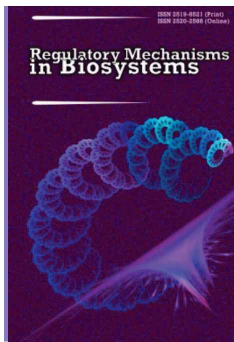

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2023, 14(1), 131–136
doi: 10.15421/022320

Dynamics of pathomorphological changes in the liver of rats at different stages of experimental alcohol damage

V. I. Didenko*, Y. A. Gaidar*, D. F. Mylostiva*, I. A. Klenina*,
O. O. Halinskyi*, O. P. Petishko*, O. I. Hrabovska*, A. M. Halinska*, **

**Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine, Dnipro, Ukraine*
***Oles Honchar Dnipro National University, Dnipro, Ukraine*

Article info

Received 07.02.2023
Received in revised form 26.02.2023
Accepted 27.02.2023

Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine, Slobozhanskya av., 96, Dnipro, 49074, Ukraine.
Tel.: +38-097-24-60-639.
E-mail: mylostivad@i.ua

Oles Honchar Dnipro National University, Gagarin av., 72, Dnipro, 49010, Ukraine.
Tel.: + 38-098-21-29-333.
E-mail: biolog.anastasia@gmail.com

Didenko, V. I., Gaidar, Y. A., Mylostiva, D. F., Klenina, I. A., Halinskyi, O. O., Petishko, O. P., Hrabovska, O. I., & Halinska, A. M. (2023). Dynamics of pathomorphological changes in the liver of rats at different stages of experimental alcohol damage. Regulatory Mechanisms in Biosystems, 14(1), 131–136. doi:10.15421/022320

Liver diseases represent one of the most common problems in gastroenterology. The liver, as the most important organ of metabolism, which plays a major role in anabolic and energy processes, takes part in the adaptive and compensatory reactions of the body under exogenous and endogenous adverse influences. Individual factors play a major role in the development of this disease, one of which is the duration of the effect of alcohol on the body. The duration of alcohol consumption affects the morpho-functional properties of the liver. When alcohol was given to the research animals, hypertrophic changes were seen in the linear dimensions of hepatocytes: in the 12th week of alcoholization, the nuclear area was 1.25 times larger, and the cytoplasmic area of hepatocytes was 1.16 times larger compared with the same indicators in the 6th week of alcoholization. Alcoholic hepatitis is characterized by protein-fatty degeneration, inflammatory lymphocytic infiltration, increase in the area of sinusoids and the size of hepatocytes, and, accordingly, the Vizotto coefficient (1.83 times in the 6th week and 2.10 times in the 12th week of the research). The alcohol consumption is accompanied by increase in the volume of the nucleus and cytoplasm of hepatocytes, decrease in the nuclear-cytoplasmic ratio with increase in pathohistological changes. With the lengthening of the terms of alcoholization, the number of binuclear hepatocytes decreased. Morphometric calculation of the number of fat cells per 100 hepatocytes showed that in the 12th week of the experiment, the number of fatty inclusions increased by 1.42 times, compared with 6 weeks of forced alcoholization. Fatty small- and large-droplet steatosis of hepatocytes was diffuse in nature. The duration of alcoholization also affects the liver vessels. The diameter of the central vein in the 6th week of the experiment was 1.52 times greater than in control rats, and 1.81 times in the 12th week of alcoholization. The bile ducts of rats of the experimental groups were also larger in diameter: by 37% after 6 weeks and 47% after 12 weeks of forced alcoholization. An increase in the area of the sinusoids and the diameter of the central vein indicates an impairment of the blood supply to the liver. The complexity of the structure of the liver and the diversity of its functions necessitate the use of a variety of diagnostic techniques and methodological approaches to assessing its activity in the normal condition, during a pathology. It will be promising to study the structure of the liver at the ultramicroscopic level of the effect of drugs on the treatment of alcoholic liver disease during different periods of alcoholization.

Keywords: hepatocytes; ethanol; hypertrophy; hepatitis; sinusoids; liver vascular system.

Introduction

Alcohol is a major factor causing a high level of morbidity and mortality in most countries of the world. Ethanol has an equally negative effect on all organs and systems of the body, but the first organ that takes on the "alcohol blow" is the liver. Because of high level of morbidity, frequent deaths and serious social and material problems, alcoholic liver disease, together with viral hepatitis and toxic lesions, are the main problems of modern hepatology. Continuous alcohol consumption is a potential trigger of alcoholic hepatitis, which may be related to the immunosuppressive effect of chronic alcohol use (Tsomaia et al., 2020; Liu et al., 2021). Liver damage of varying severity is observed in almost all persons who abuse alcohol. In almost 50% of liver-cirrhosis patients, the cause of the disease is alcohol abuse. At the same time, 80–90% of them have alcoholic steatosis, 10–35% develop alcoholic hepatitis, often transforming into cirrhosis. Up to 3.3 million deaths from alcoholic liver disease are recorded annually, which is up to 6% of the total number of deaths in the world (Singal et al., 2018; Askgaard et al., 2021). Important points in the diagnosis of alcoholic disease are morphological changes in the liver. Alcoholic hepatitis is both an acute and chronic form of liver damage, characterized by hepatic steatosis, hepatocyte hypertrophy, neutrophil-lymphocytic infiltration, and the formation of fibrotic changes. Hepatic fibrosis is induced by the activation of collagen-forming non-parenchymal liver cells under the direct action of aldehydes (Macdonald et al., 2018; Ha et al., 2022). There are two types of alcohol metabolism: oxidative and non-oxidative alcohol metabolism. Both the oxidative and non-oxidative metabolism of alcohol and its metabolites have toxic consequences for many organs, including the liver, adipose tissue, the intestine, and pancreas (Lamas-Paz et al., 2018; Hyun et al., 2021). The main factors in the development of alcoholic liver disease are toxic products of ethanol and acetaldehyde metabolism, which trigger oxidative stress, mitochondrial dysfunction, liver cell necrosis, inflammation and, as a result, fibrosis. Damage to macromolecules such as lipids, DNA, and proteins is a common toxic effect of alcohol consumption associated with cell viability, proliferation, and tumorigenesis, usually mediated by covalent modification (Argemi et al., 2020; Donne et al., 2021).

Also, from a biochemical point of view, there is a fairly strong correlation with markers of systemic inflammation and macrophage activation, which indicates massive cell death during alcoholic liver disease (Bajaj, 2019; Thomes et al., 2019). Adverse effects from the somatic sphere during the abuse of ethyl alcohol are manifested by the pathology of internal organs and entail the appearance of a complex of somatic diseases – alcoholic polyvisceropathy (Okamura et al., 2018; Slevin et al., 2020). The most sensitive marker of the severity of alcoholic polyvisceropathy is liver damage. The liver, as the most important metabolic organ, which plays an important role in anabolic and energy processes, is involved in the adaptive-compensatory reactions of the body under various exogenous and endogenous adverse effects, even in cases where damaging factors do not have a pronounced hepatotropic effect (Furuya et al., 2019; Hyun et al., 2021). Although many studies have focused on the effects of oxidative alcohol metabolites on liver damage, the importance of non-oxidative alcohol metabolites in cellular damage has also been found. Moreover, the extrahepatic effects of alcohol are critical in providing additional information needed for the progression of alcoholic liver disease. It is important to determine clear diagnostic criteria for alcoholic liver damage in order to improve the existing methods of treatment and prevention of this pathological condition. The linear dimensions of hepatocytes and their nuclei, their nuclear-cytoplasmic ratio, a number of other histological and morphometric measurements are significant indicators for assessing the state of the liver with alcohol damage. The mechanisms of morphological changes in the liver during different periods of alcohol exposure remain unclear.

The objective of this study was identifying patterns of histological and morphometric changes in the rat liver tissues during different periods of alcoholization.

Materials and methods

The experimental animals were kept in the conditions that met the requirements of the domestic legislation, the Law of Ukraine "On the Protection of Animals from Cruelty" No. 3447-IV of February 21, 2006, with the latest amendments of April 8, 2017, and Directive 2010/63/EC of the European Parliament and Council. The animals were kept in cages with 12-hours of daylight , at the air temperature of 20–23 °C with free access to food at the vivarium of the Pathophysiology Laboratory of the State Institution the Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine. The animals were allowed to be used in the studies according to the scheme agreed by the local commission on bioethics of the Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine (April 30, 2018, Protocol No. 3). Bioethical norms were not violated by forced manual drinking of a mixture of ethanol and water to simulate chronic alcohol dependence.

The experimental animals received a standard diet (13% mass fraction of moisture; 3,220 kcal metabolic energy in 1 kg; 22% mass fraction of crude protein; 5% mass fraction of crude fiber; 6% mass fraction of crude fat; 6% mass fraction of crude ash; 0.90–0.12% mass fraction of calcium; 0.6–0.9% mass fraction of phosphorus; and no less than 0.2% mass fraction of sodium), which quantitatively and qualitatively satisfied their physiological needs. In the group I of rats, modeling of chronic alcoholic liver disease with an aqueous solution of ethanol with chronicity of the pathological process was carried out during 6 weeks (n = 10); in group II – modeling of chronic alcoholic liver disease by two-phase alcoholization with an aqueous solution of ethanol with chronicity of the pathological process was carried out during 12 weeks (n = 10). The control group of animals consisted of intact rats (n = 10) kept on a standard vivarium diet with free access to water. Histological and morphometric studies of liver biopsy samples of research animals were carried out at the Laboratory of Pathomorphology of the state institution Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine, Dnipro.

The object of the study was liver samples obtained from the Wistar rats weighing 230 ± 3.2 g with induced alcoholic hepatitis. To reduce the statistically required number of animals involved in the study, they were preliminarily tested in the "Open Field" maze and tested for individual resistance to acute hypobaric hypoxia. Then, only the animals that had test results close to the average for the sample (± 30%) were used, and the others were culled. For 5 days, with the repetition after 2 days, 16% aqueous ethanol solution in 5% aqueous glucose solution was injected intraperitoneally at the rate of 4 ml of ethanol per 1 kg of animal weight. After 14 days from the start of forced alcoholization, the rats were transferred to semi-forced alcoholization, that is, the animals used 10% aqueous ethanol solution as the only source of water. The animals were lethally anesthetized with an anesthetic dose and removed from the experiment by decapitation. After that, an autopsy was performed to collect liver samples. For histological examination, parts of the liver of rats were fixed in 10% formalin, followed by dehydration in alcohols of increasing concentration. Hematoxylin-eosin staining method was used for staining histological samples (Ukrainian Market of Chemical Raw Materials, Kyiv). The materials for histological examination (peripheral lobules of the greater lobe of the liver) were fixed in 10% solution of neutral formaline (24–36 hours) with subsequent paraffin embedding. After that, the biopsies were washed in water for at least 30 minutes. For dehydration and degreasing, they were processed with ascending spirits. Then, the biopsies were kept in castor oil for 24 hours for tissue softening. They were put in chloroform for 40–60 minutes and transferred to the mixture of chloroform and paraffin (1:1) for 40–60 minutes at the temperature of 37 °C. The liver samples were transferred to heated paraffin in a thermostat (56 °C) for 2 hours for infiltration. Then, the biopsy samples were embedded in paraffin. After the solidification of the paraffin, the pieces were cut out with the paraffin and put in the freezer until solidification. The biopsies were then embedded in paraffin. The pieces were cut out from the paraffin and placed in a freezer. The 5–7 mm thick histosections were obtained using a sledge microtome RM60-EKA (Kharkov, Ukraine). The microscopy was performed using an XSZ-21 light microscope (Micro Med, Ukraine). Morphometric measurements on digital images of the biopsy specimens were made using the IMAGE J software (National Institutes of Health, USA). In the obtained histological preparations, the number of hepatocytes in the field of view (^{x}400), the number of fat drops per 100 hepatocytes (^{x}400), the nuclear-cytoplasmic ratio, and the steatosis index were determined – the ratio of the area of the adipose tissue of a biopsy sample in the field of view to the area of the liver tissue; the ratio of the area of the nucleus of the hepatocyte to the total area of a hepatocyte In each case, about 30 measurements of each histological preparation were made. The relative area of the hepatocyte parenchyma and the relative area of sinusoids was also estimated. Using these data, the Vizotto coefficient was determined (the ratio of the area of sinusoids to the area of the parenchyma of hepatocytes).

The results were statistically processed using the Statistica 6.1 software package (StatSoft Inc., USA). To evaluate the quantitative characteristics, the mean (x) and standard error (SE) were calculated. The mean values were compared using the Tukey test with Bonferroni correction. Differences between the samples were assessed using ANOVA ($P < 0.05$).

Results

In the control group of animals, the histological sections of the liver showed a clear lobular structure of the liver with a radial arrangement of the tubules, and expressed sinusoids. Hepatocytes had light pink cytoplasm and were located centrilobularly. Closer to the portal tracts in the liver cells, the cytoplasm was darker with fine granularity. The nuclei of liver cells were rounded. The tubular cytoarchitectonics of the hepatic lobule was structurally traced. We also saw a small number of binucleated hepatocytes and single hepatocytes with large polymorphic hyperchromic nuclei. Portal tracts were thin with plethorized vessels and few histiocytes. Sometimes, in the field ofview one or two cells with intensively eosinophilic cytoplasm were observed, decompensated relative to hepatic laminae. These are apoptotic cells that perform natural (physiological) destruction of hepatocytes. The described histological picture of the liver of intact rats corresponds to the normal structure of the hepatic lobule. The vessels of triads of portal tracts were moderately plethorized. Single erythrocytes and a small amount of pink masses of blood plasma were noted in the lumens of the central veins. Sinusoidal capillaries were moderately plethorized. The average area of the liver cytoplasm in the control animals was 326.4 ± 1.4 μm. Hepatocytes mostly had one round nucleus, located cen-

trally. According to the results of the studies, the average area of the nuclei was 50.7 ± 1.2 µm (Table 1).

Table 1
Morphometric changes in rat hepatocytes (µm)
at different periods of alcoholic liver damage (x ± SE, n = 10)

Indicator	Control	Experiment	
		6 weeks	12 weeks
Area of the nucleus, µm	50.7 ± 1.2[a]	60.4 ± 1.3[b]	75.4 ± 2.8[c]
Area of the cytoplasm, µm	326.4 ± 1.4[a]	353.2 ± 4.9[b]	410.6 ± 10.7[c]
Nuclear-cytoplasmic spontaneity	0.157 ± 0.006[a]	0.173 ± 0.003[b]	0.187 ± 0.004[c]

Note: different letters indicate values that differed one from another significantly within one line of the table according to the results of comparison using the Tukey test with Bonferroni correction.

When analyzing the histological preparations of the rat liver after 6 weeks of forced alcoholization, we saw that the structuring of the hepatic tubules was partially changed. The lobes were significantly enlarged, did not have clear boundaries, the sinuses were dilated, plethorized, there was a discomplexation of the hepatic tubules of the third zone. Decomplexation of hepatocytes relative to each other was clearly observed. In the periportal zone, a neutrophil-lymocyte infiltrate was detected, predominantly of a focal nature, partially extending beyond the boundary plate (Fig. 1). We noted a gradual increase in the prevalence and intensity of hepatocyte dystrophy with subtotal damage in the form of small-sized fatty (up to 50% of the total), granular dystrophy and the development of hydropic dystrophy in the 12th week of the study. Against this background, there were areas of necrobiosis and necrosis, single hepatocytes and groups of hepatocytes with leukocyte-macrophage infiltration.

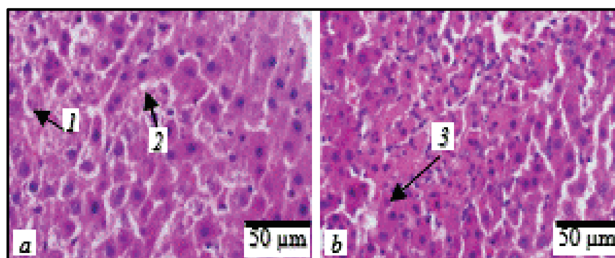

Fig. 1. Histological changes in the liver after alcoholization: *a* – after the 6th week of alcoholization, *b* – after the 12th week of alcoholization: focal necrosis (*1*); different large-droplet steatosis of hepatocytes (*2*); leuko- and lymphohistiocytic infiltration (*3*); hematoxylin-eosin

In the 12th week of the experiment, the morphological picture of the liver underwent a number of changes due to the progression of dystrophic processes under the influence of the toxic factor. Signs of protein dystrophy were manifested clearer. The cytoplasm of hepatocytes was loose, coarsely granular, and hypochromic nuclei were seen. Hydropic dystrophy was detected in the majority of liver tissue samples, which was more pronounced than in the end of the 6th week of observation. The cytoplasm of the cells was translucent with a distinctive outline of hepatocytes. In some preparations, the cytoplasm of hepatocytes was diffusely filled with vacuoles of different sizes, separated from each other by a thin layer of cytoplasm. The nuclei of hepatocytes in most preparations looked swollen, their chromatin was not visually seen. The portal tracts were moderately dilated with the remains of bile. The initial signs of edema of the liver tissue can be detected even earlier (week 6), however, they reached the maximum degree of severity by the 12th week of the experiment. Histological changes were characterized by a moderate expansion of Disse's spaces, dystrophic changes, which were expressed by the appearance of single or focal areas of dystrophic cells. Swollen endothelial cells were also found. Such a picture corresponded to acute toxic hepatitis. On the periphery of the lobules, structureless hepatocytes were noted in the form of fine-grained masses, between which there was leuko- and lymphocytic infiltration. Already in the 12th week of alcohol use, the lobular structure of the rats had almost no boundaries, the hepatic tubules were indistinct, blurred. Portal tracts were characterized by macrophage-histiocytic infiltration, the intensity of which changed over the experiment.

A morphometric study complements the qualitative characterization of hepatocytes, provides more objective and complete information about the processes occurring in the liver cells with alcohol damage and can be used in additional diagnostics and prediction of the course of pathological conditions. One of the pathological forms of alcoholic liver disease is alcohol-induced steatosis, which is defined as the accumulation of lipid droplets in the cytoplasm of hepatocytes. In our studies, fatty degeneration looked like small fat droplets, as well as large ones. Fatty infiltration was diffuse in nature, with the most pronounced changes (larger size of lipid droplets, dystrophic changes in the nuclei) observed in the centrilobular part, decreasing towards the periphery of the lobule. The number of fat drops in rats in the 12th week of the experiment (153.2 ± 2.8) had increased by 12% ($P < 0.01$), compared with this indicator (136.7 ± 3.1) in animals in the 6th week of alcoholization. Besides the increase in the number of fat droplets, the steatosis index also changed: after 12 weeks of alcoholization, it was 1.54 times higher than after 6 weeks ($P < 0.01$, Fig. 2).

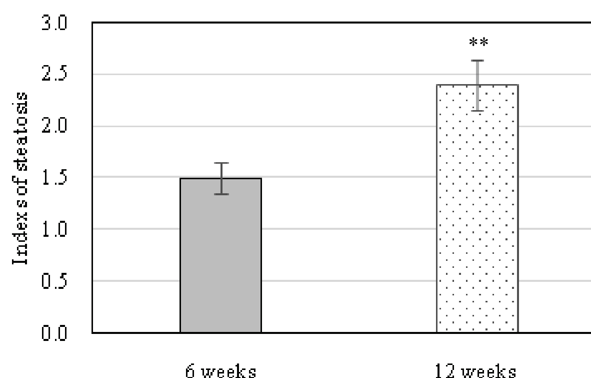

Fig. 2. Changes in the index of steatosis of the liver of rats during different periods of alcoholization (x ± SE, n = 10)

The structuring of hepatic tubules was damaged in almost all parts of the liver lobule: decomplexation of liver hepatocytes relative to one another is observed. An accumulation of fat cells was observed in the middle zones of liver particles. Fat droplets were found in ⅓ of hepatocytes, which corresponds to the first degree of steatosis. Liver cells with fatty inclusions were characterized by nuclei shifted to the periphery. In rare cases, nuclei were completely absent. Such morphological features indicate fatty transformation of hepatocytes (Fig. 3).

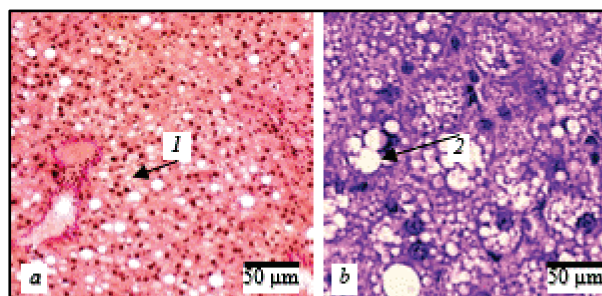

Fig. 3. Fatty degeneration of the liver due to alcohol consumption: *a* – after 6 weeks of alcoholization; *b* – after 12 weeks of alcoholization; small-droplet steatosis (*1*), large-droplet steatosis (*2*); hematoxylin-eosin

Also, under the influence of alcohol, correlations were found between the parameters of the morpho-functional state of the liver during alcohol intoxication. Morphometric measurements showed that with increase in the duration of exposure to alcohol, the volume of both hepatocyte nuclei and cytoplasm increased, and the nuclear-cytoplasmic ratio index changed accordingly. The nuclear-cytoplasmic ratio is considered to be one of the important morphological characteristics of metabolic processes in hepatocytes. In our study, when hypertrophy of cells and their nuclei was detected, positive correlations were revealed between the area of the cytoplasm of hepatocytes (326.4 ± 1.3 µm) and the area of the nucleus (50.7 ± 1.2) ($r = 0.45$, $P > 0.05$; cytoplasmic ratio $r = 0.73$; $P < 0.05$). In rats in the 12th week of alcohol consumption, the maximum significant increase in the

size of hepatocytes was experimentally determined: the area of the nucleus – by 1.25 times, the area of the cytoplasm – by 1.16 times, compared with the 6th week of alcohol intake. At the same time, the value of the nuclear-cytoplasmic ratio was the lowest in rats that had been receiving alcohol for 6 weeks (Table 2). Such hypertrophic changes of hepatocytes and their nuclei can be considered an adaptation to increased functional load, in particular, to increase in detoxification function when affected by large doses of alcohol.

Increase in the number of binucleated hepatocytes was observed against the background of toxic lesions of various etiologies and was considered as a compensatory-adaptive reaction to damage. However, in our study, the number of binucleated hepatocytes of the rats decreased by 1.43 times in the experimental group I, i.e. by 30% ($P < 0.05$), and 1.91 times (by 47%, $P < 0.01$), compared with animals of the intact group This indicates that under the influence of alcohol, the regenerative properties of the liver decreased with duration of its comsumption (Fig. 4).

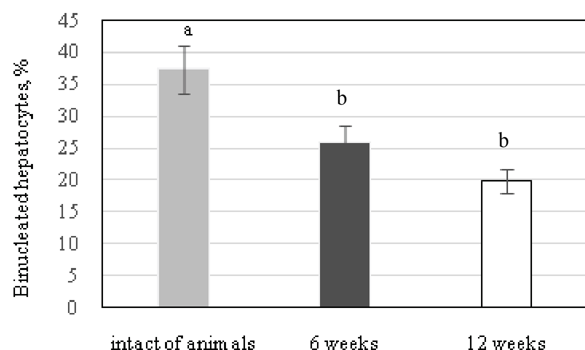

Fig. 4. The number of binucleated hepatocytes of rats at different periods of alcoholization (x ± SE, n = 10)

A prerequisite for the development of hepatocyte necrosis is the condition of hyalino-drop dystrophy of liver cells, which was especially expressed after prolonged exposure to alcohol (12 weeks of alcoholization). It is based on deep denaturation of abnormal cell proteins, followed by infiltration of the hepatocyte itself by these proteins and damage to cell ultrastructures.

After the influence of alcohol, in the rat liver biopsy specimens, clearly expressed pathological changes in the vascular system of the organ were noted. In the 6th week of experimental studies in the periportal zone, a slight lymphoid-histiocytic infiltration of hepatocytes was observed. Degenerative changes in the vascular endothelium were found in the portal tracts. The central veins were characterized by a thickened basement membrane, plethora, and the endothelial layer showed signs of desquamation. Most of the sinusoids were dilated and contained chains of lymphocytes.

The most significant plethora and thickened vessel walls were seen in the 12th week of the experiment, since the lesions of the liver increased in relation to the duration of alcohol consumption. Enlargement of the central vessels was noted, with lymphocytic inflammations near them, dystrophic changes of about 10 hepatocytes with increased fatty and ballooning degeneration (more than 70% of hepatocytes) were seen. Endothelial cells of the vessels were hypertrophic, and the loose connective tissue was dispersed. The sinusoidal capillaries were dilated, and accumulation of leukocytes was seen in their lumens. The central veins also were enlarged, with hypertrophied endothelial cells. The subendothelial layer had many fibrous structures. Alcohol intoxication is characteristic of macrophage reaction, manifesting in large masses of macrophages and lymphocytes, as well as plasma cells. Such a phenomenon occurs because of inflammatory changes in the liver tissues and necrosis of hepatocytes (Fig. 5).

At the same time, the relative area of sinusoids in the 6th week of the experiment was 34% (or 1.51 times) larger, and in the 12th week of alcoholization – by 48% (1.90 times), compared with the animals of the intact group. At the same time, the indicator of the Vizotto coefficient increased by almost 1.83 times ($P < 0.05$) in week 6 of alcohol consumption, and by 2.13 times ($P < 0.05$) in the 12th week of the experiment due to decrease in the relative area of hepatocytes (Table 2). The walls of the central veins were thickened due to the growth of fibrous structures of the subendothelial layer and the adventitia. The diameter of the central vein increased by 33% ($P < 0.001$), compared with the control, or 1.51 times after 6 weeks of alcoholisation and by 45% ($P < 0.001$) or 1.82 times after 12 weeks of ethanol consumption.

Fig. 5. Changes in the vessels of the liver:
a – after 6 weeks of alcoholization; *b* – after 12 weeks of alcoholization; expansion of the vessels of the hepatic triad (*1*); expansion of the sinusoidal capillaries of the liver (*2*); hematoxylin-eosin

Table 2
Changes in the morphometric parameters of the vascular system of the liver of rats during different times of alcoholization (x ± SE, n = 10)

Indicator	Control	Experiment	
		6 weeks	12 weeks
Central vein diameter, μm	53.5 ± 0.9^a	81.2 ± 1.3^b	97.3 ± 1.5^c
Bile duct diameter, μm	9.46 ± 1.16^a	14.52 ± 0.22^b	17.54 ± 0.46^c
Relative parenchymal area, %	89.51 ± 0.74^a	73.38 ± 0.83^b	79.02 ± 0.62^c
Coefficient Vizotto	0.144 ± 0.003^a	0.264 ± 0.017^b	0.303 ± 0.009^c

Note: see Table 1.

Increase in the Vizotto coefficient was associated with decrease in the relative area of hepatocytes and the expansion of the area of the sinusoidal channel, which, overall, may indicate imbalance in the blood flow from and to the liver. After 12 weeks of alcoholization, the inflammation-infiltrate foci of the triads were denser than in the rats in the 6th week of consuming the toxin. In the lumina of the central veins, erythrocytes, lymphocytes, dead liver cells, and peeled-off epithelium were seen (Fig. 7). The diameter of the bile duct also changed by 38% ($P < 0.01$; or 1.62 times) after 6 weeks of consuming ethanol and by 47% ($P < 0.01$; or 1.85 times) after 12 weeks, compared with the control.

Discussion

Excessive consumption of alcohol is the cause of a wide range of pathologies of various organs, particularly alcoholic liver disease. Duration of alcoholism is the critical factor determining the alcoholic liver disorder. Understanding how animal-modelled periods of alcohol consumption initiate and promote the alcoholic liver disease may help reveal molecular mechanisms and design new effective methods of treatment (Dastidar et al., 2018). Toxicity of alcohol and its metabolites causes the development of alterative (fatty and ballooning degeneration), exudative (swelling) and proliferating lesions (decrease in the fraction of binuclear hepatocytes) (Argemi et al., 2020; Li et al., 2020). Alcohol intoxication was severer with more inflammation on the tissue reactions and various pathological processes in the animals in the 12th week of exposure to the toxin (Brygadyrenko et al., 2019; Khaderi, 2019; Tsomaia et al., 2020; Ha et al., 2022).

At all stages of the experiment, polymorphic hepatocytes, swelling of the cytoplasm and indistinct cell borders, and expansion of the central veins were found in the liver of rats. The nuclei of hepatocytes were of a distinct oval shape, located mostly in the centers of the cells, but there were also cells with nucleus at the periphery because of it being shifted by lipid inclusions. There were also clearly defined singular sites of hemorrhage in the liver tissues (Birková et al., 2021; Park et al., 2022). In the liver of rats, there were morphometrically determined deviations of certain parameters (in the 6th and 12th weeks of the experiment) from the control group (increase in the area and nuclei of hepatocytes, and therefore changes in the nuclear-cytoplasmatic ratio). Against the background of a dystrophic condition, regenerative processes such as increase of nuclei occur, suggesting accumulation of chromosomal material for further reparative processes by cell division in the conditions of intoxication (Zhang et al.,

2017; Antonenko et al., 2020; Hyun et al., 2021). There was also decrease in the number of binuclear liver cells as a result of degenerative changes.

Morphological changes in the liver under the influence of alcohol were also characterized by the presence of infiltration. There were foci of inflammatory infiltrate, which consisted mainly of lymphocytes, and in some cases neutrophils. Subsequently, in the 12th week of alcoholism, the foci of inflammatory infiltration were larger, against the background of deterioration of the functional parameters of the liver. Those data are coherent with other reports (Kong et al., 2019; Ma et al., 2022), where it was proved that reactive oxygen species of neutrophils contribute to the initiation of mechanisms of liver damage and dysfunction during alcoholic liver disease. Fatty liver, as the earliest reaction of the liver to alcohol abuse, can most often fully recover if this toxic factor is removed. Excessive and constant alcohol consumption increases the ratio of reduced nicotinamide adenine dinucleotide to oxidized nicotinamide adenine dinucleotide in hepatocytes. At the same time, there occur impairment in mitochondrial oxidation of fatty acids and the onset of alcoholic liver disease (Zhang et al., 2017). In addition, alcohol activates certain biochemical factors in the development of fatty liver disease (HIF-1 – hypoxia-induced factor 1 and iNOS – induced nitric oxide synthase) (Mihm, 2018). Fatty steatosis was observed in the form of small- and large-droplet steatosis. The number of fat droplets per 100 hepatocytes was 136.7 ± 3.1, and over 12 weeks of alcohol consumption, the number of fat droplets increased by 12%. It was confirmed that the development of fatty dystophy in hepatocytes correlates with the duration of exposure to a toxic agent. Under the influence of alcohol in the blood, the level of hormones of the adrenal cortex (glucocorticoids) increases, causing greater expression of serotonin receptors 5-HT2A and 5-HT2B, tryptophangidoxylase 1 and serotonin synthesis (Gao et al., 2011; Teschke, 2018).

After longer exposure to ethanol (12 weeks), the histological picture of the liver of rats worsened due to more pronounced active hepatitis. In the 12th week of alcohol intake, the lobular structure of the liver had blurred borders, the hepatic tubules were discomplexed, areas of necroticzed hepatocytes were more frequent. The central veins were dilated, a small amount of leukocytes and plasma fluid were found in their lumina; in the precentral zone of the hepatic lobe, the sinusoids were unevenly expanded with single lymphocytes, discomplexation of the hepatic tubules was noted. Hypertrophic changes in hepatocytes were observed due to the accumulation of small and medium-sized lipid inclusions in them. Vacuolar dystrophy of hepatocytes, which is a pathognomonic sign of alcoholic liver damage, was also well expressed. In hepatocytes of the rats that had been receiving alcohol for 12 weeks, changes in morphometric indicators (decrease in the nuclear-cytoplasmic ratio, the number of binucleated hepatocytes) were more expressed, which obviously indicates a greater expression of alternative processes due to the accumulation of the toxic effect of alcohol and its metabolites (Okamura et al., 2018; Buchanan et al., 2021; Donne et al., 2021; Liu et al., 2021).

Necrotic hepatocytes were localized in the periportal and centrolobular zones. Along the periphery of the necrotic area, there were reactive and reparative processes, such as the migration of inflammatory cells (lymphocytes, neutrophilic leukocytes) from blood vessels, accumulation of a small amount of macrophage granulocytes, and regeneration of hepatocytes. This was especially noticeable on histological preparations of the liver of the rats after 12 weeks of alcohol intoxication. Also, in the 12th week of forced alcoholism, the development of fibrosis was noted between the liver lobes, which had the appearance of cords of connective tissue in the form of incomplete septa. The appearance of fibrotic changes is a negative phenomenon, as it contributes to the strengthening of hypoxia, the progression of dystrophic and atrophic changes in liver cells, and gradual development of portal hypertension (Lange et al., 2018; Elgendy et al., 2022).

Conclusions

During different times of alcoholic liver damage, the animals were observed to have changes in the morpho-functional condition of the liver varying in degrees of severity: in the 12th week of alcoholism, we registered a significant 19.9% ($P < 0.01$) increase in the area of the nuclei, 14% ($P < 0.01$) in the area of the cytoplasm, and 8.0% ($P < 0.01$) in the nuclear-cytoplasmic ratio, compared with the same indicators in the 6th week of alcoholization. The amount of fat droplets in the rats increased significantly after 12 weeks of alcohol consumption – by 12% ($P < 0.01$), compared with the 6th week. Along with the increase in the number of fat droplets, the index of steatosis also changed: after 12 weeks of alcoholization, it was 1.54 times higher than after 6 weeks ($P < 0.01$).

There were changes in the vascular system of the liver. In the 6th and 12th weeks of the experiment, there was increase in the diameters of the central vein and bile duct by 1.51 and 1.80 times ($P < 0.001$), respectively, compared with the intact rats, due to decrease in the relative area of hepatocytes. The relative amplitude of sinusoids increased by 1.51 and 1.90 times, respectively, in 6 and 12 weeks of the experiment. The indicator of the Vizotto coefficient increased by 1.83 times ($P < 0.01$) in week 6 of forced alcoholization, and by 2.10 times ($P < 0.01$) in the 12th week of the experiment due to decrease in the relative area of hepatocytes.

The authors declare that there is no conflict of interest.

The article was prepared according to the scientific research work "Study of clinical, biochemical and structural features of the formation of steatosis and fibrosis in patients with chronic diffuse liver disease" (state registration number 0118U001834). The grantor organization is the National Academy of Medical Sciences of Ukraine.

References

Antonenko, P. P., Zazharskyi, V. V., Suslova, N. I., Sklyarov, P. M., Reshetnichenko, O. P., Kostyuk, V. K., & Mylostyvyi, R. V. (2020). Efficacy of herbal essential oils at tetrachloromethane induced hepatitis in laboratory rats. World of Medicine and Biology, 16(73), 149–153.

Argemi, J., Ventura-Cots, M., Rachakonda, V., & Bataller, R. (2020). Alcoholic-related liver disease: pathogenesis, management and future therapeutic developments. Revista Española de Enfermedades Digestivas, 112(11), 869–878.

Arteel, G. E. (2020). Liver-lung axes in alcohol-related liver disease. Clinical and Molecular Hepatology, 26(4), 670–676.

Askgaard, G., Kraglund, F., Kann, A. E., Vilstrup, H., & Jepsen, P. (2021). Epidemiology for alcohol-related liver disease. Ugeskrift for Laeger, 183(14), 1–9.

Bajaj, J. S. (2019). Alcohol, liver disease and the gut microbiota. Nature Reviews Gastroenterology and Hepatology, 16(4), 235–246.

Birková, A., Hubková, B., Čižmárová, B., & Bolerázska, B. (2021). Current view on the mechanisms of alcohol-mediated toxicity. International Journal of Molecular Sciences, 22(18), 14–22.

Brygadyrenko, V. V., Lieshchova, M. A., Bilan, M. V., Tishkina, N. M., & Horchanok, A. V. (2019). Effect of alcohol tincture of *Aralia elata* on the organism of rats and their gut microbiota against the background of excessive fat diet. Regulatory Mechanisms in Biosystems, 10(4), 497–506.

Buchanan, R., & Sinclair, J. M. A. (2021). Alcohol use disorder and the liver. Addiction, 116(5), 1270–1278.

Dastidar, S. G., Warner, J. B., Warner, D. R., McClain, C. J., & Kirpich, I. A. (2018). Rodent models of alcoholic liver disease: Role of binge ethanol administration. Biomolecules, 8(1), 3–21.

Donne, R., Sangouard, F., Celton-Morizur, S., & Desdouets, C. (2021). Hepatocyte polyploidy: Driver or gatekeeper of chronic liver diseases. Cancers, 13(20), 5151.

Elgendy, S. A., Baloza, S. H., Mohammed, L. A., Nasr, H. E., El-Shaer, N. O., Ghamry, H. I., Althobaiti, S. A., Shukry, M., Soliman, M. M., & Elnoury, H. A. (2022). Ameliorative impacts of wheat germ oil against ethanol-induced hepatic and renal dysfunction in rats: Involvement of anti-inflammatory, anti-apoptotic, and antioxidant signaling pathways. Life, 12(10), 1671.

Furuya, S., Cichocki, J. A., Konganti, K., Dreval, K., Uehara, T., Katou, Y., Fukushima, H., Kono, H., Pogribny, I. P., Argemi, J., Bataller, R., & Rusyn, I. (2019). Histopathological and molecular signatures of a mouse model of acute-on-chronic alcoholic liver injury demonstrate concordance with human alcoholic hepatitis. Toxicological Sciences, 170(2), 427–437.

Gao, B., & Bataller, R. (2011). Alcoholic liver disease: Pathogenesis and new therapeutic targets. Gastroenterology, 141(5), 1572–1585.

Ha, Y., Jeong, I., & Hyun, K. T. (2022). Alcohol-related liver disease: An overview on pathophysiology, diagnosis and therapeutic perspectives. Biomedicines, 10(10), 2530.

Hyun, J., Han, J., Lee, C., Yoon, M., & Jung, Y. (2021). Pathophysiological aspects of alcohol metabolism in the liver. International Journal of Molecular Sciences, 22(11), 5717.

Khaderi, S. A. (2019). Introduction: Alcohol and alcoholism. Clinics in Liver Disease, 23(1), 1–10.

Kong, L.-Z., Chandimali, N., Han, Y.-H., & Kwon, T. (2019). Pathogenesis, early diagnosis, and therapeutic management of alcoholic liver disease. International Journal of Molecular Sciences, 20(11), 2712.

Lamas-Paz, A., Hao, F., Nelson, L. J., Vázquez, M. T., Canals, S., Gómez Del Moral, M., Martínez-Naves, E., & Cubero, F. J. (2018). Alcoholic liver disease: Utility of animal models. World Journal of Gastroenterology, 24(45), 5063–5075.

Lange, C. M., Bechstein, W. O., Berg, T., Engelmann, C., Bruns, T., Canbay, A., Moreau, R., & Trebicka, J. (2018). Acute-on-chronic liver failure. Visceral Medicine, 34(4), 296–300.

Li, W., Li, L., & Hui, L. (2020). Cell plasticity in liver regeneration. Trends in Cell Biology, 30(4), 329–338.

Liu, S.-Y., Tsai, I.-T., & Hsu, Y.-C. (2021). Alcohol-related liver disease: Basic mechanisms and clinical perspectives. International Journal of Molecular Sciences, 22(10), 5170.

Ma, J., Guillot, A., Yang, Z., Mackowiak, B., Hwang, S., Park, O., Peiffer, B. J., Ahmadi, A. R., Melo, L., Kusumanchi, P., Huda, N., Saxena, R., He, Y., Guan, Y., Feng, D., Sancho-Bru, P., Zang, M., MacGregor, C. A., Bataller, R., Tacke, F., Sun, Z., Liangpunsakul, S., & Gao, B. (2022). Distinct histopathological phenotypes of severe alcoholic hepatitis suggest different mechanisms driving liver injury and failure. Journal of Clinical Investigation, 132(14), e157780.

Macdonald, S., Andreola, F., Bachtiger, P., Amoros, A., Pavesi, M., Mookerjee, R., Zheng, Y. B., Gronbaek, H. L., Gerbes, A. L., Sola, E., Caraceni, P., Moreau, R., Gines, P., Arroyo, V., & Jalan, R. (2018). Cell death markers in patients with cirrhosis and acute decompensation. Hepatology, 67(3), 989–1002.

Mihm, S. (2018). Danger-associated molecular patterns (DAMPs): Molecular triggers for sterile inflammation in the liver. International Journal of Molecular Sciences, 19(10), 3104.

Monnig, M. A. (2017). Immune activation and neuroinflammation in alcohol use and HIV infection: Evidence for shared mechanisms. The American Journal of Drug and Alcohol Abuse, 43(1), 7–23.

Okamura, Y., Omori, A., Asada, N., & Ono, A. (2018). Effects of vitamin C and E on toxic action of alcohol on partial hepatectomy-induced liver regeneration in rats. Journal of Clinical Biochemistry and Nutrition, 63(1), 50–57.

Park, S. H., Lee, Y. S., Sim, J., Seo, S., & Seo, W. (2022). Alcoholic liver disease: A new insight into the pathogenesis of liver disease. Archives of Pharmacal Research, 45(7), 447–459.

Seitz, H. K., Bataller, R., Cortez-Pinto, H., Gao, B., Gual, A., Lakner, C., Mathurin, P., Mueller, S., Szabo, G., & Tsukamoto, H. (2018). Alcoholic liver disease. Nature Reviews Disease Primers, 16(4), 16.

Singal, A. K., Bataller, R., Ahn, J., Kamath, P. S., & Shah, V. H. (2018). ACG clinical guideline: Alcoholic liver disease. World Journal of Gastroenterology, 113(2), 175–194.

Slevin, E., Baiocchi, L., Wu, N., Ekser, B., Sato, K., Lin, E., Ceci, L., Chen, L., Lorenzo, S. R., Xu, W., Kyritsi, K., Meadows, V., Zhou, T., Kundu, D., Han, Y., Kennedy, L., Glaser, S., Francis, H., Alpini, G., & Meng, F. (2020). Kupffer cells: Inflammation pathways and cell-cell interactions in alcohol-associated liver disease. The American Journal of Pathology, 190(11), 2185–2193.

Teschke, R. (2018). Alcoholic steatohepatitis (ASH) and alcoholic hepatitis (AH): Cascade of events, clinical aspects, and pharmacotherapy options. Expert Opinion on Pharmacotherapy, 19(8), 779–793.

Thomes, P. G., Rasineni, K., Yang, L., Donohue Jr., T. M., Kubik, J. L., McNiven, M. A., & Casey, C. A. (2019). Ethanol withdrawal mitigates fatty liver by normalizing lipid catabolism. American Journal of Physiology Gastrointestinal and Liver Physiology, 316(4), 509–518.

Tsomaia, K., Patarashvili, L., Karumidze, N., Bebiashvili, I., Azmaipharashvili, E., Modebadze, I., Dzidziguri, D., Sareli, M., Gusev, S., & Kordzaia, D. (2020). Liver structural transformation after partial hepatectomy and repeated partial hepatectomy in rats: A renewed view on liver regeneration. World Journal of Gastroenterology, 26(27), 3899–3916.

Zhang, F., Little, A., & Zhang, H. (2017). Chronic alcohol consumption inhibits peripheral NK cell development and maturation by decreasing the availability of IL-15. Journal of Leukocyte Biology, 101(4), 1015–1027.