinical studies of new drugs, pathomorphological studies are important because they are a necessary step in studying the biological response of animals to the action of test substances. It was found that some pathological changes develop

in the body of white mice under conditions of experimental acute and chronic iron (IV) clathrochelate intoxication. They correlated with the dose of the test compound. During chronic intoxication, the microscopic changes in the liver and kidney of white mice treated with iron (IV) clathrochelate at a dose of 1/10 DL50 were similar to the microscopic changes in the liver and kidney of mice treated with the experimental drug at a dose of 1/5 DL50. However, the severity of these changes was lower, reflecting a lower degree of organ damage. In the myocardium of mice treated with iron (IV) clathrochelate at a dose of 1/5 DL50, as during acute iron (IV) clathrochelate poisoning, only edema was recorded on the 10th day. The prospects for further research are the study of microscopic changes in the organs of laboratory animals of other species during experimental iron (IV) clathrochelate toxicosis.

Keywords: *iron, pathomorphological studies, liver, kidneys, heart, toxicity*

Introduction

During preclinical studies of a new drug, the microscopic research of the systems and organs of experimental animals enables studying the pathogenesis of intoxication with the studied drug (Kotsyumbas et al., 2006). We have previously reported the study of acute and chronic toxicity of iron (IV) clathrochelate (based on a macrocyclic ligand of the hexahydrazide type) for laboratory animals (Dukhnisky et al., 2018; 2019; 2020), but the microscopic changes in their internal organs under experimental toxicosis of the test compound remain unexplored.

Analysis of recent researches and publications

Iron is the second most common metal in nature (after aluminum) and occurs in valencies II and III, and only in the form of compounds. However, in recent decades, such data have expanded significantly and are deepening today. In particular, studies of iron in high valencies, IV, V, VI, have become relevant (Weiss et al., 2001; Krahe et al., 2014; Shylin et al., 2019; Prakash et al., 2020; Kloß et al., 2021). Thus, Hohenberger et al. (2012) prove that oxo and nitrido complexes of high-valent iron are active intermediates in many biological and chemical processes in nature. Nam et al. (2014) studied the reactionary ability and the mechanism of the reaction of mononuclear nonheme iron (IV) oxo complexes. Machalová Šišková et al. (2016) investigated ferrates of high-valent forms (Fe (VI), Fe (V), and Fe (IV)) in aquatic environment. Under ambient conditions, high-valent iron compounds (+4, +5, +6) are not able to form spontaneously, and the synthetically obtained ones are unstable in polar organic solvents, especially in aqueous solutions, which, in turn, limits their research and use.

However, several studies have been conducted, in which more stable compounds of high-valent iron have been synthesized. Thus, Tomyn et al. (2017) proposed the synthesis of complexes of iron (IV) hexahydrazide clathrochelate in an alkaline aqueous medium from salts of iron (III), oxalodihydrazide, and formaldehyde, accompanied by air oxidation. Such combinations can exist for a long time in the environment without any signs of decomposition in water, non-aqueous solutions, and in the solid state. According to the results of pre-

clinical studies conducted by us, it was found that iron (IV) clathrochelate corresponds to class III hazard according to the classification of chemicals by degree of danger (GOST 12.1.007-76), and class IV and degree of toxicity – "low toxicity" – according to the classification of substances for toxicity (Dukhnisky et al., 2018; 2019).

In acute experimental toxicosis, the average lethal dose of iron (IV) clathrochelate was found to be 764.3 ± 32.71 mg/kg body weight for quails and 1258.3 ± 144.87 mg/kg body weight for white mice (Dukhnisky et al., 2018). The effect of iron (IV) hexahydrazide clathrochelate solutions in the various concentrations on laboratory animals (white mice, white rats, and quails) was investigated in the study of chronic toxicity. In this case, the determination of the dynamics of body weight, the relative mass coefficients of their internal organs, blood morphological parameters, and biochemical indicators of serum with repeated administration of the test substance in different doses to animals of three species was conducted (Dukhnisky et al., 2019; 2020).

During toxicological studies of drugs for laboratory animals, pathomorphological studies are quite important among other methods. They are a necessary step in the research of the biological response of animals to the action of drugs and allow to describe the nature and severity of the pathological process under the action of the studied substances. Analysis of microscopic changes in organs and tissues facilitates to determine the cause of animal death during the experiment. In this case, morphological studies in the dynamics during the experiment are mandatory, it assists to trace the development of pathological and restorative processes, to understand their nature and significance (pathology, compensation, and adaptation).

The research material is organs and tissues from specially killed animals and those who died during the experiment. The results of the effect of the veterinary medicinal product are evaluated after macroscopic examination and microscopic examination of the organs in animals of the control and experimental groups. Morphological methods can be used to test changes in various organs and tissues from minimal to obvious. Thus, under the influence of chemicals of general toxic and hepatotropic action, a typical corresponding reaction of the body is most often observed: violation of the protein, lipid, and carbohydrate metabolism, changes in the biliary and vascular systems. In general, the process may be in the nature of acute toxic dystrophy, toxic persistent hepatitis, and hepatosis. In case of intoxication with cardiotropic substances, morphologically changes are mainly diffuse in nature. Changes in the myocardium are more often registered in the form of disorders of lipid, carbohydrate, and protein metabolism in the heart muscle, which are found in eosinophilic, fuchsinophilic foci of muscle fibers, myolysis, fine-grained fatty degeneration, and coagulation necrosis. With various intoxications in the heart, there is fatty degeneration. An increase in the number of histiocytes in the stroma is often noted, but interstitial myocarditis with cellular infiltrates is rare, and its detection indicates a severe degree of intoxication. It should be noted that conducting microscopic examinations of animals in a chronic toxicological experiment has difficulties due to the need to diagnose minimal structural, structural and functional abnormalities in the organs, as well as to determine their severity (Kotsyumbas et al., 2006).

Therefore, during preclinical studies of the new drug, a detailed microscopic study of the systems and organs in experimental animals enables studying the pathogenesis of intoxication under the influence of the test substance.

Purpose. The aim of the study was to investigate microscopic changes in the internal organs of white mice in the acute and chronic experimental toxicosis with iron (IV) clathrochelate.

Materials and methods of research

The study of chronic toxicity of iron (IV) clathrochelate Na2[Fe(L-6H)]·2H2O (L – macrobicyclic hexahydrazide ligand) was performed on white mice weighing 19–25 g, formed into three groups of 15 white mice each. Mice of the 1st group (control) received water; mice of the 2nd group – a solution of iron (IV) clathrochelate at the rate of 125.8 mg/kg body weight (1/10 DL50 of the test compound); mice of the 3rd group – a solution of iron (IV) clathrochelate at a rate of 251.6 mg/kg body weight (1/5 DL50 of the test compound).

The animals were kept in the vivarium of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine, with a constant air temperature and humidity in the premises. Feeding mice provided a standard diet with constant access to water/aqueous solution of iron (IV) clathrochelate. Before the experiment, laboratory animals of all groups were kept in the adaptation period of 10 days. No deviations in the behavioral responses of white mice in both the experimental and control groups were observed.

All activities were carried out in accordance with the "General Ethical Principles of Animal Experiments" (Ukraine, 2001) and in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg: Council of Europe 18.03.1986).

During the study of the acute toxicity of iron (IV) clathrochelate, the main stages of pathomorphological research were the following: white mice that died after euthanasia (at the end of the experiment) were dissected; the macroscopic examination was performed; the organs were excised; the pieces of internal organs for histological examination were weighed and fixed.

During the study of chronic toxicity of iron (IV) clathrochelate, white mice of experimental and control groups were euthanized on days 10, 20, and 30 of the experiment; the internal organs of white mice of the 1st group (control), 2nd experimental group (1/10 DL50 of test compound), and 3rd experimental group (1/5 DL50 of test compound) were subjected to histological examination.

Histological examinations were performed in the laboratory of histology of the Academician Volodymyr Kasyanenko Department of Animal Anatomy, Histology and Pathomorphology of Faculty of Veterinary Medicine, National University of Life and Environmental Sciences of Ukraine. From each group of white mice in the established terms of research, an autopsy of 5 animals was carried out by a method of partial evisceration (Zon et al., 2009). The pieces of the liver, heart, kidneys, lungs, spleen, and stomach were taken for histological examination after pathological autopsy. The selected pieces were fixed in a 10% solution of neutral formalin, dehydrated in ethanol of increasing concentration, and poured into paraffin

through chloroform. Sections 6 ± 2 mm thick were prepared using a sled microtome and stained with Carazzi's hematoxylin and eosin. Hydropic dystrophy was differentiated from fatty dystrophy by staining frozen histological sections with Sudan III (Goralskyi et al., 2005). The study of histopreparations and their photography was performed using an OLYMPUS CX41 microscope and an OLYMPUS C-5050 camera.

Results of the research and their discussion

Histological examination of the liver in mice during acute experimental toxicosis with iron (IV) clathrochelate revealed the fragmentation of a part of liver plates and the granular dystrophy of hepatocytes. In many hepatocytes, the presence of fluid-filled vacuoles in the cytoplasm was observed, these vacuoles were not painted over in the response to the detection of lipids, which indicated the beginning of the development of hydropic dystrophy (Fig. 1).

On the 20th day, an increase in hydropic dystrophy was registered, and part of the dystrophically altered hepatocytes was destroyed (Fig. 2).

On the 30th day, complete disorganization of the structure of the liver lobes was observed and all hepatocytes were at different stages of destruction, or in a state of necrosis (Fig. 3).

Histological studies of the liver of white mice during chronic iron (IV) clathrochelate poisoning at various doses also revealed the microscopic changes but they were not as pronounced as in acute toxicity of iron (IV) clathrochelate at any time in our studies.

In the liver of mice treated with 1/5 DL50 of iron (IV) clathrochelate, granular dystrophy of hepatocytes was registered on the 10th day and in some liver cells – fluid-filled vacuoles in the cytoplasm (hydropic dystrophy). However, fragmentation of the liver plates and disorganization of the structure of the liver lobes, as observed in acute poisoning, were not observed (Fig. 4).

On the 20th day, the progression of

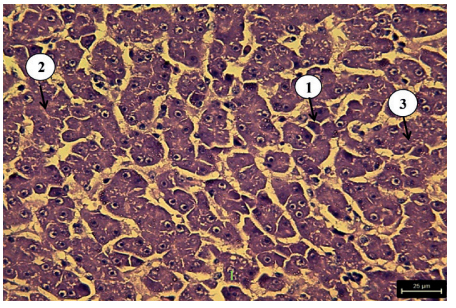

Fig. 1. The liver of a mouse during acute experimental toxicosis with iron (IV) clathrochelate on the 10th day: 1 – fragmentation of the liver plate; 2 – granular dystrophy of hepatocytes; 3 – fluid-filled vacuoles in the cytoplasm of the hepatocyte. Carazzi's hematoxylin and eosin, ×40

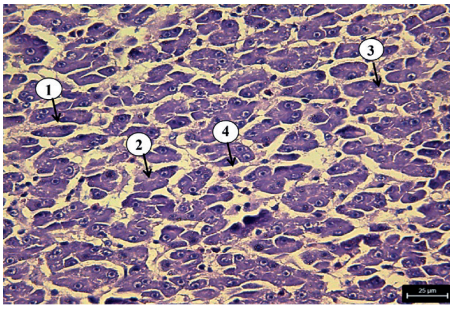

Fig. 2. The liver of a mouse during acute experimental toxicosis with iron (IV) clathrochelate on the 20th day: 1 – fragmentation of the liver plate; 2 – granular dystrophy of hepatocytes; 3 – fluid-filled vacuoles in the cytoplasm of the hepatocyte; 4 – destruction of the hepatocyte. Carazzi's hematoxylin and eosin, ×4

hydropic dystrophy in hepatocytes was detected (Fig. 5), which in some liver cells led to a complete lysis of the cytoplasm (Fig. 6).

On the 30th day, the number of hepatocytes in the state of hydropic dystrophy markedly increased but the fragmentation of liver plates and disorganization of the structure of liver lobes, as on day 10, was not detected (Fig. 7).

In the liver of mice treated with 1/10 DL50 of iron (IV) clathrochelate, granular dystrophy of hepatocytes and signs of hydropic dystrophy in some liver cells were registered on the 10th day, as well as with the use of iron (IV) clathrochelate at a dose of 1/5 DL50. However, the number of liver cells with signs of hydropic dystrophy was significantly lower (Fig. 8).

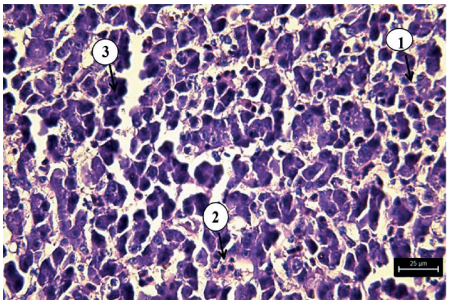

Fig. 3. The liver of a mouse during acute experimental toxicosis with iron (IV) clathrochelate on the 30th day: 1 – complete disorganization of the structure of the liver lobe; 2 – destruction of hepatocytes; 3 – necrosis of hepatocytes. Carazzi's hematoxylin and eosin, ×40

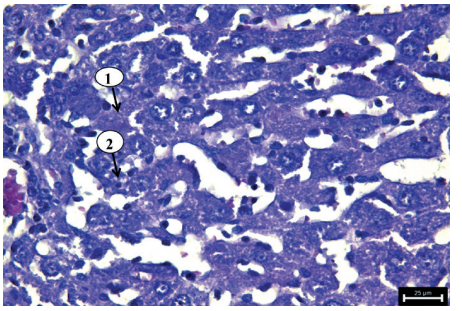

Fig. 4. The liver of a mouse during chronic experimental toxicosis with clathrochelate iron (IV) (1/5 DL50 of the test compound) on the 10th day: 1 – granular dystrophy of hepatocytes; 2 – fluid-filled vacuoles in the cytoplasm of the hepatocyte. Carazzi's hematoxylin and eosin, ×40

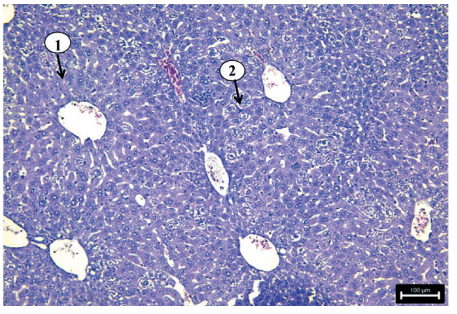

Fig. 5. The liver of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/5 DL50 of the test compound) on the 20th day: 1 – granular hepatocyte dystrophy; 2 – hydropic hepatocyte dystrophy. Carazzi's hematoxylin and eosin, ×10

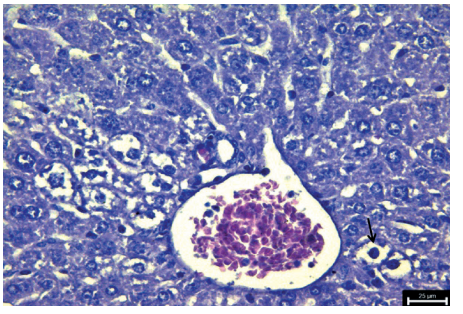

Fig. 6. The liver of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/5 DL50 of the test compound) on the 20th day: 1 – granular hepatocyte dystrophy; 2 – hydropic hepatocyte dystrophy. Carazzi's hematoxylin and eosin, ×40

On the 20th day, the number of cells with hydropic dystrophy increased slightly and on the 30th day, a small number of hepatocytes with complete lysis of the cytoplasm was detected (Fig. 9).

Thus, in the liver of mice treated with iron (IV) clathrochelate at a dose of 1/10 DL50 during chronic intoxication, the microscopic changes were similar to the microscopic changes in the liver of mice treated with the test drug at a dose of 1/5 DL50. However, the severity of these changes was lower, reflecting a lower degree of organ damage.

Histological examination of the kidneys of mice during acute experimental toxicosis with iron (IV) clathrochelate on the 10th day revealed granular dystrophy of the epithelial cells of the convoluted and straight tubules. In some tubules, the centers of the destruction of epitheliocytes were registered (Fig. 10).

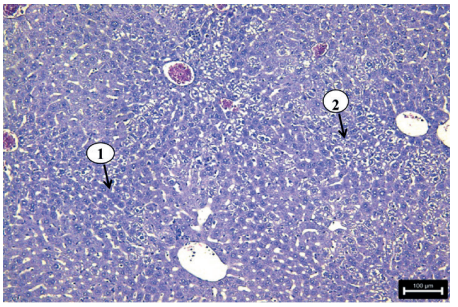

Fig. 7. The liver of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/5 DL50 of the test compound) on the 30th day: 1 – hepatic plate; 2 –hydropic hepatocyte dystrophy. Carazzi's hematoxylin and eosin, ×10

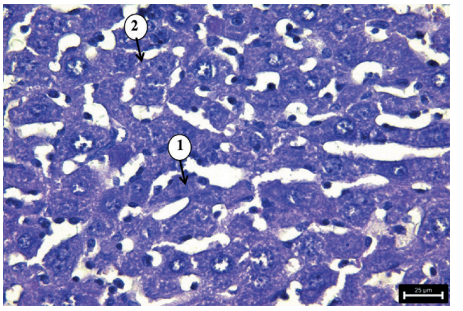

Fig. 8. The liver of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/10 DL50 of test compound) on the 10th day: 1 – granular dystrophy of hepatocytes; 2 – fluid-filled vacuoles in the cytoplasm of the hepatocyte. Carazzi's hematoxylin and eosin, ×40

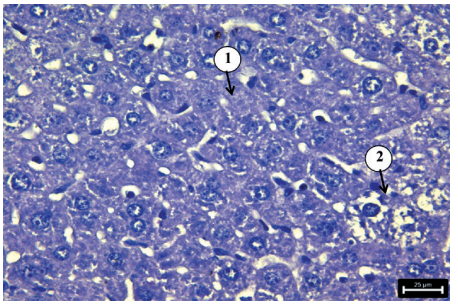

Fig. 9. The liver of a mouse during chronic experimental toxicosis with clathrochelate iron (IV) (1/10 DL50 of the test compound) on the 30th day: 1 – granular dystrophy of hepatocytes; 2 – hydropic hepatocyte dystrophy. Carazzi's hematoxylin and eosin, ×40

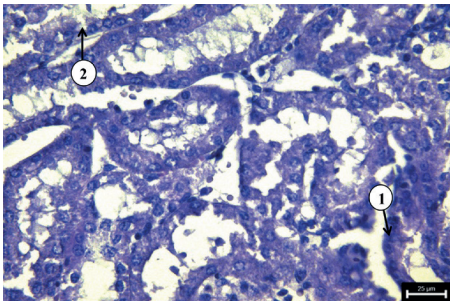

Fig. 10. The kidney of a mouse during acute experimental toxicosis with iron (IV) clathrochelate on the 10th day: 1 – granular dystrophy of the epithelium of the tortuous tubule; 2 – destruction of epithelial cells of the tortuous tubule. Carazzi's hematoxylin and eosin, ×40

On the 20th day, in addition to dystrophic changes and destruction of the epithelial cells of the renal tubules, serous extracapillary glomerulonephritis appeared (Fig. 11), and on the 30th day, necrosis of their epithelium was already registered in the tortuous tubules (Fig. 12).

Such microscopic changes indicated the rapid progression of significant renal damage in acute experimental toxicosis with iron (IV) clathrochelate.

Histological examinations of the kidneys of mice during chronic poisoning with iron (IV) clathrochelate at various doses also revealed microscopic changes but they were not as pronounced as with acute iron (IV) clathrochelate poisoning in any of the terms of research.

In the kidneys of mice treated with iron (IV) clathrochelate at a dose of 1/5 DL50 on the 10th day, as in acute poisoning, granular dystrophy of epithelial cells of tortuous and straight tubules was registered and the areas of epitheliocyte destruction were observed (Fig. 13).

On the 20th day, there was a serous extracapillary glomerulonephritis (Fig. 14).

However, later the nature of microscopic changes during chronic iron (IV) clathrochelate poisoning at a dose of 1/5 DL50 did not differ from that in acute poisoning. Necrosis of epithelial cells of the renal tubules did not develop (Fig. 15).

In the kidneys of mice treated with iron (IV) clathrochelate at a dose of 1/10 DL50, from days 10 to 30, only granular dystrophy and destruction of tubular epithelial cells were found (Fig. 16, 17).

Thus, during chronic poisoning in the kidneys of mice treated with iron (IV) clathrochelate at a dose of 1/10 DL50, the microscopic changes were similar to the microscopic changes in the kidneys of mice treated with iron (IV) clathrochelate at a dose of 1/5 DL50. However, the severity of these changes was less, reflecting a lower degree of organ damage.

Histological examinations of the heart of mice during acute experimental toxicosis with iron (IV) clathrochelate

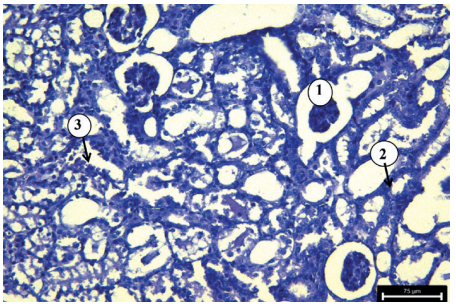

Fig. 11. The kidney of a mouse during acute experimental toxicosis with iron (IV) clathrochelate on the 20th day: 1 – serous extracapillary glomerulitis; 2 – granular dystrophy of the epithelium of the tortuous tubule; 3 – destruction of epithelial cells of the tortuous tubule. Carazzi's hematoxylin and eosin, ×20

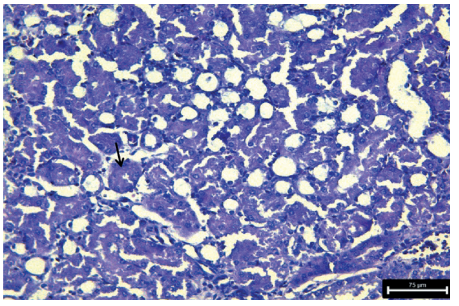

Fig. 12. The kidney of a mouse during acute experimental toxicosis with iron (IV) clathrochelate on the 30th day: necrosis of epithelial cells of the convoluted tubule (shown by the arrow). Carazzi's hematoxylin and eosin, ×20

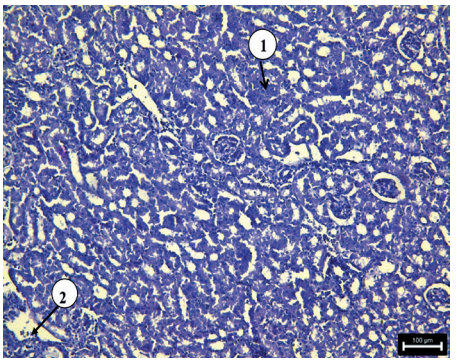

Fig. 13. The kidney of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/5 DL50 of the test compound) on the 10th day: 1 – granular dystrophy of the epithelium of the tortuous tubule; 2 – destruction of the epithelium of the tortuous tubule. Carazzi's hematoxylin and eosin, ×10

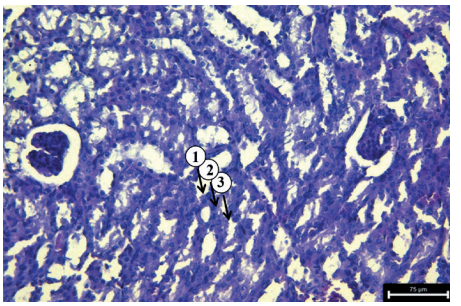

Fig. 14. The kidney of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/5 DL50 of the test compound) on the 20th day: 1 – serous extracapillary glomerulitis; 2 – granular dystrophy of the epithelium of the tortuous tubule; 3 – destruction of epithelial cells of the tortuous tubule. Carazzi's hematoxylin and eosin, ×20

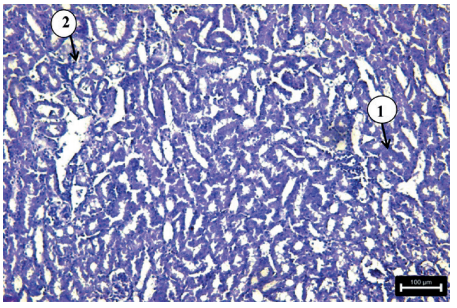

Fig. 15. The kidney of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/5 DL50 of the test compound) on the 30th day: 1 – granular dystrophy of the epithelium of the convoluted tubule; 2 – destruction of epithelial cells of the tortuous tubule. Carazzi's hematoxylin and eosin, ×10

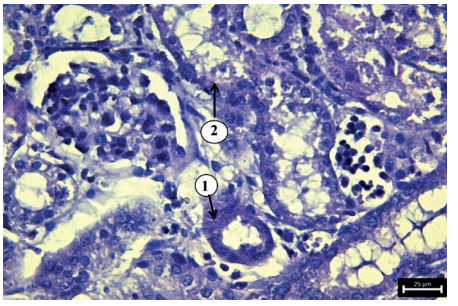

Fig. 16. The kidney of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/10 DL50 of the test compound) on the 10th day: 1 – granular dystrophy of the epithelium of the tortuous tubule; 2 – destruction of epithelial cells of the tortuous tubule. Carazzi's hematoxylin and eosin, ×40

revealed myocardial edema on the 10th day (Fig. 18).

On the 20th day, in addition to myocardial edema, granular dystrophy of myocardial cells was detected, and in part of the bundles of heart muscle fibers – granular decay of their sarcoplasm. Some of the dystrophically altered cells were destroyed (Fig. 19, 20).

On the 30th day, the presence of fairly large areas of myocardial necrosis was also found (Fig. 21).

In the epicardium and endocardium, we did not detect any microscopic

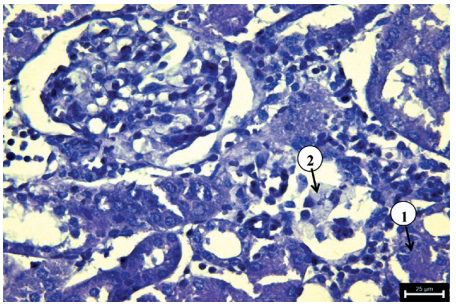

Fig. 17. The kidney of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/10 DL50 of the test compound) on the 30th day: 1 – granular dystrophy of the epithelium of the tortuous tubule; 2 – destruction of epithelial cells of the tortuous tubule. Carazzi's hematoxylin and eosin, ×40

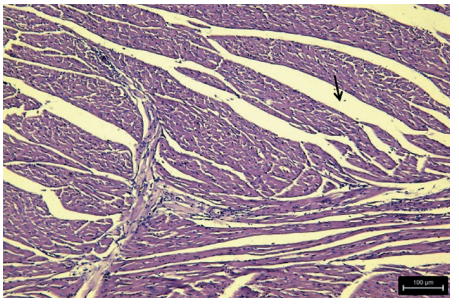

Fig. 18. The heart of a mouse during acute experimental toxicity of iron (IV) clathrochelate on the 10th day: edema between bundles of muscle fibers (shown by arrow). Carazzi's hematoxylin and eosin, ×10

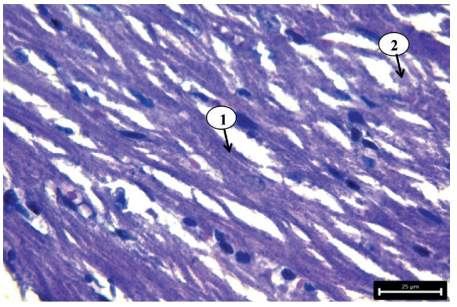

Fig. 19. The heart of a mouse during acute experimental toxicosis with iron (IV) clathrochelate on the 20th day: 1 – granular myocardial dystrophy; 2 – destruction of muscle fiber. Carazzi's hematoxylin and eosin, ×40

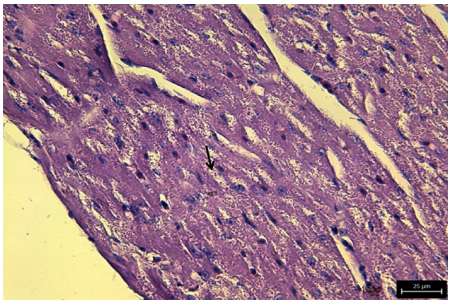

Fig. 20. The heart of mouse during acute experimental toxicosis with iron (IV) clathrochelate on the 20th day: granular disintegration of muscle fiber sarcoplasm (shown by arrow). Carazzi's hematoxylin and eosin, ×40

changes during acute experimental toxicosis with iron (IV) clathrochelate in any of our studies.

Histological examinations of mice hearts during chronic toxicosis caused by iron (IV) clathrochelate at various doses also revealed microscopic changes but they were not as pronounced as during acute poisoning with iron (IV) clathrochelate in our studies. In the myocardium of mice treated with iron (IV) clathrochelate at a dose of 1/5 DL50 on the 10th day, as in acute poisoning, edema was registered (Fig. 22).

On the 20th day, granular dystrophy of myocardial cells and destruction of some dystrophically altered cells were registered (Fig. 23), and on the 30th day, granular decay of sarcoplasm of muscle fibers was observed (Fig. 24).

On the 10th day, the myocardial edema was detected in mice treated with

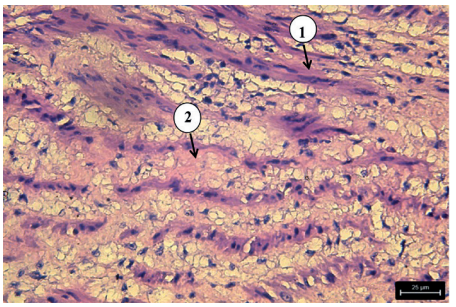

Fig. 21. The heart of a mouse during acute experimental toxicosis with iron (IV) clathrochelate on the 30th day: 1 – muscle fiber; 2 – necrosis of muscle fiber. Carazzi's hematoxylin and eosin, ×40

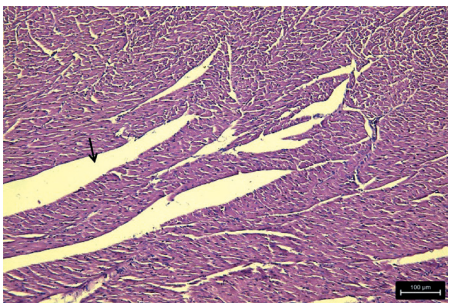

Fig. 22. The heart of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/5 DL50 of test compound) on the 10th day: edema (shown by arrow). Carazzi's hematoxylin and eosin, ×10

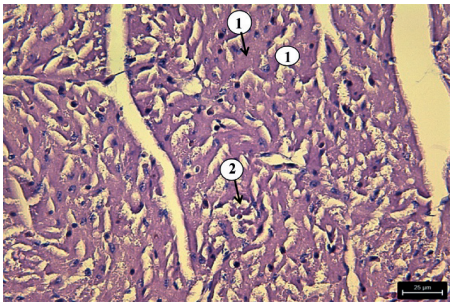

Fig. 23. The heart of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/5 DL50 of the test compound) on the 20th day: 1 – granular dystrophy of muscle fibers; 2 – destruction of muscle fibers. Carazzi's hematoxylin and eosin, ×40

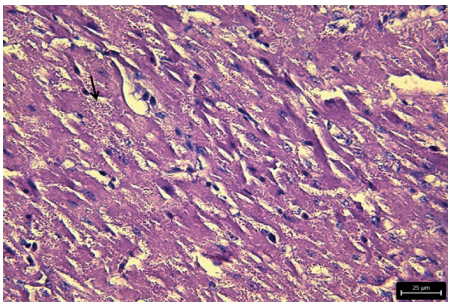

Fig. 24. The heart of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/5 DL_{50} of test compound) on the 30th day: granular disintegration of muscle fiber sarcoplasm (shown by arrow). Carazzi's hematoxylin and eosin, ×40

iron (IV) clathrochelate at a dose of 1/10 DL50, and on the 20th day – also granular dystrophy of myocardial cells (Fig. 25), and on the 30th day – the destruction of a part of the dystrophic altered heart muscle cells (Fig. 26).

As with acute poisoning, microscopic changes in the epicardium and endocardium during chronic intoxication were not found by us in any of the cases.

Thus, microscopic changes in the myocardium of mice treated with iron (IV) clathrochelate at doses of 1/5 and 1/10 DL50 during chronic intoxication were similar to microscopic changes in the myocardium of a mouse during acute poisoning with iron (IV) clathrochelate. However, the severity of these changes was lower, reflecting a lower degree of organ damage, and depended on the dose of iron (IV) clathrochelate that mice received. In the stomach, intestines, and

lungs, microscopic changes during both acute and chronic poisoning with iron (IV) clathrochelate were not detected in any of the cases (Fig. 27, 28).

In the spleen of all studied mice, the lymphoid nodules were small, fairly dense arrangement of lymphocytes, fuzzy borders, and did not contain light centers (Fig. 29), which indicated the absence of sufficiently strong antigenic stimulation.

This confirms that the animals in our studies were free of infectious agents. In addition, the red pulp had a microscopic structure characteristic of infectious diseases in mice (Fig. 30).

Therefore, the described changes indicated a violation of metabolic processes in the body of white mice. They are confirmed by previously obtained research results (Dukhnisky et al., 2019; 2020). Thus, the analysis of the coefficients of mass of the internal organs of white mice

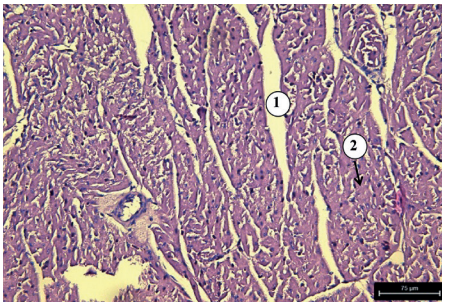

Fig. 25. The heart of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/10 DL50 of the test compound) on the 20th day: 1 – edema; 2 – granular dystrophy of myocardial cells. Carazzi's hematoxylin and eosin, ×20

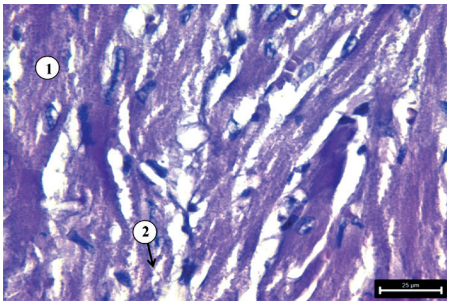

Fig. 26. The heart of a mouse during chronic experimental toxicosis with iron (IV) clathrochelate (1/10 DL_{50} of the test compound) on the 30th day: 1 – granular myocardial dystrophy; 2 – destruction of myocardial cells. Carazzi's hematoxylin and eosin, ×40

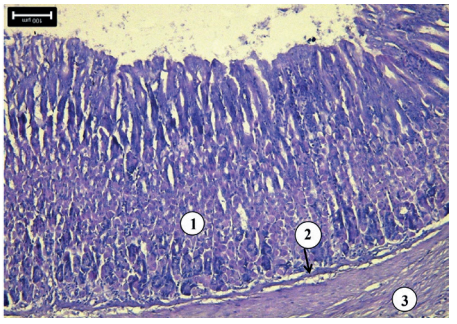

Fig. 27. The stomach of a mouse during acute experimental toxicosis with iron (IV) clathrochelate: 1 – gastric dimples; 2 – submucosal basis; 3 – muscular membrane. Carazzi's hematoxylin and eosin, ×10

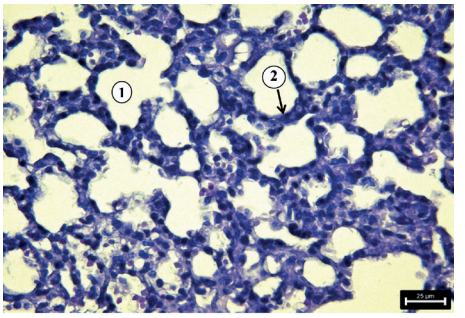

Fig. 28. The lungs of a mouse during acute experimental toxicosis with iron (IV) clathrochelate: 1 – lumen of the alveoli; 2 – the wall of the alveoli. Carazzi's hematoxylin and eosin, ×40

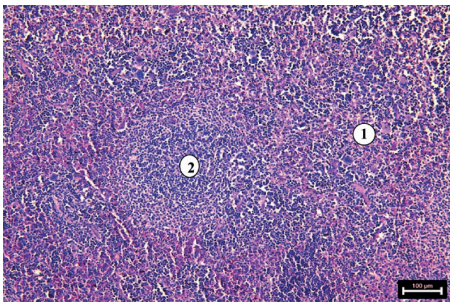

Fig. 29. The spleen of a mouse during acute experimental toxicosis with iron (IV) clathrochelate: 1 – red pulp; 2 – lymphoid nodule (white pulp). Carazzi's hematoxylin and eosin, ×10

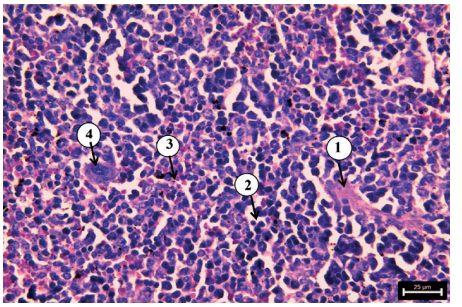

Fig. 30. The spleen of a mouse during acute experimental toxicosis with iron (IV) clathrochelate: 1 – trabeculae; 2 – lymphocyte; 3 – erythrocyte; 4 – macrophage. Carazzi's hematoxylin and eosin, ×40

on the 10th day shows an increase in the masses of all studied organs in animals of experimental groups. Moreover, under the influence of iron (IV) clathrochelate at a dose of 251.6 mg/kg body weight (3rd experimental group), these changes were more pronounced than under its influence at a dose of 125.8 mg/kg body weight. On the 20th day, only the decrease in the relative ratio of the weight of the liver by 6% and spleen by 30% ($P < 0.05$) was found in animals of the 3rd experimental group compared with animals in the control group. The indicators of relative coefficients of internal organs in mice of experimental groups on the 30th day tended to decrease compared with those of animals in the control group. The relative ratio of the weight of the liver was lower by 5% in mice of the 2nd experimental group (a dose of 125.8 mg/kg body weight) and by 13% in mice of the 3rd experimental group (a dose of 251.6 mg/kg body weight). The relative ratio of the mass of the heart was lower by 29% ($P < 0.001$) in mice of the 2nd experimental group and by 43% ($P < 0.001$) in mice of the 3rd experimental group compared with animals in the control group. The relative ratio of the mass of the kidney decreased sharply on the 30th day and was lower by 5% in mice of the 2nd experimental group and by 11% in mice of the 3rd experimental group compared with animals in the control group. The relative coefficient of the mass of the spleen was lower by 17% in mice of the 2nd experimental group and by 25% in mice of the 3rd experimental group. The described data indicate an excessive load of iron (IV) clathrochelate on these organs in white mice. Our previous results of the study of serum biochemical parameters in white mice showed that the greatest changes were in the metabolism of proteins and non-protein compounds of nitrogen, enzyme activity, glucose, and inorganic phosphorus.

Our data are confirmed by the results of studies by other researchers. Thus, the nanoparticles of iron (II) accumulate in target organs (heart, liver, and kidneys) during prolonged administration to the abdominal cavity and cause a wide range of structural and functional changes, which indicate their cardio-vasotoxic, hepatotoxic, and nephrotoxic effects. It was found that prolonged introduction of

these particles into the abdominal cavity of rats is accompanied by the accumulation of small crystalline inclusions in the cytoplasm of cardiomyocytes, hepatocytes, and nephrothelial cells. This indicates the accumulation of the nanoparticles of iron (II) in target organs, which leads to dystrophic and necrobiotic processes in cells and determines the effect of toxic action (Luhovskyi et al., 2019).

Since our studies were conducted for the purpose of preclinical studies of antianemic drugs with the active substance clathrochelate iron (IV), our attention was paid to the microscopic studies of the internal organs of animals using drugs of iron and to the microscopic changes in the body of animals suffering from the iron deficiency anemia. Antipov & Zharov (2013) established that alternative and compensatory processes develop in parenchymal and immunocompetent organs of animals suffering from iron deficiency anemia. The lesions of the liver have certain topical features that reflect the structural and functional heterogeneity of this organ. The most pronounced pathomorphological changes were observed in centrilobular hepatocytes, which were in a state of protein, fatty, and carbohydrate degeneration. Protein dystrophy develops in the epithelium of the kidney tubules. Alterative processes in the kidney tubules are accompanied by apical destruction and desquamation of nephrocytes. The results of studies of parenchymal organs indicate the initial processes of the formation of fibrosis with the possible development of renal and hepatic failure.

In the research of Chetverikova et al. (2006), the morphological studies of internal organs fully confirmed the lack of iron preserved in the body and the associated hypoxic and dystrophic phenomena. The liver had a pale color, hemosiderin was present in the form of traces diffusely, sometimes in the region of the vessels, in some sections, there were areas of necrosis of hepatocytes, the phenomena of protein and fatty degeneration were observed.

Conclusions and future perspectives

The severity of microscopic changes in the organs of white mice correlates with the dose of iron (IV) clathrochelate received by the animals. The histological changes in the liver of mice during acute experimental toxicosis with iron (IV) clathrochelate were characterized by fragmentation of a part of the liver plates, granular dystrophy of hepatocytes, and the presence of fluid-filled vacuoles was observed in the cytoplasm of many hepatocytes, and on the days 20 and 30, there was complete disorganization of the structure of the liver lobes, all hepatocytes were at different stages of destruction, or in a state of necrosis. During chronic poisoning with iron (IV) clathrochelate, microscopic changes in the myocardium of white mice were similar to microscopic changes in the myocardium of white mice during acute poisoning with iron (IV) clathrochelate. In the myocardium of mice treated with 1/10 DL50 of iron (IV) clathrochelate, only edema was detected on the 10th day, additional granular myocardial dystrophy on the 20th day, and destruction of some dystrophically altered cardiac cells on the 30th day. In the kidneys of white mice from the 10th to 30th days, we found granular dystrophy and destruction of tubular epithelial cells.

The future perspectives are the study of microscopic changes in the organs of laboratory animals of other species (white mice and quails) during experimental toxicosis with iron (IV) clathrochelate.

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Анотація. *Клатрохелат Феруму (IV) на основі макробіциклічного ліганду гексагідразидного типу – унікальна сполука, до складу якої входить Ферум у рідкісній високій валентності IV. Вона характеризується високою стабільністю за високих температур та за різних значень pH тощо. Доклінічні та клінічні дослідження цього комплексу, які розпочаті вперше в Україні, мають важливе теоретичне та практичне значення для різних наук, зокрема для галузі ветеринарної медицини, оскільки цей комплекс може бути рекомендований як діюча речовина у ферумумісних лікарських засобах із протианемічною дією. Нами було досліджено гостру та хронічну токсичність Феруму (IV) для білих мишей,*

білих щурів та перепелів. Клатрохелат Феруму (IV) відповідає III класу небезпечності згідно з класифікацією хімічних речовин за ступенем небезпечності (ГОСТ 12.1.007-76), та IV класу і ступеню токсичності «малотоксичні речовини» відповідно до класифікації речовин за токсичністю. Встановлено, що середня смертельна доза клатрохелату Феруму (IV) для білих мишей за внутрішнього введення становить 1258,3 ± 144,87 мг/кг маси тіла. За проведення доклінічних досліджень нових лікарських засобів важливе місце займають патоморфологічні дослідження, які є необхідним етапом у вивченні біологічної реакції організму тварин на дію лікарських засобів. Вони дають змогу скласти точне уявлення про характер і важкість перебігу патологічного процесу за дії досліджуваних речовин, що суттєво доповнює характеристику загальної інтоксикації за експериментального токсикозу. Установлено, що за умов експериментальної інтоксикації клатрохелатом Феруму (IV) в організмі білих мишей розвиваються патологічні зміни, які корелюють із дозою досліджуваної сполуки: чим вищою є доза, тим більш тяжкі виникають ураження. За хронічної інтоксикації у печінці та нирках білих мишей, які одержували клатрофелат Феруму (IV) у дозі 1/10 DL50, мікроскопічні зміни були подібними до мікроскопічних змін у печінці та нирках мишей, які одержували досліджуваний препарат у дозі 1/5 DL50. Проте ступінь виразності цих змін була меншою, що відображало нижчий ступінь пошкодження органу. У міокарді мишей, які одержували клатрохелат Феруму (IV) у дозі 1/5 DL50, на 10 добу, як і за гострого отруєння, реєструвався лише набряк. Виявлені зміни вказували на порушення метаболічних процесів організмі білих мишей, що підтверджується результатами досліджень, отриманими нами раніше. Перспективою подальших досліджень є вивчення мікроскопічних змін в органах лабораторних тварин інших видів за експериментального токсикозу клатрохелатом Феруму (IV).

Ключові слова: *залізо, патоморфологічні дослідження, печінка, нирки, серце, токсичність*