ORIGINAL ARTICLE

Qualitative and quantitative analysis of rat liver sinusoids under condition of a long-term experimental exposure to cannabidiol oil

Mykola M. Shevchuk[1,2], Liliya I. Volos[1], Oksana R. Kovalska[1]
[1]STATE NON-PROFIT ENTERPRISE «DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY», LVIV, UKRAINE,
[2]STATE SPECIALIZED INSTITUTION «LVIV REGIONAL BUREAU OF FORENSIC MEDICAL EXAMINATION», LVIV, UKRAINE

ABSTRACT

Aim: The study aimed to determine the structural organization and conduct a quantitative analysis of the cellular composition of rat liver sinusoids under condition of a long-term (10 weeks) experimental exposure to cannabidiol oil.

Materials and Methods: An experimental study was conducted on 20 white non-linear male rats weighing 180-230 g, in compliance with ethical principles. The modeling of prolonged cannabidiol oil exposure in 14 white rats was performed through daily per os administration at a dose of 10 mg/kg/ for 10 weeks, the control group was 6 sexually mature white male rats, that oral administration the CBD carrier solvent – hemp seed oil. Histological, immunohistochemical and morphometric studies of the cellular composition of liver sinusoids were performed with statistical processing. In all comparisons, the difference was considered statistically significant at a minimum significance level of $p < 0.05$.

Results: After 10 weeks exposure to CBD oil administration pathohistological changes in the rat liver sinusoids were not observed. There is a significant increase in the average number of Kupffer cells in the sinusoidal wall in all zones of the lobule $p < 0.001$, especially near the portal zone (1.58 times) compared to the control group, which can be explained by their activation in response to long-term exposure to CBD. Perisinusoidal cells in the experimental series in all areas of the liver lobule were the least compared to endothelial cells and Kupffer cells, $p < 0.001$.

Conclusions: Histological, immunohistochemical study with quantitative analysis of the cellular composition of rat liver sinusoids indicates the safety of long-term experimental exposure to CBD oil.

KEY WORDS: liver, rats, sinusoids, endothelial cells, Kupffer cells, perisinusoidal cells, CBD, safety

Wiad Lek. 2025;78(12):2611-2619. doi: 10.36740/WLek/215799 DOI

INTRODUCTION

Cannabidiol (CBD) is a phytocannabinoid found in the hemp plant (Cannabis sativa L.) of the Cannabaceae family [1]. As a drug, CBD is approved at appropriate therapeutic doses by the US Food and Drug Administration, the European Medicines Agency, and the European Food Safety Authority [2-4] and is currently marketed as the oral drug Epidyolex in the EU and Epidiolex outside the EU for the treatment of severe childhood epilepsy (Lennox-Gastaut syndrome, Dravet syndrome) and tuberous sclerosis [5]. In addition, preclinical and clinical trials evaluating the efficacy of CBD as a therapeutic agent have been identified for a variety of conditions, including anxiety, pain, inflammation, various substance use disorders, post-traumatic stress disorder, sleep disorders, and others [6].

In Ukraine, CBD is a legal substance and is excluded from the list of narcotic and psychotropic substances (CMU Resolution of May 6, 2000, No. 770, CMU Resolution of April 7, 2021, No. 324) [7].

Today, most consumers use CBD in the form of non-medicinal over-the-counter preparations, so-called CBD oils, which contain from 5% to 40% CBD extract dissolved in edible oil and are advertised with various health benefit claims [8]. In 2022, the European Medicines Agency and the European Food Safety Authority identified the risks associated with the use of CBD as a dietary supplement or food ingredient and summarized the possible adverse effects of CBD on human health. Possible side effects from the liver, gastrointestinal tract, endocrine and reproductive systems, metabolic disorders, genotoxicity, neurological and psychiatric disorders were noted. Regarding side effects from the liver, increased liver weight, hypertrophy of liver hepatocytes, increased levels of liver enzymes (ALT, AST, alkaline phosphatase), increased bilirubin, potential drug-induced liver damage were indicated [9, 10].

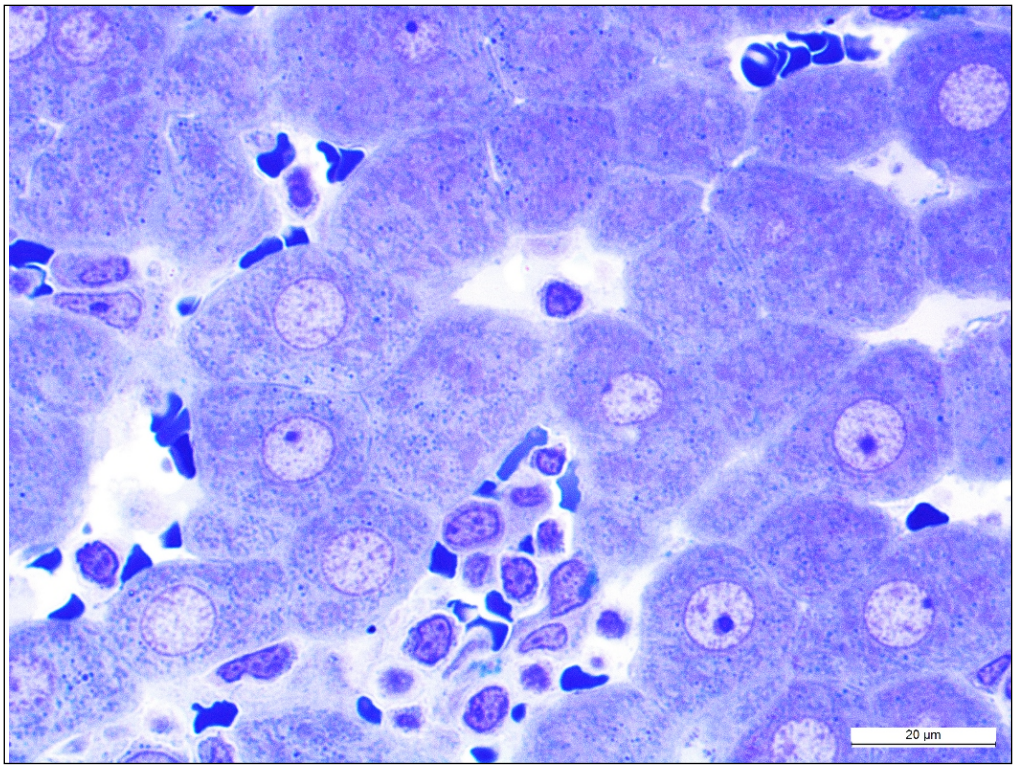

Fig. 1. Rat liver after 10 weeks of experimental use of CBD oil. Preservation of the structure and shape of hepatocytes. Individual sinusoidal capillaries are moderately dilated, full of blood. Semithin preparation (1 µm), staining with methylene blue – basic fuchsin, ×1000 (immersion) *Picture taken by the authors*

Thus, experimental studies of the liver are needed to establish the nature and severity of possible liver damage, including the stromal-vascular compartment, hemodynamic features and to determine the safety of CBD use.

AIM

To determine the structural organization and conduct a quantitative analysis of the cellular composition of rat liver sinusoids under condition of a long-term (10 weeks) experimental exposure to CBD oil.

MATERIALS AND METHODS

A series of experimental studies was conducted in vivarium conditions on 20 white non-linear male rats weighing 180-230 g, 5-7 months old at the beginning of the experiment after ethical approval by the Bioethics Commission at the Danylo Halytskyi Lviv National Medical University (protocol No. 7 dated August 29, 2022) in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council of Europe Directive 2010/63/EU, Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty to Animals" [11, 12].

The main group included 14 rats that received 10% CBD oil orally once a day at a dose of 10 mg/kg for 10 weeks. The manufacturer of the product under study is the licensed company "Aroma Extract Labs s.r.o." (Praha, Czech Republic). To administer the dose, a laboratory single-channel pipette dispenser 20 µl MicroPette Plus was used. The control group included 6 sexually mature white male rats, that oral administration the CBD carrier solvent – hemp seed oil. During the experimental study, the general condition of the rats was observed. At the end of the experiment, after euthanasia, material was collected for morphological study. The liver was used as the material for the study. Paraffin blocks were made from liver tissue samples according to the standard method [13]. Deparaffinized histological sections with a thickness of 5±1 µm were stained with hematoxylin-eosin, after which the general histological structure of the liver was examined. In addition, for a targeted study of the sinusoids of the liver lobules, serial semithin sections with a thickness of 0.5-1 µm were made from epoxy blocks according to the generally accepted method, stained with methylene blue–basic fuchsin, and studied under a light microscope at a magnification of ×1000 (immersion) [14].

We also performed immunohistochemical studies of the liver sinusoids. For this purpose, histological sections 5±1 µm thick were prepared from paraffin blocks, which were applied to highly adhesive "Super Frost" slides, and then dried according to the standard method. Unmasking was performed in citrate buffer, pH-6.0. Visualization of the IHC reaction was performed using the DAKO EnVision+System detection system with DAB chromogen (diaminobenzidine) [15]. We used

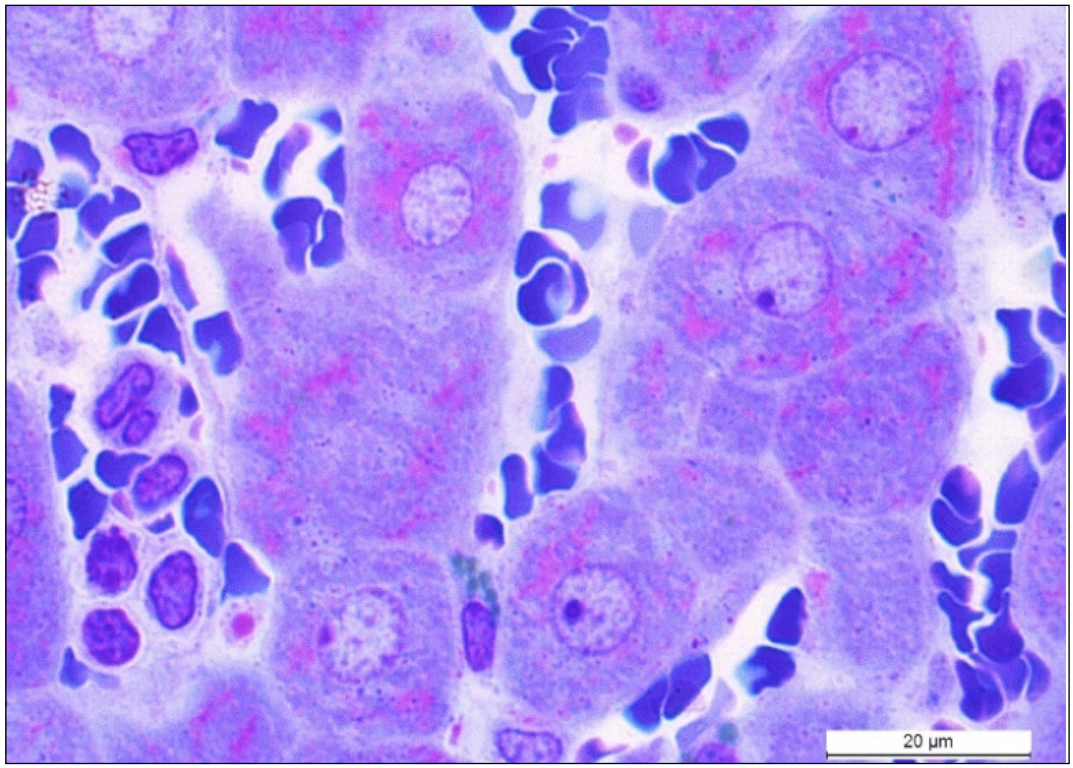

Fig. 2. Marked dilatation of sinusoids and deposition of blood in them. Semi-thin preparation (1 µm), stained with methylene blue – basic fuchsin, ×1000 (immersion)
Picture taken by the authors

monoclonal antibodies for macrophages CD68 (Clone KP1, DAKO), vascular endothelium CD31 (Clone JC70A, Thermo Fisher scientific).

The studies were performed according to the manufacturer's protocol with the necessary controls. To study histological preparations, we used a Leica DM 2500 light-optical microscope (Leica Microsystems GmbH, Germany) with a Leica DFC450 C digital camera (Germany) and licensed Leica Application Suit Version 3.8 software. For quantitative analysis, we counted the main cells of the sinusoid wall in different areas of the liver lobules (around the central vein, near the portal tracts and in the intermediate zone between the triad and the central vein) on an area of 0.01 mm^2 of the histological preparation (100 µm×100 µm).

Data processing was carried out by applied statistical methods used in medicine using the R Commander program (version 2.7-2, GNU General Public License) based on the Windows operating system. Data are presented as mean values: arithmetic mean with standard deviation (M±SD), the reliability of the difference between these indicators was determined by the Mann-Whitney (U) test. In all comparisons, the difference was considered statistically significant at a minimum significance level of $p < 0.05$ [16].

RESULTS

Microscopically, the liver tissue after 10 weeks of experimental use of CBD oil is represented by lobules in the form of hexagonal prisms, the lobular and trabecular structure is not disturbed. Hepatocyte dystrophy was not observed. Sinusoids were located between the hepatocyte beams, some of them were moderately dilated and hyperemic. The structural microscopic organization of hepatic beams and sinusoids is presented in a semi-thin section (Fig. 1).

In some histoslides, sinusoidal capillaries are significantly dilated, although unevenly (Fig. 2). The diameter of single dilated and hyperemic sinusoids reached 20 µm. The median diameter of sinusoids in the variation series was 9.96 µm. The average diameter of sinusoids was 9.78±0.58 µm and significantly exceeded the parameter of the control group (7.66±1.00 µm) ($p < 0.001$). At the same time, the parameter of the average diameter of sinusoids is averaged and relative, since in different parts of the lobule the lumen of sinusoids is uneven.

The sinusoidal wall was lined with elongated endothelial cells with hyperchromic nuclei. Individual endothelial cells protruded into the lumen of the sinusoidal capillaries, which could be well visualized by immunohistochemical typing using the CD31 marker (Fig. 3).

Kupffer cells, which were significantly larger than endothelial cells, were visualized in the wall of sinusoidal capillaries and partially at their bifurcations. IHC typing using the CD68 marker made it possible not only to visualize Kupffer cells in different areas of the liver lobule (Fig. 4), but also to conduct a quantitative study and compare them with the control group.

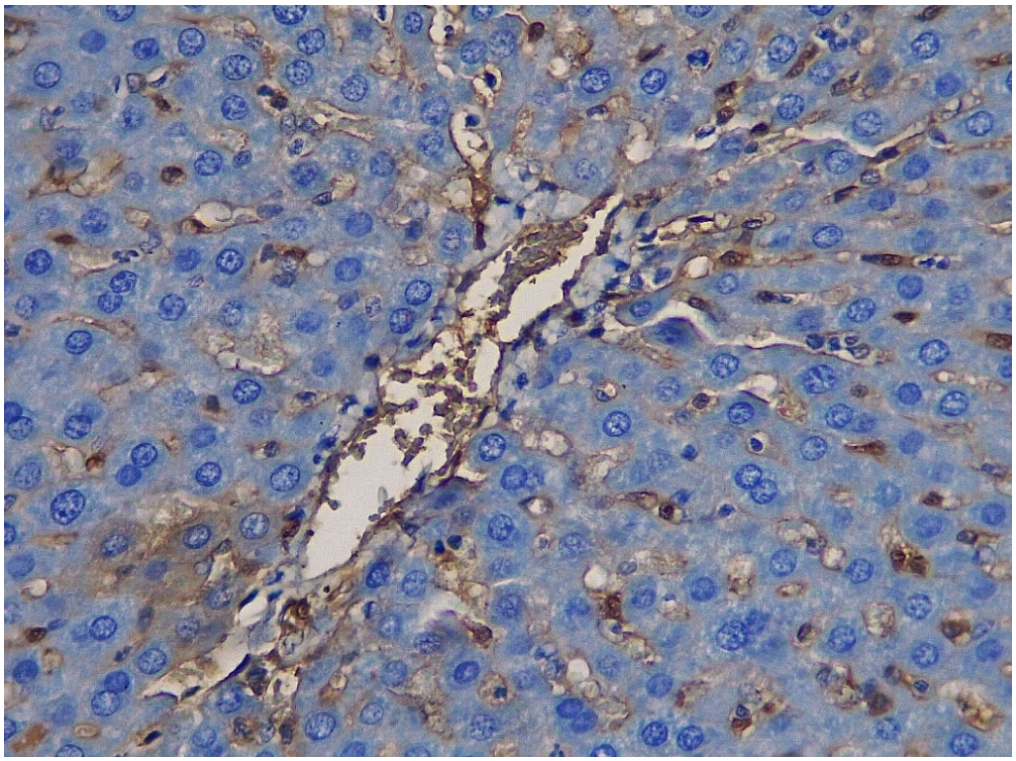

Fig. 3. Rat liver tissue after 10 weeks of experimental CBD oil administration. Representative immunohistochemistry results for CD31. CD31-positive endothelial cells of the sinusoids and central vein are stained brown. IHC typing of endothelial cells using the CD31 marker (Clone JC70A, Thermo Fisher scientific), ×400
Picture taken by the authors

Ito fat-storing cells were in the perisinusoidal space of Disse in the so-called "pockets" between hepatocytes and endothelial cells, had long processes, a rounded, slightly elongated or irregular shape, a large nucleus, and lipid droplet-like inclusions, indicating their inactive state.

For quantitative analysis of the cellular composition of rat liver sinusoids under condition of a long-term (10 weeks) experimental exposure to CBD oil, we counted the main cells of the sinusoidal wall on an area of a histological preparation of 0.01 mm^2 (100 μm × 100 μm) in different zones of the liver lobule, using the traditional classical concept, where the central vein is the central zone, the triads are the peripheral zone, and the intermediate zone is the interlobular. According to the Rappaport model, the liver acinus is divided into three acinar zones (1, 2, and 3) - periportal (1), intermediate (2), and pericentral (3) [17]. The quantitative characteristics of endothelial cells, Kupffer cells, and perisinusoidal cells in the classical liver lobule in the experimental series after 10 weeks of CBD exposure and in the control group are presented in Fig. 5, Fig. 6, Fig. 7.

Comparison of the average CD31 cell counts on the 0.01 mm^2 histological preparation area of the experimental CBD group at the end of week 10 (M±SD) with the control group is presented in Fig. 5. Analysis of the average endothelial cell counts on the 0.01 mm^2 area demonstrated a significant difference in different zones of the liver lobules. In the experimental group, in zone 1 of the liver lobules near the triads, the average number of endothelial cells was the lowest compared to other zones, amounting to 4.88±0.12 and significantly different from the control group (5.22±0.17), $p < 0.001$. In the intermediate (midzone) zone 2, the average number of endothelial cells was 5.24±0.07, which was also lower than the control group (5.79±0.18), $p < 0.001$. Around the central vein (zone 3), the average number of endothelial cells was 11.77±0.56, significantly exceeding the indicators in other zones and significantly different from the control group (13.12±0.30), $p < 0.001$.

The average CD31 cell counts (in an area of 0.01 mm^2) in different areas of the liver lobule, both in the experimental series and in the control group, significantly differing from each other, which can be explained by the different average diameter of sinusoids. Thus, around the central vein, the average diameter of sinusoids is smaller than in the midzonal zone and near the triads, as a result, the density of endothelial cells in an area of 0.01 mm^2 around the central vein in the lobule was significantly higher (11.77±0.56) than near the triads (4.88±0.12) and in the midzonal zone (5.24±0.07). The average number of CD31-positive cells around the central vein exceeded the average number near the triads by 2.41 times ($p < 0.01$). When comparing the average CD31 cell counts in an area of 0.01 mm^2 in the experimental series with the control group, it was found that in all areas of the liver lobule, the average CD31 cell count was significantly lower than the analogical parameter in the control group, which is explained by the larger diameter of the sinusoids and improved blood flow to the liver lobule under long-term exposure to CBD oil.

In the experimental series of long-term exposure to

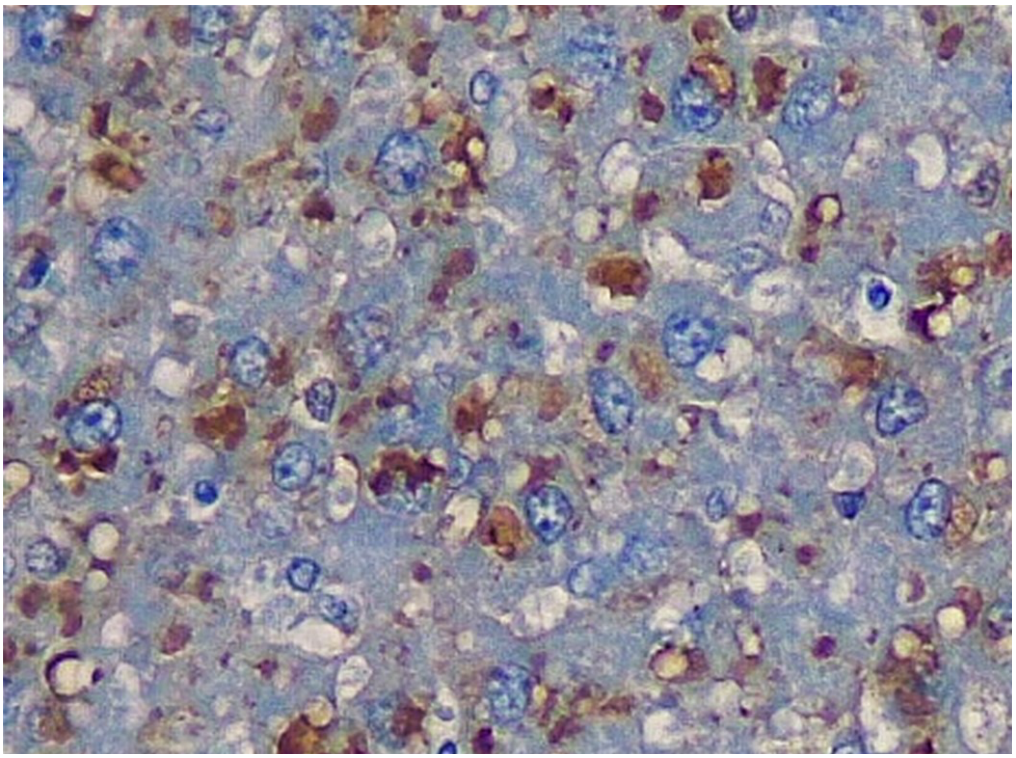

Fig. 4. Rat liver tissue after 10 weeks of experimental CBD oil administration. Representative immunohistochemistry results for CD68. Hyperplasia and hypertrophy of Kupffer cells in the sinusoids of the hepatic lobule. IHC typing of Kupffer cells using the CD68 marker (Clone KP1, DAKO), × 600
Picture taken by the authors

CBD oil, the highest average number of Kupffer cells per 0.01 mm^2 area (100 μm × 100 μm) was determined in the liver triad zone and was 6.12±0.13 cells, and significantly ($p < 0.001$) 1.58 times higher than the analogical parameter in the control group (3.88±0.10 cells). Also, the average number of Kupffer cells in the liver triad zone in a comparative analysis exceeded the parameter in the intermediate zone by 1.17 times (5.22±0.06 cells) and around the central vein by 1.79 times (3.42±0.13 cells) within the experimental group. The highest average levels of CD68-positive cells in the liver triad zone in both the experimental and control groups can be explained by the key role of Kupffer cells in immune regulation, protecting the liver from various harmful effects, toxins, and harmful substances that can enter the bloodstream from the intestine.

In the intermediate zone of the liver lobule of the experimental series, the average CD68 cell count was 5.22±0.06 and significantly exceeded the appropriate value in the control group (2.90±0.16), $p < 0.001$. In the zone of the lobule around the central vein, the average CD68 cell count was the lowest (3.42±0.13) compared to other zones of the liver lobule of the experimental series but exceeded the value of the control group (1.06±0.12) by 3.23 times, $p < 0.001$. As in the triad zone, in the intermediate zone of the lobule and around the central vein, the average CD68 cell counts significantly exceeded the appropriate values in the control, which is explained by hyperplasia of specialized macrophages and their important importance for the immune function of the liver (Fig. 6).

Perisinusoidal cells in the experimental series in all zones of the liver lobule were the least compared to endothelial cells and Kupffer cells. When comparing between zones, the average number of cells per 0.01 mm^2 area (100 μm × 100 μm) was the highest near the triads (3.02±0.08) and significantly exceeded the appropriate value in the control group (2.13±0.08), $p < 0.001$. In the intermediate zone and around the central vein, the average number of perisinusoidal cells was 2.33±0.09 and 1.37±0.06, respectively, and significantly differed from the control group, $p < 0.001$. In the intermediate zone of the control group, the average number was 1.31±0.11, and around the central vein – 0.67±0.07 (Fig. 7).

Thus, conducted histopathological, immunohistochemical and morphometric study with quantitative analysis of the cellular composition of the liver sinusoids allowed to establish their structural microscopic organization after long-term experimental exposure to CBD oil for 10 weeks. In some fields of view, the sinusoidal capillaries are unevenly dilated. The average diameter of the sinusoids was 9.78±0.58 μm and differed from the control group (7.66±1.00 μm) ($p < 0.001$).

In rare cases, in the intermediate and pericentral zones of hepatic lobules sinusoids were dilated and hyperemic, which can be explained by increased blood flow to the liver triad and a compensatory-adaptive process under the conditions of experimental exposure to CBD. We did not observe any hemodynamic disturbances, and we did not diagnose any impaired blood outflow through the central vein. In this case, slight hyperemia was part of the normal physiological process.

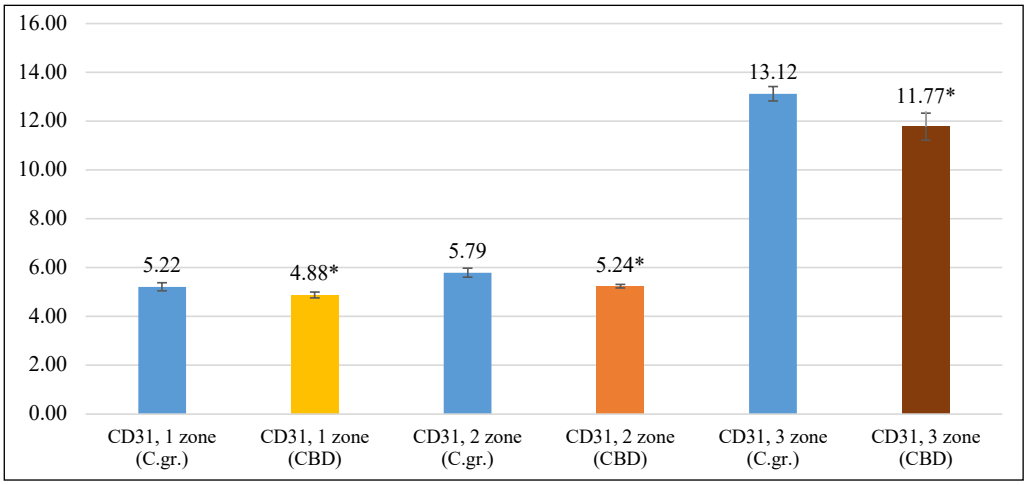

Fig. 5. Comparison of average indicator CD31 cells (in an area of 0.01 mm^2) at week 10 of experimental exposure to CBD with the control group (C.gr.) (M±SD)
Note: * - significant difference ($p < 0.05$) with the control group
Picture taken by the authors

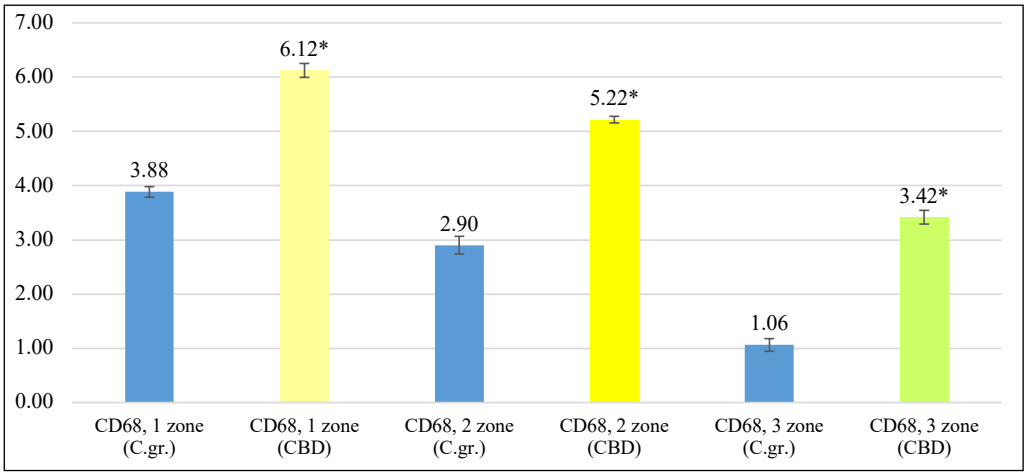

Fig. 6. Comparison of average indicator CD68 cells (in an area of 0.01 mm^2) at week 10 of experimental exposure to CBD with the control group (C.gr.) (M±SD)
Note: * - significant difference ($p < 0.05$) with the control group
Picture taken by the authors

Comparative analysis of the average values of endothelial cells, Kupffer cells and perisinusoidal cells in different zones of the lobule on the area of the histological preparation of 0.01 mm^2 with long-term use of CBD oil and in the control group demonstrated that the trend of cell distribution in different zones of the lobule was preserved. In the experimental series, in the zone near the triads (zone 1), Kupffer cells prevailed, the average value of which was 6.12±0.13, there were slightly fewer endothelial cells - 4.88±0.12 per 0.01 mm^2 of the preparation area and 3.02±0.08 - perisinusoidal cells. When compared with the control group, there were fewer endothelial cells, which can be explained by an increase in the diameter of sinusoids. However, there were significantly more Kupffer cells than in the control group, which is associated with their proliferation in response to CBD oil. There were also more perisinusoidal cells than in the control group due to their activation and proliferation. In the area around the central vein of the lobule (zone 3), endothelial cells (11.77±0.56) predominated among all the studied cells, however, the average number of endothelial cells was significantly lower ($p < 0.001$) than in the control group, which can be explained by the larger average diameter of sinusoids in the experimental group. There were fewer Kupffer cells around the central vein than in the area near the triads in the experimental series, but more than in the control group. Among the perisinusoidal cells present, there were the fewest (1.37±0.06) in the experimental series, but significantly ($p < 0.001$) more than in the control group (0.67±0.07). In the intermediate zone of the lobule (zone 2), among all the studied cells, the average number of endothelial cells (5.24±0.07) and Kupffer cells (5.22±0.06) did not differ significantly from each other ($p > 0.05$) but significantly differed from the control group ($p < 0.001$). Perisinusoidal cells were the least in zone 2 of the lobule (2.33±0.09) among all cells, but significantly more than in the control group ($p < 0.001$).

DISCUSSION

CBD is a cannabinoid with significant therapeutic potential among many non-psychoactive compounds. CBD has a broad spectrum of pharmacological actions in conditions such as pain, inflammation, epilepsy, anxiety, and others, in the treatment of various neurodegenerative diseases and neuropsychiatric conditions [18, 19]. In rodent models, CBD has also been shown to have, among others, anxiolytic, antioxidant, and

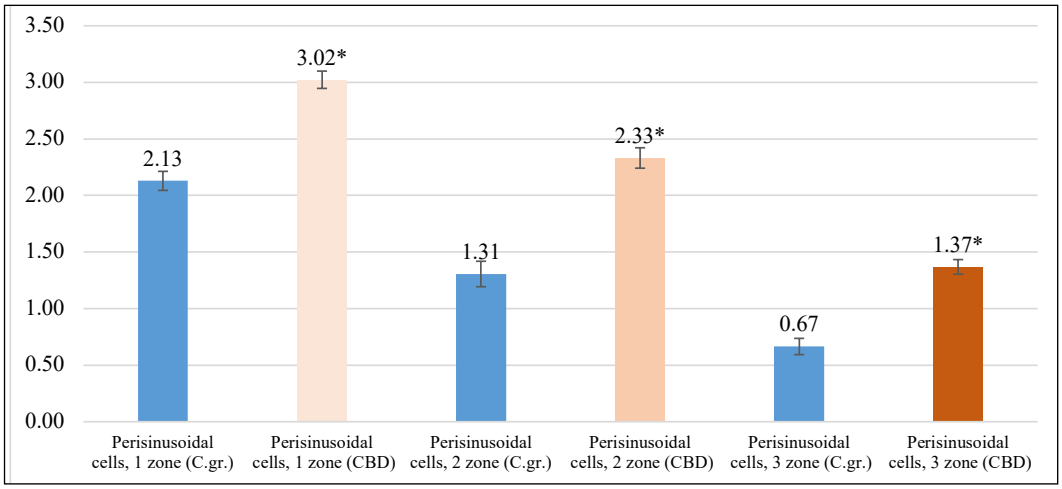

Fig. 7. Comparison of the average indicator of perisinusoidal cells (on an area of 0.01 mm^2) at week 10 of experimental exposure to CBD with the control group (C.gr.) (M±SD)
Note: * - significant difference ($p < 0.05$) with the control group
Picture taken by the authors

anti-inflammatory effects [20, 21]. The potential therapeutic effects of CBD in various liver diseases have been reported in vivo and vitro models [22]. However, in addition to the results with the therapeutic effect of CBD, there are studies in both animals and humans that have found negative effects associated with the use of CBD, including changes in liver function. Henderson R.G. et al. (2023) evaluated the toxicity of a CBD extract derived from hemp administered for 14 and 90 days to male and female Sprague Dawley rats. The first series of animals were orally administered doses of 0, 30, 70, and 150 mg/kg CBD for 14 days, and the second series of rats were administered doses of 0, 50, 80, 120, and 140 mg/kg CBD for 90 days. In neither of the first or second series of studies were there any biochemical changes in the blood associated with CBD administration, including changes in AST, ALT, alkaline phosphatase, and total bilirubin. However, histopathological examination of the liver showed dose-dependent hypertrophy of hepatocytes during the 14-day study, consistent with an increase in liver weight. Similarly, liver hepatocyte hypertrophy was also diagnosed after a 90-day experimental study, however, these histological changes disappeared after a 28-day recovery period when CBD was discontinued [23]. In our study, we did not observe hepatocyte hypertrophy, nor did we observe dystrophic-necrotic changes.

Previously, Ewing L.E. et al. (2019) investigated the hepatotoxic potential of CBD in male B6C3F1 mice after varying durations of oral administration of the cannabinoid [24]. The authors noted that a total dose of 246 mg/kg CBD extract is equivalent to 20 mg/kg CBD per day, the maximum recommended human maintenance dose of CBD in Epidiolex. In an acute toxicity study, mice were given single oral doses of 0, 246, 738, or 2460 mg/kg CBD extract, equivalent to 0, 20, 60, and 200 mg CBD/kg/day, respectively, and observed for 24 hours. Clinical and biochemical analysis revealed a modest but statistically significant ($p < 0.01–0.001$) dose-dependent increase in serum levels of both AST and ALT at single doses of 738 mg/kg and 2460 mg/kg. Administration of CBD at a dose of 2460 mg/kg resulted in a significant increase in total bilirubin (> 20-fold, $p < 0.001$). In a subacute toxicity study, animals of different series were administered single doses of 61.5, 184.5 or 615 mg/kg, which are equivalent to 5, 15 and 500 mg CBD/kg/day of CBD extract, respectively, daily for 10 days. Histopathological evaluation revealed swelling of the cytoplasm of hepatocytes in mice administered 184.5 and 615 mg/kg of CBD. Clinical and biochemical analyses showed that mice treated with 615 mg/kg CBD extract had significantly elevated total bilirubin, moderately elevated ALT and AST levels, but no significant increase in alkaline phosphatase. In contrast, lower doses of CBD extract did not significantly alter these parameters. Thus, according to the authors, oral CBD may cause liver damage, especially at high doses [24].

CONCLUSIONS

Based on the conducted experimental study, it was found that long-term exposure to 10% CBD oil at a dose of 10 mg/kg/day does not lead to the development of pathomorphological changes in the liver sinusoids. Characteristic morphological manifestations were dilation and hyperemia of single sinusoids, as a compensatory-adaptive process. Destructively altered endothelial cells and Kupffer cells were not detected.

Comparative analysis of the average values of endothelial cells, Kupffer cells and perisinusoidal cells in the sinusoidal wall on an area of histological preparation of 0.01 mm^2 in the experimental and control groups demonstrated that the trend of cell distribution in different zones of the lobule was maintained.

There is a significant increase in the average number of Kupffer cells in the sinusoidal wall in all areas of the

lobule $p < 0.001$, especially near the portal triads (1.58 times) compared to the control group, which can be explained by their activation in response to long-term exposure to CBD. When comparing the average numbers of endothelial cells (CD31 positive cells) in an area of 0.01 mm^2 in the experimental series with the control group, it was found that in all areas of the liver lobule the average number of CD31 cells was significantly lower than the corresponding number in the control group $p < 0.001$, which is explained by the larger diameter of the sinusoids. Perisinusoidal cells in the experimental series in all areas of the liver lobule were the least compared to endothelial cells and Kupffer cells, $p < 0.001$. Identification of perisinusoidal liver cells and study of their morphological features is an important task of pathomorphology and pathophysiology in predicting the consequences of experimental exposure to CBD oil on the liver.

REFERENCES

1. Castillo-Arellano J, Canseco-Alba A, Cutler SJ, León F. The Polypharmacological Effects of Cannabidiol. Molecules. 2023;28(7):3271. doi:10.3390/molecules28073271. DOI
2. FDA Cross Discipline Team Leader Review. Center for Drug Evaluation and Research. Application Number: 210365Orig1s000. Summary Review. 2018 https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210365Orig1s000SumR.pdf [Accessed 19 Nay 2025]
3. EMA (European Medicine Agency) Assessment Report, Epidyolex, International Non-Proprietary Name: Cannabidiol. EMA/458106/2019. 2019. https://www.ema.europa.eu/en/documents/assessment-report/epidyolex-epar-public-assessment-report_en.pdf [Accessed 19 Nay 2025]
4. EC Information and Notices. Off J Eur. Union. 2019;62 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:C:2019:369:FULL&from=EN [Accessed 19 Nay 2025]
5. EFSA Statement on safety of cannabidiol as a novel food: Data gaps and uncertainties; Panel on Nutrition, Novel Foods and Food Allergens. EFSA J. 2022;20:e07322. doi:10.2903/j.efsa.2022.7322. DOI
6. Sholler DJ, Schoene L, Spindle TR. Therapeutic Efficacy of Cannabidiol (CBD): A Review of the Evidence from Clinical Trials and Human Laboratory Studies. Curr Addict Rep. 2020;7(3):405-412. doi:10.1007/s40429-020-00326-8. DOI
7. Pro vnesennya zmin do pereliku narkotychnykh zasobiv, psykhotropnykh rechovyn i prekursoriv. vid 7 kvitnya 2021 r. N 324. Kyyiv. [On introducing amendments to the list of narcotic drugs, psychotropic substances and precursors. dated April 7, 2021 N 324. Kyiv]. https://ips.ligazakon.net/document/KP210324?an=1&hide=true [Accessed 19 May 2025] (Ukrainian)
8. Engeli BE, Lachenmeier DW, Diel P et al. Cannabidiol in Foods and Food Supplements: Evaluation of Health Risks and Health Claims. Nutrients. 2025;17(3):489. doi:10.3390/nu17030489. DOI
9. EFSA Statement on safety of cannabidiol as a novel food: Data gaps and uncertainties; Panel on Nutrition, Novel Foods and Food Allergens. EFSA J. 2022;20:e07322. doi:10.2903/j.efsa.2022.7322. DOI
10. FDA. Non-Clinical Review: Application number 210365Orig1s000. Non-Clinical Review: Application number 210365Orig1s000. 2017, p.89. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210365Orig1s000PharmR.pdf [Accessed 19 Nay 2025]
11. European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. Strasbourg: Council of Europe. Strasbourg, 18.III.1986. https://rm.coe.int/168007a67b [Accessed 19 May 2025]
12. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes. Off J Eur Union. 2010;53(L276):33–79. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF [Accessed 19 May 2025]
13. Suvarna SK, Layton C, Bancroft GD et al. Bancroft's Theory and Practice of Histological Techniques, 8th Edition. Elsevier. 2019, p.558. doi:10.1016/B978-0-7020-6864-5.00008-6. DOI
14. Hayat MA. Principles and techniques of electron microscopy: Biological applications [4th ed.]. Cambridge: Cambridge University Press. 2000, p.543. doi:10.1006/anbo.2001.1367. DOI
15. Nguyen T. Immunohistochemistry: A Technical Guide to Current Practices. Cambridge: Cambridge University Press. 2022, p.264.
16. Hruzieva TS, Lekhan VM, Ohniev VA et al. Biostatystyka. [Biostatistics]. Vinnytsia: New Book. 2020, p.384. (Ukrainian)
17. Suriawinata AA, Thung SN. Liver pathology: an atlas and concise guide. – N.Y.: demosMEDICAL. 2011, p.260.
18. Cassano T, Villani R, Pace L et al. From Cannabis sativa to cannabidiol: promising therapeutic candidate for the treatment of neurodegenerative diseases. Front. Pharmacol. 2020. doi: 10.3389/fphar.2020.00124. DOI
19. Campos AC, Fogaça MV, Scarante FF et al. Plastic and Neuroprotective Mechanisms Involved in the Therapeutic Effects of Cannabidiol in Psychiatric Disorders. Front. Pharmacol. 2017;8:269. doi: 10.3389/fphar.2017.00269. DOI
20. Campos AC, Guimarães FS. Involvement of 5HT1A Receptors in the Anxiolytic-like Effects of Cannabidiol Injected into the Dorsolateral Periaqueductal Gray of Rats. Psychopharmacology. 2008;199:223–230. doi: 10.1007/s00213-008-1168-x. DOI
21. Atalay S, Jarocka-karpowicz I, Skrzydlewskas E. Antioxidative and Anti-Inflammatory Properties of Cannabidiol. Antioxidants. 2019;9:21. doi:10.3390/antiox9010021. DOI

22. Chen S, Kim J-K. The Role of Cannabidiol in Liver Disease: A Systemic Review. Int J Mol Sci. 2024;25(4):2370. doi:10.3390/ijms25042370. DOI
23. Henderson RG, Lefever TW, Heintz MM et al. Oral toxicity evaluation of cannabidiol. Food Chem Toxicol. 2023;176:113778. doi:10.1016/j.fct.2023.113778. DOI
24. Ewing LE, Skinner CM, Quick CM et al. Hepatotoxicity of a Cannabidiol-Rich Cannabis Extract in the Mouse Model. Molecules. 2019;24(9):1694. doi:10.3390/molecules24091694. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Liliya I. Volos
Danylo Halytsky Lviv National Medical University
69 Pekarska St, 79010 Lviv, Ukraine
e-mail: liliya.volos@gmail.com

ORCID AND CONTRIBUTIONSHIP

Mykola M. Shevchuk: 0000-0001-7852-5980 A B C D
Liliya I. Volos: 0000-0002-1733-589X C E F
Oksana R. Kovalska: 0000-0001-5242-601X C E

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 04.08.2025
ACCEPTED: 30.11.2025