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Original research

Morphofunctional characteristics of liver in rat exposed to high-fat diet and Lemon balm

M. A. Lieshchova, A. V. Oliyar, A. A. Bohomaz
Dnipro State Agrarian and Economic University, Dnipro, Ukraine

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Dnipro State Agrarian and Economic University, Serhii Efremov Str., 25, 49600, Dnipro, Ukraine

Tel.: +38-067-256-24-86
E-mail: *lieshchova.m.o@dsau.dp.ua*

[#]**Corresponding author:**
M. A. Lieshchova
lieshchova.m.o@dsau.dp.ua

Abstract. Herbal preparations used in the treatment protocols of metabolic disorders are highly effective and less toxic than chemically synthesized ones. *Melissa officinalis* is a plant that is an example of phytotherapy and functional nutrition, which is associated with many physiological and functional effects, including antioxidant, sedative, anti-inflammatory, antibacterial, antifungal, hypoglycemic and anxiolytic. The purpose of this work was to determine the effect of *Melissa officinalis* dry leaves on the morpho-functional state of liver during the consumption of high-fat diet by rat. The study was designed with two groups of white laboratory rats (n=6) while control group of animals consumed a high-fat diet for 30 days. The experimental group diet was additionally supplemented with 5% of *Melissa officinalis* in the form of dry crushed herb. Absolute and relative liver mass, the liver histostructure, and blood biochemical parameters were analyzed to estimate the main characteristics of the liver functional activity. There was established that a high-fat diet caused the progress in fatty liver disease, which was manifested by large droplet vacuolization of hepatocyte cytoplasm. The supplementation with *Melissa officinalis* did not stop this process, but reduced the intensity of the manifestation (small droplet cytoplasm vacuolization). The suplementation of *Melissa officinalis* into a high-fat diet caused an increase in absolute and average daily body weight gain, weight of the liver, significantly affects lipid metabolism, the changes in blood enzymes activity, causes a decrease in the blood glucose and total bilirubin level. Thus, *Melissa officinalis* can potentially be used as a dietary and phytotherapeutic agent.

Keywords: medicinal plants; lipid metabolism; absolute and relative organs mass; blood biochemical parameters; hepatocytes; histostructure.

Морфофункціональний стан печінки щурів за впливу *Melissa officinalis* на тлі високожирового раціону

Анотація. Рослинні препарати, що застосовують у схемах лікування порушень обміну речовин – високоефективні та менш токсичні, ніж хімічно синтезовані. *Melissa officinalis* – рослина, що є прикладом фітотерапії та функціонального харчування, з якою пов'язано багато фізіолого-функціональних ефектів, зокрема антиоксидантний, седативний, протизапальний, антибактеріальний, протигрибковий, гіпоглікемічний та анксіолітичний. Мета роботи – визначити вплив сухого листя *Melissa officinalis* на морфофункціональний стан печінки лабораторних щурів на тлі раціону з надлишковим вмістом жиру. Для цього сформовано дві групи білих лабораторних щурів (n=6), які упродовж 30 діб споживали високожировий раціон. Дослідній групі додатково задавали 5% сухої подрібненої трави *Melissa officinalis* у складі раціону. По завершенню досліду визначали абсолютну і відносну масу та гістоструктуру печінки тварин, а також проводили біохімічні дослідження крові із визначенням основних показників функціональної активності цього органу. Встановили, що високожировий раціон викликав розвиток жирової дистрофії печінки, що проявлялася крупнокраплинною вакуолізацією цитоплазми гепатоцитів, а додавання *Melissa officinalis* не припинило цей процес, проте знизило інтенсивнійсть прояву (дрібнокраплинна вакуолізація цитоплазми). Введення до високожирового раціону *Melissa officinalis* викликало збільшення абсолютного і середньодобового приросту маси тіла; абсолютної маси печінки; суттєво впливає на ліпідний обмін, змінює активність ферментів крові, зумовлює зниження рівня глюкози і загального білірубіну. Таким чином, *Melissa officinalis* потенційно може використовуватися як дієтичний та фітотерапевтичний засіб.

Ключові слова: лікарські рослини; ліпідний обмін; абсолютна і відносна маса органів; біохімічні показники крові; гепатоцити; гістоструктура.

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Introduction

Diseases caused by metabolic disorders (obesity, diabetes, etc.) have a complex etiology and are characterized by a combination of genetic predisposition and environmental factors, primarily diet and lifestyle. An imbalanced diet, in particular with an excessive fat and carbohydrates content, is the main cause of the obesity in human. Cases of obesity in pets, according to veterinary hospital data, are 24-44% in dogs, and more than 22% in cats. Obesity can increase the risk of cardiovascular system diseases, gastrointestinal system complications, diabetes, and cancer (German, 2006; Hoenig, 2012; Bilyi, & Khomutenko, 2022; Logvinova & Kravtsova, 2022).

Currently, there are a number of drugs that can successfully control the level of glucose or cholesterol in the blood, but their use is often associated with serious side effects. Thus, the development of new drugs is necessary for better treatment of metabolic diseases (Hernández Bautista et al., 2019). Herbal medicines can often be used alongside traditional medications, and sometimes even instead of them, especially for complex or chronic conditions (Wynn & Fougère, 2007). Herbal medicines are less toxic, environmentally friendly and usually have a low cost, so the study of their pharmacological impact is an urgent task for scientists.

Lemon balm (*Melissa officinalis* L.) is a well-known medicinal plant that has long been widely used in alternative medicine in various countries. Lemon balm leaves and stems, which contain up to 1% essential oil rich in citral, citronellal, myrcene, geraniol, cineol, and aldehydes, are used as medicinal raw materials. The plant contains many biologically active compounds such as: terpenes (monoterpenes, sesquiterpenes and triterpenes) and phenolic compounds (phenolic acids, flavonoids and tannins) (Ribeiro et al., 2001). A brief description of the botanical characteristics, traditional uses, phytochemistry, pharmacological activity, pharmacokinetics, and toxicity of *M. officinalis*, as well as unanswered questions and future research opportunities for this plant, is provided in the review by Shakeri et al. (2016). Lemon balm herb has a wide range of pharmacological effects. Thus, scientific literature describes its sedative, antispasmodic, hypotensive, analgesic, antimicrobial, antihistaminic, mild laxative and diaphoretic effects. Water-spirit extract of lemon balm is recommended as an alternative or complementary medicine in the treatment of diabetes, as its activity in preventing damage to β-cells of pancreatic islets has been shown (EL-Kassaby et al., 2019). Lemon balm essential oil exhibits hypoglycemic effect in an experiment and shows a positive effect in non-alcoholic fatty liver disease caused by a high-fat diet. It has also been proven that the active substance isolated from *M. officinalis*, the angiogenesis inhibitor ALS-L1023, when administered to obese mice, reduces their weight gain, causes a decrease in the amount of adipose tissue and the size of adipocytes (Park et al., 2015; Kim et al., 2017). It has been shown that lemon balm in the form of a 10% aqueous tincture has protective properties on liver tissue and normalizes the blood glucose level in rats that consumed an unbalanced diet (cafeteria diet) (Da Silva et al., 2022). Therefore, the goal of our work was to reveal the effect of lemon balm (*M. officinalis*) on liver morphofunctional state in outbred laboratory male rats during their consumption of a high-fat diet.

Materials and methods

The protocol of the study was reviewed and approved by the local ethical committee of Dnipro State Agrarian and Economic University (Dnipro, Ukraine). Two groups of young white male laboratory rats weighing 150 ± 20 g were formed for the study, with six animals per group (n=6). The control group of animals received a high-fat diet, made on the basis of standard one with the addition of 15% vegetable (sunflower) oil. In addition to the high-fat diet, the experimental group received 5% crushed dry young shoots of *Melissa officinalis* (JSC "Liktravy", Zhytomyr, Ukraine). Animals received food and water ad libitum while the both of them were recorded as the amount for food and water consumed by every group per day and the total amount for the entire period. The animals were observed daily, weighed, and absolute and average daily body weight gain as well as relative weight gain were measured (Lieshchova & Brygadyrenko, 2021).

After 30 days of the exposure to aforementioned diets total bleeding carried out from the heart under general anesthesia and the animals were euthanized with ethyl ester and. After the autopsy the liver state was assessed to assess the presence of pathological changes. The organ was removed, weighed and histological studies were performed. The absolute weight of the organs was determined on an analytical balance (Metrinco AB224, China) with an accuracy of 0.0001 g. The relative liver weight was calculated according to animals' body weight (Lieshchova et al., 2022). For histological analysis pieces of liver tissue were taken from animals under general anesthesia which were fasted overnight before. Then those tissues were fixed with 10% formalin solution, embedded in paraffin, and sections of 5–7 μm thickness were made. The sections were stained with hematoxylin-eosin (H&E) (Horalskiy et al., 2019). Photomicrographs were obtained using a computer morphometric setup (Micromed XC-3330 microscope; Micromed MDC500 digital camera; personal computer).

Before the euthanasia of the rat blood samples were taken for biochemical studies. Biochemical parameters were measured in the blood serum to assess liver functional state (total protein, activity of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, total bilirubin, urea), as well as carbohydrate (glucose) and lipid (cholesterol, triglycerides, low- and high density lipoprotein cholesterol, atherogenicity index) with using device Miura 200 automatic analyzer and Spinreact S.A., High Technology reagent kits were used.

Digital data obtained during the experiment were calculated by univariate analysis of variance with determination of the mean value (x) and standard deviation (SD), the difference between groups was considered significant at $p \leq 0.05$.

Table 1 – The weight of control and exposed rat groups (x ± SD, n = 6)

Parameter	Control group	*Melissa officinalis* exposed group
Body weight at the beginning of the experiment, g	158.8 ± 5.49	166.7 ± 7.12
Body weight at the end of the experiment, g	178.7 ± 2.16	223.5 ± 5.54*
Absolute body weight gain, g	24.83 ± 12.83	56.83 ± 2.32*
Average daily body weight gain, g/day	0.83 ± 0.43	1.89 ± 0.07*
Relative body weight gain,%	15.67 ± 8.07	34.18 ± 2.77*

Note: in this and subsequent tables * – $p \leq 0.05$, the difference is credible compared to control group animals

Results

According to the results of weighing the rats at the beginning of the experiment, the average weight in the control group was 158.8 g, in the experimental group – 166.7 g. After 30 days of the experiment, the weight of animals in the control group increased by 15.7%, while the absolute increase in body weight was 24.8 g, and the average daily increase in weight did not exceeded 0.83 g. In rats that consumed dry herb of *M. officinalis* in addition to a high-fat diet, their weight increased by 34%, and the absolute and average daily weight gain in animals was significantly twice as high as the indicators of the control group (Table 1).

Examining rats' internal organs revealed that they are placed anatomically correctly, have smooth, shiny, serous coverings of cavities and organs without layering. The liver had an elastic consistency, sharp edges, red-brown color. However, there were areas with a lighter color. The characteristic lobular structure of the organ is preserved in the section. The weight of the liver at the end of the experiment was 7.36 ± 0.31 g in rats that received a high-fat diet. This indicator was significantly higher in animals that received dry herb of *M. officinalis* added to their diet (8.47 ± 0.88 g). The relative weight of the liver in animals of the control group was 4.11%, while in the experimental group it was only 3.79% (Table 2).

Histological analysis of liver tissue from rat group that consumed a high-fat diet for 30 days has shown the changes which characterized the progress in protein and fatty dystrophy. These abnormalities were manifested by hypertrophy of hepatocytes and the accumulation of excessive fat content in them. Most of the liver lobules had a typical structure – a hexagonal shape, the central vein was located in the center, beams of hepatocytes were located radially, the borders between cells and contours of trabeculae were visible, sinusoidal capillaries were dilated (Fig. 1a). However, a violation of the beam structure and hepatocytes radial arrangement was observed in some of the liver lobules. The hepatocytes were placed irregularly, in groups, tightly adhering to each other, which led to the narrowing of sinusoidal capillaries lumens. A significant number of hepatocytes had signs of hypertrophy, the change in the shape of cells in combination with reduced basophilia and cytoplasmic vacuolization. In such cells, the cytoplasm was cloudy, had oxyphilic grains, small and large vacuolar cavities, large hypochromic nuclei, which were often located eccentrically (Fig. 1b).

Liver histostructure in animals that consumed the lemon balm herb in addition to the high-fat diet did not differ significantly (Fig. 2). The lobules had a hexagonal shape with a central vein in the middle and radially arranged beams of hepatocytes. Sinusoidal capillaries were dilated and filled with blood in some places. Hepatocytes were with cloudy cytoplasm and mainly with small vacuoles. However, among hepatocytes with hypochromic, cloudy and small vacuole cytoplasm, there were large hepatocytes (often binucleated) with intensively stained nuclei and cytoplasm. Fatty dystrophy of hepatocytes was less pronounced. Fatty inclusions in hepatocytes cytoplasm were small, diffusely located in the cytoplasm, and no cell destruction was observed. Vascular disorders and inflammatory reaction were not detected.

Well-known fact, that biochemical parameters are the main diagnostic criteria in clinical practice. Analysing protein metabolism, it was established that the high-fat diet caused an increase in total protein level above reference range (Shayakhmetova et al., 2020), but did not affect albumin and urea level in the blood of rats. The addition of *M. officinalis* dry herb to the high-fat diet did not cause significant changes in the protein metabolism of experimental group animals (Table 3). It should be noted that the consumption of the high-fat diet led to a sharp increase in blood total bilirubin level in rats (compared to reference range), and the supplementation with dry lemon balm significantly reduced this indicator (by 37.7%).

High-fat diet in animals of the control group induced a violation in lipid metabolism, that was manifested by an increase in blood

Table 2 – Absolute and relative liver weight of control and exposed rat groups (x ± SD, n = 6)

Parameter	Control group	*Melissa officinalis* exposed group
Absolute weight, g	7.36 ± 0.31	8.47 ± 0.88*
Relative weight, %	4.11 ± 0.13	3.79 ± 0.31*

Note: see Table 1.

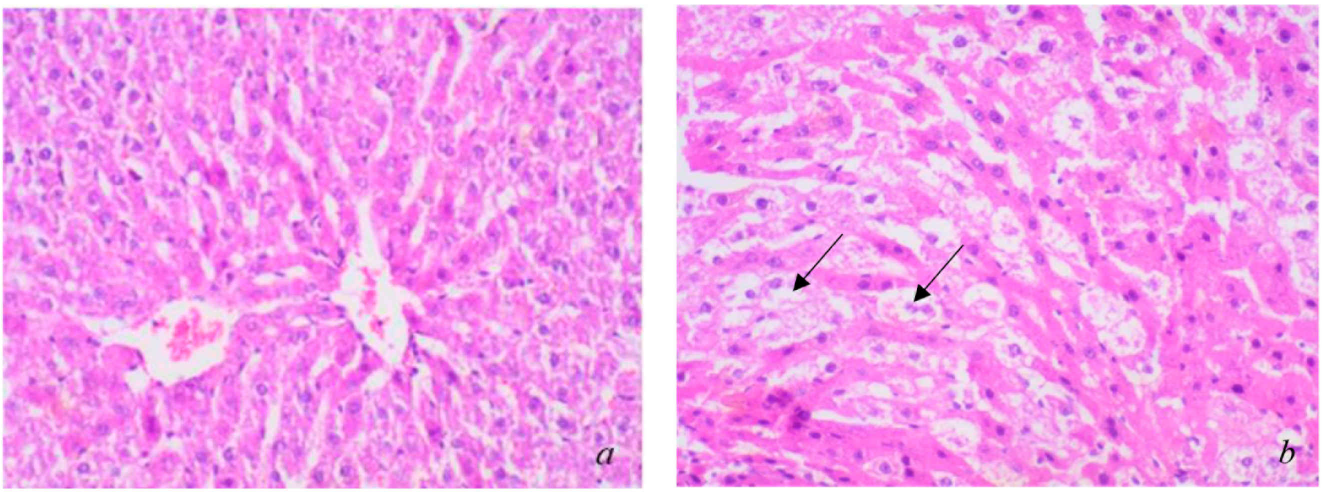

Fig. 1. Histostructure of rat liver that consumed a high-fat diet for 30 days: *a* – preserved beam structure, expanded sinusoidal capillaries, ×100; *b* – hepatocytes hypertrophy with unevenly colored, vacuolated cytoplasm and hypochromic nuclei, ×400. Hematoxylin and eosin.

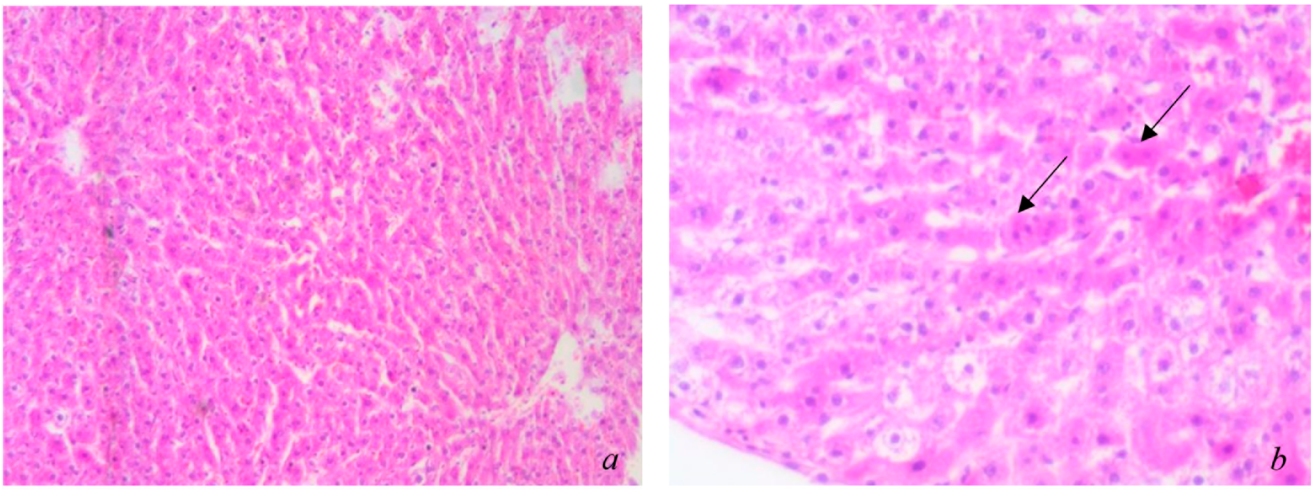

Fig. 2. Histostructure of rat liver that consumed dry herb of *M. officinalis* and a high-fat diet for 30 days: *a* – preserved liver lobular structure, radial arrangement of hepatocyte beams, ×100; *b* – large hepatocytes with intensively stained nucleus and cytoplasm, ×400. Hematoxylin and eosin.

triglycerides level (2.13 ± 0.55 mmol/l) almost three times more in compare to the reference parameters (Shayakhmetova et al., 2020). The total cholesterol level (1.27 ± 0.14 mmol/l) was within the normal range for this age group of rats, and high-density lipoprotein cholesterol (0.65 ± 0.13 mmol/l) and low-density lipoprotein cholesterol (0.52 ± 0.30 mmol/l) were reduced, therefore, the indicator of the atherogenicity coefficient did not exceed 1.04 ± 0.45 Units (Table 3). The supplementation of *M. officinalis* dry herb to the animal diet caused a significant and reliable decrease in the level of triglycerides by 37%, compared to the control group. At the same time, the indicator of high-density lipoprotein cholesterol increased by 23%, low-density lipoprotein cholesterol was at the same level as the indicator of the control group. The total cholesterol level in the animals of the experimental group was only 12.6% higher than that of the animals of the control group (Table 3). Adding dry herb of *M. officinalis* to the high-fat diet showed a 23% decrease in blood glucose levels on 30th day of the study.

The exposure to high-fat diet for 30 days caused an increase in aspartate aminotransferase activity (186 ± 61 U/l), alanine aminotransferase (131 ± 41 U/l), and gamma-glutamyl transferase (9.29 ± 2.6 U/l) in the blood above the reference range for this age groups of animals, and the indicator of alkaline phosphatase activity (129 ± 64 Units/l) was even below the limits of physiological values (Shayakhmetova et al., 2020). When dry herb of *M. officinalis* was added to the high-fat diet, the alkaline phosphatase activity increased sharply and reliably by 4.6 times, the activity of gamma-glutamyl transferase (by 28%) and alanine aminotransferase (by 15%), while aspartate aminotransferase activity did not change significantly (Table 3).

Table 3 – Blood biochemical parameters of control and exposed rat groups (x ± SD, n = 6)

Parameters	Control group	*Melissa officinalis* exposed group
Protein metabolism		
Total protein, g/L	77,0 ± 5,3	80.0 ± 3.7
Albumins, g/L	39.6 ± 2.6	41.3 ± 2.2
Urea, mmol/L	6.8 ± 1.0	5.4 ± 0,6
Total bilirubin, μmol/L	6.1 ± 1.7	3.8 ± 1.4*
Lipid and carbohydrate metabolism		
Cholesterol, mmol/L	1.27 ± 0.14	1.43 ± 0.18
Blood triglycerides, mmol/L	2.13 ± 0.55	1.34 ± 0.31*
Low density lipoprotein (LDL) cholesterol, mmol/L	0.52 ± 0.30	0.51 ± 0,11
High density lipoprotein (HDL) cholesterol, mmol/L	0.65 ± 0.13	0.80 ± 0.44
Atherogenic Index of Plasma (AIP), U	1.04 ± 0.45	1.30 ± 0.91
Glucose, mmol/L	7.39 ± 0.04	6.40 ± 0.55*
Enzyme activity		
Aspartate aminotransferase (AST), U/L	186 ± 61	182 ± 33
Alanine aminotransferase (ALT), U/L	131 ± 41	111 ± 18
Alkaline phosphatase, U/L	129 ± 64	601 ± 149*
Gamma-glutamyl transferase (GGT), U/L	9.29 ± 2.6	6.7 ± 0.7

Note: see Table 1.

Discussion

In this study, we evaluated the effect of dry lemon balm on the morphofunctional state of rat liver via detection the body weight dynamics, blood biochemical parameters, and liver histostructure. The obtained results have shown that a more intensive body weight gain in animals exposed to lemon balm dry herb was detected in compare with the control group that consumed only high-fat diet. Opposite results were obtained by Da Silva and coauthors (Da Silva et al., 2022), where rats consuming a complete diet and rats on an unbalanced diet (cafeteria diet) had higher average daily body weight gains than animals supplemented with *M. officinalis*.

Well known fact that the organ mass is an index that is often used as a sensitive indicator to assess the toxic effect of various substances, including medicinal ones (Balogun et al., 2014; Varcholyak & Gutyi, 2019). The relative weight of the liver, along with blood biochemical parameters, are indicators that can characterize the functional state of the body. Amid the additional consumption of lemon balm, the absolute liver weight in experimental rats was significantly higher, and its relative weight was lower than the corresponding indicators of the control group. On the contrary, the absolute liver weight was higher in rats that received a standard diet and a cafeteria diet, compared to animals that additionally consumed *M. officinalis* in their diet in the form of an aqueous tincture, while the relative weight of the organ did not differ (Da Silva et al., 2022).

There was considered that the main reasons for fat accumulation in the cytoplasm of hepatocytes are an increase in the synthesis of fatty acids and a decrease in their oxidation (Reddy & Sambasiva Rao, 2006). Morphologically, this is manifested by fatty liver dystrophy (steatosis), in which lipid droplets accumulate in the cytoplasm of hepatocytes and even in the intercellular space. The initial degree of liver damage is determined by the number, size, and localization of lipid droplets accumulation (cytoplasmic vacuolization). At the same time, small-droplet and large-droplet forms are distinguished (Brunt, 2012). Against the background of the high-fat diet consumed by animals for 30 days, in the liver we observed signs of fatty dystrophy development of hepatocytes in the form of large droplet cytoplasm vacuolization. Addition of *M. officinalis* in the form of dry grass to the high-fat diet reduced the intensity of this pathology manifestation, as the accumulation of small vacuoles was noted in the hepatocytes. Recent research carried out to assess the imbalanced with both carbohydrate lipid excessive content diet there was observed development of non-alcoholic fatty liver disease in the form of vacuolization of the cytoplasm. Lemon balm supplementation in the diet did not have a pronounced hepatoprotective effect, but it reduced the hepatocyte cytoplasm vacuolization (Da Silva et al., 2022). Similar results on the effect of *M. officinalis* extract on liver histostructure during hyperlipidic diet were obtained by Bolkent et al. (2005). It was shown that in the hepatocytes of rats with hyperlipidaemia, numerous vacuoles of various sizes, mononuclear cell infiltration, pyknotic nuclei, endothelium ruptures of some central veins were detected, while the same degenerative changes in the liver cells of animals that received lemon balm extract were minimal or absent at all.

There was reported that obesity is the main risk factor for the development of dyslipidaemia and hyperlipidaemia, and the lipid profile of obese animals is characterized by an increased triglycerides level and impaired lipoprotein metabolism. Insulin resistance, hyperinsulinemia, impaired glucose tolerance, and type 2 diabetes usually develop in the body when lipid metabolism is disturbed, and type 1 diabetes in dogs (Hoenig, 2012). There was proposed that the excessive fatty acids content in the diet certainly causes an increase in lipid metabolism including total cholesterol, triglycerides level, the ratio of cholesterol to different densities lipoproteins, which is expressed by the indicator – atherogenic index of plasma (AIP). In our study, this index was 1.04 U in rats consuming the high-fat diet for 30 days, and the addition of lemon balm to the diet caused a slight increase in atherogenic index to 1.30 U, due to an increase in the level of high-density lipoprotein cholesterol.

M. officinalis caused a significant decrease in serum lipid levels in hyperlipidaemic rats was first reported by Bolkent et al. (2005). An increase in blood total cholesterol level amid the high-fat diet (2% cholesterol and 20% sunflower oil) was detected in rats only on the 42nd day of the experiment, while in the group of rats that additionally supplemented with *M. officinalis* extract, this indicator was significantly lower (Bolkent et al., 2005). In our experiment, we did not find a dramatic increase in total cholesterol level both in the group of animals consuming the high-fat diet and with the supplementation of *M. officinalis* dry herb, compared to the reference values for this age group of rats (Ihedioha et al., 2011). The treatment with *M. officinalis* significantly modulated just the content of triglycerides in rat blood and at the trend level on high-density lipoprotein cholesterol level.

The effect of *M. officinalis* on rat body weight can be dependent on both direct and indirect effect. Indirect effect can be mediated by the suppression of certain microorganism types in the animal intestine. Thus, adding 5% of crushed dry young shoots of *M. officinalis* to the high-fat diet of laboratory rats significantly changed the quantitative ratio of *Escherichia coli* with normal and altered enzyme activity in the intestinal system (Bilan et al., 2023). The results of Zazharskyi et al. (2019) showed that the ethanol extract of *M. officinalis* significantly inhibited *Salmonella typhimurium* colonies growth, slightly inhibited the growth of *Escherichia coli*, *Klebsiella pneumonia*, and *Corinebacterium xerosis*, and did not affect *Proteus mirabilis*, *Listeria monocytogenes*, and *Candida albicans*. Therefore, medicinal plants, in particular lemon balm which potent to modulate intestinal microflora can indirectly determine the physiological state of experimental animals.

The results obtained in Bolkent and coauthors study have shown that serum activity of AST, ALT and AP - enzymes that confirmed as the markers of liver damage – were markedly increased in animals with hyperlipidaemia compared to normal rats. The use of *M. officinalis* L. extract at a dose of 2 g/kg significantly prevented the hyperlipidaemic increase of AST, ALT and AP in blood serum (Bolkent et al., 2005). In our study, we also observed an increase in the main enzymes activity in rat blood that received the high-fat diet for 30 days, compared to the normal values for this age and sex group of animals (Shayakhmetova et al., 2020). Supplementation with dry lemon balm herb caused a significant and reliable increase in AP activity in rats. It is known that this enzyme in the body hydrolyses phosphate ethers in an alkaline environment. Besides, observed in our study increase in similar activity may indicate a violation of mineral metabolism (damage to the bone system) or damage to the hepatobiliary system. The fact of a sharp increase in the activity of alkaline phosphatase in rat blood of high-fat diet plus lemon balm exposed group requires further additional research.

The diet imbalanced in terms of carbohydrates and fat can cause hyperglycaemia and dyslipidaemia (Da Silva et al., 2022). A number of scientific sources indicate the hypoglycaemic effect of lemon balm and its active components (Hasanein, & Riahi, 2014; Asadi et al., 2018; EL-Kassaby et al., 2019). Moreover, the consumption of dry lemon balm herb caused a decrease in the blood glucose level of animals that received a "cafeteria diet" compared to rats that consumed a standard diet and with the addition of lemon balm extract instead of water (Bolkent et al., 2005). Our studies confirmed the hypoglycaemic effect of lemon balm, since a high-fat diet caused an increase in glucose levels above physiological values, and when adding 5% dry herb *M. officinalis*, this indicator decreased. This effect may be related to the rosmarinic acid contained in lemon balm, as a study by Ngo and coauthors. (Ngo et al., 2018) demonstrated the potent role of this active component in controlling blood glucose level and increasing insulin sensitivity in hyperglycaemia (Hitl et al., 2020).

Conclusion

Morphological and biochemical studies confirm the effectiveness of lemon balm use in the correction of the high-fat diet to reduce dyslipidaemia and hyperglycaemia in the blood and protect liver tissue. The high-fat diet consumed by rats for 30 days causes a metabolic disorder, which is manifested by a change in the blood biochemical parameters. Supplementation with dry herb of *M. officinalis* to the diet causes a significant change in lipid metabolism indicators: a decrease in the level of triglycerides with a simultaneous increase in the level of high-density lipoprotein cholesterol; changes enzymes activity: the activity of alkaline phosphatase increases and the activity of gamma-glutamyl transferase and alanine aminotransferase decreases, and also causes a decrease in the level of glucose and total bilirubin.

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The authors declare no conflict of interest.

References

Asadi, A., Shidfar, F., Safari, M., Hosseini, A. F., Fallah Huseini, H., Heidari, I., & Rajab, A. (2018). Efficacy of Melissa officinalis L (lemon balm) extract on glycemic control and cardiovascular risk factors in individuals with type 2 diabetes: A randomized, double-blind, clinical trial. Phytotherapy Research, 33(3), 651–659.

Bilan, M. V., Lieshchova, M. A., & Brygadyrenko, V. V. (2023). Impacts on gut microbiota of rats with high-fat diet supplemented by herbs of Melissa officinalis, Lavandula angustifolia and Salvia officinalis. Regulatory Mechanisms in Biosystems, 14(2), 155-160.

Bilyi, D. D., & Khomutenko, V. L. (2022). Canine mastopathy (Overview). Theoretical and Applied Veterinary Medicine, 10(4), 3-11.

Bolkent, S., Yanardag, R., Karabulut-Bulan, O., & Yesilyaprak, B. (2005). Protective role of Melissa officinalis L. extract on liver of hyperlipidemic rats: A morphological and biochemical study. Journal of Ethnopharmacology, 99(3), 391–398.

Brunt, E. M. (2012). Histological assessment of nonalcoholic fatty liver disease in adults and children. Clinical Liver Disease, 1(4), 108–111.

Da Silva, P. J., Marcon Borges, L., Augusto Piva, P., Moreno Frederico, G., Guimarães Drummond e Silva, F., Maria Netto, F., Alexandre Vessaro Silva, S., & Miotto Bernardi, D. (2022). Impacto do consumo de Melissa officinalis L. (Lamiaceae) em ratos wistar alimentados com dieta de cafeteria. Revista Fitos, 16(4), 479–489.

EL-Kassaby, M., Salama, AbeerA. A., Mourad, H., & Abdel-Wahhab, K. (2019). Effect of lemon balm (Melissa officinalis) aqueous extract on streptozotocin-induced diabetic rats. Egyptian Pharmaceutical Journal, 18(4), 296.

German, A. J. (2006). The growing problem of obesity in dogs and cats. The Journal of Nutrition, 136(7), 1940-1946.

Hasanein, P., & Riahi, H. (2014). Antinociceptive and Antihyperglycemic Effects of Melissa officinalis Essential Oil in an Experimental Model of Diabetes. Medical Principles and Practice, 24(1), 47–52.

Hernández Bautista, R. J., Mahmoud, A. M., Königsberg, M., & López Díaz Guerrero, N. E. (2019). Obesity: Pathophysiology, monosodium glutamate-induced model and anti-obesity medicinal plants. Biomedicine & Pharmacotherapy, 111, 503–516.

Hitl, M., Kladar, N., Gavarić, N., & Božin, B. (2020). Rosmarinic Acid–Human Pharmacokinetics and Health Benefits. Planta Medica, 87(04), 273–282.

Hoenig, M. (2012). The Cat as a Model for Human Obesity and Diabetes. Journal of Diabetes Science and Technology, 6(3), 525–533.

Horalskiy, L. P., Khomych, V. T., & Kononsky, A. I. (2019). Histological techniques and morphological methods in normal and pathological conditions. Zhitomir, Polissia (in Ukrainian).

Ihedioha, J. I., Noel-Uneke, O. A., & Ihedioha, T. E. (2011). Reference values for the serum lipid profile of albino rats (Rattus norvegicus) of varied ages and sexes. Comparative Clinical Pathology, 22(1), 93–99.

Kim, J., Lee, H., Lim, J., Oh, J., Shin, S. S., & Yoon, M. (2017). The angiogenesis inhibitor ALS-L1023 from lemon-balm leaves attenuates high-fat diet-induced nonalcoholic fatty liver disease through regulating the visceral adipose-tissue function. International Journal of Molecular Sciences, 18(4), 846.

Lieshchova, M. A., Bilan, M. V., Evert, V. V., Kravtsova, M. V., & Mylostyvyi, R. V. (2022). Morphofunctional state of the rat's liver under the influence of Aralia elata alcohol tincture during the high-fat diet. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, 24(108), 75–81.

Lieshchova, M. A., & Brygadyrenko, V. V. (2021). Influence of Lavandula angustifolia, Melissa officinalis and Vitex agnus-castus on the organism of rats fed with excessive fat-containing diet. Regulatory Mechanisms in Biosystems, 12(1), 169–180.

Lieshchova, M. A., Oliyar, A. V., & Evert, V. V. (2022). Influence of Lavandula angustifolia on metabolic indicators and morphofunctional state of rat organs with a high-fat diet. The Animal Biology, 24(4), 21–26.

Logvinova, V. V., & Kravtsova, M. V. (2022). Pathomorphological changes in the liver and internal organs in obese cats. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, 2022, 24(108). 101–106.

Ngo, Y. L., Lau, C. H., & Chua, L. S. (2018). Review on rosmarinic acid extraction, fractionation and its anti-diabetic potential. Food and Chemical Toxicology, 121, 687–700.

Park, B. Y., Lee, H., Woo, S., Yoon, M., Kim, J., Hong, Y., Lee, H. S., Park, E. K., Hahm, J. C., Kim, J. W., Shin, S. S., Kim, M. Y., & Yoon, M. (2015). Reduction of adipose tissue mass by the angiogenesis inhibitor ALS-L1023 from Melissa officinalis. PloS One, 10(11), e0141612.

Reddy, J. K., & Sambasiva Rao, M. (2006). Lipid Metabolism and Liver Inflammation. II. Fatty liver disease and fatty acid oxidation. American Journal of Physiology-Gastrointestinal and Liver Physiology, 290(5), 852–858.

Ribeiro, M. A., Bernardo-Gil, M. G., & Esquível, M. M. (2001). Melissa officinalis, L.: study of antioxidant activity in supercritical residues. The Journal of Supercritical Fluids, 21(1), 51–60.

Shakeri, A., Sahebkar, A., & Javadi, B. (2016). Melissa officinalis L. – A review of its traditional uses, phytochemistry and pharmacology. Journal of Ethnopharmacology, 188, 204–228.

Shayakhmetova, G. M., Kovalenko, V. M., Basovska, O. G., & Vozna, A. V. (2020). Clinical biochemical parameters of healthy adult white male rats blood serum (retrospective assessment). Pharmacology and Drug Toxicology, 2019, 13(6), 428–433.

Varcholyak, I. S., & Gutyi, B. V. (2019). Determination of the chronic toxicity of preparation "Bendamin" on laboratory animals. Theoretical and Applied Veterinary Medicine, 7(2), 63–68.

Wynn, S. G., & Fougère, B. J. (2007). Veterinary herbal medicine: A systems-based approach. Veterinary Herbal Medicine, 291–409.

Zazharskyi, V. V., Davydenko, P. O., Kulishenko, O. M., Borovik, I. V., & Brygadyrenko, V. V. (2019). Antimicrobial activity of 50 plant extracts. Biosystems Diversity, 27(2), 163–169.