Impacts on gut microbiota of rats with high-fat diet supplemented by herbs of *Melissa officinalis*, *Lavandula angustifolia* and *Salvia officinalis*

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Medicinal plants are used in traditional, folk medicine and veterinary practice to treat and prevent exacerbations of chronic diseases, diseases of the cardiovascular and nervous systems, respiratory and digestive organs, liver, bile ducts, kidneys, urinary tract, to regulate metabolism and to boost immunity, etc. The therapeutic effect on the body is exerted by biologically active substances (alkaloids, glycosides, tannins, essential oils, and others) that are present in various parts of plants. Understanding the issue of interaction between the intestinal microbiota and drugs based on medicinal plants will contribute to the development of treatment protocols for various diseases, including chronic ones, by the normalization of impaired functions due to plant-origin substances. In this study, we determined the effect of *Melissa officinalis*, *Lavandula angustifolia*, *Salvia officinalis* on the intestinal microbiota of white rats fed a high-fat diet. The addition of 5% crushed young dry shoots of *S. officinalis*, *L. angustifolia*, *M. officinalis* to the high-fat diet of laboratory rats significantly changed the quantitative ratio of *Escherichia coli* with normal and altered enzymatic properties in the intestinal content. The number of typical *E. coli* in rats fed with *S. officinalis* and *L. angustifolia* decreased by 1.7 and 1.6 times; non-lactose fermenting form of *E. coli*, on the contrary, increased by 1.8–2.1 times in rats fed with any of the medicinal plants compared to the control. Amid the addition of medicinal plants to the diet, it was not possible to isolate opportunistic enterobacteria of the genus *Citrobacter*; however, an increase in the number of the genus *Candida* fungi was observed.

Keywords: medicinal plants; Lamiaceae; high-fat diet, gut microbiota, dysbiosis, male white rats.

Introduction

The use of herbal medicines is an important area of therapy that is used to treat diseases of various etiologies through a wide therapeutic spectrum and with minimal or no side effects. Medicinal plants are used as an independent type of treatment, and auxiliary, in combination with other drugs (Behrmanamesh et al., 2013; Ghorbani & Esmaeilzadeh, 2017; Asadi et al., 2018). Currently, medicinal plants are used to improve the health and well-being of people, especially those with chronic inflammatory processes, for the prevention and treatment of obesity, the treatment of osteoporosis, headache, migraine, dizziness, epilepsy, seizures in children, tetany, etc. (Guo et al., 2014; Iundaghi et al., 2016; Zhan et al., 2016; Chen et al., 2017; Piersch-Wenzig et al., 2022; Ying et al., 2022). In veterinary medicine, medicinal plants and biologically active additives of plant origin as part of feed are used to normalize the digestive process, treat diseases of the gastrointestinal tract, nervous and circulatory systems, regulate metabolism, strengthen immunity and increase productivity (Ashour & Astal, 2005; Martynov et al., 2019; Atea & Hassan, 2020; Krizhak et al., 2020; Salomon et al., 2021). A promising direction of research is the creation of drugs based on medicinal plants that could replace antibiotics. Antibiotic residues in animal products pose a health risk to consumers, and uncontrolled use of antibiotics contributes to the development of antibiotic resistance in microorganisms (Witte, 2000; Salman et al., 2016; Melidi et al., 2018; Kogut, 2019). New drugs and complementary or alternative medicines from different herbal ingredients could increase productive potential, keep productive animals healthy and would be more affordable than synthetic ones (Foltinová et al., 2017). Kogut (2019) and Salamon & Hrytsyna (2019) report on the use of essential oils with antimicrobial activity, extracted from organo (Oregano vulgare L.), thyme (Thymus vulgaris L.), dandelion (Taraxacum officinale L.) Weber ex F. H. Wigg) for protecting health of productive animals.

In maintaining the health of the macroorganism, the barrier function of the intestine and its microbiota play a decisive role. The intestinal mucosal barrier retains the ability to digest, absorb nutrients and prevent pathogen invasion. The gut microbiota plays a role in nutrient metabolism, innate immunity development, and pathogen clearance (Yang et al., 2009; Salman et al., 2016; Kogut, 2019). It is known that nutrients, psychochemicals and drugs that enter the macroorganism digestive system first interact with the intestinal microbiota, then the substances biotransformed by the enzymes of the macroorganism and microorganisms are absorbed and localized in various tissues (Dey, 2019). The interaction of plant components with the intestinal microbiota can lead to their breakdown with the formation of metabolites that have altered biological activity, or cause a change in the composition and ratio and functional capacity of the microbial community, which in turn can affect the homeostasis of the macroorganism itself (Chen et al., 2010; Feng et al., 2019; Thammann et al., 2019).

Preclinical and clinical studies have shown that, after ingestion, medicinal plants interact with the intestinal microbiota, which can model its composition and metabolism. Botanicals (e.g. ginsenoside, hesperidin, baicalin, daidin, and glycyrrhizin) may have therapeutic effects through gut microbiota-mediated bioconversion (Chen et al., 2016; Feng et al., 2019; Illiano et al., 2020). The strongest evidence exists about using inulin as a prebiotic, and in this context, the prebiotic activity of chicory root has been extensively studied. Gong & Yang (2012) indicate that beneficial modulation of the gut microbiota may occur through flaxseed supplementation.
Goncalves et al. (2019) prove that the human gut microbiota is involved in the metabolism of rosmarinic acid. The authors established the inhibitory effect of raw and dried aqueous extracts of rosemary on methicillin-resistant Staphylococcus aureus (MRSA), methicillin-sensitive S. aureus (MSSA), Listeria monocytogenes. However, fermented rosemary extract was moderately effective against methicillin-resistant S. aureus and methicillin-sensitive S. aureus.

Van Tilburg et al. (2014) reported on the use of ginger root for the treatment of irritable bowel syndrome: 46.7% of patients improved with 1 g of ginger root and 33.3% with 2 g. In addition, ginger is classified as a broad-spectrum antiemetic, which can relieve pain and influence intestinal motility (Grzanna et al., 2005; Terry et al., 2011).

In India, Punica granatum is commonly used as a traditional medicine for the treatment of diseases caused by pathogenic bacteria. Pai et al. (2011) investigating the antibacterial activity of pomegranate peel extracts (alcoholic and aqueous) against various intestinal pathogens, found a significant inhibitory effect of its ethanol extract against Shigella flexneri and Aeromonas hydrophila.

Oso et al. (2019) examined the effect of nutritional supplements with the herbal mixture (mountain knotgrass (Aerva lanata), betel (Piper betle), Bermuda grass (Cynodon dactylon) and black pepper (Piper nigrum) on indicators of the caecal microflora, they found an increase in the number of caecal bifidobacteria (P = 0.053) with an increase of supplements in the feed at the level of 1%. In addition, an improvement in growth rates, intestinal morphology and digestibility of organic substances and tryptophan in the ileum was revealed.

According to Qureshi et al. (2015), it is known that dandelion leaves (Taraxacum officinale) and seeds of fenugreek (Trigonella foenum-graecum L.) have antibacterial properties against Escherichia coli on Mueller-Hinton agar; dandelion leaf extract showed a zone of growth retardation of 2 mm, and fenugreek seeds – 2.1 mm at concentrations of 0.5 and 0.05 mg/mL of the extract, respectively.

Qiao et al. (2022) prove that Firmicutes and Bacteroidetes are the most dominant types of microorganisms in the caecum of birds and account for 71.4% and 23.4% in fatty chickens and 53.4% and 41.1% in lean chickens, respectively. Dietary supplementation with Astragalus membranaceus Lam. polysaccharide and Chinese liquorice (Glycyrrhiza uralensis Frisch. ex DC.) polysaccharides increased the Firmicutes to Bacteroidetes ratio as well as body weight in lean chicks compared to controls. Consumption of such dietary supplements can markedly increase levels of Prevotella, Parabacteroides, and Ruminococcus, according to Zhang et al. (2017) and Chen et al. (2019), are involved in the degradation of polysaccharides and the production of short-chain fatty acids. The decrease in Bacteroides after supplementation is associated with a decrease in the abundance of Bacteroides.

Herbal preparations are able to maintain a balanced intestinal microecosystem. Bofutsushoan, curcumin, and Morinda officinalis F. C. How contributed to a decrease in a large number of pathogenic Escherchia coli and Enterococcus in vivo and an increase in Lactobacillus and Bifidobacterium (McFadden et al., 2015; Fujisaka et al., 2020).

Liquorice extract promotes an increase in Lactobacillus, Bifidobacterium, and Enterococcus and can be used to treat gut microbiota disorders and inhibit the growth of Clostridium and Brevibacillus, which may help treat colon cancer (Qiao et al., 2018). Ramiah et al. (2014) found that dietary supplementation with garlic (Allium sativum L.) and water-starwort (Callitriche) can improve growth performance in broilers. By adding garlic and pennnyroyal (Mentha pulegium L.) to the main diet, a decrease in the number of Escherichia coli and an increase in the number of lactobacilli in the small intestine was noted. Such dietary supplements can be an alternative to growth-promoting antibiotics.

Therefore, the expansion of modern views on the study of the interaction of medicinal plants and intestinal microbiota for health promotion and disease prevention is relevant and has potential in research. The lack of information on the effect of Salvia officinalis, Melissa officinalis, and Lavandula angustifolia on the intestinal microbiota of white rats fed a high-fat diet aroused interest and indicated the feasibility of conducting a study. We found only a few reports on the study of the antimicrobial and anti-nematode activity of these medicinal plants and their extracts (Zazharskyi et al., 2019a; 2019b; Boyko & Brygadyrenko, 2017, 2021).

Material and methods

The protocol of the study was agreed upon with the Ethics Committee of the Dnipro State Agrarian and Economic University. The studies were carried out on the basis of the clinic and laboratories of this university. In the study, 20 adult outbred laboratory male rats weighing 200 ± 10 g were used. The rats were divided into a control group and three experimental groups of 5 animals in each. The rats were kept in polycarbonate cages with steel lattice lids and feeding recesses, 5 individuals per cage at a temperature of 20–22 °C and relative air humidity of 50–65%. The light regime was 12 hours of light and 12 hours of darkness. Ventilation was carried out in accordance with the regime. The diet of all animals had an excess fat content (3600 kcal/g). The high-fat diet was based on a standard diet (75% grain mixture (corn, sunflower grain, wheat, barley), 8% root crops (potatoes, carrots), 2% meat and bone meal, 2% mineral-vitamin complex) with the introduction of 15% sunflower oil. The control group of animals received a high-fat diet, the experimental groups received a high-fat diet with the addition of crushed dry medicinal plants to the granulated animal feed. The first experimental group was supplemented with a high-fat diet plus 5% crushed dry young shoots of Salvia officinalis, the second – 5% Melissa officinalis, the third group – 5% Lavandula angustifolia. The main components of the diet were crushed in a mill (grain, meat and bone meal, mineral-vitamin complex, dry shoots of medicinal plants) and mixed, then oil was added and granules were made for the entire period of the experiment (30 days). Root crops in the appropriate amount in fresh form were additionally given every day. The animals had free access to food and water (Leshchova & Brygadyrenko, 2023).

To study the qualitative and quantitative indicators of the intestinal microbiota of animals, fecal samples were taken into sterile bottles immediately after slaughter, using the principles of asepsis, the intestine was cut and the contents were taken out.

In a sterile bottle, following asepsis and antisepsis rules guidelines, 1 g of feces was placed and 9 mL of sterile saline was then added. Thus, a tenfold dilution (10⁻¹) was obtained. To prepare the necessary dilutions, 9 mL of sterile saline was used and 1 mL of the contents of the preliminary tube was added. This was done until a dilution of 10⁻⁶ was obtained (Bilan et al., 2022a).

After preparing all the dilutions, 0.1 mL of the solution was taken from each test tube with a sterile pipette and added to a Petri dish with the appropriate elective medium, increasing each dilution by another 10 times. Bifidobacterium agar (HiMedia, India), lactobac agar, Enterococcus agar, Endo’s medium, bismuth sulfite agar, Wilson & Blair medium, Baird-Parker agar, Sabouraud dextrose agar (LTD Farmaktiv, Ukraine), 5% blood agar (Biomerieux, France) were used. Cultivation was carried out in a thermostat at a temperature of 24–37–43 °C for 24–72 hours.

Colonies were counted in all media dishes. CFU/g (colony forming units per 1 g of intestinal content) was calculated and multiplied by the appropriate dilution (Bilan et al., 2022b). Anaerobic conditions for bifidobacteria, lactobacilli, clostridia were achieved in anaerostats (7 L) using GENbox anaer anaeropackets (Biomerieux, France). Control of anaerobism was performed using Anaer Indicator (Biomerieux, France).

Identification and differentiation of individual species of enterobacteria was carried out by determining the enzymatic properties on the media of Hiss, Olkenistsky, Christensen, Simons, etc., tests API 20 RE 20 600, API Staph REF 10 20 500, API 20 E REF 20 100 / 20 160, API 20 NE REF 20 050, API Candida REF 10 500 (Biomerieux, France) taking into account their biological properties according to the Bergey’s Manual of Systematic Bacteriology (1997). Morphological features and tinctorial properties of isolated microorganisms were studied after smear staining by Gram and microscopy with a MICROMedXS-3330 microscope.

Data analysis was carried out using the Statistica 6.0 program (StatSoft Inc., USA). Differences between control and study groups values were determined using the Tukey test, where the differences were considered probable at P < 0.05 (taking into account Bonferroni’s correction).

Results

As a result of the conducted studies, it was established that bacteria of the genera Bifidobacterium and Lactobacillus formed the base of all iso-
lateral microorganisms from the control and experimental groups of rats (14% each). Pathogenic microflora (Shigella and Salmonella) and hemolytic strains of bacteria were not detected in any group. The number of bifidobacteria in animals of the control group mainly reached $10^{10}$ and lactobacilli – $10^{7}$-$10^{9}$ CFU/g, which corresponded to the reference values of laboratory rats’ fecal biopsies. In the same animals’ group, among the facultative microflora representatives, almost 13% of isolates were representatives of the typical Escherichia coli. The number of other microorganisms of the facultative and transient microflora was from 2% to 6% (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Gut microbiota</th>
<th>Reference range</th>
<th>Control</th>
<th>Salvia officinalis</th>
<th>Melissa officinalis</th>
<th>Lavandula angustifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacterium spp.</td>
<td>$10^{-7}$</td>
<td>8.60 ± 0.40</td>
<td>8.20 ± 0.07</td>
<td>7.90 ± 0.55</td>
<td>8.00 ± 0.63</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>$10^{-7}$</td>
<td>8.20 ± 0.20</td>
<td>8.41 ± 0.41</td>
<td>8.88 ± 0.15</td>
<td>7.84 ± 0.26</td>
</tr>
<tr>
<td>Escherichia coli typical form</td>
<td>$10^{-7}$</td>
<td>7.78 ± 0.28</td>
<td>4.64 ± 0.26***</td>
<td>7.08 ± 0.64</td>
<td>4.81 ± 0.85*</td>
</tr>
<tr>
<td>Escherichia coli weakly fermenting form</td>
<td>$&lt;25%$</td>
<td>3.60 ± 0.24</td>
<td>6.51 ± 0.73***</td>
<td>7.55 ± 0.62***</td>
<td>7.26 ± 0.51***</td>
</tr>
<tr>
<td>Escherichia coli non-lactose fermenting form</td>
<td>$10^{5}$</td>
<td>1.34 ± 0.26</td>
<td>1.52 ± 0.43</td>
<td>0.36 ± 0.36</td>
<td>1.11 ± 0.68</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>$10^{-7}$</td>
<td>6.26 ± 0.52</td>
<td>3.25 ± 1.33</td>
<td>3.54 ± 1.49</td>
<td>4.46 ± 0.62</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>$10^{7}$</td>
<td>1.58 ± 0.40</td>
<td>1.20 ± 0.49</td>
<td>0.40 ± 0.40</td>
<td>1.20 ± 0.49</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>$10^{-7}$</td>
<td>3.04 ± 0.07</td>
<td>2.08 ± 0.08</td>
<td>2.78 ± 0.15</td>
<td>2.04 ± 1.61</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>$10^{-10}$</td>
<td>2.80 ± 0.20</td>
<td>1.99 ± 0.52</td>
<td>2.58 ± 0.70</td>
<td>3.08 ± 0.55</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>$10^{-7}$</td>
<td>2.58 ± 0.24</td>
<td>2.65 ± 0.71</td>
<td>2.94 ± 1.81</td>
<td>2.06 ± 1.31</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>$10^{-7}$</td>
<td>1.10 ± 0.45</td>
<td>0.00 ± 0.00*</td>
<td>0.00 ± 0.00*</td>
<td>0.00 ± 0.00*</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>$10^{-7}$</td>
<td>2.99 ± 0.32</td>
<td>4.28 ± 0.37</td>
<td>4.96 ± 0.65</td>
<td>4.09 ± 0.89</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>$10^{-7}$</td>
<td>3.26 ± 0.28</td>
<td>4.23 ± 0.24</td>
<td>4.87 ± 0.24</td>
<td>4.53 ± 0.28</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>$10^{-4}$</td>
<td>1.80 ± 0.49</td>
<td>2.28 ± 0.60</td>
<td>1.93 ± 0.81</td>
<td>0.72 ± 0.72</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>$10^{5}$</td>
<td>3.30 ± 0.19</td>
<td>4.66 ± 0.05***</td>
<td>5.07 ± 0.52*</td>
<td>5.21 ± 0.77***</td>
</tr>
<tr>
<td>Candida albicans spp.</td>
<td>$10^{5}$</td>
<td>2.54 ± 0.22</td>
<td>3.40 ± 0.93</td>
<td>2.06 ± 1.27</td>
<td>4.37 ± 1.10</td>
</tr>
</tbody>
</table>

**Notes:** * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$ compared to the control group of animals using ANOVA with the Bonferroni correction.

It should be noted that no opportunistic enterobacteria of the *Citrobacter* genus were found in all experimental animals that consumed medicinal plants ($P < 0.05$), but an unreliable increase in the number of *Klebsiella* spp. was observed. Eating the flowers of *Salvia officinalis* by rats caused a probable decrease in the number of typical *Escherichia coli* ($P < 0.001$) and an increase in poorly fermenting *E. coli* ($P < 0.01$). The number of *Candida* fungi also increased ($P < 0.001$). However, an unreliable decrease in *Enterococcus* spp., *Proteus* spp., and *Staphylococcus aureus* was recorded.

Addition of *Melissa officinalis* to the diet of model animals did not change the number of typical *Escherichia coli* and reduced the number of poorly fermenting strains of this bacterium type ($P < 0.001$). As with the influence of *Salvia officinalis*, an increase in the number of *Candida* fungi in the intestines of rats was established up to 8% ($P < 0.05$). Against this background, there was unreliable decrease in lactose-negative *Escherichia coli*. *Enterococcus* spp., *Clostridium* spp., *Candida* spp., *Proteus* spp., and *Staphylococcus aureus* were supplemented.

Feeding rats with *Lavandula angustifolia* remarkably reduced the number of *Enterococcus* spp., *Proteus* spp., *Pseudomonas* spp. and increased the number of *Staphylococcus aureus, S. epidermidis, Candida albicans*.

Thus, medicinal plants in the diet of laboratory animals significantly changed the quantitative ratio of *E. coli* with normal and altered enzymatic properties. In all experimental groups, it was not possible to isolate opportunistic enterobacteria of the genus *Citrobacter* ($P < 0.05$); in the experimental groups to which *Salvia officinalis* and *Lavandula angustifolia* were supplemented, an increase in the number of *Candida* fungi was observed ($P < 0.05$ and $P < 0.001$, respectively).

Addition of medicinal plants (*Salvia officinalis, Melissa officinalis, Lavandula angustifolia*) to the diet of laboratory animals significantly changed the quantitative ratio of *E. coli* with normal and altered enzymatic properties. The number of typical *E. coli* in rats fed with *S. officinalis* and *L. angustifolia* decreased by 1.7 and 1.6 times; on the contrary, the low-fermenting form of *E. coli* increased by 1.8-2.1 times in rats fed any of the medicinal plants compared to animals that consumed a high-fat diet.

No opportunistic enterobacteria of the genus *Citrobacter* were detected ($P < 0.05$), but a significant increase in the number of *Candida* fungi was observed ($P < 0.05$ and $P < 0.001$). Among other representatives of the intestinal microbiota, no reliable changes were found. Thus, the addition of medicinal plants (*Salvia officinalis, Lavandula angustifolia, Melissa officinalis*) to the high-fat diet of laboratory animals significantly changed the quantitative ratio of *E. coli* with normal and altered enzymatic properties in the intestinal contents.

### Discussion

In recent years, the importance and value of medicinal plants in the treatment of various diseases has been actively discussed. Medicinal plants are naturally endowed with valuable biologically active compounds, which are the basis of traditional medicine. Medicinal plants are reported to be widely used in the treatment of obesity, diabetes, hypertension, cardiovascular diseases, nephropathy, and to stimulate digestion (Surh, 2003; Mohamed et al., 2011; Saad et al., 2013; Sipos et al., 2021; Mattera et al., 2023). Currently, plant-based dietary supplements are becoming increasingly popular and widespread, but information on the risks associated with their use is limited and conflicting (Said et al., 2011; Zaid et al., 2015; Ramadan & Ibrahim, 2021). In addition, the chemical composition is not completely known, the active compounds have not been fully established, and the mechanisms of their action have not been clarified (Seol et al., 2010; Sani et al., 2020). Therefore, herbal medicines for clinical use have not gained wide recognition.

Back in 1959, Masek & Fabry (1959) first reported the effect of a “high-fat diet” on macroorganism obesity. Other researchers (Ahren et al., 1999; Lingohr et al., 2002) found that a high fat content in the diet contributes to hyperglycemia, the formation of insulin resistance in the body, affects muscle and liver physiology, and insulin signal transmission. The consequence of diabetes and obesity can be cardiovascular diseases, obesity, oncology, the number of cases of which is increasing annually worldwide (Saad et al., 2022).

Previous studies have established that the medicinal plants studied in this experiment have a significant effect on the body weight gain of laboratory rats. Lieschova et al. (2021) in experiments on rats which were kept for 30 days on a high-fat diet and supplemented with two types of *Salvia* established a sharp increase in the body weight of the animals when *S. officinalis* was added and a slowdown in weight gain when *S. sclarea* L. was added to the diet, compared to the control. It should be noted that at the same time in the group of rats that consumed *S. officinalis*, a decrease in the need for feed was observed, compared to the control group and animals that consumed *S. sclarea*. Supplementing the diet of model animals with *Lavandula angustifolia* dried herbs and *Melissa officinalis* resulted in intensive weight gain with lower feed intake compared to a control group that only consumed a high-fat diet. Dry crushed shoots of

Salvia officinalis when added to a high-fat diet caused a significant decrease in the physical activity of experimental rats. Addition of medicinal plants to the diet led to an increase in average daily weight gain, significantly and reliably when lavender and lemon balm were consumed, less significantly and unreliable when vetex was consumed (Lieschova & Brygadyrenko, 2021).

Hancianu et al. (2008) report on the use of Melissa officinalis for the treatment of gastric diseases. The authors established a higher degree of antibacterial action of lemon balm oil than lavender oil against gram-positive strains of microorganisms. In addition, the high activity of these oils against Candida albicans and the ineffectiveness of lemon balm oil against gram-negative bacteria have been found. Abdellatif et al. (2014) published a higher degree of antibacterial action of lemon balm oil against gram-negative bacteria (P < 0.05), but an increase in the number of genus Candida fungi was observed (P < 0.05 and P < 0.001). This work was supported by the Ministry of Education and Science of Ukraine (grant 0122/200975).


