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Shevchuk M. M.[1], Volos L.I., Kovalska O.R.
State Nonprofit Company "Danylo Halytsky Lviv National Medical University", Lviv
[1]State Specialized Institution "Lviv Regional Bureau of Forensic Medical Examination", Lviv

COMPARATIVE MORPHOLOGICAL AND MORPHOMETRIC CHARACTERISTICS OF THE LIVER MICROCIRCULATORY BED IN RATS UNDER LONGTERM USE OF CANNABIDIOL OIL AND HEMP SEED OIL

e-mail: Liliya.volos@gmail.com

The study investigates the effect of long-term administration of cannabidiol oil and hemp seed oil on the state of the microcirculatory bed of the rat liver. The experiment was conducted on 80 male rats that received a cannabidiol oil and hemp seed oil as dietary supplements for 8 and 10 weeks. The article presents a comparative morphological characteristic with a morphometric assessment of the microcirculatory bed of the rat liver. The structural organization of the vascular compartment of the rat liver under long-term exposure to cannabidiol oil and hemp oil did not undergo dystrophic and necrotic changes. A significantly higher average diameter of interlobular veins and sinusoids was established in the cannabidiol oil experimental group compared to the hemp oil group ($p < 0.0001$) and the control group ($p < 0.0001$). The average diameters of the interlobular artery in the experimental groups of cannabidiol oil and hemp oil in the two series of the experiment did not differ significantly from each other ($p > 0.05$). Dilatation of sinusoids and slight hyperemia of single central veins in the cannabidiol oil group in both series did not indicate impaired blood outflow from the hepatic lobule, morphological changes were mainly adaptive nature. Thus, the experimental administration of cannabidiol oil and hemp seed oil indicates the safety of use for 8 and 10 weeks.

Key words: liver, rats, vessels, histology, morphometry, cannabidiol oil, hemp seed oil, safety.

Шевчук М.М., Волос Л.І., Ковальська О.Р.

ПОРІВНЯЛЬНА МОРФОЛОГІЧНА І МОРФОМЕТРИЧНА ХАРАКТЕРИСТИКА МІКРОЦИРКУЛЯТОРНОГО РУСЛА ПЕЧІНКИ ЩУРІВ ЗА УМОВИ ДОВГОСТРОКОВОГО ЗАСТОСУВАННЯ ОЛІЇ КАНАБІДІОЛУ ТА ОЛІЇ НАСІННЯ КОНОПЕЛЬ

Дослідження присвячено вивченню впливу тривалого введення олії канабідіолу та олії насіння конопель на стан мікроциркуляторного русла печінки щурів. Експеримент проведено на восьмидесяти щурах-самцях, які отримували впродовж 8-ми і 10-ти тижнів в якості дієтичних добавок олію канабідіолу та олію насіння конопель. У дослідженні надано порівняльну морфологічну характеристику з морфометричною оцінкою мікроциркуляторного русла печінки щурів. Структурна організація судинного компартменту печінки щурів за умови довготривалого впливу олії канабідіолу і конопляної олії не зазнавала дистрофічно-некротичних змін. Встановлено достовірно більший середній показник діаметра міжчасточкових вен, синусоїдів у дослідній групі олії канабідіолу відносно групи конопляної олії ($p < 0,0001$) і контрольної групи ($p < 0,0001$). Середні показники діаметрів міжчасточкової артерії у дослідних групах олії канабідіолу і конопляної олії у двох серіях експерименту достовірно не відрізнялися між собою ($p > 0,05$). Дилатація синусоїдів і незначна гіперемія одиничних центральних вен у групі олії канабідіолу в обидвох серіях не вказували на утруднений відтік крові з печінкової часточки, морфологічні зміни мали переважно адаптивний характер. Таким чином, експериментальне застосування олії канабідіолу та олії насіння конопель вказує на безпечність застосування впродовж 8-ми і 10-ти тижнів.

Ключові слова: печінка, щури, судини, гістологія, морфометрія, олія канабідіолу, олія насіння конопель, безпека.

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Cannabis sativa L. (cultivated or common hemp) has been known to mankind for several millennia and has been used and valued for the multifaceted properties of the plant of the genus *Cannabis* of the *Cannabaceae* family [11]. *Cannabis sativa* is used not only in industry, but also in medicine, for recreational purposes, and in food as a source of nutrients [12]. Today, there are a significant number of new products on the market that contain various components of *Cannabis sativa*. First and foremost, this depends on which part of the hemp plant is processed: the stems, leaves, flowers, or seeds. Therefore, a definition of the various components of *Cannabis sativa* is needed [8]. Phytocannabinoids such as tetrahydrocannabinol (THC), cannabidiol (CBD), and terpenoids are derived from the flowers, stems, and leaves of industrial hemp and interact with the body's endocannabinoid system. CBD is a non-psychoactive phytocannabinoid obtained by extraction with ethanol or carbon dioxide, and the resulting CBD oil is ideal for therapeutic use due to its high CBD content without any THC effects [15]. In Ukraine, CBD is a 100 % legal substance because, as a substance, it is excluded from the list of narcotic and psychotropic substances (Resolution of the Cabinet of Ministers of Ukraine No. 770 of May 6, 2000, Resolution of the Cabinet of Ministers of Ukraine No. 324 of April 7, 2021).

CBD has attracted attention due to its properties, such as anti-inflammatory antioxidants, analgesic and neuroprotective. It shows therapeutic potential for preventing or slowing the progression of various diseases, ranging from malignant tumors and viral infections to neurodegenerative disorders and ischemic diseases [1, 2]. CBD is available as a

prescription drug (Epidiolex) and is used in moderately high doses to treat severe forms of epilepsy [19]. In addition, CBD is widely available in the form of over the counter, for example, in the form of dietary supplements, oils, gummies, lotions or creams, or even in the form of vaping solutions.

Hemp seeds (*Cannabis sativa L.*) contain insignificant amounts of phytocannabinoids or none and are a valuable ingredient for producing high-quality food products and dietary supplements. According to M. Majewski and A. Jurgoński (2021), hemp seeds contain more than 26 % protein, more than 27 % dietary fiber, and 33 % polyunsaturated fatty acids, also linoleic (omega-6), α-linolenic (omega-3), and gamma-linolenic acids (omega-6) [9]. Hemp oil is produced only from seeds using the "cold pressing" method to preserve the nutritional content of the seeds. This oil is excellent for cooking or food supplements, which is why hemp oil is called hemp seed oil [4, 14]. Hemp seed oil contains almost 90 % unsaturated fatty acids with a high content of polyunsaturated fatty acids [3] and an optimal ratio of linoleic acid and alpha-linolenic acid of 3:1, which is recommended for human nutrition [6, 13]. In addition, hemp seed oil contains biologically active substances with different positive properties, that as anti-inflammatory, antioxidant and others [13].

Clinical trials and experimental studies of the effects of CBD oil and hemp seed oil on organs and systems, as well as studies of therapeutic effects and side effects, are important today. In particular, it is important to conduct in-depth research on the effects of CBD oil and hemp seed oil on the microcirculatory bed of the liver, to study the state of blood flow and the morphometric indicators of the vascular bed depending on the dose and duration of use of CBD oil and hemp seed oil.

The purpose of the study was to establish the effect of 10 % cannabidiol oil from industrial hemp *Cannabis sativa* L. and hemp seed oil *Cannabis sativa* L. as dietary supplements on the state of the liver microcirculation in rats when used for 8 and 10 weeks.

Materials and methods. The experimental study was conducted in the vivarium of the Danylo Halytsky Lviv National Medical University. All experiments were conducted in the autumn period from September 12 to November 7 (8 weeks) and to November 21 (10 weeks) of 2022. The duration of quarantine for rats that entered the vivarium was 10 days.

All animals during the experiment were kept in spacious metal cages measuring 80x50x80 cm, with a solid plastic pallet and strong metal bars, the distance between the bars did not exceed 1-1.5 cm to avoid getting their heads stuck.

According to ARRIVE 2.0 recommendations, rats were kept in groups. The number of individuals in a cage did not exceed 6 rats, based on an area per animal of 200-250 cm^2. The vivarium premises had central heating, natural and artificial lighting. The temperature in the rooms for keeping rats was 18-22 ° C, relative humidity 50-65 %. Illumination was 200 lux at a level of 1 m from the floor, the photoperiod was 12 hours light/12 hours dark. All animals were provided with standard complete granular feed in feeders, the daily rate per animal was 20 g, and with unlimited access to water. Cannabidiol oil was administered in the morning hours using a special intragastric probe.

Experimental studies were conducted on 80 white non-linear sexually mature male rats weighing 180-230 g and aged 5-7 months at the beginning of the experiment. The choice of male rats is primarily due to the avoidance of hormonal changes and the lack of interaction of hormones with the endocannabinoid system, since the response to cannabinoids in males and females can differ significantly.

The experiment was carried out under standard vivarium conditions in accordance with 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" and after ethical approval by the Bioethics Commission at the Danylo Halytsky Lviv National Medical University (protocol No. 7 dated August 29, 2022).

Depending on the duration of application of CBD oil and hemp seed oil, the animals were divided into 2 series of 40 individuals in each series. The first series (8 weeks) included the following experimental groups: Group 1 (experimental) – 14 rats, which were orally dripped with 10 % CBD oil once a day for 8 weeks (dose 10 mg/kg/day) using a special intragastric probe; Group 2 (experimental) – 14 rats, which were orally dripped with hemp seed oil once a day for 8 weeks, the dose of the oil was 0.5 ml/kg/day (average dose); Group 3 (control) – 6 rats, which were orally dripped with the CBD carrier solvent – hemp seed oil, the dose was 0.1 ml/kg/day (low dose); Group 4 (intact) – 6 sexually mature white male rats. Similarly, the 2nd series (10 weeks) included the following experimental groups: Group 1 (experimental) – 14 rats, which were orally dripped with 10 CBD oil once a day for 10 weeks (dose 10 mg/kg/day) using a special intragastric probe; Group 2 (experimental) – 14 rats, which were orally dripped with hemp seed oil once a day for 10 weeks, the dose of the oil was 0.5 ml/kg/day (average dose); Group 3 (control) – 6 rats, which were orally dripped with the CBD carrier solvent – hemp seed oil, the dose was 0.1 ml/kg/day (low dose); Group 4 (intact) – 6 sexually mature white male rats.

The design of the study from the beginning provided for the formation of smaller control and intact groups compared to the experimental ones. This decision was made for reasons of optimizing the use of laboratory animals and rationally reducing their total number, which fully complies with modern principles of bioethics. Despite the smaller number of rats in the control and intact groups, the formed sample is representative and sufficient to obtain reliable results. There was no change in the number of animals at the beginning and end of the experiment.

The manufacturer of 10 % CBD oil is the licensed company "Aroma Extract Labs s.r.o." (Prague, Czech Republic). A laboratory single-channel pipette dispenser of 20 μl was used to administer the dose. The manufacturer of Extra Virgin Golden Kings of Ukraine hemp oil 0.24l is Ukraine (TM Golden Kings of Ukraine). Hemp oil is obtained by the method of the first cold pressing of industrial hemp seeds without heat treatment.

During the entire experiment, the general condition of the rats was observed, their behavior, coat condition, food and water consumption, the nature of the stools for the presence of diarrhea, and the reaction to the experimental conditions were assessed. After 8 weeks (series 1) and 10 weeks (series 2), the animals were removed from the experiment. For euthanasia, rats were placed in a sealed container containing a cotton ball moistened with diethyl ether. The animals were kept in the sealed container for 15 minutes after respiratory arrest. Subsequently, material was collected for morphological examination.

The material for the study was liver tissue samples. The procedure for making histological preparations included cutting pieces of liver tissue and fixation, then dehydration and compaction of the tissue, embedding and making paraffin blocks, and then making histological sections with a thickness of 5±1 μm and staining with hematoxylin and eosin. The final step in preparing a microscope slide was embedding tissue slides with Canada Balsam and to apply a coverslip [18]. For careful study of the hepatic sinusoids were made from epoxy blocks semi-thin sections 0.5-1 μm thick, stained with methylene blue and methylene blue-basic fuchsin, and studied under a light microscope at a magnification of ×1000 (Oil immersion).

Morphometric study of vessel diameter with determination of indicators was performed using Aperio ImageScope v12.3.3 software (Leica biosystems, Wetzlar, Germany). For general study of histological preparations, morphometric study, and microphotography, a Leica DM 2500 light-optical microscope (Leica Microsystems GmbH, Germany) with a Leica DFC450 C digital camera (Germany) and Leica Application Suit Version 3.8 software were used.

Data processing was performed using 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. The sample distribution was checked for normality to justify the choice between parametric and nonparametric statistical methods. The average diameters of the liver microcirculatory bed are presented as the arithmetic mean with the standard deviation (M±SD). The significance of the difference between the groups in the corresponding terms in the two series was tested using the Mann-Whitney p(U), Pearson p ($\chi 2$) criteria. The difference was considered statistically significant at a minimum significance level of $p<0.05$.

Results of the study. According to the results of macroscopic examination of the rat liver at the end of 8 and 10 weeks of the experiment, it was found that no differences in the anatomical structure of the liver were found in the experimental groups of both series. The liver was dark red-brown in color, the surface was smooth, shiny, the connective tissue capsule was thin, unchanged, and did not look stretched. The consistency of the liver was soft, and the liver was uniform and homogeneous on section with moderate blood supply. Thus, the macroscopic structure of the liver after 8 and 10 weeks of experimental exposure to CBD oil and hemp seed oil was not disturbed. A detailed histological examination of the liver in two series of experiments revealed that the lobular structure was not disturbed, and portal triads were located at the at the corners of liver lobules, in which interlobular veins, arteries, bile ducts, and lymphatic vessels were visualized without pathological changes.

The interlobular veins of the liver triads in both series of the experiment were histologically unchanged, had a wide rounded or oval lumen and a thin wall. The interlobular veins of the portal triads had the largest lumen diameter in both series of the experiment. The interlobular arteries of the portal triads are of the muscular type with an inner elastic membrane, the arteries are round, endothelium of the inner membrane is preserved. The tunica media of the arteries is formed by concentrically arranged smooth muscle cells, without morphological changes. The tunica adventitia of the interlobular arteries is represented by delicate collagen fibers, thin, but in isolated cases the tunica externa was similar in thickness to the middle membrane, consisted of collagen and elastic fibers, and in such cases the thickness of the artery wall appeared greater.

When comparing the average diameters of interlobular veins in the two experimental series, a significantly larger diameter was found in the CBD oil group than in the hemp seed oil group ($p<0.0001$). Thus, in the experimental series (8 weeks), the average diameter in the CBD oil group was 56.64±2.24 μm and was 1.23 times higher than the average diameter in the hemp seed oil group (45.90±0.99 μm). In the experimental series (10 weeks), the average diameter in the CBD oil group was 57.41±2.29 μm and was 1.18 times higher than the average diameter in the hemp seed oil group (48.77±1.5 μm). Also, the average diameter of interlobular veins when using CBD oil significantly exceeded the corresponding indicator of the control group in both series of the experiment.

In the morphological study of interlobular veins in different series of the experiment, attention was paid to the features of hemodynamics, particularly the presence of hyperemia. It was found that after 8 weeks of experimental exposure to CBD, in 11 (78.57 %) of 14 observations, hyperemia was observed in single interlobular veins, and only in 3 (21.43 %) of 14 observations, hyperemia was diagnosed in most interlobular veins. In contrast, in the hemp oil group, hyperemia was not observed in 11 (78.57 %) of 14 observations, and in 3 (21.43 %) of 14 observations, hyperemia was diagnosed in single interlobular veins. When comparing the hyperemia of interlobular veins

scores in the CBD oil group with the hemp seed oil group and the control group, the Pearson test significance level was p =0.002.

After 10 weeks of experimental exposure to CBD oil, hyperemia of single interlobular veins was determined in 8 (57.14 %) of 14 cases, hyperemia of most interlobular veins was diagnosed in 6 (42.86 %). When using hemp oil, in 13 (92.86 %) of 14 observations, no hyperemia was observed, and only in 1 (7.14 %) of 14 observations, hyperemia was diagnosed in single interlobular veins. Hyperemia of most interlobular veins was not observed in the experimental hemp oil groups of both series. Thus, the relative rates of hyperemia in single and multiple interlobular veins in the series of experimental exposure to CBD (10 weeks) were significantly different from the indicators in the hemp oil group, p=0.005 and p=0.01, respectively. Therefore, the moderate hyperemia of single and multiple interlobular veins could be explained by the increased blood flow in the triads under the conditions of experimental CBD oil exposure.

When comparing the mean interlobular artery diameters in the CBD oil and hemp seed oil groups in the 8-week experimental series, it was found that the values did not differ significantly from each other or from the control group (p>0.05). In the 10-week experimental series, the mean interlobular artery diameters in the CBD oil and hemp oil treatment groups also did not differ from each other (p>0.05), but the mean diameter in the CBD oil group significantly exceeded the similar parameter in the control group (p=0.003).

When studying the relative rates of hyperemia of interlobular arteries, it was found that in the CBD oil group in the 8-week experimental series, hyperemia in single interlobular arteries was observed in 11 (78.57 %) of 14 observations, and in 3 (21.43 %) of 14 observations, hyperemia was detected in most interlobular arteries. A similar pattern was observed in the study of interlobular veins. When using hemp oil, hyperemia of interlobular arteries was not observed in 13 (92.86 %) of 14 observations, and only in 1 (7.14 %) of 14 observations hyperemia was diagnosed in single interlobular arteries. In the experimental series of 10 weeks of using CBD oil, hyperemia of interlobular arteries was diagnosed identically to that of interlobular veins, i.e. hyperemia of single interlobular arteries was determined in 8 (57.14 %) of 14 cases, hyperemia of multiple interlobular arteries was diagnosed in 6 (42.86 %). In the hemp seed oil experimental group (10-week series), hyperemia was not observed in 13 (92.86 %) of 14 observations, and only in 1 (7.14 %) of 14 observations was hyperemia diagnosed in single interlobular arteries, like the relative indices of interlobular veins. Therefore, the relative indices of hyperemia in single and multiple interlobular arteries in the two series of experimental CBD exposure were significantly different from the indices in the hemp oil group and the control group, like the hyperemia of interlobular veins (p <0.05).

In the experimental groups of the two series, the sinusoid wall is thin and fenestrated, and the basement membrane is absent. The wall is formed by flat endothelial cells of elongated shape with an oval hyperchromic nucleus and Kupffer cells, large, irregularly stellated in shape. The sinusoid diameter indicators are averaged, since the lumen of the sinusoids is uneven in different zones of the hepatic lobule, both in the control and experimental groups. In the two series of experiments, the average sinusoid diameter indicator in the experimental group of CBD oil was larger and significantly different from the group of hemp oil and the control group, p <0.05.

Thus, in the experimental series (8 weeks) with the use of CBD oil, the average diameter of sinusoids was 9.72±0.33 μm and was 1.25 times higher than the average diameter in the hemp seed oil group (7.76±1.07) and 1.27 times higher than the average diameter of the control group (7.66±1.00) (p <0.0001). In the experimental series (10 weeks), the average diameter in the CBD oil group was 9.78±0.58 μm and was 1.37 times higher than the average diameter in the hemp seed oil group (7.15±1.42 μm) and 1.28 times higher than the average diameter of the control group (7.65±1.12) (p <0.0001). However, between the experimental series, the average diameter values in CBD oil, hemp oil, and control groups did not differ significantly, p >0.05.

During the study of histological slides, our attention was focused on the presence of dilated and hyperemic sinusoids in the CBD oil and hemp seed oil experimental groups of two series. In the experimental series (8 weeks) of CBD exposure, single dilated and hyperemic sinusoids were diagnosed in 10 (71.43 %) of 14 cases and were significantly different from the control group, p=0.003. When compared with the hemp seed oil group, the relative rates of the presence of single dilated and hyperemic sinusoids were not significantly different, p=0.06. We did not diagnose multiple dilated and hyperemic sinusoids in the hemp seed oil group and in the control group. We diagnosed multiple dilated and hyperemic sinusoids only in the CBD oil group in 4 (28.57 %) of 14 cases. Similarly, in the experimental series (10 weeks), single dilated and hyperemic sinusoids predominated in the CBD oil group but did not differ significantly from the hemp oil group (p=0.25). Thus, in 7 (50.00 %) of 14 cases, single dilated and hyperemic sinusoids were diagnosed in the CBD oil group and in 4 (28.57 %) of 14 cases in the hemp seed oil group. We diagnosed multiple dilated and hyperemic sinusoids only in the CBD oil group in 7 (50.00 %). Dilation of sinusoids did not indicate impaired blood flow from the hepatic lobule. Single and in some cases multiple dilated and hyperemic sinusoids indicate the vasodilating effect of CBD and increased blood flow after long-term use of the cannabinoid. Liver tissue sample after 8 and 10 weeks of experiment are presented below on Fig. 1 and Fig. 2.

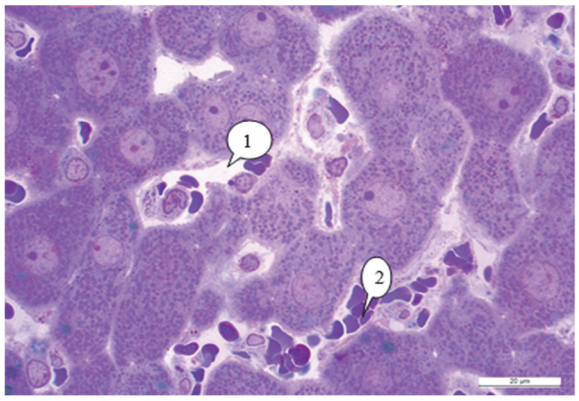

Fig. 1. Liver tissue sample after 10 weeks of experiment, CBD oil group. Single sinusoids are dilated (1) and hyperemic (2). Semi-thin section (1 μm), methylene blue-basic fuchsin stain. ×1000 (Oil immersion).

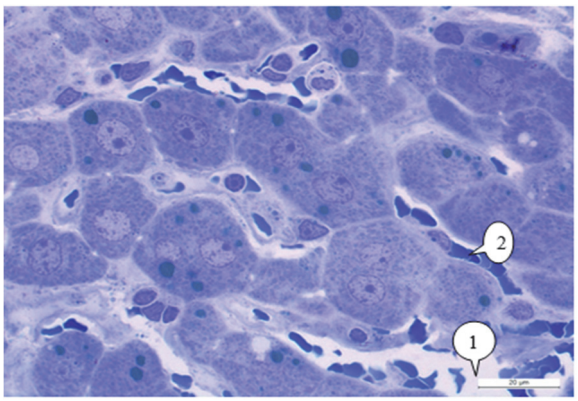

Fig. 2. Liver tissue sample after 8 weeks of experiment, hemp seed oil group (0.5 ml/kg/day). Single sinusoids are dilated (1) and hyperemic (2). Semi-thin section (1 μm), methylene blue stain. ×1000 (Oil immersion).

The central veins are the initial link of the venous drainage system of the liver. When microscopy of the central veins in the experimental groups of two series, no histological changes were noted. Endotheliocytes were normal on morphological examination, the nuclei were oval, hyperchromic without alterative changes. The adventitia was interrupted by sinusoidal inlets; the layer was formed thin collagen fibers. We determined the average diameters of the central vein in two series of the experiment and compared them in the experimental groups of CBD oil and hemp oil in each series. Thus, in the 8-week series in the CBD oil group, the average central vein diameter was 85.66±5.67 μm and was 1.24 times greater than the average in the hemp oil group (68.88±5.12 μm) ($p< 0.0001$) and 1.19 times greater than the control group (71.92±6.04 μm), $p < 0.0001$. Similarly, in the 10-week series in the CBD oil group, the average central vein diameter was 86.04±7.33 μm and was 1.24 times greater than the average in the hemp oil group (69.19±5.48 μm) ($p< 0.0001$) and 1.23 times greater than the control group (70.13±6.04), ($p< 0.0001$). However, in both the CBD oil group and the hemp oil group, we did not find a significant difference between the experimental series, $p >0.05$.

In each series of experiments, attention was paid to morphological signs of central vein hyperemia in the experimental groups. In most observations of all experimental groups, morphological signs of hyperemia were not diagnosed, however, in isolated cases, signs of hyperemia were visualized. Thus, in the experimental series (8 weeks) of CBD exposure, single hyperemia central veins were diagnosed in 3 (21.43) of 14 cases and did not significantly differ from the hemp oil group, $p=0.062$. In the hemp oil group, single hyperemia central veins were diagnosed in 2 (14.29) of 14 cases. Similarly, in the experimental series (10 weeks) in the CBD oil group, single hyperemia central veins were diagnosed in 4 (28.57) of 14 cases, and in the hemp oil group, the relative rates were like those in the 8-week experimental series. When comparing the two experimental groups in the 10-week experimental series, no significant difference was found, $p=0.36$. Slight hyperemia in single central veins in both experimental exposure to CBD oil and hemp oil indicated the absence of congestive phenomena in the liver lobule (Fig. 3, Fig. 4).

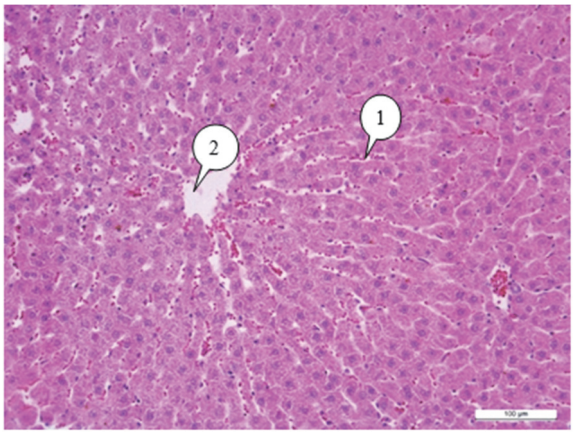

Fig. 3. Liver tissue sample after 10 weeks of experiment, CBD oil group. Single sinusoids are dilated and hyperemic (1). Absence of central vein hyperemia (2). Hematoxylin and eosin stain. ×200.

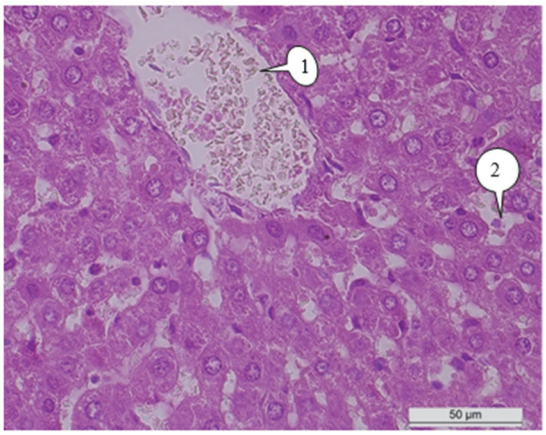

Fig. 4. Liver tissue sample after 8 weeks of experiment, Hemp seed oils group. Central vein (1) and single sinusoids (2) are dilated and hyperemic. Hematoxylin and eosin stain. ×400.

Thus, based on the morphological study of the vessels of the microcirculatory bed of the liver of rats under the experimental application of CBD oil and hemp oil for 8 and 10 weeks, it was established that the structural organization of the vascular compartment did not demonstrate pathohistological

changes. When comparing the average diameters of interlobular veins in two series of the experiment, a significantly larger diameter was found in the group using CBD oil than in the group using hemp seed oil ($p<0.0001$) and the control group ($p<0.0001$). The average diameters of the interlobular artery in the experimental groups of CBD oil and hemp oil in the two series of the experiment did not differ significantly from each other ($p>0.05$).

The relative rates of hyperemia in single and multiple interlobular veins and arteries in the two series of experimental CBD exposure were significantly different from those in the hemp oil group and the control group ($p < 0.05$). Therefore, the moderate hyperemia single and multiple interlobular veins and arteries could be explained by the increased blood flow in the triads under the conditions of experimental CBD oil exposure.

In two series of the experiment, the average diameter of the sinusoids in the CBD oil experimental group was larger and significantly different from the hemp oil group and the control group, $p <0.05$. Multiple dilated and hyperemic sinusoids were diagnosed only in the CBD oil group in the 10-week experimental series. Dilation of the sinusoids did not indicate impaired blood outflow from the hepatic lobule. Single and in some cases multiple dilated and hyperemic sinusoids indicate the vasodilation effect of CBD and increased blood flow after long-term use of the cannabinoid. In two series of the experiment, the average diameter of the central vein significantly prevailed in the CBD oil group, $p <0.05$. Slight hyperemia of single central veins in both series of experimental exposure to CBD oil and hemp oil did not indicate congestive phenomena in the liver lobule.

Discussion. Today, interest in *Cannabis sativa* L. is growing in many countries around the world due to its potential uses in medicine and the food industry. CBD and hemp seed oil are non-toxic and potentially beneficial phytocannabinoids that continue to grow in popularity. As patient interest of CBD and hemp seed oils increases, additional research is being conducted to better understand potential safety, efficacy use and positive effect on the health of CBD and hemp seed oils [8]. Significant are the results of an experimental study on the short-term effects of hemp seeds (*Cannabis sativa* L.) and hemp seed oil on vascular dysfunction in male rats with induced obesity [9]. The authors found that hemp seeds and hemp seed oil reduced the level of high-density lipoproteins and triglycerides in blood plasma. In addition, hemp seeds significantly reduced the level of uric acid and urea in plasma, and the use of hemp seed oil significantly reduced the level of total protein in plasma. A study of acetylcholine-induced vasodilation demonstrated its reduction with the use of hemp seeds, but not hemp seed oil. And as a conclusion, the authors say that hemp seeds were a much more useful food supplement than hemp seed oil [9].

Our study found that the structural organization of the vascular compartment of the liver of rats under long-term exposure to CBD oil and hemp seed oil did not undergo pathohistological changes. However, the average diameter of sinusoids in the CBD oil experimental group was larger and significantly different from the hemp seed oil group and the control group, $p <0.05$. Dilated and hyperemic sinusoids were diagnosed in the CBD oil group in the 10-week experimental series. Dilation of sinusoids and slight hyperemia of single central veins in both series of experimental exposure to CBD oil did not indicate hyperemia in the liver lobule and did not indicate impaired blood outflow from the hepatic lobule. Single and in some cases multiple dilated and hyperemic sinusoids indicate the vasodilation effect of CBD and increased blood flow after long-term use of the cannabinoid.

Of great importance are studies of the safety of CBD effects on the liver during its long-term use in the treatment of various pathological conditions and the study of the potential risk of hepatotoxicity due to the use of large doses of CBD. According to the authors, the effect of CBD on the liver depends on the dose of CBD at the beginning of treatment, the level of transaminases and the time of CBD administration [7]. L.E. Ewing et al. (2019) studied the hepatotoxicity of CBD in an experiment, causing acute or subacute toxicity, depending on the administered dose. With the development of acute and subacute toxicity, liver enzymes increased significantly and the animals sub-acute study developed a severe moribund condition on the third or fourth day of the experiment. A study of the expression of hepatotoxicity genes demonstrated that CBD regulates genes associated with oxidative stress, lipid metabolism, and enzymes. Therefore, according to the authors, pathways related to lipid and xenobiotic metabolism in the liver raise concerns about the safety of CBD [5].

As CBD becomes increasingly popular in the consumer market, there is a need to better understand the safety risks associated with CBD. CBD is available in many forms, such as oil, sublingual tablets, capsules, sublingual sprays, nasal sprays, and creams, and is found in food and dietary supplements and cosmetics [17]. CBD dosage and long-term safety are important. However, studies have mostly focused on short- and medium-term use, while CBD is typically used for long-term treatment. Experimental studies published today indicate that oral use of low doses of CBD isolates or CBD-rich preparations (dosing 1 to 2 mg/kg CBD every 12 hours) may be safe in dogs in the long term for the treatment of osteoarthritis [16]. S. Mcgrath et al. (2018) indicated that the use of 20 mg/kg/day of CBD isolate for 6 weeks was also safe in dogs, but additional studies evaluating different long-term doses are needed [10].

Limitations. Our study was limited to an experimental dose of CBD oil (10 mg/kg/day) and a relatively long observation period (8 and 10 weeks), which may not fully reflect the long-term effects of the cannabinoid on the liver microcirculation.

Conclusion

Morphological examination of rat liver with morphometric assessment of microcirculatory vessels under experimental application of CBD oil and hemp oil for 8 and 10 weeks demonstrated that the structural organization of the vascular compartment did not undergo pathohistological changes.

The average diameter of sinusoids in the CBD oil experimental group was larger and significantly different from the hemp oil group and the control group; however, sinusoid dilatation did not indicate impaired blood outflow from the hepatic lobule. Single and in some cases multiple dilated and hyperemic sinusoids indicate increased blood flow after long-term use of the cannabinoid and vasorelaxant efficacy of CBD. Slight hyperemia of single central veins in both series of experimental exposure to CBD oil and hemp oil did not indicate congestive phenomena in the hepatic lobule.

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Conflict of interest. The authors have no conflicts of interest to declare.

ORCID: Shevchuk M.M.: https://orcid.org/0000-0001-7852-5980, Volos L.I. https://orcid.org/0000-0002-1733-589X, Kovalska O.R. https://orcid.org/0000-0001-5242-601X.

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