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Results of the Study of the Influence of Viral Liver Damage in White Rats under Experimental Conditions on Liver Tissue

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ABSTRACT

This article is supposed to determine the level of activity of viral (including SARS-CoV-2) damage to the liver tissue and the stage of liver fibrosis resulting from experimental viral damage to the liver. A viral lesion (including SARS-CoV-2) was also detected in the liver tissue, as a result of which the expression of the CD 68 marker in the liver tissue of normal and experimentally infected liver tissue was determined.

Relevance. Currently, the number of infections caused by viruses and the atypical course of clinical symptoms of diseases caused by these viruses cause increasingly severe complications, which leads to an increase in the number of complications and an increase in disability and morbidity rates. a decrease in the quality of human life causes an increase in the amount of excess expenses for the study of pathologies that can be observed in the liver as a result of exposure to various viruses and the development of measures to prevent these pathological processes is a time requirement, despite the fact that many medical professionals, scientists, research institutes have conducted and are conducting scientific and practical work, the consequences for human health remains in danger. Therefore, the development of pathological conditions in the organs of the human body, especially in the liver, and measures to prevent them are not only the dictates of time, but also requires us to develop a new view of diseases, new modern measures.

Goals and objectives. The purpose of this study was to determine liver damage caused by viral infections and the reaction of Kupfer cells to this damage using the CD 68 marker, which was used as an immunohistochemical method, as well as to study the expression of this marker in normal and experimental viral liver damage, and at the same time to determine the liver reaction to viral infection (including CoV- 2), aimed at determining the level of fibrosis that occurs in the liver as a result of the lesion.

Material and methods. In total, 30 white mongrel rats were isolated for the study, divided into two groups. 10 mongrel rats were selected for the control group and 20 mongrel rats for experimental liver damage. For general morphology, slices of 1.5x1.5 cm in size were cut from each liver, i.e. a large slice, a small slice and a medium slice, and frozen in 10% neutral formalin.

After washing in running water for 2 hours, they were dehydrated in alcohol of increasing concentration, placed in xylene paraffin for 4 hours, then filled with paraffin and paraffin blocks were prepared. Sections 5-8 microns thick were prepared from paraffin blocks, stained with Masson trichrome and examined immunohistochemically with CD 68 marker. During the examination, on the basis of morphological changes in liver cells and tissues, the necessary information was obtained, liver hepatocyte cells and pathological processes observed in blood vessels were photographed using a trinocular microscope.

Results and conclusions. CD 68 is a glycoprotein belonging to the LAMP family and expressed on the surface of macrophages and monocytes, in connection with which it is used as a marker of macrophages.Macrophages located in liver tissue were identified by K. R. Kupfer in 1876, therefore macrophages in the liver are called Kupfer cells. Kupfer cells are located in the space of the liver sinusoids. Kupfer cells are located in the endothelial cells of the sinusoids and are involved in the formation of the sinusoidal vessel wall. Kupfer cells have an amoeba-like shape, have microvilli and pseudopodia on their surface, with which they ingest and phagocytize iodine corpuscles.Kupfer cells are located in the centrolobular and periportal regions of the liver lobe.Kupfer cells in the periportal region are larger and contain more lysosomes and are characterized by high phagocytosis. The cells of the centrolobular region are adapted to the generation of superoxide radicals. There are two groups of macrophages in the liver: lipid metabolism, cleaves protein complexes and small particles phagocytizes apoptotic cells. The cells of this group differ from monocytic macrophages in that they have proliferative properties and restore their number.The second group consists of cells formed in the bone marrow and transformed into macrophages after the transition to the liver.The function of Kupfer cells is the cleavage of foreign bodies trapped in the liver, phagocytosis, and produce cytokines that increase inflammation and stop inflammation.At the same time, biologically active substances produced by Kupfer cells activate Ito cells, resulting in fibrosis. Based on the CD68 marker, the expression level of macrophage cells can be calculated. In total, 500 cells were counted, and it was determined how many of them expressed this marker at a positive level, and what percentage of all cells were positive. 1) 10% is a low level, 2) 10-20% is an average level, 3) more than 20% is considered a high level of expression.

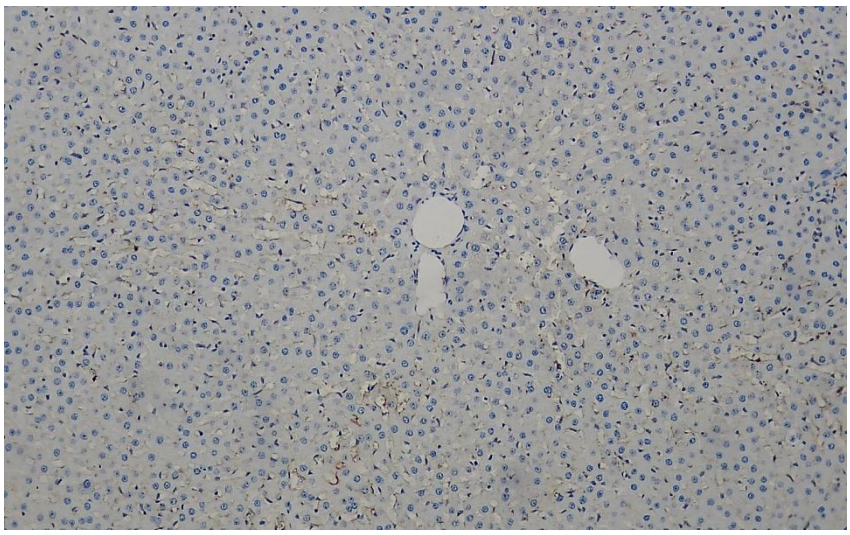

Figure 1. Low expression of the CD68 marker in the sinusoidal space, the periportal region and the central vein region in the liver of a white mongrel rat of the control group. Immunohistochemical staining. Size 10x4

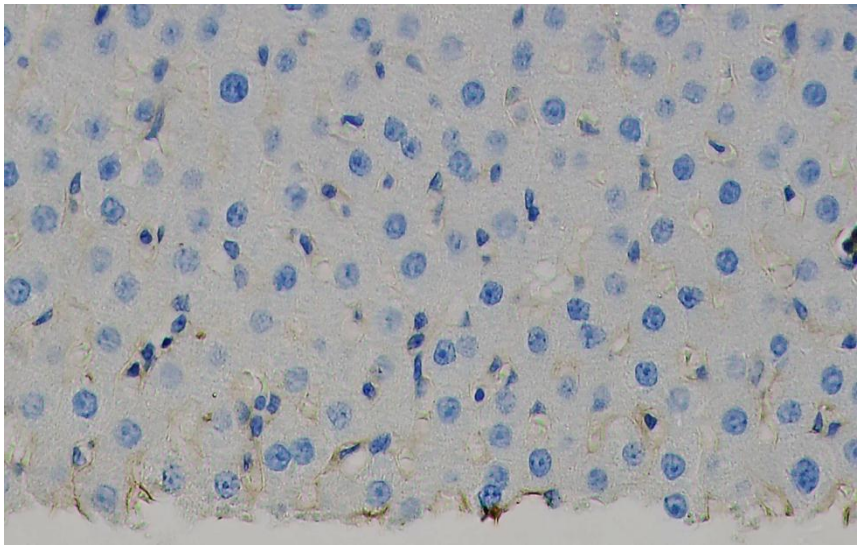

Figure 2. Liver of a white mongrel rat of the control group. Low expression of the CD68 marker in the sinusoidal cavity. Immunohistochemical staining. Size 10x4

Taking into account the above, the purpose of this study was to determine the level of expression of the immunohistochemical marker CD 68 in normal experimental viral liver lesions and the level of liver damage. In the study of micro-preparations prepared from the liver of white rats of the control group, the expression of the CD68 marker was less than 10%. It was found that expression occurs in the periportal region and the centrolobular region of the liver sinusoids (Fig. 1, 2). In experimental viral liver damage, micro-preparations prepared from the liver of 20 mongrel rats were studied.In 45% of cases, the CD 68 marker was expressed in a low amount, in 35% of cases it was expressed at an average level, and in 20% of cases it was expressed at a high level.The severity was high in the portal vein, in the perinatal region, in the bile ducts.Circumference and in the area of the central vein of the liver (Fig. 3, 4).

Another goal of ours was to determine the level of fibrosis that occurs in the liver during control and experimental viral liver damage. Masson trichromic staining is a widely used method in histology and pathology. It is used to identify connective tissue, muscle fibers and cells in biological tissues. Using this method, it is possible to determine the level of fibrosis in tissues. Collagen turns blue, cytoplasm, keratin, muscle fibers are red, red blood cells are yellow, colored, fibrosis in the liver was evaluated by metavir. (IASL, Butts-Ludwig and Metavir).

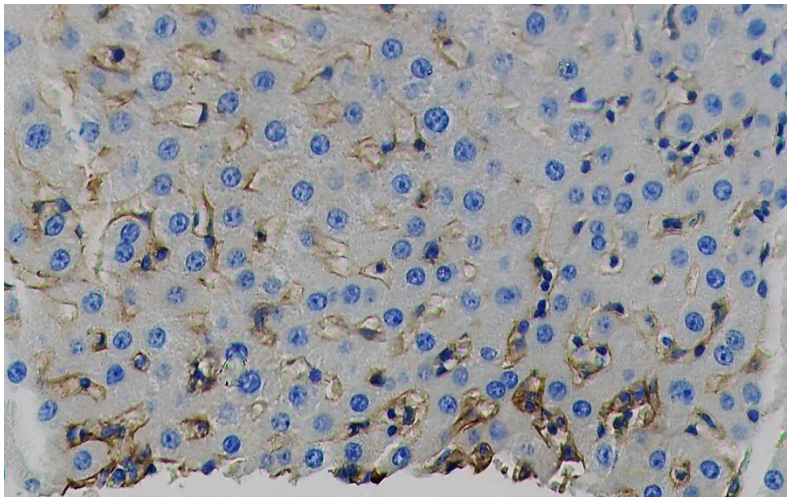

Figure 3. High expression of the CD68 marker in the sinusoidal spaces of the liver in experimental viral liver damage.The color is immunohistochemical. Size 10 x 40

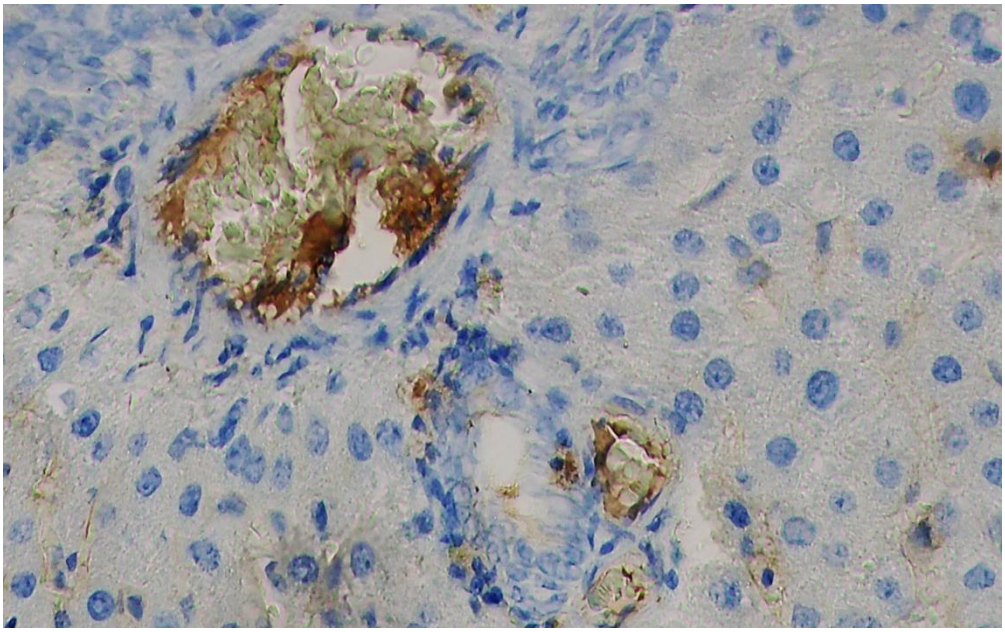

Figure 4. High expression of CD 68 marker in the area of the collar, around the central vein and around the bile ducts in experimental viral liver damage.The color is immunohistochemical. Size 10 x 40

Batts-Ludwig is a widely used system for determining the degree of fibrosis, and the results obtained using this system were evaluated. According to the Butts-Ludwig metavir, the degree of fibrosis is estimated from 0 to 4 stages. 0 or F0- fibrosis was not detected, 1 or F1-fibrous dilation of the subcutaneous vein, 2 or F2-fibrosis with a small number of obstruction coming out of the portal tract, 3 or F3 fibrosis with numerous obstruction protruding from the portal tract.4 or F4 is the stage of cirrhosis, in which regenerative nodes are surrounded by fibrous tissue. The results of viral liver damage in the control and experimental groups were obtained and evaluated by metavir. Micropreparations taken from the liver of white rats of the control group were stained with trichrome Masson, a normal amount of collagen was found around the portal tract, it is clear that the central vein of the liver section was stained to a minimal extent (Figure No. 5, 6).

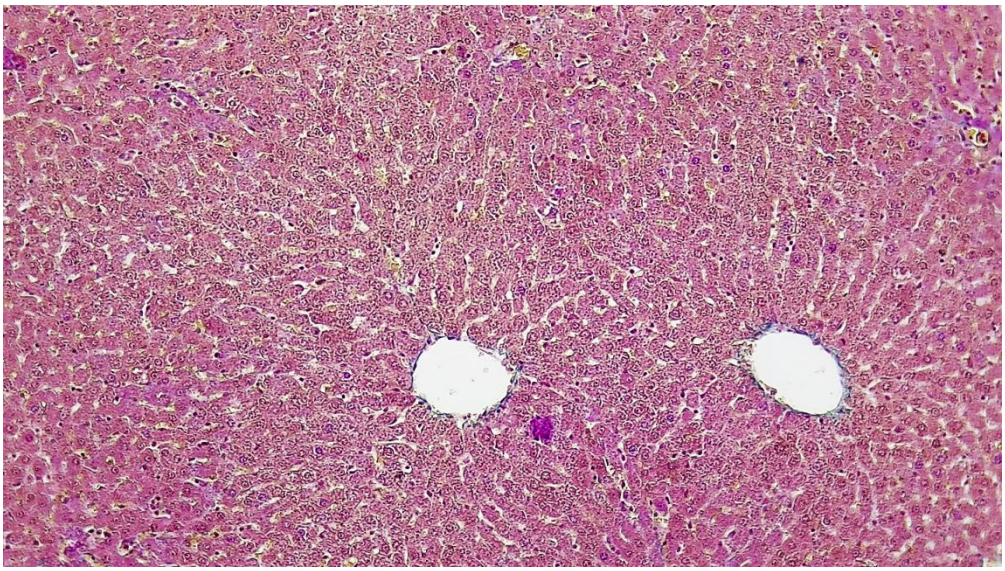

Figure 5. Liver of white mongrel rats of the control group, stained with Masson trichrome, with a normal amount of blue collagen around the portal tract and central vein. Size 10x4.

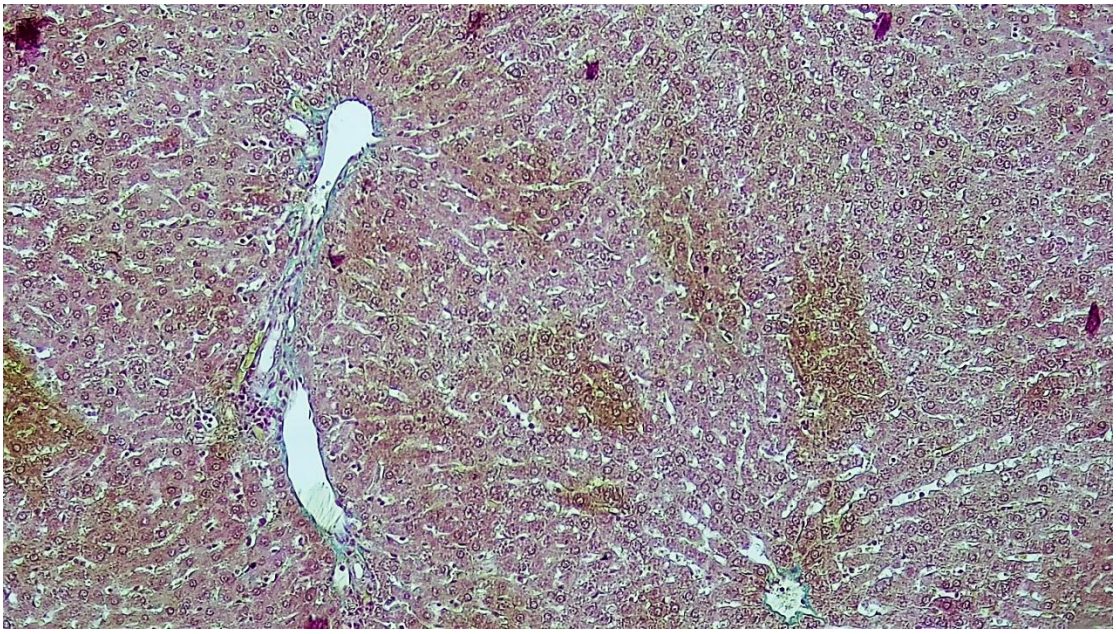

Figure 6. Liver of white mongrel rats of the control group, stained with Masson trichrome, with a normal amount of blue collagen around the portal tract and central vein.

Micropreparations obtained from the group of experimental viral liver damage were evaluated with Metavir and the results obtained as a percentage were determined as follows: 30% F0 was detected, of which 50% F 1, i.e. expansion of portal vein fibrosis. 17% of them had F2, i.e. a small number of short obstructions around the portal tract of fibrosis, and in 3% of cases there was a large number of obstructions from the portal tract of fibrosis F3 (photo No. 7,8).

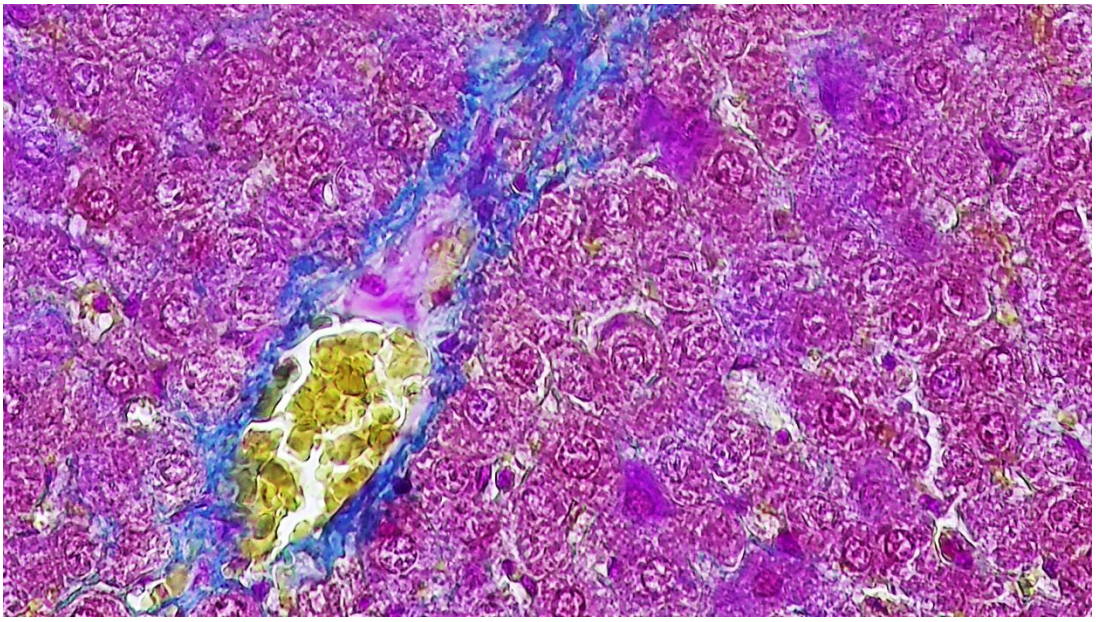

Figure 7. Liver of an outbred rat of the experimental viral liver lesion group b, stained with Trichrome Masson, increases the level of fibrosis around the portal tract and central vein, blue collagen.

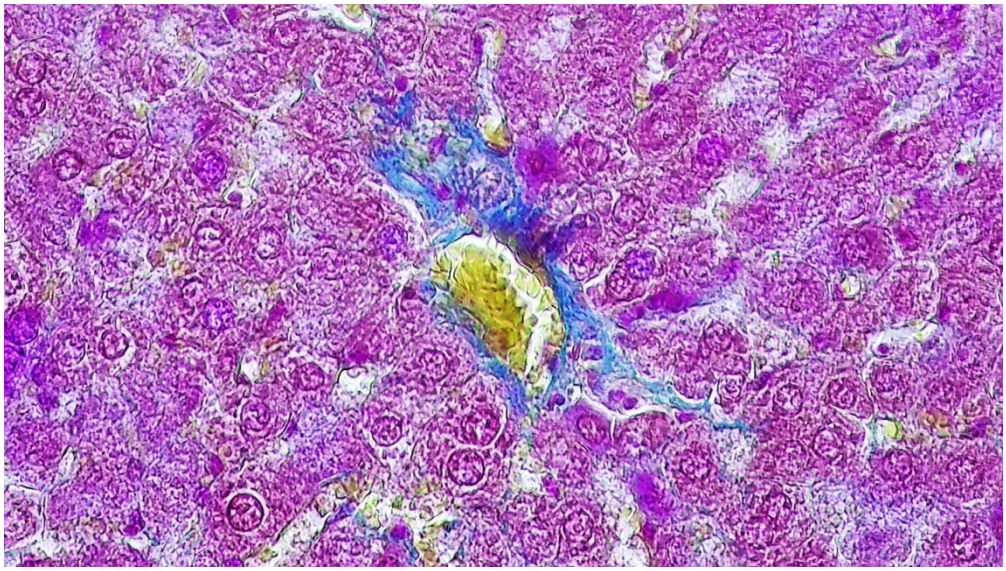

Figure 8. Liver of a bred rat of the experimental viral liver lesion group stained with Trichrome Masson, the level of fibrosis around the portal tract and central vein, blue collagen increases.

Conclusions.

- In our study, when studying micro-preparations prepared from the liver of white rats of the control group, the expression of the CD 68 marker was 10% lower, expression was detected in the periportal and centrolobular sinusoidal regions of the liver.
- When studying microsections prepared from the liver of mongrel white rats with experimental liver damage, the expression of the CD 68 marker was detected at a low level in 45% of cases, at an average level in 35% of cases and at a high level in 20% of cases.Expression was high in the portal vein area, in the peripartal region, around the bile ducts and in the central vein area.
- When staining micro-preparations obtained from the liver of a white mongrel rat of the control group with Masson trichrome, it is clear that a normal amount of collagen is located around the portal tract, minimal staining is observed around the central vein of the liver slice.
- Micro-preparations obtained from the group of experimental viral liver damage were evaluated by Metavir and the results obtained were determined as a percentage as follows: 30% F0 was detected, of which about 50% F 1, i.e. portal vein fibrosis. Of these, 17% had F2, i.e. several short obstructions around the portal tract of fibrosis, and 3% of cases had a large number of obstructions coming out of the portal tract of fibrosis F3.

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