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THE STUDY OF HISTOLOGICAL PARAMETERS IN THE DEVELOPMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE IN RATS

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease over the world, and it persists at a high prevalence. NAFLD is characterized by a spectrum of histopathological findings, ranging from simple fatty liver through non-alcoholic steatohepatitis (NASH) to fibrosis and ultimately cirrhosis, which may progress to hepatocellular carcinoma. Understanding the pathophysiology and treatment of NAFLD in humans has currently been limited by the lack of satisfactory animal models. The ideal animal model for NAFLD should reflect all aspects of the intricate etiopathogenesis of human NAFLD and the typical histological findings of its different stages. The choice of a suitable animal model for this disease while respecting its limitations may help to improve the understanding of its complex pathogenesis and to discover appropriate therapeutic strategies.

KEYWORDS: Animal Model, High-Fat Diet, Non-Alcoholic Fatty Liver Disease, Non-Alcoholic Steatohepatitis.

To date, non-alcoholic fatty liver disease is one of the most common diseases of the hepatobiliar system. In the US, it accounts for 20-30% of the population structure, in Asian countries - 12-24%. Over the past decades, the prevalence of non-alcoholic fatty liver disease around the world has increased significantly due to the consumption of high-calorie food and a decrease in physical activity in developed countries.[1,2,3] At the same time, there are very few effective approaches to solving the problems of prevention and treatment of non-alcoholic fatty liver disease, which is hindered by the lack of a clear understanding of the etiology and mechanisms of the pathogenesis of the development of this disease.[2,4] A high-fat diet is the most likely determinant of the development of non-alcoholic fatty liver disease in humans. Based on the characteristics of nutrition and lifestyle in Uzbekistan, non-alcoholic fatty liver disease is a very frequent pathology, regardless of gender and age of people. This makes extremely urgent the development of their own effective drugs that contribute to the prevention of development and treatment of this pathology.

The purpose of the study: to study the histological parameters in rats in the norm and in the process of formation of non-alcoholic fatty liver disease in the experiment.

MATERIALS AND METHODS

The study was conducted on the basis of the Republican Scientific Center of Immunology of the Ministry of Health of the Republic of Uzbekistan and on the basis of the TMA vivarium. To reproduce the experimental model of non-alcoholic fatty liver disease, rats, mature individuals, with an average mass of 170.4 ± 2.4 g were used. During the development of the model, the reproduction of similar experimental states was processed; the data were analyzed on the basis of histological studies of liver tissue. The method for modeling non-alcoholic steatohepatitis is carried out as follows.

Animals for 120 days (4 months) are on a special hyper caloric hepatogenous diet, saturated with animal fats (Table 1).

Diet
Table 1

Daily ration of rats (g / 100 g of animal mass)	**Experimental ration**	**Generalized ration**
Ingredients		
Beef bacon	5	0,5
Sunflower oil	0,5	0,5
Mixture of cereals	5	5
Bread, wheat (biscuit)	2	20
Groats of oatmeal	1,3	1,3
Beef meat of the second category	2	2
Cottage cheese skimmed	0,8	0,8
Carrot	3,3	3,3
Greenery	3,3	3,3
Yeast	0,05	0,05
Cooking salt	0,1	0,1

For the study, four groups of rats were distinguished
Control group - intact rats contained on the standard ration of the vivarium (10 heads).
Experimental Group 1 - rats, contained in the experimental hyper caloric diet for 30 days (10 heads).
Experimental Group 2 - rats contained in the experimental hyper caloric diet for 90 days (10 heads).
Experimental Group 3 - rats, contained in the experimental hyper caloric diet for 120 days (10 heads).

The animals were inspected daily; feed intake was taken into account. During the experiment there were no cases of cases and diseases of animals. Animal slaughter was carried out by decapitation under ether anesthesia in accordance with the requirements of the European Convention for the Protection of Experimental Animals 86/609 EEC.

For histological examination of liver tissue, small fragments of the central part of the right lobe of the organ were fixed in a 10% neutral formalin solution prepared on 0.07 M phosphate buffer (pH = 6.98). Dewatering of the tissues was carried out in increasing concentration in ethyl alcohol and poured into the paraplast. Then, with the help of a microtome, sections of the investigated organs with a thickness of 7 μm were prepared. For histological studies, liver tissue sections were stained with hematoxylin-eosin by Romanovsky, picrofuxin by Van Gieson.

RESULTS OF THE STUDY
As a result of our studies, the data presented in Table 2 were obtained.

In all rats of experimental groups, in comparison with an intact group, an increase in body weight, a two-fold increase in the relative and absolute mass of visceral adipose tissue and liver were revealed.

Table 2

Weight parameters of rats during NASH, M ± m	**Duration of experimental diet**			
Indicators	**Control group, n=10**	**30 days (test group 1), n=10**	**90 days (test group 2), n = 10**	**120 days (trial group 3), n = 10**
Rat weight before experiment, g	168,0±2.2	171,7±3,5	170,3±2,25	171,5±1,7
Rat weight after the end of the experiment, g	251,9±12,3	335,7±21,4**	364±9,1***	420,7±18,8**
Weight gain, g	83,9±10,3	164±21,9***	193,7±8,5***	249,2±17,6***
	7,1±0,1	14,1±1,0***	15,7±0,8***	24,2±3,1***
Absolute weight of liver, g	2,8±0,1	4,2±0,3***	4,3±0,2***	5,8±0,7***
Relative mass of liver,%	1,6±0,6	15,0±1,9***	13,8±1,2***	12,1±1,2***
Absolute weight of visceral fat, g	0,6±0,2	4,5±0,6***	3,8±0,3***	2,9±0,3***

Note: (*) is the statistical significance of the differences with respect to the control group: * - p <0.05; ** - p <0.01; *** - p <0.001.

The development of a model of nonalcoholic steatohepatitis in animals was served by the study of histological sections of liver tissue stained with hematoxylin-eosin by Romanovsky and picrofuxin according to Van Gieson. An analysis of the results of the experimental study showed that in the control group of animals within a period of 90 days, a typical morphological structure of the liver is found. The organ

is represented by segments that do not have clear boundaries and are separated by a thin layer of connective tissue. Interlobular connective tissue of the liver of the rat is poorly developed, the outline of the lobules can be judged from the location of the central vein and portal tracts. The parenchyma of the lobules is formed by the hepatic beams radially arranged around the central vein. Leukocyte infiltrates and connective tissue fibers in the parenchyma are not detected.

In the research group of animals, to the 90th day after the experiment in the liver, pronounced dystrophic processes were detected. In all the preparations, a picture of parenchymal fatty degeneration was revealed: hepatocytes appeared enlarged in size, swollen, with many small colorless vacuoles in the cytoplasm, giving it a vacuolar type characteristic of this type of dystrophy. In some cases, the nucleus had a poorly distinguishable chromatin structure, in other cases only the shadows of the nuclei, colored in a bluish color, were determined. The boundaries between the cells were determined indistinctly. In addition to fat, hydropic (balloon) dystrophy was revealed: sharply swollen hepatocytes with optically empty and discharged cytoplasm. The contours of the cells are well defined; the nuclei are located mainly centrally with the phenomena of karyopicosis and karyolysis. The remains of a weakly eosinophilic granular cytoplasm were located around the nuclei or along cell membranes. Hydropic dystrophy was found mainly in the pericentral segments of the lobule.
When animals are kept on a diet that includes 70% fructose, on the 30th day of the experiment they develop macro vesicular steatosis and intra-lobular inflammation, the weight of the liver increases. However, the distribution of fatty hepatosis in the liver and lobules of the liver is found in the 1st acene zone, while in the histological samples taken from people with non-alcoholic fatty liver disease, the predominance of steatized hepatocytes is noted in the third zone.

By the 90th day of the experiment, hepatocytes of all rats contained lipid droplets of various diameters, mostly small ones. Well defined small inclusions of neutral fat, located mainly perinuclear, the cytoplasm of cells seemed to be uniformly "dusted" with small grains giving a reaction to fat. The nucleus remained in the center of the hepatocyte. This type of fat degeneration is usually called fine-grained or dusty. In the majority of samples of liver tissue, there were other signs of dystrophic changes - weak staining of nuclei, decay of some cells. Fig.1.

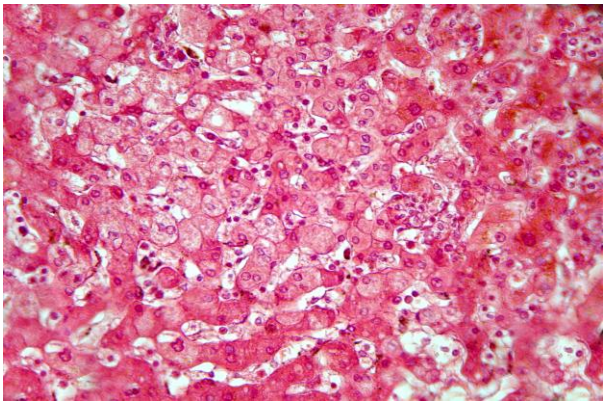

Figure. 1. Hydropic dystrophy of individual groups of hepatocytes. Coloring of hematoxylin - eosin. Magnification: ocular.10, lens.20.

In all animals, fatty degeneration, having a diffuse character, was somewhat more pronounced in the periportal zone. Fat vacuoles were detected in 1/3 of the hepatocytes on the 30th day, which corresponds to the first degree of steatosis. According to A.I. Abrikosova et al, fine-grained obesity should be interpreted as degenerative, since the process in this case is caused by damage to the liver cells. Already on the 90th day of the experiment, foci of inflammatory infiltration appeared in the liver tissue of rats, more often in the periportal zone, represented by the accumulation of lymphocytes and plasma cells. This histological pattern is characteristic of chronic hepatitis with minimal activity. There are no signs of fibroticization of the liver at these dates. Histoarchitectonics of the organ retained its normal structure. Attention was paid to the expansion of sinusoids, which can be considered as a sign of serous edema. Erythrocytes often formed columns, typical for stasis phenomena.

Thus, already on the 90th day after modeling non-alcoholic fatty hepatosis in the liver of rats, gross dystrophic changes were clearly observed-protein-fatty degeneration with signs of chronic hepatitis with minimal activity against serous edema, intracellular respiration and phosphorylation. On the 90th day of the experiment, the pathological process in the liver underwent a number of changes in connection with the progression of the dystrophic processes. Signs of fatty dystrophy, while remaining, had a greater degree of severity. The cytoplasm of hepatocytes was sharply rarefied, coarse-grained, the hypochromia of the nuclei was determined. In most liver samples, hydrophilic degeneration was detected. The cytoplasm of cells is transparent, characterized by an underlined contour of hepatocytes due to well-distinguishable membranes. Against the background of persistent pulverized dystrophy of hepatocytes, cells were found whose cytoplasm was completely filled with neutral fat. Most drugs showed a picture of chronic hepatitis of a minimal degree of activity, but in the specified time after the experiment, its severity was greater.

On the 120th day of exposure to rats with a hepatogenous diet, the histological structure of the liver underwent significant changes, which resulted in an sincrease in the area of necrotic areas (10-15% of the total area of the liver sites) surrounded by a lymphomacrofagal infiltrate, a violation of the lobular structure of the lobules of the liver. Hepatocytes did not form trabeculae, were located randomly, sinusoids were not traced. It was noted the expansion of large portal tracts due to the proliferation of the stroma and filling it with an inflammatory infiltrate. Some portal tracts were with necrotic vessel changes. The walls of such vessels were slightly different; in some cases they were violated. The destruction of the bile ducts was detected, accompanied by large foci of an inflammatory infiltrate. On preparations stained by Van Gieson, collagen fibers were clearly detected in the periportal zone, which is a direct evidence of the formed liver fibrosis.

CONCLUSION

Thus, it was possible to work out a model of nonalcoholic steatohepatitis, characterized by obesity, a violation of structural organization, tissue necrosis, and the formation of liver fibrosis.

The obtained model of nonalcoholic steatohepatitis also makes it possible to study the staging of the development of fatty liver disease, characterized by the formation of 3 stages - steatosis, steatohepatitis and fibrosis.

The development of the model of non-alcoholic fatty liver disease takes into account the majority of clinically significant liver damage factors that are most common in the pathogenesis of non-alcoholic fatty liver disease.

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