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MODELING OF LIVER FIBROSIS IN SMALL LABORATORY ANIMALS

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ABSTRACT

Chronic toxic liver damage resulting in cirrhosis was caused by intraperitoneal administration of a 30% solution of CCl_4 in olive oil at a dose of 1 ml/kg of body weight 2 times a week for 30 days, followed by the introduction of a 50% solution in olive oil at a rate of 1 ml/kg. kg into the abdominal cavity for up to 50 days. To potentiate the hepatotoxic effect of CCl_4 , the animals received orally 5 ml of 5% ethanol solution once a day, starting from the 3rd day of the experiment. The duration of the experiment was 60 days. Histological studies of the liver of experimental animals were carried out on the 20th, 30th, 40th, 50th and 60th days from the start of the experiment. Modeling of chronic toxic hepatitis with the transition to cirrhosis by the introduction of carbon tetrachloride with ethyl alcohol from the 20th day was accompanied by the development of massive necrosis in the central, third functional zone of the liver lobules. In the subsequent periods of the experiment, the development of a diffuse inflammatory reaction in the form of lymphohistiocytic infiltration was noted, mainly along the portal tracts and around the central veins. On the 60th day, the formation of dense cell infiltration and bundles of connective tissue in the form of portal cirrhosis around the vascular triads and central veins was noted, which was confirmed by staining the collagen fibers with picrofuchsin red.

Key words: morphology, liver, fibrosis, model

INTRODUCTION

Liver fibrosis (LF) is a key link in the development of a pathological process in the liver tissue, and the degree of fibrosis is a rather sensitive nonspecific marker of pathological changes in the liver under the influence of various etiological factors [4, 8, 13]. According to WHO forecasts, over the next 10-20 years,

mortality from liver diseases will increase by 2 times [6, 9]. According to data for 2018, in the world, the death rate from end-stage liver fibrosis (cirrhosis) ranks 9th among all causes of death and 6th among people of working age, i.e. it is more typical for persons of mature age [12]. According to the authors, in Russia this figure is higher and averages 60.5 cases per 100,000 population. Every year, more than 50 million people become infected with hepatitis viruses, approximately 10% of those who have been ill develop chronic viral hepatitis (CVH), in 20% of cases, CVH ends with cirrhosis, and 5% develop hepatocellular carcinoma. According to some authors, there are currently approximately 200 million patients with chronic liver diseases in the world, about 30% of them are cirrhosis of the liver (LC) [2, 5, 9]. According to the latest WHO data published in 2017, deaths from liver diseases in Uzbekistan reached 7.936 or 4.7% of total mortality [10]. According to the authors, the age-adjusted mortality rate is 32.38 per 100,000 population, Uzbekistan ranks 27th in the world.

In the region of Central Asia, liver lesions of various origins occupy a leading place among pathologies. The frequency of liver pathologies remains stably high, tends to become chronic and cause irreversible disturbances in the functioning of all body systems. Most of the chronic liver diseases can be attributed to the group of hepatitis - diffuse liver diseases of various etiologies, characterized by hepatocellular necrosis, inflammation and fibrosis of the portal tracts, occurring for 6 months or more. One of the outcomes of chronic hepatitis is cirrhosis of the liver. In clinical practice, in recent years, the number of patients with non-alcoholic fatty liver disease (NAFLD) has been increasing, as well as alcoholic liver disease (ALD), which is an important medical and social problem, because, despite the seeming favorable course, these diseases are accompanied by the development of inflammatory changes in the liver with an outcome in cirrhosis and liver failure. Although alcohol consumption has declined significantly in recent years, the statistics do not inspire optimism. More than 2 million people with a predisposition to alcoholism have liver damage and about 14 thousand die annually from liver cirrhosis [7]. The combined effects of toxicants, viruses and alcohol accelerate the chronicity of the disease and the transition to cirrhosis.

For decades, many researchers have sought to develop an adequate experimental model of acute and chronic liver pathology, including cirrhosis, for an objective assessment of the therapeutic and preventive efficacy of hepatoprotective drugs that are promising for clinical practice. To date, there are models based on chronic intoxication with formaldehyde, carbon tetrachloride (CCl_4), sovtol-1, ethanol, heliothrin, dimethylnitrosamine, paracetamol, heliothrin and other toxicants, on a chronic deficiency of essential nutrients, which, in fact,

provide more the development of toxic liver damage - acute toxic hepatitis or acute dystrophy, and to a lesser extent - chronic hepatitis and cirrhosis of the liver. An analysis of the literature indicates that the clinical and morphological manifestations of liver damage when modeling liver cirrhosis in an experiment in animals do not fully meet the criteria for liver cirrhosis in humans [1]. Therefore, in recent years, a combination of several hepatotoxins with a synergistic effect has been used to reproduce fibrotic changes in the liver with a subsequent transition to cirrhosis. So, Golofeevsky V.Yu. et al. (2017) recommend the use of dimethylnitrosamine at a dose of 5–10 mg/kg in combination with ethanol at a dose of 3 mg/kg every other day for 6 weeks [3]. However, this model has some drawbacks; in particular, DMNA is a carcinogenic compound, its toxicity increases with the induction of cytochrome P-450. Models based on the use of paracetamol are widely used in experimental hepatology, especially in combination with ethanol. However, the drug leads to the development of methemoglobinemia, leading to hemodynamic hypoxia. Most authors use CCl_4 to reproduce the model of liver cirrhosis, the mechanism of action of which is associated with the formation of the electrophilic radical CCI_3 [14]. Therefore, it was of interest to improve the model of reproduction of liver cirrhosis with carbon tetrachloride and ethanol. **The purpose of the study: to** improve the model of liver cirrhosis in experimental animals.

Material and research methods

Chronic toxic liver damage resulting in cirrhosis was caused by intraperitoneal administration of a 30% solution of CCl_4 in olive oil at a dose of 1 ml/kg of body weight 2 times a week for 30 days, followed by the introduction of a 50% solution in olive oil at a rate of 1 ml/kg. into the abdominal cavity 2 times a week for up to 50 days. To potentiate the hepatotoxic effect of CCl_4, the animals received orally 5 ml of 5% ethanol solution once a day, starting from the 3rd day of the experiment. The duration of the experiment was 60 days. Histological studies of the liver of experimental animals were carried out on the 20th, 30th, 40th, 50th and 60th days from the start of the experiment. Liver fragments for histological examination were fixed in a mixture of 10% formalin, ethyl alcohol and acetic acid, embedded in paraffin. Sections 4–5 μm thick were stained with hematoxylin and eosin (assessment of the general structure) and picrofuchsin according to Van Gieson (assessment of the connective tissue). The preparations were studied using Polyvar and Leica DMRE research microscopes, a digital video surveillance system, and the Videotest-4 image analysis program. The preparations were subjected to stereomorphometry with computer image processing for a more

distinct selection of red-stained collagen fibers. The digital material was processed by the method of variation statistics.

Results and its discussion

The results of macroscopic studies showed that on the 20th day of the experiment, signs of portal hypertension were revealed, manifested by pronounced venous plethora of the organs of the gastrointestinal tract, opening of anastomoses and the formation of roundabout blood flow paths. The size of the spleen is enlarged, the tissue looked flabby and plethoric. The presence of a small amount of fluid was noted in the pockets of the peritoneum. The liver is slightly enlarged, dark red, flabby, plethoric. In subsequent periods, the identified changes increased. The centralization of collateral circulation, the development of hepato- and splenomegaly with an uneven and granular surface were visually noted.

The results of microscopic examination showed that on the 20th day of modeling chronic toxic hepatitis with the transition to cirrhosis in the liver, massive necrosis was observed in the central, third functional zone of the lobules. At the same time, a pronounced delimitation of the central sections of the liver lobules from the peripheral part is revealed in the form of a deep protein hyaline droplet degeneration of hepatocytes with signs of necrosis and necrobiosis. In the central part of the liver lobules, where necrobiotic changes are noted, pronounced dystrophic changes are detected in hepatocytes in the form of vacuolar and hyaline-droplet protein dystrophy, which are manifested by a violation of the histotopography of the beam structure and histotopography of hepatocytes in the form of the disappearance of nuclei, vacuolization and hyalinization of the cytoplasm of hepatocytes. Nuclear structures of hepatocytes in a state of karyorrhexis, karyolysis (Fig. 1a). Kupffer cells are hypertrophied and have phagocytosed particles in the cytoplasm. On the part of the vascular-stromal tissue, the development of necrobiotic changes is also noted in the form of decay and fibrinoid necrosis of the wall of the sinusoids and the connective tissue of the Disse space.

In the peripheral, periportal zone of the liver lobules, the histostructure of both the parenchyma and the stroma of the liver tissue is preserved. The vessels of the portal tract are somewhat dilated, plethoric, in the perivascular zone development of edema and tissue vacuolization is noted. Hepatocytes of this zone have different histomorphological structures (Fig. 1b). Most of the hepatocytes are swollen due to hyaline droplet protein dystrophy with slight vacuolization of the karyoplasm of the nuclei. Among them there are hepatocytes with severe vacuolar degeneration of the cytoplasm and swelling of the nuclei. Some of them are turned

into balloon cages. Between the beams of hepatocytes, the sinusoids and the space of Disse are expanded, in the lumen it contains an eosinophilic proteinaceous mass.

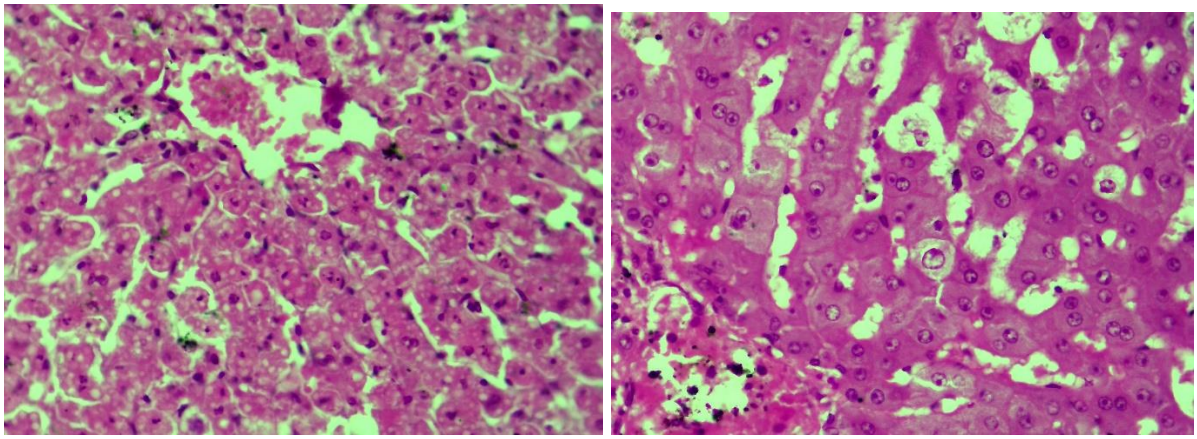

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Figure 1. 20th day of the experiment. The central part of the liver lobule (a): expansion of the central vein and necrobiotic changes in hepatocytes; periportal zone of the liver lobule (b): hyaline droplet and vacuolar degeneration of hepatocytes. Coloring: G-E. SW: 10x40.

On the 30th day of the experiment, the overview image of the liver tissue shows the separation of two zones different in histotopography. The periportal zone differs from the central zone of the lobules in its density and preservation of cellular elements. In the central lobular zone of the liver lobules, resorption of the necrotic mass is noted with the formation of multiple cavities of various shapes and sizes. Between the cavities, there is a preservation of surviving hepatocytes and Kupffer cells, which, due to stretching, have acquired elongated shapes. In places, hepatocytes are located in groups that are intensely stained with eosin, nuclear structures are also hyperchromic and have different shapes and sizes (Fig. 2a). Kupffer cells are also elongated, hypertrophied in the cytoplasm with multiple phagocytosed inclusions.

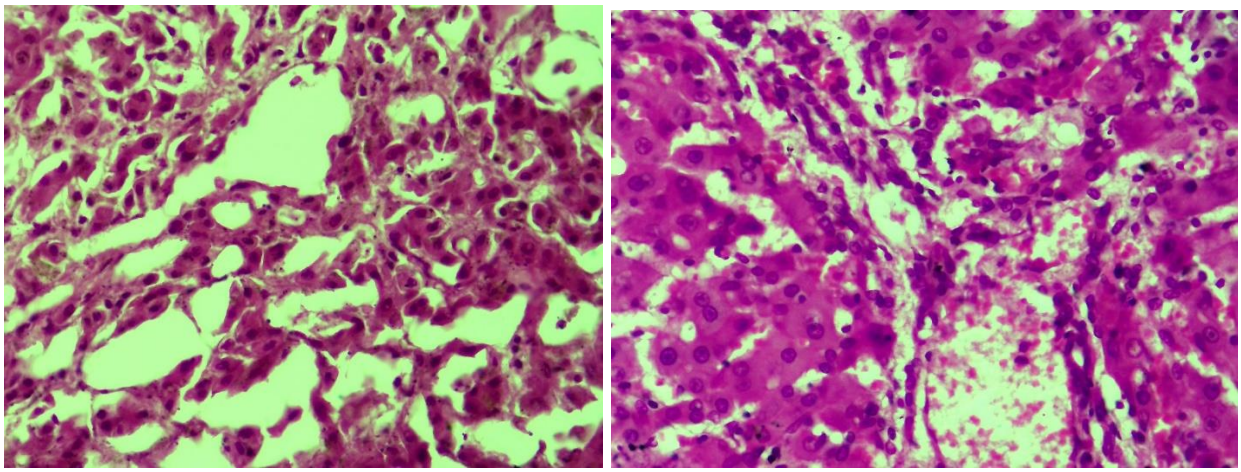

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Figure 2. 30-day experiment. The central part of a lobule of liver tissue with many vacuoles, preserved hepatocytes and Kupffer cells (a). Periportal zone of the liver lobules, vascular plethora, interstitium edema, hyaline droplet degeneration of hepatocytes (b). Coloring: G-E. SW: 10x40.

In the periportal part of the tissue, vasodilatation, plethora, and perivascular diapedetic hemorrhages are noted. At the same time, loosening and edema of the cellular and tissue elements of the vessel wall are noted. On the part of the liver parenchyma, edema of the sinusoids and the space of Disse is noted, in the lumen of the latter, the presence of single leukocytes and lymphocytes is determined. The hepatic beams are swollen due to hyaline-drop dystrophy of the cytoplasm, the nuclei of the hepatic cells are randomly located with clarification of the karyoplasm and hypertrophy of the nucleoli (Fig. 2b).

On the 40th day of the experiment, the overview image of the liver tissue shows the development of diffuse proliferative inflammatory infiltration around both the portal tracts and the central veins. Inflammatory infiltration consists of lymphoid and histiocytic cells, which are located directly around the vessels and the interstitium of the hepatic beams. In places, infiltration penetrates into deep sections of the liver parenchyma. Liver parenchyma or hepatocytes in a state of vacuolar dystrophy, in some places with the formation of balloon cells.

The wall of the central vein is thickened due to infiltration with hemato-histiocytic cells, which are located loosely in the form of increased permeability and diapedetic hemorrhages (Fig. 3a). The lumen of the vein contains an eosinophilic homogeneous plasma mass with pigment inclusions. Hepatocytes adjacent to the central vein are in a state of histotopography disturbance due to severe vacuolar dystrophy of both the cytoplasm and nuclear structures. Some nuclei of hepatocytes are in a state of karyolysis and karyopyknosis.

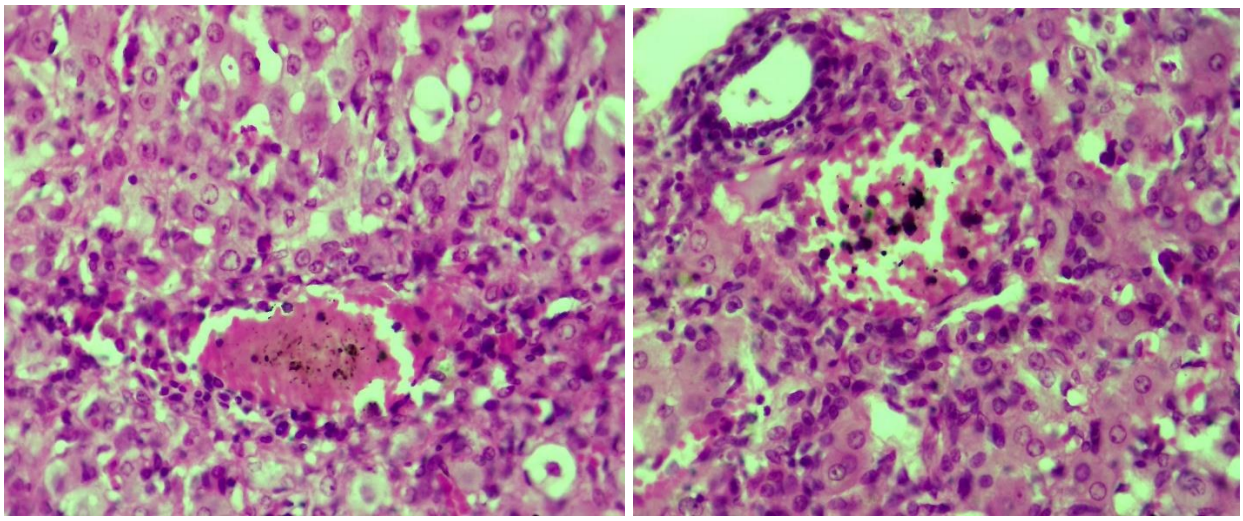

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Figure 3. 40th day of the experiment. Lymph-histiocytic infiltration around the central vein (a). Periportal lymphohistiocytic infiltration (b). Coloring: G-E. SW: 10x40.

In the periportal zone of the liver tissue, there is also a pronounced lymphohistiocytic inflammatory infiltration directly around the vessels of the

portal tract, and the spread of infiltration in the interstitium of the liver trabeculae is noted (Fig. 3b). The composition of inflammatory infiltration is dominated by histiocytic cells, which indicates the development of connective tissue. Hepatocytes are also in a state of vacuolar dystrophy and necrobiosis.

The results of the morphological study showed that on the 50th day of the experiment, signs of cirrhosis appeared in the liver tissue, which were manifested by the growth of inflammatory infiltration and connective tissue around the portal tracts and around the central vein. Along the portal tracts, the presence of proliferative inflammatory infiltration from lymphohistiocytic cells persists, in the thickness of which connective tissue layers appear in the form of fibrous structures and bundles of histiocytic cells (Fig. 4a). It is noted that connective tissue bundles grow around the hepatic lobules and reach the circumference of the central vein. The proliferation of connective tissue leads to the development of degenerative and dysregenerative changes in hepatocytes. Histochemical examination by the van Gieson method showed the presence of thick and thin bundles of fibrous structures stained red with picrofuchsin (Fig. 4b), which spread between the cellular elements of the liver parenchyma.

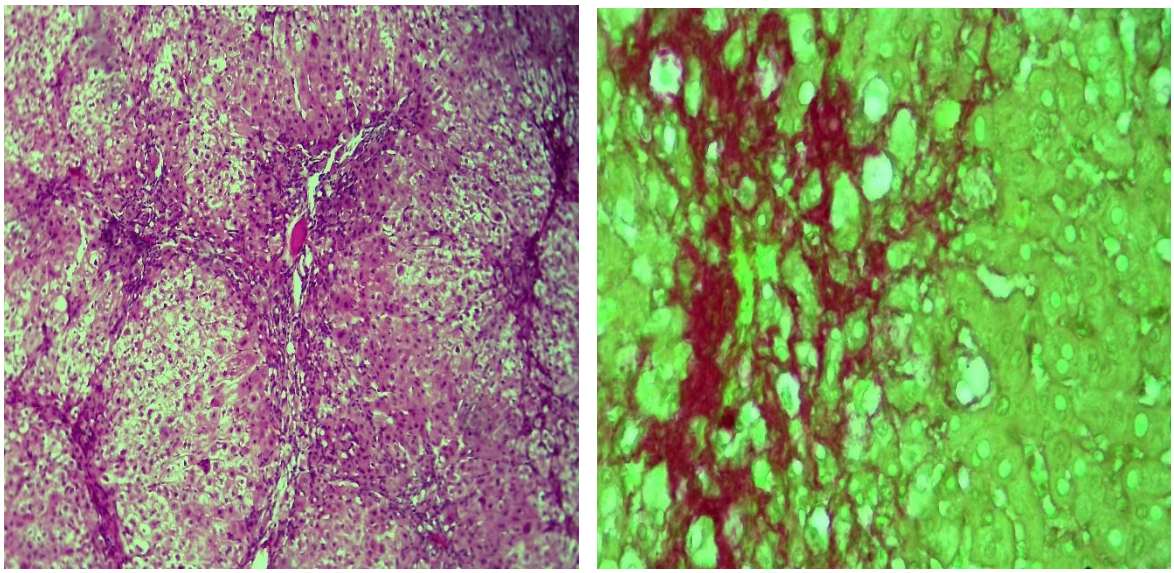

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Figure 4. 50 days of the experiment. The appearance of signs of cirrhosis in the form of periportal and perivenous layers of connective tissue. Coloring: G-E. UV: 10x20 (a). The presence of fibrous structures of connective tissue around the portal tracts. Colour: Van Gieson. UV: 10x40 (b).

The results of a microscopic examination of the liver tissue on the 60th day of the experiment showed that, in contrast to the previous periods, there was an overgrowth of connective tissue along the portal tracts and central veins. Particularly pronounced changes are determined by the region of the portal tracts in the form of dense lymphohistiocytic infiltration with the formation of

connective tissue layers. As part of the connective tissue bundles, densely packed hepatic cells and bile ducts are determined. Cellular infiltrates and bundles of connective tissue penetrate into the spaces between the beams and some of them reach the central vein. The liver parenchyma is represented by vacuolized hepatocytes; in the central part of the liver lobules, vacuolar dystrophy reaches ballooning transformation and necrobiotic destruction of liver cells (Fig. 5a). When staining histological preparations by the van Gieson method, collagen fibers are detected around the portal tracts and central veins.

The vessels of the portal tracts are narrowed due to pronounced cellular infiltration and proliferation of connective tissue. The infiltrate bundles consist of activated histiogenic cells with hyperchromasia of both the cytoplasm and nuclear structures. Cellular infiltrate and bundles of connective tissue densely pack blood vessels and liver cells. Hepatocytes are in different histotopographic states. With a large microscope objective, dense and thick bundles of collagen were revealed, located around the central vein and penetrating into the interstitium of the hepatic parenchyma along the periphery (Fig. 5b).

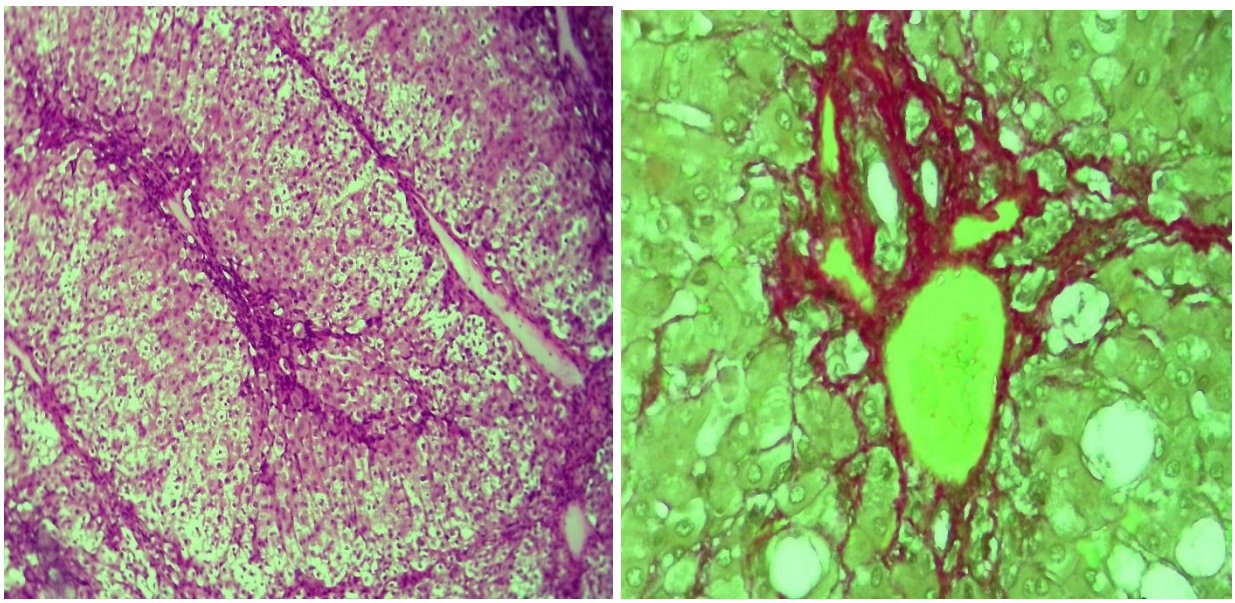

Figure 5. Liver parenchyma in a state of vacuolization of hepatocytes. Coloring: G-E. UV: 10x10 (a). Bundles of collagen fibers around the central vein stained with picrofuchsin. Colour: Van Gieson. UV: 10x40 (b).

It should be said that normally collagen metabolism in the extracellular matrix is a regulated process and there is no excessive accumulation of collagen due to its proteolysis under the influence of specific matrix metalloproteinases [11]. Increased apoptosis of damaged hepatocytes stimulates the fibrogenic activity of liver myofibroblasts, and the accumulation of lymphocytes or polymorphonuclear cells activates collagen-synthesizing Ito cells [11]. The inducers of these processes are the products of hepatocyte necrosis; cytokines,

products of alcohol metabolism, lipid peroxidation and excess iron in liver tissue. Cytokines, chemokines, growth and angiogenesis factors, peroxisomal proliferation-activating receptors, acute phase proteins, caspases, and components of the renin-angiotensin-aldosterone system serve as important regulators of fibrosis and are being investigated as potential targets for antifibrotic therapy [5, 15].

Thus, the modeling of chronic toxic hepatitis with the transition to cirrhosis by the introduction of carbon tetrachloride with ethyl alcohol from the 20th day was accompanied by the development of massive necrosis in the central, third functional zone of the liver lobules. In the subsequent periods of the experiment, the development of a diffuse inflammatory reaction in the form of lymphohistiocytic infiltration was noted, mainly along the portal tracts and around the central veins. On the 60th day, the formation of dense cell infiltration and bundles of connective tissue in the form of portal cirrhosis around the vascular triads and central veins was noted, which was confirmed by staining the collagen fibers with picrofuchsin red.

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