Influence of Lavandula angustifolia, Melissa officinalis and Vitex angus-castus on the organism of rats fed with excessive fat-containing diet

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Plant food additives are becoming more and more popular and broadly applied products, though the information on risks they pose to the organism is limited and contradictory. Obesity and overeating are some of the commonest health issues around the world, and people are increasingly consuming workability-enhancing preparations as a simple and fast method of weight control. The plant-based preparations are considered less harmful than the synthetic chemical ones. Lavandula angustifolia Mill., Melissa officinalis L. and Vitex angus-castus L. are broadly used as food additives and medicinal plants, despite the fact that their complex physiological assessment on model animals in the conditions of obesity has not yet been performed. We carried out a 30-day experiment on white male rats. All the animals were given high-fat diet, and the experimental animals, in addition to this diet, received 5% crumbled dry herbs of L. angustifolia, M. officinalis or V. angus-castus. Taking into account the overall amount of consumed food, the mean daily gain in body weight; at the end of the experiment, we determined the index of the weight of the internal organs, biochemical and morphological blood parameters. At the beginning and the end of the experiment, the rats were examined for motor and orienting activities, and emotional status. Rats on high-fat diet gained up to 112% body weight by the end of the experiment, while rats that had received V. angus-castus gained up to 119%, M. officinalis – 135%, L. angustifolia – 139%, compared with the initial body weight. Addition of medicinal plants to the diet led to increase in average daily weight increment, significantly and reliably after consuming lavender and lemon balm, less significantly and unreliably after eating Vitex. L. angustifolia and M. officinalis reduced the relative brain weight, and ingestion of L. officinalis and M. officinalis caused notable decrease in the relative mass of the thymus (down to 58% and 47% of the relative weight of thymus in animals of the control group respectively). Also, these plants decreased the motor and orienting activities of the rats by the end of the experiment. As for the biochemical parameters of blood, the activity of alkaline phosphatase significantly increased to 406% following consumption of Melissa, to 350% after consuming lavender, and to 406% after Vitex, compared to the control group. Furthermore, all the groups were observed to have increased AST and ALT activities. Intake of lavender led to increases in cholesterol (to 125%) and LDL cholesterol (to 228%), whereas the groups that consumed lemon balm were observed to have decreases in urea nitrogen (to 79%), totalbilirubin (to 63%) and triglycerides (to 63%). Addition of Vitex led to increase in the index of aterogeneity against the background of notable fall in HDL cholesterol (to 52% of the control group). The medicinal plants also contributed to the normalization of the glucose level. Morphological analysis of blood revealed no significant changes, except heightened content of monocytes in blood, which is characteristic of all groups, including the control. Effects of L. angustifolia, M. officinalis and V. angus-castus on the organism of rats on excessive-fat diet require additional histological, histochemical and immunological surveys.

Keywords: relative mass of the organs; increase in the body weight; high-fat diet; high-calorie diet; obesity; phytopreparations; motor activity; orienting activity; emotional status; biochemical blood parameters.

Introduction

Metabolic diseases such as diabetes mellitus and dyslipidemia occur due to a complex of genetic predisposition and environmental factors. Lifestyle and diet contribute to their development as well, causing significant complications, malfunctioning and failure of brain, heart and other organs, and likely death. Despite the fact that the authorized medicines may be efficiently used to control blood glucose and cholestery levels, they also may cause deleterious side effect. Thus, treating metabolic diseases requires seeking new agents for development of novel preparations (Hughes et al., 2020). Against the background of obesity, metabolic diseases such as dyslipidemia, atherosclerosis and type 2 diabetes have become health problems at the global level (Shin & Yoon, 2020). Course of obesity and overeating is attributed to angiogenesis and extracellular matrix (ECM) remodeling. Angiogenesis develops in adult adipose tissues (Arika et al., 2019; Lieshchova et al., 2019, 2020). Adipose tissue is closely related with the blood vessels. In fact, adipocytes tissue contains have extensive systems of capillaries. Adipocytes generate endothelial growth factor A and fibroblast growth factor 2, both proangiogenic factors driving the neovascularization of the tissue. Moreover, development of the adipose tissue and maturation of microvessels is greatly contributed by matrix metalloproteinases (MMPs), including MMP-2 and MMP-9, which modify the ECM. Therefore, modulating angiogenesis and MMP activity could likely be therapeutic means of controlling obesity and accompanying impairments (Shin & Yoon, 2020).

Application of medicinal plants may help to decrease body weight during obesity and other metabolic disorders (Martin, 2019; Bulvicki et al., 2020). Most often, for those purposes, the treatment involves plants of Lamiaceae family (Michel et al., 2020). Zvezdina et al. (2020) have made a review of 71 species from 30 genera of Lamiaceae family, and
drew the conclusion that the immense potential of plants of this family is still unexplored. These valuable medicinal plants could help in development of neurotropic preparations. Biologically active substances of Lamiaceae plants comprise phenolic compounds, chiefly phenoliccarboxylic and cinnamic acids and their derivatives, flavonoids, including flavones, isoflavones, flavanones, flavanones, flavans, flavans 3,4-diols, catechins, flavonoids and proanthocyanidins (Milewskaja et al., 2019). Rich in biologically active compounds (BAC), species of Lamiaceae family are broadly used in pharmacology.

Melissa officinalis L. is a perennial herbaceous plant of Lamiaceae family. It can reach 150 cm in height. Its is considered to have been originated from the territory spanning from the Eastern Mediterranean to Iran, Central Asia, the Black Sea and Western Asia, and also North Africa. Currently, this plant is cultivated ubiquitously for its essential oil. Content of essential oil ranges 0.02–0.20%, only seldom reaching 0.80%. Content of essential oil in the herbs is 0.06–0.13%, and 0.39–0.44% in the leaves. Leaves and young shoots of lemon balm that had been cut before blooming are used as spices in the European and American cuisines. Fresh and dried, they are added to salads, cheese, soups, meat, fish dishes, mushrooms, tea, vinegar, liquor, salting of cucumbers and tomatoes (Shakeri et al., 2016). Furthermore, essential oil of Melissa, similarly to other species of plants considered in this article, has for a long time been used to eliminate or scare off storage pest insects (Martynov et al., 2019a, 2019b). The essential oil’s most distinctive constituents are monoterpenes citral (geranial + neral), geraniol, nerol, citronellol, citronellal. Essential oil of lemon balm also contains linalool, geraniolacetate, myrcene, para-cimol, β-caryophyllene oxide, β-caryophyllene and other terpenoids. More than 200 constituents of the essential oil have been described, including neral and geraniol, which are responsible for lemon odour. Their proportions is 3:4, and the presence of 6-methyl-5-hepten-2-one and β-caryophyllene are the criteria of identification of lemon balm oil. The second group of the substances of lemon balm are phenylpropanoids, including rosmarinic acid as the most distinctive ones (Nogouchi-Shimohara et al., 2015). Phenylpropanoids are also represented by ethyl oil of rosmarinic acid, caffeic acid, chlorogenic acid, para-coumaric acid, ferulic acid and sinapinic acid. Using the method of liquid chromatography, we determined that content of rosmarinic acid in the leaves of lemon balm accounts for 0.54–1.59%. Among the phenol substances, antioxidant activity was exhibited by flavonoids apigenin, cosminolin, luteolin, cynaroside, and also tannin-citrus (7-methoxy kaempferol) and iso-quereticin (3-quinolinic glucoside), rhodanzin (3,7 dimetoxycamphor). Moreover, the plant contains phenol-carboxylic acids: gentisic, salicylic, p-hydroxybenzoic, vanillic, syringic, isoflavones apigenin, cosmosiin, luteolin, cynaroside, and also ramno-citrin (peppermint) (Clifford, 1999; Sik et al., 2019). Rosmarinic acid is a secondary metabolite of plants, which they synthesize to protect themselves against fungi and bacteria, as well as herbivorous organisms. Plant contains rosmarinic acid in the vacuoles, separately from oxidase enzymes. In case of plant’s trauma, oxidases influence the rosmarinic acid, and the phenol hydroxyl group of rosmarinic acid becomes oxidized to orto-chinons. They bind with proteins of bacteria, fungi or herbivorous animal, thus inactivating them (Häusler et al., 1993). Rosmarinic acid of Lamiaceae plants exerted inhibition of chloride esterase, and was reported to be effective in dementia intervention. Shinjyo & Green (2017) reviewed the reports on efficiency of these herbs, finding seven out of eight articles on lemon balm indicating its positive effects on mood and cognition, while one study observed no effect (Shinjyo & Green, 2017). The summary by Shakiri et al. (2016) describes the botanical characterization, traditional uses, phytochemistry, pharmacological activities, pharmacokinetics and toxicity of M. officinalis, and discusses blanks in the data and perspectives of surveying this plant.

M. officinalis and its major constituent – rosmarinic acid – exhibit powerful antioxidant and anti-inflammatory activities. Likewise, studies demonstrated that M. officinalis and rosmarinic acid mitigates the effects of memory loss caused by Alzheimer’s disease (Eivani & Khosronezhad, 2020). Rosmarinic acid is considered to be metabolized by gut microbiota, thus providing phenolic elements that may be absorbed more easily. In the human organism, molecules of rosmarinic acid alter their structure, undergo conjugation reactions, and are removed with excrements (Hitl et al., 2021).

Lavandula angustifolia Mill. is a perennial shrub of Lamiaceae family. Height of cultivated plants reaches 100–200 cm, and the plants in the nature grow up to 50–70 cm. The leaves are opposite, elongated-linear, with bent margins, 2–6 cm in length, grey-green from the indumentum. All the parts of the plant contain essential oil: leaves – up to 0.4%, the stems – to 0.2%, inflorescences – 3.5–4.5%. The main constituents of the essential oil (30–60%) are complex ethers of L-linalool alcohol and acids (acetic, butyric, valeric, and capric acids). Furthermore, it was found to...
contain cineol, geraniol, borneol (Kanabagias et al., 2019). The gas chromatography revealed the shares of the main components to equal as follows: linyl acetate (25–46%), linalool (20–45%), terpinen-4-ol (1.2–6.0%), lavandulaldehyde (1.0%), 1,8-cineole (2.5%), 3-octanone (<2.5%), camphor (<1.2%), limonene (<1.0%), and alpha-terpineol (<2.0%) (Koriem, 2021). Flowers and oil of lavender are used as a culinary spice. It is particularly popular in Spanish, French and Italian cuisines. Sedative effect of lavender during neurasthenia and heightened pulse is after surgeries (Wang et al., 2012; Yu & Seol, 2017; Mekonnen et al., 2019). Lavender oil is used to improve the odor of medicines. In folk medicine, the alcohol solutions of oil of lavender and inflorescence are applied to treat migraines, neurasthenia, stress (Kennedy & Wightman, 2011; Lundstrom et al., 2017; Uritu et al., 2018), rheumatism, cardiovascular diseases, kidney-stone disease and pyelonephritis, for medical treatment of rhinites, laryngitis; it is applied to speed up the wound healing and neuralgias, bruises and paralyses (Zaee et al., 2015; Saghazraddeh et al., 2017; Samarth et al., 2017; Xu et al., 2017; Cardia et al., 2018; Boulkhatem et al., 2020). In households, the flowers of lavender are used to scare off mosquitoes, blackflies and no-see-ums, and protect fur goods against moths. Similarly to other species of Lamiaceae family, lavender is a good nectar-beaver whose honey is considered healing. Lavender hybrids are called lavandins. Hybrids between L. angustifolia and L. latifolia (spike lavender) are called Lavandula × intermedia. They bloom later than the common English lavenders. Based on lavender, complex medicinal nano particle-containing preparations are developed (Shokri et al., 2017; Belova et al., 2019).

For centuries, the most commonly used species of Lavandula genus have been L. angustifolia, L. latifolia, L. stoechas and L. × intermedia (Cavanagh & Wilkinson, 2002; Woronuk et al., 2011). Despite the research data on this subject oftentimes being inconclusive and controversial, the benefits of lavender have nonetheless been confirmed by a number of studies (Cavanagh & Wilkinson, 2002). The surveys mainly focused on its effect on pain, anxiety, learning, memory, attention, arousal, relaxation, sedation and sleep (Dobosberger & Buchbauer, 2011). Constituents of lavender essential oil have immune-modulating activity, increase phagocytic activity of macrophages toward the bacteria (Peterfalvi et al., 2019). Likewise, it is being considered for treatment of epilepsy, stress, dementia and Alzheimer’s disease (Dobetsberger & Buchbauer, 2011; Boukhatem et al., 2020). In households, the flowers of lavender are used as a culinary spice. It is particularly popular in Spanish, French and Italian cuisines.
pregnancy. In the studies by Maleki-Saghooeni et al. (2018), V. agnus-castus and M. officinalis mitigated symptoms of premenstrual syndrome (PMS).

Safarabadi et al. (2018) consider L. officinalis and V. agnus-castus some of the most important analgesic plants. The research notes that the herbs have anti-nociceptive effects, inhibition of the release of arachidonic acid, synthesis of prostaglandins and action toward the opioid system, with peripheral anti-nociceptive mechanism and cholinergic pathways, stimulation of GABA A receptors, COX-1 and 5-LO and central and environmental mechanisms (Safarabadi et al., 2018).

Nonetheless, topically applied herbal medicinal preparations made of L. angustifolia may lead to such side-effects as contact dermatitis (Gan-geni et al., 2015). Biological properties of linalool, namely sedative, anxiolytic, analgesic, anticonvulsant, anti-inflammatory, local anaesthetic, are discussed in the context of the molecule’s chirality influence, the mechanisms of activity and type of study (in vitro, in vivo, clinical studies) (Aprotosoaie et al., 2014). The recently obtained data on properties of linalool to skin synthesis are considered in report by Aprotosoaie et al. (2014).

Despite the relatively thorough degree of study of the chemical composition and application of those plants during separate human and animal diseases (Wynn & Fougère, 2007; Lee et al., 2014; Zarei et al., 2014; Kubo et al., 2015; Saberi et al., 2016; Dolatabadi et al., 2018; EFSA Panel et al., 2020; Torki et al., 2021), no complex impact of these three species of medicinal plants during high-fat diet and excessive consumption of food was found. Therefore, the objectives of this study were overall effects of M. officinalis, L. angustifolia, V. agnus-castus on weight gain, changes in index of body weight, biochemical and morphological blood parameters, orienting-motor activity and emotional status of white laboratory rats against the background of excessive fat diet.

Materials and methods

Selection of animals for the experiment, the study protocols, euthanasia of animals were approved by the local ethics committee of the Dnipro State Agrarian-Economical University. Content, feeding, care for the animals and withdrawal of the animals from the experiment were performed following the principles formulated in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, March 18, 1986, ETS No. 123) and the order No. 3447-IV as of 21.02.2006 “On protection of animals against abuse” (Ukraine).

In the experiment, we used 32 adult white outbred laboratory male rats of 200 ± 10 g weight. The rats were divided into the control and experimental groups with 8 animals in each. The rats were kept in polycarbonate cells with steel grid covers, food pit, 4 individuals per a cell. The rats were maintained in the room with the temperature of 20–22 °C and relative air moisture of 50–65%. Light regime was 12 h of light and 12 h of dark. The ventilations were performed according to the regime. The animals received water ad libitum.

The diet of all animals had excessive fat content (3,600 ccal/kg). High-fat diet was composed based on the standard diet (75% of grain mixture (maize, sunflower seeds, wheat, barley), 8% of root vegetables (potatoes, carrot), 2% of meat and bone meal, 2% of mineral-vitamin complex) with introduction of 15% of sunflower oil. The control group of animals received high-fat diet, while the experimental group was fed with high-fat diet supplemented with the medicinal plants. The first experimental group, in addition to high-fat diet, was given 5% dry crumbled young shoots of L. angustifolia; the second experiment – 5% M. officinalis; the third experimental – 5% V. agnus-castus. The main ingredients of the diet were crushed in the mill (grain, meat and bone meal, mineral-vitamin complex, dry shoots of medicinal plants) and mixed. Then we have added sunflower oil, and prepared granules assessing the amount equaling 4,200 g for each group for the whole period of the experiment (30 days). Fresh root vegetables in the corresponding amount were given additionally daily. The animals had free access to the food. During the experiment, we recorded the amount of food consumed by each group a day and its total amount throughout the experiment.

Morphometric parameters (live mass, belly volume) were determined on the first and the 30th days of the experiment (Lieshchova et al., 2018, 2019, 2020). The calculated parameters were the overall increase in live mass and daily weight gains.

Orienting-motor activity and emotional status of the organisms of the experimental animals were studied in the “open field” test using an installation of 1 m² square area divided into 16 squares and limited non-transparent 20 cm-high wall. The experiment was performed in complete silence with intense light on the field itself. An experimental animals had been taken from the cage from previously shadowed compartment and placed in the center of the field. The exposure time was 2 min. The animals were tested for 4 days (1–4th days) at the beginning of the experiment and 4 days at the end (26–30th). We counted the number of squares the animals passed: peripheral and central ones – we assessed moving activity; peripheral (with reliance on the wall) and central (without reliance on the wall) stances – orienting activity; the amount of acts of grooming, defecation and urination – emotional status (Fig. 1).

The animals were euthanized on the 30th day of the experiment under narcosis (80 mg/kg of cetamine and 12 mg/kg of xylazine, intraperitoneal injection) by cardiac exsanguination. After the autopsy, we visually assessed the condition of the internal organs on the presence of pathological changes. The extraction of the organs and the tissues (heart, liver, lungs, thymus, spleen, stomach, thin and large intestines, kidneys) was carried out using surgical tools. The weight of the internal organs was determined with the accuracy of ± 0.01 g.

Fig. 1. Behaviour of rats in the “open field” test: a – crossing of peripheral squares, b – central stance, c – act of grooming.
Blood samples taken during te euthanasia were then used for biochemical and morphological assays. Biochemical parameters were determined using Micra automated analyzer (I.S.E. Sri, Italy) and a set of High-Technology reagents (USA), PZCorny S.A. (Poland) and Spinreact S.A. (Spain). The erythrocytes and leukocytes in stabilized blood were counted using automated BC-2800Vet analyzer (Mindray, China). For the leukogram, we prepared blood smears according to Pappenheim with subsequent Romanovsky-Giemsa staining. The numbers of erythrocytes and leukocytes in stabilized blood of mice were determined using automatic haematological analyzer BC-2800Vet and Mindray (Lieshchova et al., 2018, 2019, 2020; Brygadyrenko et al., 2019).

The data were analyzed using Statistica 8.0 program (StatSoft Inc., USA). The tables demonstrate the results as \( x \pm SD \) (standard deviation). Differences between the values of the control and experimental groups were determined using the Tukey test, where the differences were considered significant at \( P < 0.05 \).

**Results**

The median of the body weight on the 11th day increased to 110.2% compared with the initial weight in the control group of animals (Fig. 2a). By the end of the experiment (by the 30th days), the body weight did not exceed 112.0% of the initial weight. Stems of *L. angustifolia* caused almost even gain in body weight of the animals to 139.2% by the 30th day of the experiment, this parameter remained within the values of the norm. Activity of hepatic enzymes in the control group was significantly higher than the reference values, indicating somewhat damage to hepatocytes, while the general functional condition of the liver was good, for the rest parameters (relative and absolute weight of the organ, absence of macroscopic signs of damage and the rest biochemical parameters: total bilirubin, urea, total protein) were within the norm.

Intake of lavender shoots stimulated notable increase in alkaline phosphatase (to 349.7% of the levels of the control group), concentration of LDL cholesterol (to 227.7%) and moderate significant increase in the concentration of the total cholesterol (124.7%) in blood of rats (Table 3). Consumption of crumbled shoots of lemon balm caused great increases in the activity of alkaline phosphatase (to 465.7% of the activity of the enzyme in the control group), decrease in the concentration of uric nitrogen (to 79.0%), total bilirubin (to 63.3%) and triglycerides (to 63.1%) in blood of rats. Shoots of vetex highly increased the activity of alkaline phosphatase (to 406.2% of the control group), index of aterogenecity (to 524.0% of the control group) first of all due to decrease in the concentration of LDL cholesterol (to 51.9% of the control group). Also, there was seen decreases in the concentration of triglycerides (to 56.4% of the control group) and glucose (to 80.9% of the control group, Table 3).

**Table 1**

| Parameter                | Control | *L. angustifolia* | *L. angustifolia* compared to the control, % | *M. officinalis* | *M. officinalis* compared to the control, % | *V. angus-castus* | *V. angus-castus* compared to the control, % |
|--------------------------|---------|------------------|--------------------------------------------|------------------|--------------------------------------------|------------------|--------------------------------------------|
| Consumption of food       | 20.69   | 16.67            | 83.0                                       | 16.90            | 84.1                                       | 18.81            | 93.6                                       |
| by animals, g/day         |         |                  |                                             |                  |                                            |                  |                                            |
| Consumption of liquid     | 18.42   | 18.50            | 100.5                                      | 19.05            | 103.4                                      | 18.93            | 102.8                                      |
| by animals, g/day         |         |                  |                                             |                  |                                            |                  |                                            |
| Change in body weight, g/day | 700 ± 271 | 1943 ± 406***       | 277.6                                      | 2024 ± 393***     | 289.1                                      | 1171 ± 417       | 167.3                                      |
| Change in body weight, %/day | 136 ± 5.9 | 35.7 ± 8.0***       | 261.7                                      | 36.7 ± 7.9***     | 269.3                                      | 18.8 ± 6.5       | 138.1                                      |
| ОБЪЕМ ЖИВОТНОГО, CM          | 140 ± 0.5 | 140 ± 0.9            | 999                                        | 13.8 ± 0.4       | 98.9                                       | 14.8 ± 1.0       | 106.0                                      |

Note: *P < 0.05, **P < 0.01, ***P < 0.001, significant differences within one line of the table according to the results of ANOVA with Bonferroni correction.

**Table 2**

| Organ                     | Control | *L. angustifolia* | *L. angustifolia* compared to the control, % | *M. officinalis* | *M. officinalis* compared to the control, % | *V. angus-castus* | *V. angus-castus* compared to the control, % |
|---------------------------|---------|------------------|--------------------------------------------|------------------|--------------------------------------------|------------------|--------------------------------------------|
| Heart                     | 0.352 ± 0.023 | 0.362 ± 0.036           | 103.0                                      | 0.330 ± 0.025    | 93.7                                       | 0.352 ± 0.033    | 100.2                                      |
| Liver                     | 4.08 ± 0.17   | 4.32 ± 0.36            | 105.8                                      | 3.87 ± 0.36      | 94.8                                       | 3.71 ± 0.26      | 91.0                                       |
| Lungs                     | 0.979 ± 0.169 | 0.980 ± 0.190           | 98.9                                       | 0.872 ± 0.138    | 89.2                                       | 0.967 ± 0.365    | 98.8                                       |
| Brain                     | 0.867 ± 0.002 | 0.700 ± 0.073***        | 80.8                                       | 0.738 ± 0.075**  | 85.2                                       | 0.698 ± 0.142    | 80.5                                       |
| Thymus                    | 0.285 ± 0.046 | 0.166 ± 0.077***        | 58.3                                       | 0.133 ± 0.040*** | 46.7                                       | 0.221 ± 0.043    | 77.6                                       |
| Splenic                   | 0.370 ± 0.036 | 0.473 ± 0.159           | 128.0                                      | 0.412 ± 0.150    | 111.5                                      | 0.470 ± 0.070*   | 127.0                                      |
| Stomach                   | 0.699 ± 0.006 | 0.769 ± 0.211           | 110.0                                      | 0.764 ± 0.206    | 109.4                                      | 0.847 ± 0.266    | 121.3                                      |
| Small intestine           | 2.58 ± 0.52   | 2.16 ± 0.35            | 83.8                                       | 2.20 ± 0.25      | 85.0                                       | 2.44 ± 0.25      | 94.7                                       |
| Cecum                     | 0.509 ± 0.176 | 0.457 ± 0.099           | 89.9                                       | 0.422 ± 0.077    | 82.9                                       | 0.509 ± 0.262    | 100.1                                      |
| Large intestine           | 0.374 ± 0.085 | 0.397 ± 0.121           | 106.2                                      | 0.400 ± 0.082    | 107.0                                      | 0.431 ± 0.089    | 115.4                                      |
| Rectum                    | 0.398 ± 0.073 | 0.307 ± 0.077           | 77.2                                       | 0.203 ± 0.104**  | 51.0                                       | 0.366 ± 0.063    | 92.0                                       |
| Right kidney              | 0.338 ± 0.031 | 0.321 ± 0.031           | 89.6                                       | 0.317 ± 0.031    | 88.4                                       | 0.310 ± 0.026    | 86.4                                       |
| Left kidney               | 0.332 ± 0.040 | 0.319 ± 0.022           | 85.7                                       | 0.321 ± 0.039    | 86.4                                       | 0.308 ± 0.030    | 82.8                                       |

Note: see Table 1.
Fig. 2. Changes in the body weight of the rats in the control variant of the experiment (a) and when adding ground seeds of *Lavandula angustifolia* Mill. (b), *Melissa officinalis* L. (c) and *Vitex angus-castus* L. (d) into the diet: on the abscissa axis – 24 h of the experiment, on the ordinate axis – body weight of the animals (% of the initial body weight before the experiment, considered 100% for each experimental animal); small square – median, upper and lower borders of the square – 75% and 25% of quartiles, the upper line – minimum and maximum values, circles – emissions; n = 8
Analysis of protein metabolism revealed that high-fat diet did not increase the concentration of the total protein. Addition of vitex and lemon balm to high-fat diet elevated the level of total protein beyond the limits of the normal values, while lavender has not. At the same time, in all groups, we observed slight increase in globuline fraction, especially noted at addition of Vitex.

High-fat diet did not significantly affect the morphological composition of blood of the experimental animals. Almost all the parameters were within the reference values. Exception was the level of monocytes in blood, which in all the groups was 1.5-2.0 times above the normal parameters. Intake of dry shoots of *L. angustifolia* and *M. officinalis* contributed to significant increase in the concentration of leukocytes in blood (to 165.4% and 199.9% of the concentration in the control group, respectively), but did not exceed the thresholds of the reference values (Table 4). Consumption of dry herbs of *V. angus-castus* stimulated decrease in concentration of band neutrophils in blood of rats (four times lower than in the control group, Table 4). General analysis of blood and leukogram of male rats revealed no other significant changes.

Physical activity (Fig. 3a) of the animals was significantly reduced by the end of the experiment after consumption of *L. angustifolia* and *M. officinalis*. Under the influence of these plants, orienting activity of the rats also decreased significantly (Fig. 3b). No significant changes in emotional status (Fig. 3c) were seen during the experiment when the animals were fed with all three species of medicinal plants. Addition of the shoots of *V. angus-castus* led to no changes in physical and oriented activity of animals (Fig. 3). Significant changes in the "open field" test at the beginning and the end of the experiment were observed between and inside the groups of rats (Table 5) that consumed the shoots of *L. angustifolia* and *M. officinalis* for the quantity of the attended peripheral squares and the number of stances in the peripheral squares.

### Table 3

| Parameters                      | L. angustifolia compared to the control, % | M. officinalis compared to the control, % | V. angus-castus compared to the control, % |
|--------------------------------|------------------------------------------|------------------------------------------|-------------------------------------------|
| Total protein, g/L             | 77.0 ± 4.9                               | 76.0 ± 4.0                               | 80.0 ± 3.7                                |
| Albumins, g/L                  | 39.6 ± 2.6                               | 38.7 ± 2.5                               | 41.3 ± 2.2                                |
| Globulins, g/L                 | 37.4 ± 3.9                               | 37.3 ± 3.3                               | 38.7 ± 3.3                                |
| Protein coefficient, U         | 1.10 ± 0.15                              | 1.06 ± 0.12                              | 1.09 ± 0.14                               |
| Urea, mmol/L                   | 6.84 ± 1.02                              | 6.00 ± 0.74                              | 5.40 ± 0.58                               |
| Urea nitrogen, mg/100 g        | 13.1 ± 2.0                               | 11.5 ± 1.4                               | 10.3 ± 1.1*                               |
| Creatinine, µmol/L             | 63.0 ± 4.4                                | 61.0 ± 7.4                               | 74.9 ± 12.8                               |
| Aspartate aminotransferase (AST), U/L | 186.6 ± 61                               | 166.0 ± 48                               | 182.3 ± 35                                |
| Alanine aminotransferase (ALT), U/L | 131.4 ± 41                               | 129.3 ± 39                               | 111 ± 18                                  |
| De Ritis ratio (AST/ALT), U     | 1.63 ± 0.78                              | 1.37 ± 0.45                              | 1.64 ± 0.30                               |
| Alkaline phosphatase, U/L      | 129.6 ± 46                               | 451.9 ± 94***                           | 601.6 ± 140***                            |
| Total bilirubin, µmol/L        | 6.1 ± 1.7                                | 4.1 ± 2.8                                | 3.8 ± 1.4*                                |
| Glicose, mmol/L                | 7.39 ± 1.04                              | 6.36 ± 0.63                              | 6.40 ± 0.55                               |
| Totalcalcium, cmol/L           | 2.53 ± 0.09                              | 2.51 ± 0.11                              | 2.59 ± 0.14                               |
| Non-organic phosphorus, mmol/L | 3.07 ± 0.58                              | 3.67 ± 0.40                              | 3.46 ± 0.29                               |
| Ratio Ca/P                     | 0.843 ± 0.129                            | 0.686 ± 0.09                              | 0.743 ± 0.09                              |
| Gamma-glutamyltransferase (GGT), units/L | 9.3 ± 2.6                                | 9.1 ± 4.4                                | 6.7 ± 0.7                                 |
| Cholesterol, mmol/L            | 1.27 ± 0.13                              | 1.59 ± 0.20*                             | 1.43 ± 0.18                               |
| Triglicerides, mmol/L          | 2.13 ± 0.55                              | 1.36 ± 0.38                              | 1.34 ± 0.31*                              |
| high-dense lipoprotein cholesterol (HDL cholesterol), mmol/L | 0.65 ± 0.13                              | 0.66 ± 0.19                              | 0.80 ± 0.44                               |
| Low-dense lipoprotein cholesterol (LDL cholesterol), mmol/L | 0.52 ± 0.29                              | 1.18 ± 0.08***                           | 227.7 ± 0.11                              |
| C-reactive protein, mg/L       | 12.5 ± 5.4                               | 13.2 ± 5.2                               | 10.2 ± 1.4                                |
| Atenogenecity index, units      | 1.04 ± 0.45                              | 1.85 ± 1.41                              | 1.77 ± 1.30                               |

Note: see Table 1.

### Table 4

| Parameter                                    | Control | L. angustifolia compared to the control, % | M. officinalis compared to the control, % | V. angus-castus compared to the control, % |
|----------------------------------------------|---------|------------------------------------------|------------------------------------------|-------------------------------------------|
| Hemoglobin, g/L                              | 126.8 ± 70 | 119.3 ± 7.1                              | 130.4 ± 6.9                              | 126.7 ± 11.8                              |
| Hematocrit, %                                | 40.5 ± 2.7 | 38.6 ± 2.4                               | 42.0 ± 1.9                               | 40.3 ± 4.0                               |
| Erythrocytes, 10⁶/L                          | 6.93 ± 0.29 | 7.13 ± 0.30                              | 7.03 ± 0.91                              | 7.32 ± 0.67                              |
| Erythrocyte sedimentation rate (ESR), mm/h   | 1.17 ± 0.37 | 1.33 ± 0.47                              | 1.00 ± 0.00                              | 1.00 ± 0.00                              |
| Thrombocytes, 10⁶/L                          | 339 ± 66   | 351 ± 87                                 | 336 ± 66                                 | 284 ± 72                                 |
| Leukocytes, 10⁶/L                            | 8.6 ± 1.6  | 14.2 ± 2.3***                            | 17.1 ± 5.9***                            | 113.6 ± 6.1                              |

Leukocytic formula

| Basophils, %                                 | 0.0 ± 0.0  | 0.0 ± 0.0                                 | 0.0 ± 0.0                                 | 0.0 ± 0.0                                 |
| Eosinophils, %                               | 1.50 ± 0.76 | 1.17 ± 0.37                              | 1.57 ± 0.73                              | 1.06 ± 0.35                              |
| Macrophats, %                                | 0.0 ± 0.0  | 0.0 ± 0.0                                 | 0.0 ± 0.0                                 | 0.0 ± 0.0                                 |
| Neutrophils, %                               | 0.0 ± 0.0  | 0.0 ± 0.0                                 | 0.0 ± 0.0                                 | 0.0 ± 0.0                                 |
| young                                        | 0.0 ± 0.0  | 0.0 ± 0.0                                 | 0.0 ± 0.0                                 | 0.0 ± 0.0                                 |
| band                                          | 1.17 ± 0.69 | 1.17 ± 1.07                              | 0.71 ± 0.45                              | 0.29 ± 0.45*                             |
| with segmented nuclei                        | 23.0 ± 8.2  | 22.3 ± 5.1                               | 25.6 ± 6.4                               | 21.0 ± 4.4                               |
| lymphocytes, %                               | 68.8 ± 8.6  | 67.2 ± 6.3                               | 65.9 ± 6.9                               | 72.1 ± 3.6                               |
| Monocytes, %                                 | 5.5 ± 1.3  | 8.2 ± 3.3                                | 6.3 ± 2.3                                | 5.6 ± 1.7                                |

Note: see Table 1.
Changes in the behaviouristic characteristics of the three groups of rats during 120 seconds of the experiment when crumbled shoots of Lavandula angustifolia Mill, Melissa officinalis L. and Vitis angus-castus L.: on abscissa axis – groups of animals (n = 8) on the diet with excessive fat content and addition of crumbled shoots of the plants (in parentheses there are indicated day after the experiment: beginning – 1–4th or the end – 26–30th days), on ordinate axis – absolute number of markers of this type of behavior during 120 seconds of the experiment: for the motor activity – the number of attended squares of the “open field”, for the orienting activity – number of stances, for the emotional status – number of acts of grooming, defecation and urination; small square – median, the upper and lower line of the rectangle – 75% and 25% quartiles, the upper line – minimum and maximum values, circles – emissions; different letters within each figure indicate significant differences between the groups (P< 0.05) according to the results of Tukey test.

Table 5
Changes in the behaviouristic characteristics of the three groups of rats during 120 seconds of the experiment when crumbled shoots of Lavandula angustifolia Mill, Melissa officinalis L. and Vitis angus-castus L. were added to the diet (x ± SD, n = 32, duration of the experiment was 30 days)

| Characteristic                      | Control, 1–4th days | Control, 26–30th days | L. angustifolia, 1–4th days | L. angustifolia, 26–30th days | M. officinalis, 1–4th days | M. officinalis, 26–30th days | V. angus-castus, 1–4th days | V. angus-castus, 26–30th days |
|-------------------------------------|---------------------|-----------------------|-----------------------------|-----------------------------|---------------------------|-----------------------------|-----------------------------|-----------------------------|
| Number of attended periphery squares | 28.13 ± 18.04a      | 24.33 ± 14.45a        | 13.64 ± 8.59a               | 3.71 ± 7.96b               | 16.11 ± 14.29a            | 4.57 ± 9.32a               | 13.54 ± 12.78a              | 8.96 ± 11.30a               |
| Number of attended central squares  | 1.00 ± 2.341        | 0.292 ± 1.042         | 0.00 ± 0.00                 | 0.821 ± 1.887              | 0.00 ± 0.00               | 0.107 ± 0.416              | 0.00 ± 0.00                 | 0.00 ± 0.00                 |
| Number of stances in peripheral squares | 5.58 ± 4.53a       | 3.79 ± 3.13b          | 3.04 ± 2.13a                | 0.79 ± 1.03a               | 3.75 ± 2.74b              | 1.00 ± 1.44a               | 2.93 ± 3.33b                | 1.86 ± 2.21b                |
| Number of stances in the central squares | 1.292±1.429        | 0.708±0.999           | 0.607±0.994                 | 0.036±0.189                | 0.750±1.602               | 0.000±0.000                | 0.222±0.641                 | 0.000±0.000                 |
| Number of acts of grooming | 0.583±0.830        | 0.583±0.929          | 1.000±1.122                 | 0.536±0.744                | 1.071±1.464               | 0.643±1.224                | 0.393±0.994                 | 0.393±0.786                 |
| Number of fecal bolus | 2.250±2.027        | 2.375±1.555          | 0.536±1.071                 | 0.964±1.478                | 0.571±1.317               | 1.679±2.056                | 2.679±2.568                 | 2.179±2.195                 |
| Number of urinations | 0.333±0.482        | 0.375±0.495          | 0.107±0.416                 | 0.026±0.189                | 0.036±0.189               | 0.036±0.189                | 0.000±0.000                 | 0.000±0.000                 |

Notes: no significant differences between the groups were found according to the Tukey test; differences between the number of attended peripheral squares and the number of stances in the peripheral squares are indicated by different Latin letters (P< 0.05).

Discussion

Plant-based food supplements are currently gaining popularity, but the data about the risk they pose are rare and controversial. Lamiaceae family contains herbs of high socio-economic significance, several horticultural and ornamental species, culinary herbs, having broad range of application because of richness in phenolic compounds (Trivellini et al., 2016). Natural phenols are less harmful to the environment and health than components used in cosmetics, pesticides and preservatives (Trivellini et al., 2016). Obesity and overeating are some commonest health issues around the world, and many people see easy solution in the performance and image-enhancing drugs (PIEDs). Nonetheless, those preparations may exert toxicity and impair metabolism, despite the manufacturers’ claims about safety of the natural receiptes of their medicinal drugs (Bersani et al., 2015). Identifying the composition of biologically active compounds in medicinal herbs is complicated because there are no unified methods for this purpose (Milevska et al., 2019). Therefore, in our study, we chose to add dry crumbled plants to granulated feed of animals.

In our study, addition of lavender and lemon balm to the diet was followed by more intense weight gain while consuming less food than the control group that received high-fat diet.

Valuable medicinal plant M. officinalis is native to the eastern Mediterranean Region and Western Asia. Its main constituents are citral (geraniol and neral), citronellal, geraniol. In the experiments, M. officinalis notably decreased body weight (Vailzadeh et al., 2016); nonetheless, the review emphasizes that there are needed randomized trials of higher quality to confirm the results. Abilities to improve memory had been also demonstrated by some other plants like M. officinalis, and the mechanisms of action were determined (Shojaii et al., 2016), but for many medicinal herbs there is not a sufficient amount of studies on their efficiency in improving memory and learning (Shojaii et al., 2016).

Many pharmacological effects have been reported for crude extracts and pure compounds isolated from M. officinalis, but only anxiolytic, antiviral, antispasmodic activities, as well as effects on mood, cognition and memory were confirmed in the clinical experiments. The major mechanisms of this plant’s neurological effects, which are the subject of discussion worldwide, are AChE inhibitory activity, stimulation of the acetylcholine and GABAA receptors, as well as inhibition of matrix metalloproteinase-2 (Shakeri et al., 2016). Lemon balm is applied during discussion worldwide, are AChE inhibitory activity, stimulation of the acetylcholine and GABAA receptors, as well as inhibition of matrix metalloproteinase-2 (Shakeri et al., 2016). Lemon balm is applied during improving memory and learning (Shojaii et al., 2016).
neurotoxicity and gastro-intestinal symptoms. The symptoms in most cases were mild (Lude et al., 2016).

High content of fat in diet is considered to inevitably cause increase in the parameters of lipid metabolism such as total cholesterol, level of triglycerides, content and proportion of hyperliproteins of different density, which are expressed by such a parameter as aterogenecity index. In our experiments, in rats, the consumption of the diet with heightened content of fat during 30 days caused no elevation of the level of total cholesterol, which remained within the reference values. As known, the parameters of lipid metabolism in rats were lower than such in human due to production of specific bile acids – α- and β-muricholic acids, absent in humans (Thomas et al., 1984). Bile acids in particular are those considered responsible for fast removal of cholesterol from the rats’ organism. Difference lies in the fact that rats are very resistant to the level of serum cholesterol, unlike human. Moreover, the animals are hardly vulnerable to development of plaques in the arteries as a result of intake of cholesterol-rich food (Stieh- bens, 1986).

Gross et al. (2019) reported that M. officinalis was clinically effective against symptoms related to anxiety and displayed no signs of toxicity. The review by Swieder et al. (2019) analyzed the literature data on the chemical composition of M. officinalis and the possibilities of using it in medicine and food. Heshmati et al. (2020) indicated the relationship between consumption of M. officinalis and decreased total cholesterol and reduced systolic blood pressure. Intake of M. officinalis was not observed to be related to statistically significant changes in triglycerides, low-density lipoprotein, diastolic blood pressure, high sensitivity c-reactive protein levels, fasting blood sugar, HbA1c, insulin or high-density lipoprotein levels. No serious side effects were reported. According to the study by Heshmati et al. (2020), M. officinalis is safe beneficial supplement. In our experiment, addition of lemon balm to high-fat diet of rats reduced the intensity of increase in the level of triglycerides and HDL cholesterol compared with the control, and at the same time the indicator of total cholesterol, LDL cholesterol, did not change significantly.

In a 21-day experiment on rats that received high-fat diet and various doses of extract of melissa, Zarei et al. (2014) observed significant decrease in the activity of hepatic enzymes. In our experiment, by the 30th day, the rats fed with fat diet were seen to AST, ALT and alkaline phosphatase exceeding the reference values, indicating damaged cellular membrances of hepatocytes. Addition of lemon balm to the diet led to decrease in only ALT activity, and AST activity remained the same as in the animals on high-fat diet, and the activity of alkaline phosphatase was significantly higher. This may be related to either longer duration of our experiment or lower dose of active agents.

Benny & Thomas (2019) analyzed the literature reporting anti-amyloid, antioxidiant, anticholinesterase, and memory-enhancement activities of essential oils from M. officinalis, L. angustifolia in preclinical and clinical studies of Alzheimers disease.

Treatment of neurodegenerative diseases with M. officinalis and rosmarinic acid – its major constituent – has been reported in many scientific and non-scientific articles, but clinical trial of ethanol extract from M. officinalis was only made so far toward Alzheimer disease (Mahboubi, 2019). Action mechanisms of M. officinalis comprise inhibitory effects against beta-amyloid, reactive oxygen species, and acetylcholine esterase. M. officinalis can mitigate psychological symptoms in the patients undergoing operation (Shahbani et al., 2019). Use of medicinal herbs before and after surgery alleviates anxiety, depression, aggressive and impulsive behavior, stress, delirium and cognitive dysfunction. Trials on children with attention deficit hyperactivity disorder (ADHD) who consumed M. officinalis preparations showed low evidence for their effectiveness (Anheyer et al., 2017); but no concrete recommendations could be made while there is still a lack of sufficient numbers.

In many studies, M. officinalis exerted high antioxidant activity through flavonoids, roscuric acid, gallic acid, phenolic contents. A number of studies confirmed the antioxidative action of M. officinalis, and its effect in preventing and treating oxidative stress-related diseases might be reliable (Mirij et al., 2017). Moradi et al. (2016) report that more comprehensive studies using more advanced methods are needed to develop promising anti-HSV drugs based on bioactive compounds isolated from M. officinalis.

Rosmarinic acid is considered to have notable pharmacological effects and was recently surveyed as a therapeutic drugs in treatment of diabetes (Ngo et al., 2018). Earlier researches confirmed that rosmarinic acid can control the plasma glucose level and heighten insulin sensitivity in hyperglycemia. Rosmarinic acid is quickly absorbed in the human body, but its mechanism remains unclear (Ngo et al., 2018). Against the background of high-fat diet, glucose level in blood plasma of the studied rats increased, whereas addition of medicinal plants to the diet decreased the glucose level to the normal values.

M. officinalis and L. angustifolia are commonly considered to take generally calming effect. Experimental pharmacology using L. angustifolia includes anesthetic, anticonvulsant, sedative, anti-inflammatory, anti-microbial, antispasmodic, antispasmodic, central nervous system depres- sant effects; clinical pharmacology includes anxiolytic, analgesic, and cardio-vascular effects (Korien, 2021). In the quantitative synthesis, inhalati- on of lavender decreased levels of anxiety, according to any validated scale and sign of anxiety (Donelli et al., 2019), but caused no reduction of blood pressure, a physiological parameter of anxiety. Investigation of effects of inhalation of lavender oil aroma in sleep needs more in-detail surveys (Fiser & Pilkington, 2012). Some studies have shown the ef- ficiency of oral lavender supplements, but independent replications are needed to draw conclusions (Perry et al., 2012). In the “open field” me- thod, addition of lavender and lemon balm perorally with food significantly decreased motor activity of animals, compared with the control group (high-fat diet). Also, these animals exhibited decrease in orienting activity. Despite some data on vexes manifesting calming effect (Mehlhorn, 2016), it exhibited no inhibition of motor and orienting activities in our experi- ment. Moreover, all the studied plants caused no changes in the emotional status of the experimental rats.

V. angus-castus is rich in phytoestrogens and is traditionally applied in the treatment of premenstrual syndrome (Arzi et al., 2019). In the rat groups, no anti-anxiety effects were manifested by tamoxifen or a combi- nation of tamoxifen and a high dose of V. angus-castus. Extract from V. angus-castus displayed anti-anxiety activity and may be used to treat anxiety (Mehlhorn, 2016). Interaction between phytoestrogens from V. angus-castus and estrogen receptors could be the mechanism that de- termines the plant’s anxiolytic activity (Arzi et al., 2019).

Effects of the plants we tested on the organism of rats were both direct and indirect. By inhibiting certain species of microorganisms in the intest- ine of animals (Bilan et al., 2019). In our earlier experiments, ethanol ext- ract from M. officinalis powerfully inhibited growth of colonies of bacteria of Salmonella typhimurium, poorly inhibited such of Escherichia coli, Klebsiella pneumonia and Corinobacterium xerosis, and caused no effect on Proteus mirabilis, Listeria monocytogenes and fungus of Candida albicans (Zahzarsky et al., 2019). Similar effects were observed for ethyl extract of the leaves of L. angustifolia: it notably inhibited growth of colo- nies of Salmonella typhimurium and Klebsiella pneumonia, weakly af- fected Escherichia coli, Proteus mirabilis and fungus of Candida albicans and inhibited no growth of cells of bacteria of Listeria monocytogenes and Corinobacterium xerosis (Zahzarsky et al., 2019). We saw broader range of antibacterial activity in in vitro experiments exhibited by ethyl extract from V. angus-castus that notably inhibited growth of Corinobacterium xerosis, Serratia marcescens, Salmonella typhimurium, Proteus mirabilis; weakly affected growth of colonies of Rhodococcus equi, Pseudomonas aeroginosa, Yersina enterocolitica, Klebsiella pneumonia, Enterococcus faecalis, Escherichia coli, and took no inhibitory effect on growth of colo- nies of Enterobacter aerogenes, Listeria ivanovii, L. innocua, L. monocytogenes, Campylobacter jejuni and fungus of Candida albicans (Zahzars- kyi et al., 2020).

Also, in our previous studies, we determined that aqueous tincture of V. angus-castus in in vitro experiment had weak lethal effect on larvae of parasitic intestinal nematodes of Strongyloides papillosum (Wedel, 1856), though mortality of nematodes of Haemonchus contortus (Rudolphi, 1803) in aqueous tincture of this plants was no different from the control (Boyko et al., 2020). Essential oil from L. officinalis had similar effect on these species of nematodes, causing 4-fold increase in mortality of larvae of S. papillosum, but took no effect on larvae of H. contortus (Boyko & Brygadyrenko, 2021). Thus, possible effect of medicinal plants of Lamiaceae family may likely occur through various species of parasitic nema-
todes of Strongyloides genus, specific various species of model animals and human.

Conclusion

Against the background of high-fat diet, lemon balm and lavender manifested similar influences. Addition of these plants to the diet led to significant decrease in food intake, and at the same time the intensity of weight gain was greater than in the animals that consumed high-fat diet supplemented by vitrox. Taking into account that during consumption of lavender and lemon balm, the motor and orienting activities of the animals decreased by the end of the experiment, we consider it as manifestation of calming effect taken by the plant, which was not observed in the control group and with addition of vitrox. Also, lemon balm and lavender, by the end of the experiment, led to significant decrease in the relative weights of the brain and the thymus.

High-fat diet caused impairment of metabolism of animals. Addition of medicinal plants to the diet with high content of fat alleviates the disorders in the metabolisms of fat (increase in the level of triglycerides) and carbohydrates (increase in glucose level), but takes negative effect on protein metabolism (hyperproteinemia as a result of hyperglobulinemia).

Additional of medicinal plants to high-fat diet led to impaired activity of blood enzymes – alkaline phosphate, AST and ALT; increase in triglycerides, LDL cholesterol against the decrease of decrease in HDL cholesterol and normal value of total cholesterol. Also, all the groups were manifested similar influences. Addition of these plants to the diet led to enhanced activity of enzymes responsible for therapy against inflammation and cancer. Molecules, 25(16), 3671.

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