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OXIDATIVE STRESS AND MORPHOLOGICAL CHANGES IN THE BRAIN ON A HIGH-FAT DIET

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ABSTRACT

The study examined the morphological changes, intensity of lipid peroxidation, and the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase in the brain during a high-fat diet. The high-fat diet caused perivascular and pericellular edema, a reduction in the neuronal density of the molecular and pyramidal layers, as well as a decrease in antioxidant enzyme activity and an increase in lipid peroxidation processes in the brain. The obtained results indicate a disruption of the structural-functional capabilities of the brain due to enhanced lipid peroxidation and decreased activity of antioxidant systems in fatty liver disease.

Key words: steatosis, hippocampus, cerebral cortex, MDA, dienes, SOD, catalase, high-fat diet.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disorder globally, affecting almost a quarter of the world's population [5]. NAFLD represents a metabolic syndrome that affects other organs and is a multisystemic disease. In addition to its hepatic manifestations, NAFLD is associated with significant extrahepatic complications, including alterations in brain morphology and function [7]. In the decompensated stages of NAFLD, liver failure and hepatic

encephalopathy develop. NAFLD can lead to brain damage through several mechanisms, including inflammation, oxidative stress, and metabolic disturbances. These changes may affect cognitive functions and increase the risk of dementia. Existing studies show that NAFLD can cause inflammation and oxidative stress in the brain. Inflammatory cytokines and reactive oxygen species (ROS) in the brain may lead to neuronal damage. These processes can result in cognitive impairments, memory decline, and an increased risk of neurodegenerative diseases. Furthermore, NAFLD can lead to various morphological changes in the brain, including cortical atrophy, microhemangiomas, cerebral atrophy, and microaneurysms. Inflammation and oxidative stress associated with NAFLD may contribute to these changes, affecting cognitive functions and increasing the risk of neurodegenerative diseases [8].

The mechanisms of pathogenesis of liver lesions of various origins are based on processes that damage the integrity of hepatocyte membranes due to the peroxidative oxidation of membrane lipids (LPO). Oxidative stress processes also develop in fatty liver disease. During oxidative stress, the accumulation of products from lipid peroxidation is observed, and their concentration is important for determining the severity of the disease. This is because their concentration provides information about the depth and degree of the pathological process. In various pathological conditions of the hepatobiliary system, morphological changes in the brain and the role of the antioxidant-oxidant system in the brain have not been fully studied.

Purpose of the research: To study the morphological changes in the brain, the intensity of lipid peroxidation, and the activity of antioxidant systems in the brain in non-alcoholic fatty liver disease (NAFLD).

MATERIAL AND METHODS OF RESEARCH

As research subjects, 8-10-week-old male Wistar rats were used. Before the start of the study, the rats were kept in vivarium conditions for 2 weeks. To achieve the research goals, the rats were given a high-fat diet and a glucose-fructose mixture instead of water, and fatty liver disease was modeled. On the relevant days of the study, the rats were decapitated in a cold room at 0°-+2°C. The levels of malondialdehyde (MDA) [1] and dien conjugates (DC) [9], as well as the activity of superoxide dismutase (SOD) [14] and catalase [10], were determined in brain homogenates on the 12th, 16th, and 20th weeks, and morphological examinations were performed on the brain sections during these periods.

RESULTS AND DISCUSSION

The results of the study on lipid peroxidation and antioxidant system activity in an experimental fatty liver disease model showed that the process of lipid

peroxidation in the hippocampal region of the brain accelerated, leading to a decrease in the activity of antioxidant system enzymes (Figure 1). In particular, the MDA level in the corresponding periods is 1.31; 1.34, and 1.4 times higher, while the dienic conjugates increased by 1.63; 1.66, and 1.67 times. At the 12th, 16th, and 20th weeks of the experiment, the SOD activity in the hippocampus of the brain significantly decreased by 1.23, 1.34, and 1.34 times, and the catalase activity decreased by 1.19, 1.36, and 1.39 times compared to the intact rat values.

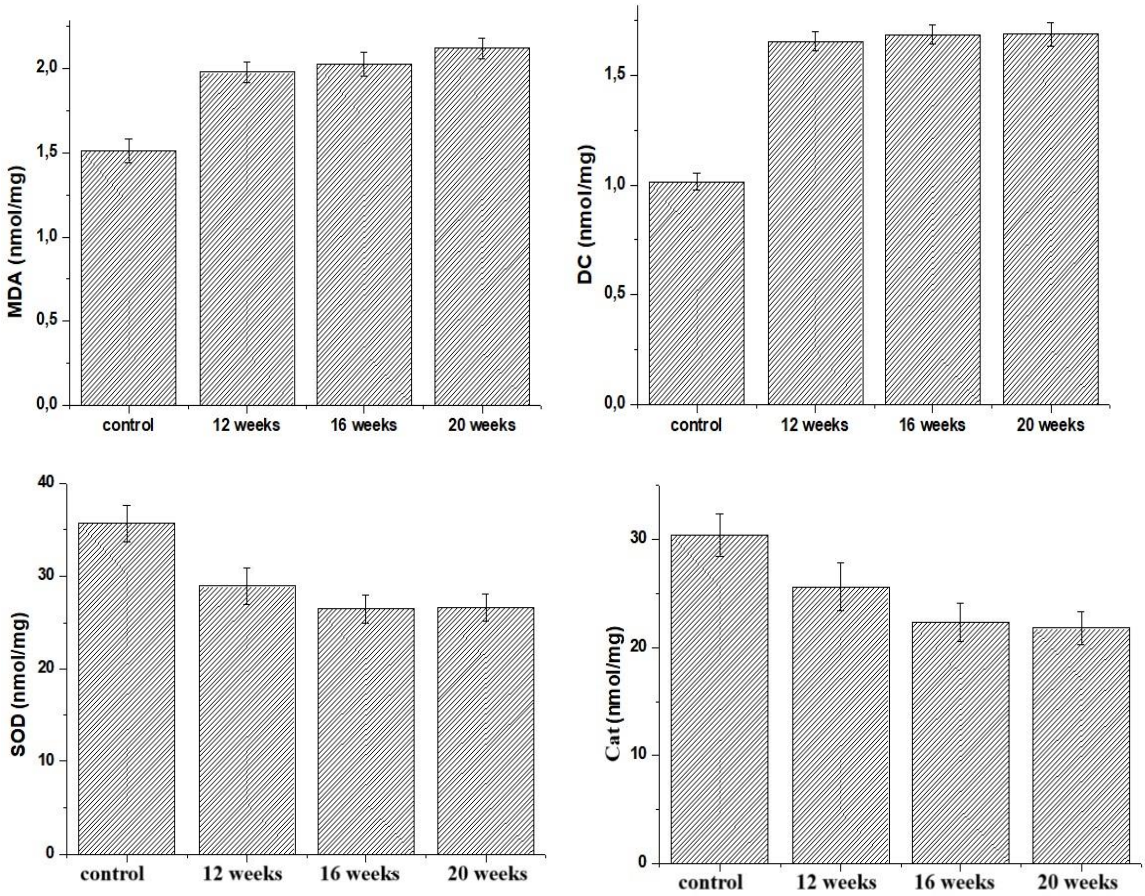


Figure 1. The levels of MDA, dienes, and the activity of SOD and catalase in the hippocampus in experimental fatty hepatosis

In our next study, the process of lipid peroxidation and the activity of the antioxidant system in experimental animals were examined. The results showed that in the area of the brain cortex of the experimental animals, the lipid peroxidation processes were accelerated, resulting in a decrease in the activity of the antioxidant system enzymes (Figure 2). Specifically, the MDA levels increased 1.25, 1.28, and 1.3 times at the corresponding time intervals, while the levels of dienes increased 1.58, 1.6, and 1.63 times. The SOD activity decreased by 1.24, 1.27, and 1.27 times in the brain cortex area during the 12th, 16th, and 20th weeks of the experiment, respectively. The catalase activity also decreased by 1.27, 1.35, and 1.36 times compared to the intact rats at the corresponding time intervals.

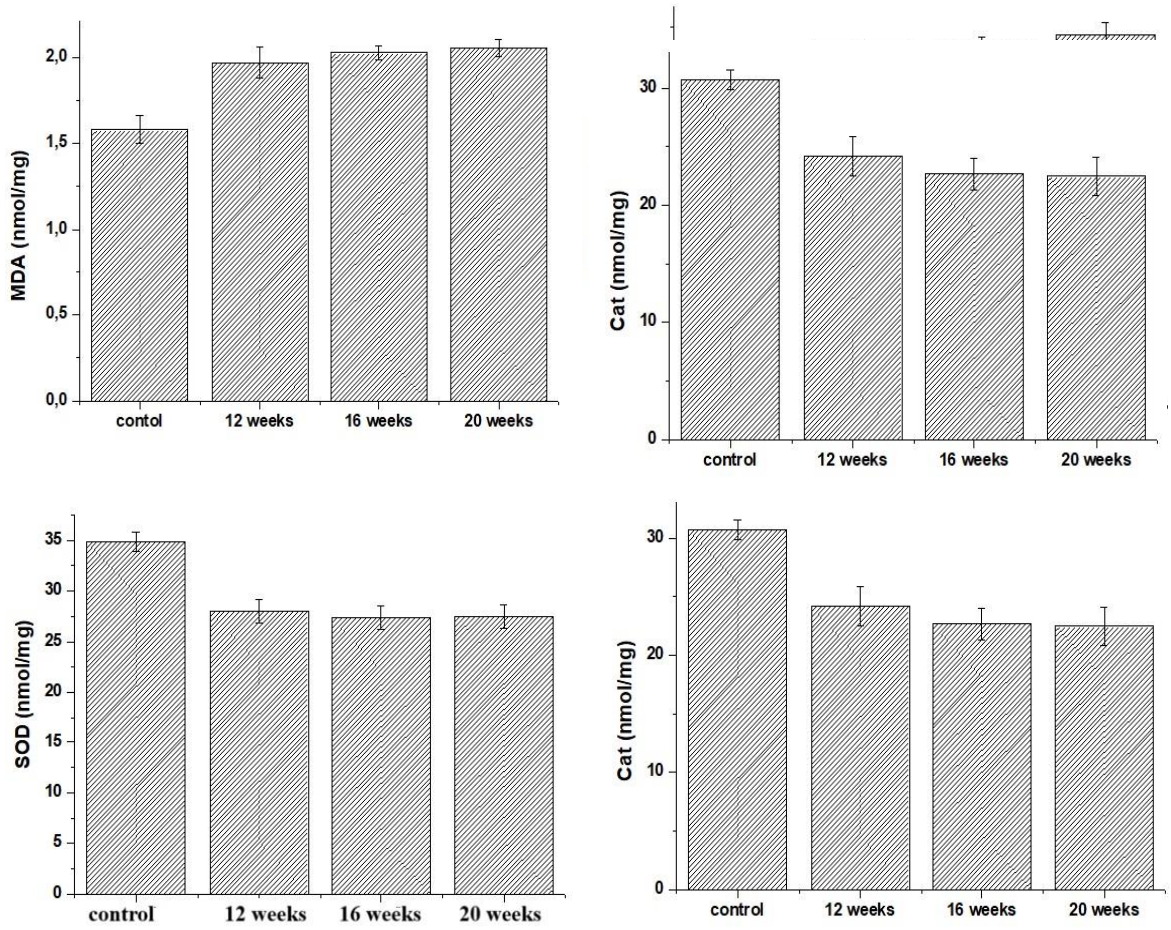


Figure 2. The levels of MDA, dienes, and the activity of SOD and catalase in the brain cortex in experimental fatty hepatosis,

The histological structure of the brain cortex and cerebellum of rats fed regular food exhibited normal cytoarchitecture. Except for mild signs of pericellular and perivascular edema, the structure was unremarkable and corresponded to the normal condition (Figures 3 and 4).

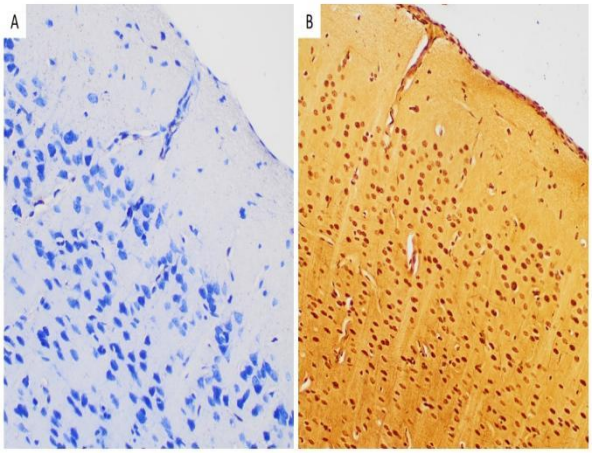


Figure 3. Histopathological image of the brain cortex in rats fed regular food. Staining with thionin according to Nissl,

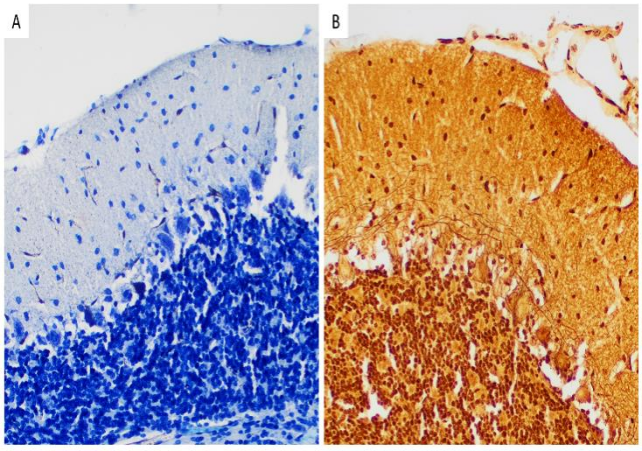
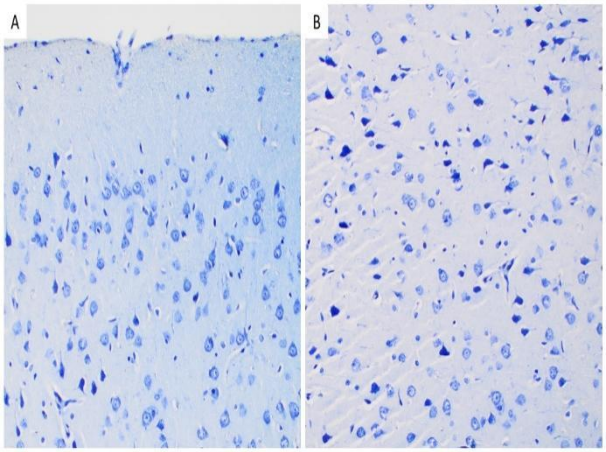


Figure 4. Histopathological image of the cerebellum in rats fed regular food. Staining with thionin according to Nissl, magnification

magnification x200 (A); B. Staining with silver impregnation, magnification x200 (B).	x200 (A); B. Staining with silver impregnation, magnification x200 (B).

In the brain tissue of rats that were fed a high-fat diet for 8 weeks, more pronounced signs of pericellular and perivascular edema were observed. Some pyramidal cells in the cortex were shriveled, with hyperchromatic nuclei and a reduction in the area and volume of the perikaryon (Figure 5).



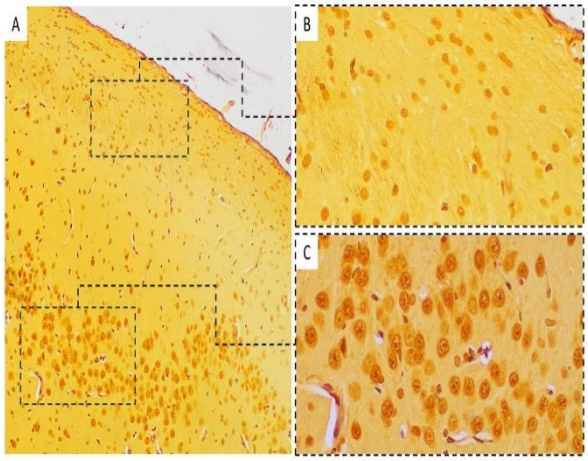


Figure 5. Histopathological image of the brain cortex in rats fed a high-fat diet for 8 weeks. Staining with thionin according to Nissl, magnification x200.	**Figure 6.** Histopathological image of the brain cortex in rats fed a high-fat diet for 8 weeks. Silver impregnation staining, magnification x100-400.

In histological preparations stained with silver impregnation, the overall structure of the brain cortex was normal. However, in individual cells, the nuclear material was not visualized, and signs of karyorexis were observed (Figure 6).

The structure of the cerebellum showed no significant changes.

The histological structure of the hippocampus in rats fed a high-fat diet for 8 weeks generally corresponded to the normal structure. Neurons in the pyramidal layer of the CA1 zone predominantly exhibited round-shaped nuclei and nearly round-shaped perikarya, with a uniform distribution of Nissl substance in the cytoplasm. In a few cells, the nuclear contours were erased due to focal chromatolysis. In the CA2-3 zones, the pyramidal neurons were more dispersed, having polygonal (triangular) shapes with clearly visible nuclei and a single nucleolus. Some cells were moderately shrunken, with pyknotic nuclei (Figure 7).

In histological preparations stained with silver impregnation, against the background of mild pericellular and perivascular edema, pyramidal neurons in the CA1-4 zones had dense arrangement and typical shapes, with clear contours and well-defined initial parts of lateral and apical dendrites, visible due to the chromophilic substance (Figure 8).

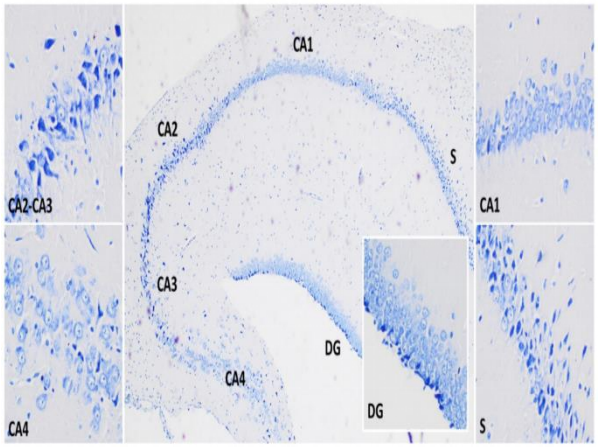


Figure 7. Overall structure, topography, and detailed pathomorphology of the studied areas of the hippocampus in rats fed a high-fat diet for 8 weeks: CA1-4 – hippocampal fields, DG – dentate gyrus, S – subiculum. Staining with thionin according to Nissl, magnification x100-400.

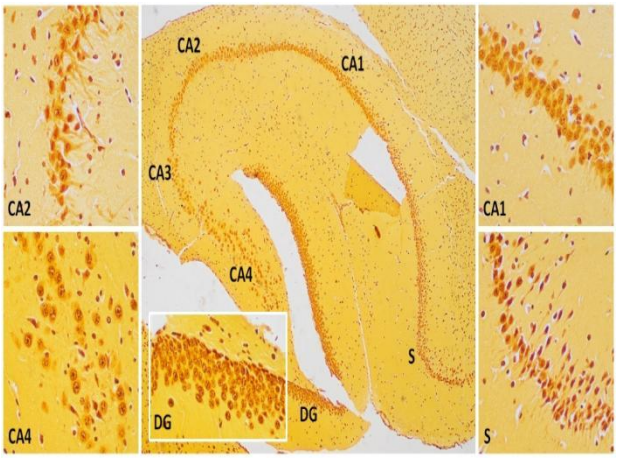


Figure 8. Overall structure, topography, and detailed pathomorphology of the studied areas of the hippocampus in rats fed a high-fat diet for 8 weeks: CA1-4 – hippocampal fields, DG – dentate gyrus, S – subiculum. Silver impregnation staining, magnification x100-400.

In the brain cortex tissue of rats fed a high-fat diet for 12 weeks were also noted signs of pericellular and perivascular edema. Some pyramidal cells in the cortex were shriveled, with hyperchromatic nuclei and a reduction in the area and volume of the perikaryon. Single foci of satellitosis (the "adhesion" of microgliocytes to the surface of dead neurons) were found (Figure 9).

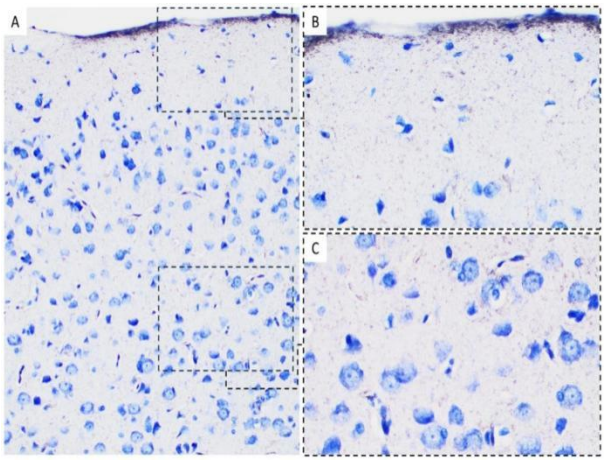


Figure 9. Histopathological image of the brain cortex in rats fed a high-fat diet for 12 weeks. Staining with thionin according to Nissl, magnification x200.

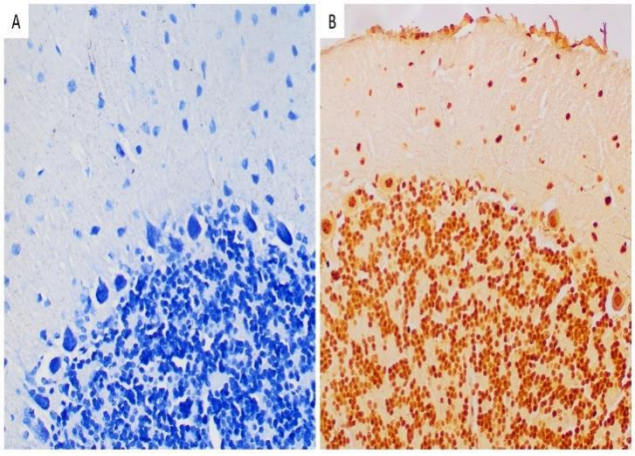


Figure 10. Histopathological image of the cerebellum in rats fed a high-fat diet for 12 weeks. Staining with thionin according to Nissl, magnification x200 (A); B. Silver impregnation staining, magnification x200 (B).

The structure of the cerebellum showed no significant changes (Figure 10).

The histological structure of the hippocampus in rats fed a high-fat diet for 12 weeks was characterized by a relative decrease in the width of the pyramidal layer

and a reduced density of neurons, with moderate thinning of the neuronal layers. Areas of focal loss of neurocytes, as well as mild signs of pericellular and perivascular edema, were observed (Figures 11 and 12).

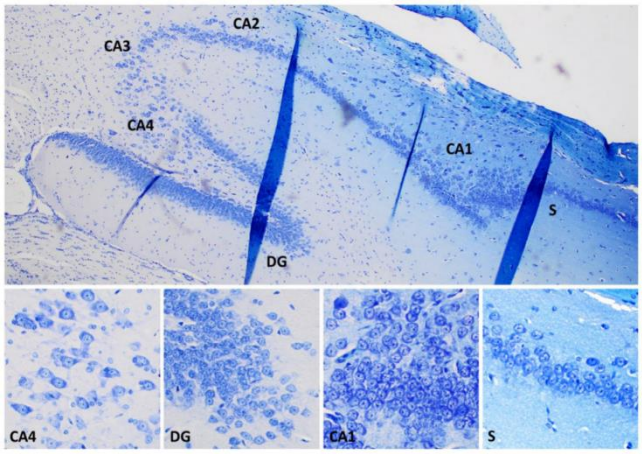


Figure 11. Overall structure, topography, and detailed pathomorphology of the studied areas of the hippocampus in rats fed a high-fat diet for 12 weeks: CA1-4 – hippocampal fields, DG – dentate gyrus, S – subiculum. Staining with thionin according to Nissl, magnification x100-400.

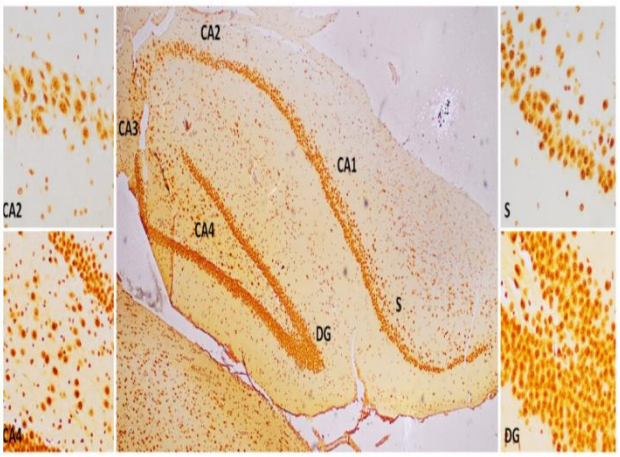


Figure 12. Overall structure, topography, and detailed pathomorphology of the studied areas of the hippocampus in rats fed a high-fat diet for 12 weeks: CA1-4 – hippocampal fields, DG – dentate gyrus, S – subiculum. Silver impregnation staining, magnification x100-400.

In the brain cortex tissue of rats fed a high-fat diet for 16 weeks, signs of pericellular and perivascular edema were most pronounced. Moderate reactive gliosis was observed. A large number of pyramidal cells in the cortex were shriveled, with hyperchromatic nuclei and a reduction in the area and volume of the perikaryon (Figure 13).

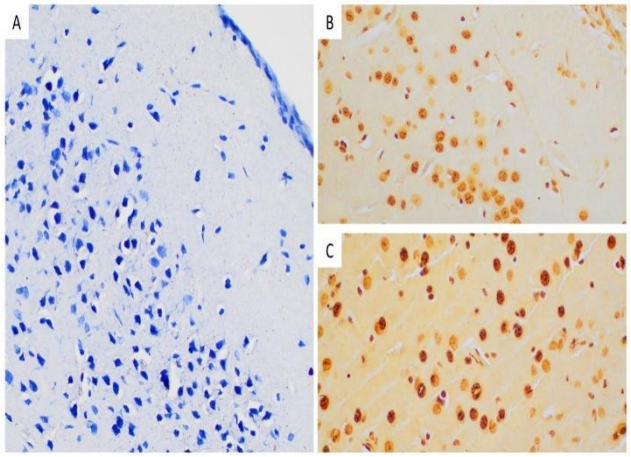


Figure 13. Histopathological image of the brain cortex in rats fed a high-fat diet for 16 weeks. Staining with thionin according to Nissl, magnification x200 (A); B. Silver impregnation staining, magnification x200 (B-C).

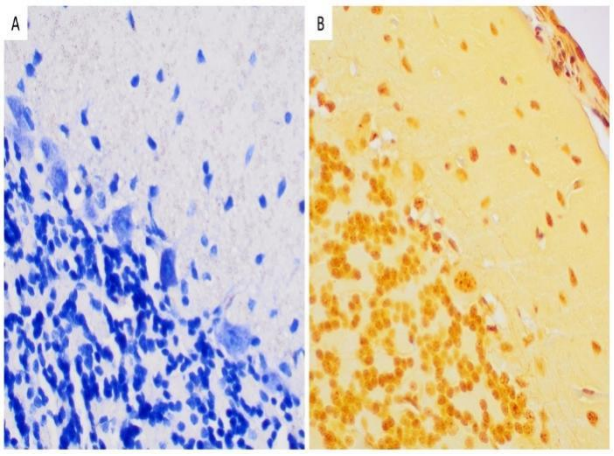


Figure 14. Histopathological image of the cerebellum in rats fed a high-fat diet for 16 weeks. Staining with thionin according to Nissl, magnification x200 (A); B. Silver impregnation staining, magnification x200 (B).

Perivascular and pericellular edema were also noted in histological preparations of the cerebellum, along with small-vesicular spongiosis in the white matter of the brain. (Figure 14).

The histological structure of the hippocampus in rats fed a high-fat diet for 16 weeks was characterized by a relative decrease in the width of the pyramidal layer and reduced density of neurons, with moderate thinning of the neuronal layers. The CA2 and CA3 layers showed uneven thickness, with focal areas exhibiting a sharp reduction in thickness to one or two neurons and occasional loss of pyramidal cells, as well as focal gliosis (Figure 15).

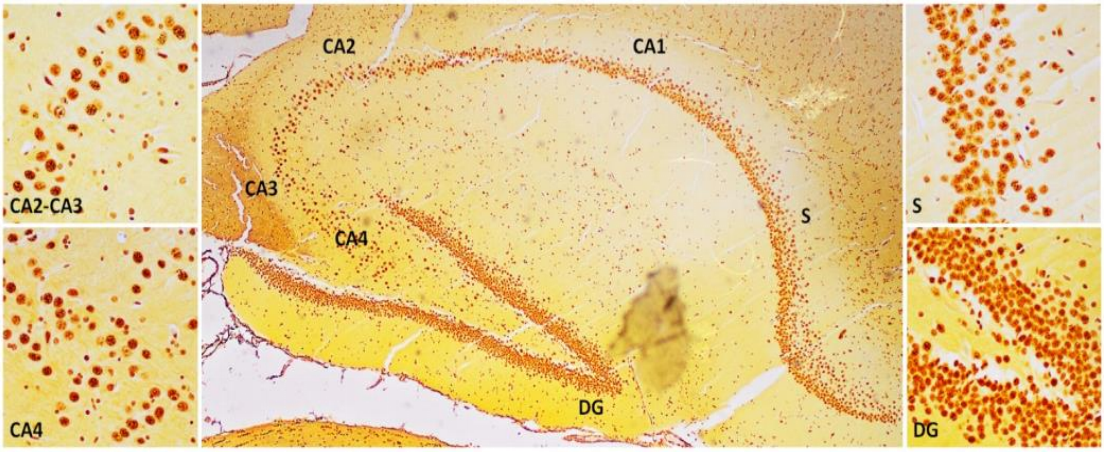


Figure 15. Overall structure, topography, and detailed pathomorphology of the studied areas of the hippocampus in rats fed a high-fat diet for 16 weeks: CA1-4 – hippocampal fields, DG – dentate gyrus, S – subiculum. Silver impregnation staining, magnification x100-400.

Signs of pericellular and perivascular edema were also observed in the brain cortex tissue of rats fed a high-fat diet for 20 weeks. Some pyramidal cells in the cortex were shriveled, with hyperchromatic nuclei and a reduction in the area and volume of the perikaryon. In some cells, the nuclear material was not visualized, and signs of karyorexis were observed (Figure 16).

In the histological preparations of the cerebellum, perivascular and pericellular edema, hyperchromia, and shrinkage of individual Purkinje cells were noted (Figure 17).

The histological structure of the hippocampus in rats fed a high-fat diet for 20 weeks was characterized by a decrease in the relative density of neurons in the molecular and pyramidal layers, with areas showing reduced thickness and loss of neurons. In the perikarya, pronounced discoloration was observed, with areas of chromatolysis and, conversely, hyperchromatosis, forming homogeneous hyperchromatic fusiform and globular structures. The dendritic areas, visible due

to the chromophilic substance, were deformed and often had an "amputated" appearance (Figure 18).

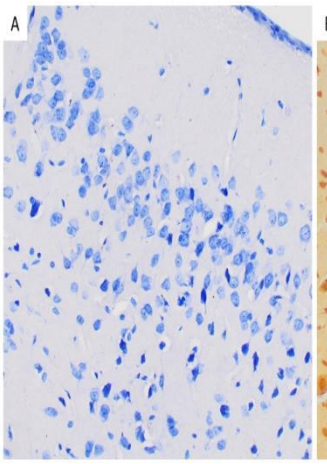


Figure 16. Histopathological image of the brain cortex in rats fed a high-fat diet for 20 weeks. Staining with thionin according to Nissl, magnification x200 (A); B.Silver impregnation staining, magnificationx200 (B).

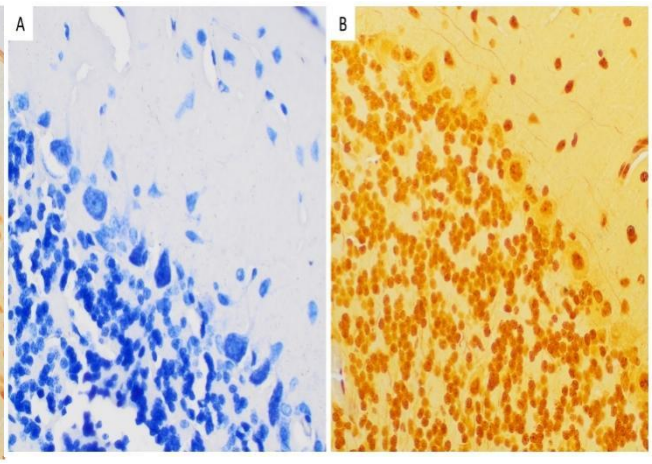


Figure 17. Histopathological image of the cerebellum in rats fed a high-fat diet for 20 weeks. Staining with thionin according to Nissl, magnification x200 (A); B.Silver impregnation staining, magnificationx200 (B).

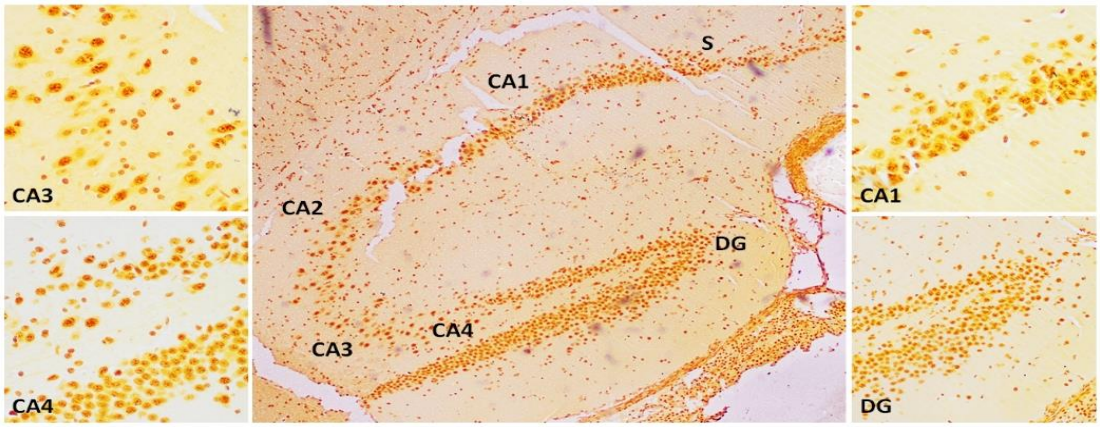


Figure 18. Overall structure, topography, and detailed pathomorphology of the studied areas of the hippocampus in rats fed a high-fat diet for 20 weeks: CA1-4 – hippocampal fields, DG – dentate gyrus, S – subiculum. Silver impregnation staining, magnification x100-400.

Our study showed elevated levels of lipoperoxidation in the brain during hepatic steatosis. This is accompanied by structural changes in the hippocampus and cerebral cortex, as well as cognitive impairments. Furthermore, there is a decrease in the levels of antioxidant enzymes, such as superoxide dismutase and catalase.

The results of our research confirm the hypothesis that NAFLD (Non-Alcoholic Fatty Liver Disease) negatively affects brain functions through oxidative

stress mechanisms. These findings emphasize the need to develop treatment strategies aimed at reducing inflammation and restoring antioxidant balance in the brain in the case of NAFLD.

Existing studies show that fat accumulation in the liver leads to a reduction in the supply of oxygen to the brain and inflammation of brain tissue [4]. The progressive lipid deposition in the liver leads to alterations in lipid metabolism, oxidative stress, lipid peroxidation, insulin resistance, and inflammatory damage [13].

Researchers have studied the impact of high-fat diets on the body, particularly on the liver and brain. It was found that all mice developed obesity, NAFLD, insulin resistance, and brain dysfunction. Additionally, it was revealed that the brains of mice with NAFLD suffered from a lack of oxygen, and they were more anxious and showed signs of depression [11; 12].

Excessive intake of fructose, commonly used as a sweetener in soft drinks, along with systemic metabolic disturbances, has been shown to induce oxidative stress. This stress causes lipid peroxidation and protein nitrosylation in the hippocampus, reduces the expression of synaptic proteins, impairs synaptic function, and subsequently affects learning and memory in a long-lived animal model [15].

Histological examination of the cerebella in patients with NAFLD showed parenchymal microthrombi, neurodegeneration in the Purkinje cell layer, glial alterations in the molecular layer, and activation of microglia and astrocytes in the white matter [3].

Studies have found that NAFLD increases the risk of dementia by 38% – acquired dementia due to organic brain damage. Additionally, steatosis can be associated with the development of cardiovascular diseases [16].

Neurodegeneration is closely linked with oxidative stress due to heightened production of reactive oxygen species. Oxidative damage begins at the earliest stages of neurodegeneration [17]. Various conditions, such as hypoxia, ischemia, and insulin resistance, can trigger oxidative stress.

Thus, it has been shown that mice with NAFLD develop brain hypoxia. Hypoxia is associated with the development of thickened vascular walls, reduced blood flow, and inflammation in the brain, as the need for oxygen increases under inflammatory conditions. In NAFLD, microglial and astrocytic morphological and metabolic changes occur, which are signs of developing encephalopathy. Importantly, a link has been established between hypoxia and the development of neurodegenerative diseases. It has been found that hypoxia not only directly causes neuronal damage but also leads to the development of cerebral inflammation.

Toxic inflammatory mediators produced by glial cells under hypoxic conditions play a key role in the development of cerebral inflammation, which exacerbates further neuronal damage, synaptic remodeling, and neurodegeneration [2; 6].

The results of our studies confirm that NAFLD has a significant impact on brain morphology. Oxidative stress is a key mechanism contributing to these changes. These data highlight the need to develop treatment strategies aimed at reducing inflammation and oxidative stress in patients with NAFLD.

Morphological changes in the brain associated with NAFLD may have significant consequences for cognitive functions and the overall health of patients. Future research should focus on further studying these changes and developing effective methods of prevention and treatment.

CONCLUSION

A high-fat diet resulted in perivascular and pericellular edema, hyperchromia, a decrease in the neuronal density of the molecular and pyramidal layers, as well as a reduction in antioxidant enzyme activity and an increase in lipoperoxidation processes in the brain.

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