Hematological parameters and protein metabolism in the blood of pregnant rats under the effect of vanadium citrate

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Klymets, H. V., Iskra, R. Y., & Svarchevska, O. Z. (2021). Hematological parameters and protein metabolism in the blood of pregnant rats under the effect of vanadium citrate. Regulatory Mechanisms in Biosystems, 12(1), 145–152. doi:10.15421/02212

Dose-dependent changes in protein metabolism in the blood and hematological parameters of pregnant rats under the effect of vanadium citrate are presented in the article. The animals were divided into five groups: group I – non-pregnant females, II – pregnant females consuming pure water without additives, III, IV, V – females which during the mating and pregnancy period received the solution of vanadium citrate at concentrations of 0.03, 0.125 and 0.50 μg V/mL water. The research findings show that in pregnant animals of group II, the level of urea and alkaline phosphatase activity increased, meanwhile aspartate aminotransferase activity decreased, as compared to the non-pregnant females of group I. The levels of total protein and albumin decreased; however, the content of β-globulins increased in the pregnant animals of group II, as compared with that in group I. Also, in the rats of group II, there was a decrease in hemolysis time, total content of erythrocytes and hemoglobin, the content of old and mature erythrocytes, while the content of young erythrocytes increased, as compared to group I. The platelet content and thrombocrit in rats of group II increased in comparison with group I. The content of leukocytes and lymphocytes in pregnant animals of group II decreased, while the content of granulocytes increased, in contrast to non-pregnant rats. Under the effect of vanadium citrate at concentrations of 0.03–0.50 μg V/mL, there was a significant increase in the maximum number of prohemolysed erythrocytes, the time of maximum hemolysis was delayed by 0.4–0.6 min, as compared with the pregnant rats of group II. This did not affect the time of total hemolysis in rats of groups III and V, as compared with the pregnant animals in group II. Under the effect of vanadium citrate, an increase in the content of young erythrocytes was observed, as compared with group II. The hemoglobin content decreased at the concentration of 0.125 μg V/mL, while at the concentration of 0.50 μg V/mL, it increased, as compared to the pregnant animals of group II. Also, under the effect of vanadium citrate there was a decrease in the mean hemoglobin concentration in the erythrocyte. In pregnant animals fed with vanadium citrate solutions, the platelet content and thrombocrit, the relative width of platelet distribution by volume decreased, as compared with the pregnant rats of group II. The content of leukocytes, lymphocytes and granulocytes under the effect of vanadium citrate increased, as compared with the pregnant animals in group II. Under the effect of vanadium citrate at the concentration of 0.03 μg V/mL, the level of albumin, creatinine and aspartate aminotransferase activity increased in blood plasma in comparison with group II. Meanwhile, at the concentration of 0.125 μg V/mL, the relative content of γ-globulins and aspartate aminotransferase activity increased, alkaline phosphatase activity and urea level decreased in comparison with group II. However at the concentration of 0.50 μg V/mL, the relative α- and γ-globulins content and aspartate aminotransferase activity increased, at the same time, the relative β-globulins content and urea level decreased in comparison with group II. Therefore, vanadium citrate normalizes the indicators of protein metabolism during pregnancy, thus it can be considered as a potential dietary drug for the pregnant.

Keywords: rats; pregnancy; hematology; protein metabolism; vanadium citrate.

Introduction

To ensure the growth and development of the fetus, the mother’s body undergoes compensatory changes in almost all systems, which leads to a state of unstable stress balance at homeostasis. In particular, pregnant women have anatomical, biochemical, physiological and endocrine changes that are necessary to support and regulate embryonic development (Salisu, 2009). Some authors point to certain changes in the protein and lipid profile of blood, kidney and liver dysfunction, and carbohydrate metabolism disorder in the body of pregnant women (Sánchez, 2011; Kolla et al., 2012). Also, during pregnancy many hematological changes take place (Sanci et al., 2017). In particular, erythrocytes change shape and size more often, and are more prone to abnormalities, in contrast to their condition in non-pregnant animals. These changes occur independently of the content of iron, folic acid and vitamin B12 (Lesesve, et al., 2019). An increase in mean corpuscular volume is common during pregnancy. This may be due to an increase in the content of reticulocytes in the blood (Lurie, 1993). It is important to establish and maintain a positive pregnancy outcome, which implies the state of selective immune tolerance, immunosuppression and immunomodulation in the presence of strong antimicrobial immunity. The mammalian immune system is adapted to these needs. It regulates the reduction of potentially dangerous T-cell-mediated immune responses activating some components of the innate immune system, including monocytes and neutrophils. This unique dysregulation between different components of the immune system plays a central role in the mother’s adaptation to pregnancy (Luppi et al., 2003). The proper functioning of the monocyte-macrophage system, an important unit of innate immunity, ensures the normal course of pregnancy. Normal pregnancy is also associated with phenotypic and metabolic changes in granulocytes. However, the innate immune response is not maximally activated during normal pregnancy (Naccasha et al., 2001). Therefore, the use of dietary drugs, based on trace elements that will strengthen the immune system in pregnant women, is necessary and will stop the occurrence of complications. It is known that trace elements act by participating in the

Regul. Mech. Biosyst., 2021, 12(1)
transmembrane transport of immunocompetent cells. Since vanadium and its derivatives have antioxidant properties, their use results in an increase in antioxidant enzymes activity, reduces the side effects of statins on the heart tissues, liver and kidneys, as well as on the function of these organs (Crans et al., 2018). Due to the ability of vanadium to pass through the placental barrier, it can affect the development of the fetus and accumulate in its skeleton (Aureliano et al., 2014). In animal experiments, the deficiency of vanadium caused deformation of the skeleton extremities and their edema (Haenlein & Anke, 2011; Aureliano et al., 2018), disorders of protein metabolism and hematological indicators, occurrence of fermentsopathies, impairment of reproduction, growth and development of the organism. In the body, vanadium compounds act as cofactors that modulate enzyme activities, play an important role in metabolic processes, including glucose metabolism in the liver, glycogen, cholesterol, and triacylglycerols metabolism (Gunasinghe & Kim, 2018). They interact with amino acids, in particular with L-cysteine, L-histidine and L-glutamic (Levina et al., 2015).

Vanadium simulates some effects of insulin in adipocytes due to stauros- porin-susceptible cytosolic protein tyrosine kinase (CytPTK) (Mohammadi & Yazdanparast, 2010; Aureliano & Ohlin, 2014; Gunasinghe & Kim, 2018). The studied element is involved in numerous physiological responses, particularly as an inhibitor of phosphate-mobilizing enzymes, such as tyrosine phosphatase, ribonuclease. ATPase (Crans et al., 2018). Vanadium enhances phosphorylation of proteins, inhibits intracellular protein tyrosine phosphatase, which causes dephosphorylation of the insulin receptor β-subunit, which contributes to the growth of numerous metabolic effects of insulin (Gunasinghe & Kim, 2018). This element is able to form complexes with proteins. The protein compounds with vanadium have similar properties to compounds with ferrum. In particular, hemoglobin is a protein that binds the cytoplasmic ions of vanadyl (Cantdium have similar properties to compounds with ferum. In particular, vanadium caused deformation of the skeleton extremities and their edema (Aureliano et al., 2014). In animal experiments, the deficiency of vanadium caused deformation of the skeleton extremities and their edema (Haenlein & Anke, 2011; Aureliano et al., 2018), disorders of protein metabolism and hematological indicators, occurrence of fermentsopathies, impairment of reproduction, growth and development of the organism. In the body, vanadium compounds act as cofactors that modulate enzyme activities, play an important role in metabolic processes, including glucose metabolism in the liver, glycogen, cholesterol, and triacylglycerols metabolism (Gunasinghe & Kim, 2018). They interact with amino acids, in particular with L-cysteine, L-histidine and L-glutamic (Levina et al., 2015).

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Preparation of gel for electrophoresis

Table 1

| Component                  | 7.5% separating gel, cm | Concentrating gel, cm |
|----------------------------|-------------------------|-----------------------|
| Solution of acrylamide     | 4.130                   | 0.500                 |
| 1.5 M Tris                 | 4.125                   | 0.500                 |
| Water                      | 13.600                  | 3.800                 |
| Persulfate ammonium, 10%   | 0.136                   | 0.100                 |
| Tetramethylenediamine, ready to use | 0.014 | 0.005 |
| HCl concentrated           | 0.000                   | 0.030                 |

Solution (25 cm³) – add MBA (0.35 g) to AA (0.65 g) and make it up to 25 cm³ with distilled water: 1.5 M tris (25 cm³) – add HCl concentrated (0.60 cm³) to tris (4.54 g) make it up to 25 cm³ with distilled water (pH = 8.8). PSA is prepared on the day of the study. PSA (10%) – make up PSA (0.10 g) to 1 cm³ with distilled water.

Preparation of the separating gel: according to the table, add AA solution + 1.5 M Tris and water to the flask, stir well, then add PSA (10%) and TEMED, stir again and pour between the cassettes. Pour a little distilled water on the top to level the surface. The polymerization time is 30–45 minutes. After polymerization, remove water with filter paper.

Preparation of the concentrating gel: prepare quickly to prevent polymerization; according to the table, add AA solution + 1.5 M tris + water to the flask, stir well, quickly add PSA (10%) and TEMED, stir again and pour over the separating gel. Put a comb with an appropriate number of holes for samples on the top. The polymerization time is 20–30 minutes. After polymerization of the concentrating gel, remove the comb. Put 0.04 cm³ of prepared samples in the formed holes. Add a little electrode buffer on the top of the samples. Put the cassette with the gel in the chamber. Fill the chamber with electrode buffer so that the buffer covers the sample holes. Connect the electrodes to the power device (UVIP) to supply current. The example for a cassette for 20 samples: for the concentrating gel 20–30 mA at 220 V, for the separating gel 40–50 mA at 220 V. When the front of the samples reaches the separating gel (visually visible), switch to 50 mA. When the front of the samples reaches the bottom of the cassette, turn off the UVIP. Carefully peel the gel from the glass cassettes and transfer it to the baths for painting. Staining: preparation of stain for fixation: dilute amido black 10B (0.01 g) in 6% acetic acid (100 cm³). Stain for 20–30 minutes. Washing: wash the gel in 6% acetic acid until complete discoloration of the gel. The gel is ready for further work.

Resistance of erythrocytes to the action of acid hemolytic (Gitelezov & Terskov, 1959). The principle of the method is based on the use of a direct relationship between the time of hemolysis of erythrocytes and the action of hemolytic used for their hemolysis. The analysis of hemolytic erythrograms was performed according to such parameters as: time of maximum hemolysis (min); the percentage of hemolyzed erythrocytes at the time of maximum hemolysis (% max); duration of total hemolysis (min). Washed erythrocytes were diluted with 0.150 mmol NaCl in a ratio of 1:1000. The received suspension was diluted to achieve extinction in the range of 0.10 g to 1 cm³ with distilled water.

Preparation of gel for electrophoresis

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0.700-0.750 at a wavelength of 630 nm. The studies were performed in a thermostat cuvette to maintain a constant temperature of the samples, as the hemolysis kinetics depends on the temperature of the solution. The optimal temperature for the study is +24 °C. Erythrocyte suspension 2 mL was put to the cuvette, and a similar volume of hemolysing solution 0.004 n HCl prepared in 0.150 mmol NaCl solution was added. From the moment the hemolytic was added, changes in extinction at a wavelength of 630 nm were recorded every 30 s. Monitoring was performed until the complete cessation of changes in extinction values. Saline 2 mL was placed in the control cuvette, and 2 mL 0.004 N HCl solution prepared on 0.150 mm NaCl was added. The percentage distribution of erythrocytes by the percentage of hemolyzed erythrocytes and the time of hemolysis. Population distribution of erythrocytes in the density gradient of sucrose (Table 2). Firstly, erythrocytes were washed by 0.9% NaCl solution. The obtained erythrocytes were diluted with the saline in a ratio of 1:10. Erythrocytes from different age populations were separated by the cell fractionation method in a seven-step density gradient of sucrose. Then, 0.5 mL of erythrocytes diluted in a sucrose gradient were applied to the column.

Table 2
Preparation of sucrose gradients

| Concentration of sucrose gradients, % | Content of sucrose gradients | sucrose, g | saline, mL |
|--------------------------------------|-----------------------------|-----------|------------|
| 30                                   | 15                          | 50        | 30         |
| 26                                   | 13                          | 50        | 22         |
| 22                                   | 10                          | 50        | 18         |
| 18                                   | 9                           | 50        | 14         |
| 14                                   | 7                           | 50        | 10         |
| 10                                   | 5                           | 50        | 6          |
| 6                                    | 3                           | 50        | 6          |

After applying erythrocytes to the column, it was rotated by 75°, and 2 mL of each sucrose solution, starting with the solution with the highest density of 30% and then respectively: 26, 22, 18, 14, 10 and 6%, was slowly applied to the wall of the column. The column was again fixed vertically. Thus, seven cell fractions were obtained. Each fraction of erythrocytes was collected in a separate tube and diluted with the saline to 10 mL. Fractions of old erythrocytes (1–3) were obtained first, mature erythrocytes (4–5) followed, and fractions of young erythrocytes (6–7) went last. Photometer measurement was performed at 520 nm. All extinctions were put together to get 100%. To calculate the percentage of each fraction, they were related to E.

Statistical processing of the survey results was performed using the computer software package Statistica 8 (StatSoft Inc., USA, 2014). The arithmetic mean value and the standard deviation of the arithmetic mean (x ± SD) were determined. The differences between the values in the control and experimental groups were determined using the ANOVA, where the differences were considered significant if P-value less than 0.05 (with Bonferroni correction).

Results

Our results showed that the total protein in the blood plasma of the pregnant females in group II decreased by 9.7%, as compared to group I (Table 3). The studies showed an increase in the mean levels of β-globulins in pregnant females of group II (10.7%), as compared to non-pregnant rats, which may be due to their physiological state. Also, in the females of group II, there were no changes in the α- and γ-globulins fraction. The total protein reduction in group III by 8.3% was observed, as compared to group I. Stimulation of the body immune system by the vanadium compound is caused by the γ-globulins level growth in the animals of group IV by 4.7% and in those of group V by 9.7%, as compared to the animals of group II. The fraction of α-globulins in the blood plasma of the females in group V increased by 15.9% as compared to the animals of group II.

The performed studies showed that in the pregnant females of group II, the growth of urea content reached 27.3%, as compared to the non-pregnant females in group I (Table 4).

Table 3
Protein plasma spectrum of blood of pregnant female rats under the effect of vanadium citrate (x ± SD, n = 7)

| Number of group | Animal groups | Total protein, g/L | Albuminas, % | Globulins, % |
|-----------------|---------------|--------------------|--------------|--------------|
|                 |               |                    | α            | β            | γ            |
| I Non-pregnant  | 60.24 ± 1.00  | 26.17 ± 0.63       | 24.60 ± 0.44 | 26.10 ± 0.93 | 23.14 ± 0.69 |
| II Pregnant     | 54.36 ± 0.72  | 23.73 ± 0.78       | 23.93 ± 1.13 | 28.90 ± 0.48 | 23.40 ± 0.23 |
| III Pregnant + 0.03 μg/Vml | 55.26 ± 1.93 | 26.61 ± 0.32 | 21.80 ± 0.32 | 27.44 ± 0.49 | 24.16 ± 0.57 |
| IV Pregnant + 0.125 μg/Vml | 61.20 ± 3.53 | 23.90 ± 1.71 | 23.88 ± 0.99 | 28.24 ± 0.53 | 24.50 ± 0.34 |
| V Pregnant + 0.50 μg/Vml | 65.59 ± 5.48 | 23.12 ± 1.35 | 27.74 ± 0.47 | 24.15 ± 0.82 | 25.67 ± 0.27 |

Note: *P < 0.05; **P < 0.01; ***P < 0.001, reliable for group I; "#P < 0.05; ""P < 0.01; "##P < 0.001, reliable for group II.

Table 4
Biochemical parameters of blood plasma in pregnant female rats which consumed vanadium citrate (x ± SD, n = 7)

| Number of group | Animal groups | Creatinine, kmol/L | Urea, mmol/L | Aspartate aminotransferase, Units/L | Alkaline aminotransferase, Units/L | Alkaline phosphatase, Units/L |
|-----------------|---------------|-------------------|-------------|-----------------------------------|-----------------------------------|-------------------------------|
| I Non-pregnant  | 56.25 ± 1.54  | 0.216 ± 0.005     | 178.19 ± 0.78 | 83.40 ± 0.77 | 313.4 ± 11.0                     |
| II Pregnant     | 51.35 ± 1.69  | 0.275 ± 0.005     | 140.00 ± 1.28 | 76.60 ± 4.17 | 500.4 ± 17.7                     |
| III Pregnant + 0.03 μg/Vml | 56.61 ± 0.87 | 0.288 ± 0.017**  | 146.80 ± 0.66** | 74.93 ± 0.32 | 460.2 ± 20.3                     |
| IV Pregnant + 0.125 μg/Vml | 54.63 ± 2.30 | 0.229 ± 0.002**  | 163.20 ± 0.13** | 83.50 ± 2.43 | 394.2 ± 39.8**                    |
| V Pregnant + 0.50 μg/Vml | 53.08 ± 1.03 | 0.211 ± 0.004**   | 172.81 ± 3.42** | 77.90 ± 4.75 | 379.4 ± 81.7                     |

Note: see Table 3.

A decrease in ASAT and ALAT activity by 21.4% and 8.2% respectively was observed in the blood plasma of animals in group II in comparison with group I. As a result of the study, it was found that in the rats of group II the AP activity increased by 62.5%, as compared to that in group I. Under the condition of feeding rats in experimental groups with vanadium citrate, the level of creatinine in the animals of group III increased by 10.3%, while the level of urea decreased in the blood plasma of the animals in groups IV and V by 16.7% and 23.3%, respectively, as compared to the pregnant females of group II which consumed water only. It was found that ASAT activity increased in rats of groups III, IV and V by 4.8%, 16.6% and 23.4%, respectively, as compared to group I.

A significant 9.0% increase in ALAT activity was seen in the IV group compared with non-pregnant animals in group I. At the same time, the AP activity decreased in animals of group IV by 22.6%, as compared to the pregnant females of group II which consumed water only. The studies did not show significant differences in hemolysis of erythrocytes in non-pregnant and pregnant rats (groups I and II), but the time of hemolysis in pregnant rats decreased by 0.4 min, as compared with non-pregnant, and was 8.4 min (Fig. 1, Table 5).

In groups III and IV of pregnant animals fed with vanadium citrate, there was a significant increase in the maximum number of prohemolized erythrocytes by 5.6% and 4.8%, as compared with the pregnant rats in group II. In the animals of group IV, there was a decrease in the total hemolysis time as compared with non-pregnant rats (group I) and pregnant animals (group II). And in groups III, IV and V, the time of maximum hemolysis was delayed by 0.4, 0.5 and 0.6 min, respectively, compared with group II of pregnant rats, and was 3.4, 3.5 and 3.6 minutes. This did not affect the time of total hemolysis in the rats of groups IV and
V, as compared with the pregnant animals in group II. Besides, this indicates that vanadium citrate at a concentration of 0.03 and 0.50 μg V/mL has the most favourable effect on the resistance of erythrocyte membranes to acid hemolytic. The erythrograms obtained as a result of the experiment are single-vertex, which indicates the normal erythropoiesis and the absence of pathologies in the formation of red blood cells. Erythrograms of the animals of experimental groups III, IV and V are slightly shifted to the right (Fig. 1), as compared with groups I and II, which indicates the regenerative effect of vanadium citrate on erythropoiesis and the appearance of young erythrocytes in pregnant animals consuming sample solutions.

Under the effect of vanadium citrate, there was a slight increase in the content of old and mature erythrocytes by 10.6% and 3.8%, respectively, while the content of young erythrocytes increased by 14.5%, as compared with the non-pregnant animals of group I (Table 6).

In the pregnant animals, fed with vanadium citrate solutions, the number of erythrocytes and their functional properties in the peripheral blood of pregnant rats under the effect of vanadium citrate (x ± SD, n = 7) increased in group III by 11.7%, IV – 9.7% and V – 14.5%, as compared with the non-pregnant animals in group I.

In particular, we found a decrease in erythrocyte content in the pregnant rats of group II by 14.5%, as compared with the non-pregnant animals of group I. The concentration of hemoglobin in the blood of the animals of group II decreased by 9.0%, as compared with the non-pregnant animals of group I (Table 7). The mean hemoglobin concentration in the erythrocytes of the animals of group II increased slightly by 3.1%, as compared with the non-pregnant animals of group I. The content of erythrocytes increased by 5.1% and 6.1% in the animals of groups III and IV, respectively, which consumed vanadium citrate, whereas the changes in the study group V were insignificant, as compared with the pregnant group of pregnant animals. The decrease in the mean hemoglobin concentration in the erythrocytes in group IV decreased by 5.1%, in group V it increased by 5.8%, as compared with the pregnant animals of group II. Also, under the effect of vanadium citrate there was a decrease in the mean hemoglobin concentration in the erythrocytes in group III by 6.4%, IV – 3.3%, while in group V there was a slight increase, as compared with the pregnant rats of group II.

In pregnant animals of group II, there was an increase in platelet count by 14.3%, as compared with the control (Table 9). The value of thrombocrit in the rats of group II increased by 88.8%, as compared with the non-pregnant animals of group I. This is due to an increase in platelet count in the pregnant rats of group II. The relative platelet distribution width by volume in the pregnant animals of group II decreased by 4.0%, as compared with the non-pregnant females of group I.

In the pregnant animals, fed with vanadium citrate solutions, the platelet count increased, in group III – by 25.7%, IV – by 37.9% and V – by 21.3%, respectively, as compared with the pregnant rats in group II. Platelets also decreased in three experimental groups: III – by 28.0%, IV – by 43.7% and V – by 21.0%, as compared with the pregnant females in group II. In group III, the relative platelet distribution width by volume decreased by 3.2%, and in group V increased by 1.3%, as compared with group II.

### Table 5

| Number of group | Animal group | Maximum hemolysis, min | Hemolysis time, min | Maximum hemolysis, % |
|-----------------|--------------|------------------------|---------------------|----------------------|
| I               | Non-pregnant | 3.10 ± 0.10            | 8.80 ± 0.12         | 35.35 ± 1.30         |
| II              | Pregnant     | 3.00 ± 0.12            | 8.40 ± 0.10         | 33.89 ± 0.45         |
| III             | Pregnant + 0.03 μg V/mL | 3.40 ± 0.40          | 8.40 ± 0.44         | 39.47 ± 2.26         |
| IV              | Pregnant + 0.125 μg V/mL | 3.50 ± 0.21           | 8.10 ± 0.14         | 38.74 ± 3.42         |
| V               | Pregnant + 0.50 μg V/mL | 3.60 ± 0.13           | 8.30 ± 0.36         | 31.30 ± 3.70         |

### Table 6

| Number of group | Animal group | Percentage of erythrocyte populations, % |
|-----------------|--------------|-----------------------------------------|
|                 |              | old, %                                  |
| I               | Non-pregnant | 17.52 ± 0.34                           |
| II              | Pregnant     | 6.90 ± 0.16                             |
| III             | Pregnant + 0.03 μg V/mL | 8.70 ± 0.25   |
| IV              | Pregnant + 0.125 μg V/mL | 5.42 ± 0.20   |
| V               | Pregnant + 0.50 μg V/mL | 9.50 ± 0.21   |

### Table 7

| Number of group | Animal groups | Erythrocytes, 1×10⁶/L | Hemoglobin, g/L | Hematocrit, % | Mean corpuscular volume, μm³ | Mean corpuscular hemoglobin in absolute quantities, pg | Mean corpuscular hemoglobin concentration, mg/dL |
|-----------------|---------------|-----------------------|----------------|----------------|-------------------------------|-------------------------------------------------|----------------------------------|
| I               | Non-pregnant  | 7.34 ± 0.25           | 150.5 ± 3.2    | 0.34 ± 0.023  | 46.40 ± 3.14                  | 20.50 ± 2.27                                 | 442.00 ± 5.22                     |
| II              | Pregnant     | 6.27 ± 0.30           | 140.3 ± 1.3    | 0.31 ± 0.014  | 49.10 ± 2.52                  | 22.37 ± 1.37                                 | 455.50 ± 1.72                     |
| III             | Pregnant + 0.03 μg V/mL | 6.59 ± 0.16 | 137.5 ± 2.4   | 0.32 ± 0.010  | 48.95 ± 2.57                  | 20.85 ± 1.75                                 | 426.50 ± 2.70                     |
| IV              | Pregnant + 0.125 μg V/mL | 6.65 ± 0.16 | 133.2 ± 6.1   | 0.33 ± 0.024  | 47.15 ± 1.76                  | 21.97 ± 1.64                                 | 440.50 ± 7.06                     |
| V               | Pregnant + 0.50 μg V/mL | 6.15 ± 0.28   | 174.0 ± 4.6   | 0.32 ± 0.017  | 52.00 ± 3.64                  | 23.93 ± 2.00                                 | 460.75 ± 3.31                     |

Note: see Table 3.

### Fig. 1

Acid erythrograms (after Gietlzon) in the blood of pregnant female rats under the effect of vanadium citrate (x ± SD, n = 7)

![Image of erythrograms](image-url)
The ten-bulins produced by the lymphoid tissue in response to antigenic stimulation are signals of the state of this organ (Alberghina et al., 2010). According to the literature, reduced albumin concentration during pregnancy affects liver function and the immune system functioning (Alberghina et al., 2010).

The concentration of leukocytes and their fractions in the peripheral blood of pregnant rats under the action of vanadium citrate (x ± SD, n = 7)

| Number of group | Animal groups | Leukocytes, 1×10^9/L | Lymphocytes, 1×10^9/L | Monocytes, 1×10^9/L | Granulocytes, 1×10^9/L |
|----------------|---------------|----------------------|-----------------------|---------------------|------------------------|
| I              | Non-pregnant  | 19.20 ± 0.77         | 14.10 ± 0.19          | 2.40 ± 0.27         | 2.75 ± 0.21            |
| II             | Pregnant     | 13.95 ± 0.33         | 9.10 ± 0.13           | 1.77 ± 0.18         | 4.39 ± 0.35            |
| III            | Pregnant + 0.03 μg V/mL | 15.20 ± 0.25        | 9.75 ± 0.26           | 2.15 ± 0.16         | 3.30 ± 0.24            |
| IV             | Pregnant + 0.125 μg V/mL | 16.45 ± 0.57       | 11.25 ± 0.21          | 2.35 ± 0.26         | 1.85 ± 0.20            |
| V              | Pregnant + 0.50 μg V/mL | 15.00 ± 0.54        | 11.57 ± 0.65          | 1.98 ± 0.14         | 2.30 ± 0.23            |

Note: see Table 3.

The content of total leukocytes and lymphocytes, including in the pregnant animals of group II decreased by 27.3% and 35.5%, respectively, while the concentration of γ-globulins increased by 59.6%, as compared with the control group I (Table 9). Under the action of vanadium citrate, the content of leukocytes increased in group III by 9.0%, in group IV – by 18.0% and in group V – by 7.5%; the content of lymphocytes also increased in the experimental groups: in III – 7.1%, IV – 23.6% and in V – by 27.2%, as compared with the pregnant animals of group II. The content of granulocytes in the pregnant animals consuming vanadium citrate solutions decreased in group III by 25.8%, IV – 57.9% and in V – by 47.6%, as compared with the pregnant animals of group II.

**Discussion**

Blood plasma proteins carry out important functions: nutrition, pH maintenance, osmotic balance, regulation of cellular functions, transport of substances, reserve of amino acids (Dai et al., 2017). Pregnancy affects the expression of protein in the maternal plasma of blood and urine, manifested by quantitative differences in its content (Kolla et al., 2012; Gloria et al., 2018). The total protein in the blood plasma of the pregnant rats (Table 3) decreased, which is related to a physiological adaptation to pregnancy (Faught et al., 1995). Quantitative determination of albumins and globulins is essential for the diagnosis of diseases, including the problems of the liver and the immune system functioning (Alberghina et al., 2010).

The concentration of α-globulins in the blood plasma of the females at concentrations 0.50 μg V/mL increased. Its content growth in the blood may be a sign of increased activity of kininogen and plasmin, the normalizing effect of vanadium on the antioxidant defense system, the ability of this microelement to reduce the number of free radicals in the blood and to inhibit the lipids peroxide oxidation processes (Gloria et al., 2018). At the concentration of 0.50 μg V/mL, a reduction of the β-globulin fraction was observed, which probably indicates the ability of vanadium to affect ferrum transport in iron proteins and possibly delay the onset of iron deficiency anemia (Sánchez et al., 2010).

The level of urea and creatinine in the blood is important for the diagnosis and understanding of the intensity of the pathological processes in the body, as well as for the assessment of the applied corrective therapy efficacy (Table 4). The level of albumin in the pregnant rats’ blood performs a marker function and may indicate kidney disorder. The observed increase in the level of urea in the blood plasma of pregnant females is often due to the changes in renal function caused by the increase in urine production and its excretion (Mohammadi & Yazdanparast, 2010). Creatinine, as a urea metabolite, is an intermediate of muscle metabolism, and the direct correlation between the level of this metabolite and muscle mass is indicated by Baba et al. (2017). In the females of group II, there is a tendency to the decreased level of creatinine, compared to group I, which is also a result of the increased kidney function and the total blood volume growth in females during pregnancy. This intensifies urination and the increased excretion of creatinine with urine, which results in an inverse correlation between the content of urea and creatinine.

Studies of the ASAT and ALAT activity in pregnant females permit one to detect possible heart and liver complications. A decrease in ASAT and ALAT activity in the blood plasma of pregnant animals in group II may be caused by the reduced levels of B6 vitamin in pregnant animals. Grown of the AP activity in pregnant animals may be due to intensified synthesis of its isoenzymes in the fetal tissues, the growth of bone tissue and the placenta development (Crans et al., 2018; Gloria et al., 2018). It is known that vanadium has hepatoprotective effect (Mohammadi & Yazdanparast, 2010), it causes stabilization of the ASAT and ALAT activity, normalizes the urea and creatinine levels. Alkaline phosphatase is a vanadium-sensitive phospho-hydrolyase. According to the literature data, vanadium has the ability to inhibit enzymes-phosphatases, which are important for the phosphorylation of proteins in the process of osteoblast differentiation (Haemen & Anke, 2011; Crans et al., 2018).

The studies did not show significant differences in hemolysis of erythrocytes in non-pregnant and pregnant groups (I and II), but the time of hemolysis in pregnant rats decreased by 0.4 min, as compared with non-pregnant, and was 8.4 min (Fig. 1; Table 5). The erythrograms obtained as a result of the experiment are single-vertex, which indicates the normal erythrotopiosis and the absence of pathologies in the formation of red blood cells. Erythrograms of the animals of experimental groups III, IV and V are slightly shifted to the right (Fig. 1), as compared with groups I and II, which indicates the regenerative effect of vanadium citrate on erythrocytes in non-pregnant and pregnant females.
erethropoiesis and the appearance of young erythrocytes in pregnant animals consuming sample solutions. The identified differences in the resistance of erythrocytes to the action of acid hemolytic can be explained on the basis of the properties of the structure of the erythrocyte membrane, erythrocyte life expectancy, changes in metabolic processes in these cells and the effect of vanadium citrate (Dudlo et al., 2016). Vanadium in complex compounds has a direct effect on the membrane organization of lipids. This is one of the possible mechanisms of enhancing the action of insulin. Also, it is known that vanadium is insulin-mimetic. Such changes in the organization of lipids contribute to the distribution of insulin receptors and other receptors on the membrane microdomains, which contributes to their (receptors) optimal functioning. Therefore, the use of vanadium citrate affects the resistance of erythrocyte membranes to hemolytic action (Roess et al., 2008).

The lifespan of erythrocytes in pregnant rats is shorter than in non-pregnant rats (Table 6). This causes an increase in the content of young erythrocytes in pregnant females. The reduced lifespan of erythrocytes can be explained by the fact that erythrocytes, formed under the conditions of enhanced erethropoiesis or increased metabolic rate, accelerate the aging process. This indicates a reduction in the lifespan of erythrocytes in late pregnancy, may contribute to a better understanding of increased erythropoiesis, on the one hand, and decreased hemoglobin, on the other hand, which often occurs in late pregnancy. Also, the increase in reticulocyte content in pregnant rats is due to organogenesis in the fetus (Mizoguchi et al., 2010). In our studies, a decrease in erythrocytes was observed in the animals of group II, which is also a consequence of the increase in the content of reticulocytes during the physiological pregnancy of the animals. These results indicate the ability of vanadium citrate to increase reticulocyte content at all investigated concentrations (Hogan, 2000). Studies by other authors showed a two-phase increase in reticulocytes under the effect of the compound vanadium – sodium orthovanadate (Aguirre et al., 2005).

During physiological pregnancy, the formation of erythrocytes and erethropoietin increases, while the mass of erythrocytes per unit of body-weight remains unchanged throughout pregnancy. Hemoglobin and hematocrit constantly decrease until the third trimester of pregnancy. Erythrocyte life expectancy decreases during normal pregnancy due to “emergency hematopoiesis” in response to elevated erethropoietin levels (Lurie, 2000). However, the function of hematopoietic organs lags behind the rate of the increase in the volume of circulating blood. Therefore, autohemodilution takes place during pregnancy, which is accompanied by a decrease in hemoglobin and a decrease in the number of erythrocytes per unit volume of blood. This is a significant factor that contributes to the occurrence of anemia (Lyman’ska et al., 2020). We found a decrease in erythrocyte content and concentration of hemoglobin in the pregnant rats (Table 7). The decreased hemoglobin during pregnancy is a common phenomenon, which is consistent with the results of other researchers (Feleke & Feleke, 2020). Changes in hemoglobin, occurring from early pregnancy to mid-pregnancy or late pregnancy, were inversely related to fetal weight at birth and placental weight (Owa et al., 2015). The mean hemoglobin concentration in the erythrocytes of pregnant animals increased slightly. An increase in the mean percentage of reticulocytes was observed in mid-pregnancy, after which it remained at a high level until the delivery. The red blood cell distribution width (RDW) also increases in mid-pregnancy and then decreases before the delivery. The continuous change in the age distribution of erythrocytes in relation to the young cell population occurs from early pregnancy and lasts until the delivery (Lurie, 1993). In the maternal circulation, according to the results of Belo et al. (2002), the number of both damaged erythrocytes and young erythrocytes increases. This also causes a decrease in the number of erythrocytes, hemoglobin and hematocrit during pregnancy and the postpartum period (Belo et al., 2002). Under the effect of vanadium citrate at concentrations of 0.03–0.125 μg V/mL, the content of erythrocytes increased. The concentration of hemoglobin at concentrations of 0.125 μg V/mL decreased, at concentrations of 0.50 μg V/mL increased. Also, under the effect of vanadium citrate there was a decrease in the mean hemoglobin concentration in the erythrocytes at concentrations of 0.03–0.125 μg V/mL. Such changes in the number of erythrocytes and their functional properties in the peripheral blood of pregnant rats under the effect of vanadium citrate may be due to the ability of vanadium to some extent to delay the maturation of erythrocytes, as compared to non-pregnant animals fed with only water. This can be expressed by a decrease in the number of erythrocytes, hemoglobin levels and an increase in the number of reticulocytes and also polychromatophilic cells in the peripheral blood (Zaporowska & Wasilewski, 1989).

Platelets (thrombocytes) are the smallest elements of the blood, being the key players in hemostasis and thrombosis. The defects affecting platelets during pregnancy can lead to heterogeneous complications such as thrombosis, miscarriage in the first trimester (early pregnancy) and postpartum haemorrhage. The incidence of complications increases if there are inherited platelet function disorders (Valera et al., 2010). In pregnant animals there was an increase in platelet count (Table 8). Increasing platelet count is one way to protect the body from the development of gestational diabetes in pregnant animals (Yang et al., 2015). It is known that physiological pregnancy is characterized by an increase in platelet activation and a decrease in the total number of circulating platelets (Szklanu et al., 2019). The value of thrombocrit in the pregnant rats increased, which is due to an increase in platelet count in the pregnant rats of group II. The relative platelet distribution width by volume in the pregnant animals decreased, which may be due to low blood agglutination in pregnant animals. In the pregnant animals, fed with vanadium citrate solutions, the platelet content decreased, at all investigated concentrations (0.03–0.50 μg V/mL). The decrease in the increased content of platelets and thrombocrit in the blood of pregnant animals under the effect of vanadium citrate can prevent thrombosis during pregnancy, which is a consequence of blood thinning. The results of González-Villalva et al. (2011), who investigated the effect of vanadium pentoxide on the platelets of mice and humans, showed the inhibition of platelet aggregation in platelet-rich plasma under a four-week influence. The platelet condition returned to normal after eight weeks (González-Villalva et al., 2011). Normal pregnancy is a complex process that involves many immuno-regulatory mechanisms that protect the fetus from activating the maternal immune system. This involves qualitative and quantitative changes in lymphocyte function. The content of total leukocytes and lymphocytes in the pregnant animals of group II decreased, while the content of granulocytes increased (Table 9). The decrease in the content of leukocytes and lymphocytes, including in the pregnant animals of group II may indicate the suppression of cellular immunity during gestation (Pramanik et al., 2007). Also, the lymphocyte content decreases due to the susceptibility of CD3(+) CD8(+) T cells to apoptosis as a protective mechanism in early pregnancy (Darmochwal-Kolarz et al., 2014). A significant decrease in the phagocytic function of monocytes and neutrophil granulocytes in healthy pregnancy may be a part of the mother’s immune suppression, which is important for fetal protection (Lampi et al., 2015). Activation of granulocytes, NK-cells and extrathymic T-cells is essential for pregnancy preservation, but their excessive activation can cause pregnancy disorders. Pregnancy is associated with temporary changes in granulocyte surface markers, such as lower CD16 expression and higher CD64, partially imitating the protective response (Elghetaty & Lacombe, 2004). Under the effect of vanadium citrate, the content of leukocytes and lymphocytes increased in all investigated groups. The increase in the content of blood lymphocytes may be due to the increase in DNA synthesis of these cells under the effect of vanadium citrate (Sharma et al., 1981). The effect of vanadium citrate on the increase of T-lymphocytes has a desensitizing effect, increases the nonspecific resistance of the organism and has a normalizing effect on the indicators of humoral immunity (Tsiclaurs, 2010). The content of granulocytes in the pregnant animals consuming vanadium citrate solutions decreased at all concentrations (0.03–0.50 μg V/mL). In the study by Di Gioacchino et al. (2002), it was suggested that vanadium may have an important effect on the body’s immune system. This is proved by the fact that under the action of 10−5 M NaVO3, the formation of granulocytes decreased by about 70%, while under the influence of 10−4 M vanadium, their formation also decreased, but to a lesser extent (Di Gioacchino et al., 2002).

Conclusion

The results of the study show that in pregnant animals, urea levels and alkaline phosphatase activity increase, while aspartate aminotransferase activity decreases. The total content of protein and albumin decreases,
however, the content of β-globulins increases. Also, in pregnant animals there was a decrease in hemolysis time, the total content of erythrocytes and hemoglobin, the content of old and mature erythrocytes, while the content of young erythrocytes increased. Platelet content and thrombocrit increased in pregnant rats. The total content of leukocytes and lymphocytes in pregnant females decreased, while the content of granulocytes increased, in contrast to non-pregnant animals.

Under the effect of vanadium citrate at the concentration of 0.03 μg V/mL, the level of albumin, creatinine and aspartate aminotransferase activity increased in blood plasma in comparison with group II. And under the effect of vanadium citrate at the concentration of 0.125 μg V/mL, the relative content of γ-globulins and aspartate aminotransferase activity increased, whereas alkaline phosphatase activity and urea level decreased, in comparison with group II. As well under the effect of vanadium citrate at the concentration of 0.50 μg V/mL, the relative α- and γ-globulins’ content and aspartate aminotransferase activity increased, while the relative β-globulin content and urea level decreased, in comparison with group II. Also, under the effect of vanadium citrate at concentrations of 0.03–0.50 μg V/mL, there was a significant increase in the maximum number of prohemolysed erythrocytes, the time of maximum hemolysis was delayed by 0.4–0.6 min, as compared with the pregnant rats of group II. However under the effect of vanadium citrate, the increase in the content of young erythrocytes was observed, as compared with group II. The hemoglobin content decreased at a concentration of 0.125 μg V/mL, but increased at a concentration of 0.50 μg V/mL. Also, under the effect of vanadium citrate there was a decrease in the mean hemoglobin concentration in the erythrocytes. In the pregnant animals fed with vanadium citrate solutions, platelet content and thrombocrit, the relative platelet distribution width by volume decreased, as compared with the pregnant rats of group II. Further under the effect of vanadium citrate, the content of leukocytes, lymphocytes and granulocytes increased, as compared to the pregnant animals in group II.

To sum up, vanadium has normalizing properties for certain indicators of protein metabolism during pregnancy. It is able to normalize the hematological profile during pregnancy, increase the resistance of erythrocyte membranes to hemolytic action. This ensures a healthy pregnancy. Therefore, vanadium citrate can potentially be used as a dietary agent to stop the development of pregnancy complications.

The authors would like to thank s.r.f. A. Z. Pylypov for performing the methodology of electrophoresis in polyacrylamide gel.

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Features of modern winter wheat varieties in terms of winter hardiness components under conditions of Ukrainian Forest-Steppe

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Article info
Received 10/02/2021
Received in revised form 15/03/2021
Accepted 16/03/2021

Introduction

In recent years, there has been a significant change in climatic conditions affecting the cultivation and yield of winter wheat. Therefore, the creation of winter varieties with high adaptive potential is one of the main tasks of modern breeding. A significant component of the overall adaptive potential of winter wheat is winter hardiness, which is determined by a set of factors enabling plants to overwinter. To a large extent, winter hardiness is determined by gene systems that control vernalization requirement duration, photoperiod sensitivity, and frost resistance. The research is aimed at determining the features of modern winter wheat varieties developed at the V. M. Remeslo Myronivska Institute of Wheat of the National Academy of Agrarian Sciences of Ukraine in terms of winter hardiness components and adaptive potential in the environment of the Central part of the Ukrainian Forest-Steppe. Winter broad wheat varieties Estafeta myronivska, Hratsia myronivska, MIP Assol, and Balada myronivska were studied. They also were crossed on incomplete diallel scheme with three near-isogenic lines derived from Erythrospermum 604 with different alleles of Vrd genes 1) Vrd1Vrd1vrd2vrd2, 2) vrd1vrd1Vrd2vrd2, and 3) vrd1vrd1Vrd1vrd2. It was established that vernalization requirement duration in the varieties Estafeta myronivska and Balada myronivska was short whereas in the varieties Hratsia myronivska and MIP Assol it was medium. All the varieties studied have medium photoperiod sensitivity. The results of the hybridological analysis indicate the absence of the Vrd1 and Vrd2 genes in the varieties. Frost tolerance of these varieties is at the same level and higher than in the highly tolerant to the low temperatures variety Myronivska 808. Thus, the results indicate the possibility of recombining different levels of expression of these traits in genotypes by breeding efforts. This has great practical importance in farming, because in recent years the areas of crops harvested late (corn, sunflower, etc.) in the production conditions has significantly increased. It causes a shift in sowing dates of winter wheat to a later period. In this case, varieties Estafeta myronivska, Hratsia myronivska, MIP Assol, and Balada myronivska are able to undergo sufficient hardening, to satisfy the vernalization requirement, and to form a high level of winter hardiness. Their relatively medium photoperiod sensitivity allows vegetation to be restored a little earlier in the spring and winter reserves of moisture to be used more effectively.

Keywords: Triticum aestivum; frost tolerance; vernalization requirement; photoperiod sensitivity; hybridological analysis.
genes are represented by recessive alleles. However, the presence of only one dominant allele of Vrn-A1 gene provides complete insensitivity of plants to vernalization. Dominant alleles of the Vrn-B1 and Vrn-D1 loci only partially reduce the vernalization requirement (Pugsley, 1971; Pugsley, 1972). Genes determining plant growth habit are localized on different chromosomes: Vrn-A1 (previous designation Vrn1) on 5A, Vrn-B1 (Vrn2) on 5B, Vrn-D1 (Vrn3) and Vrn-D4 (Vrn4) on chromosome 5D.

The Vrn-B3 gene (earlier Vrn5) is located in the short arm of chromosome 7B (Worland, 1996; Yan et al., 2006; Yoshida et al., 2010). The efficacy of Vrn and Ppd gene alleles marking for early diagnosis of plant response to vernalization and photoperiod has been reported (Cockram et al., 2009; Yang et al., 2009). The study of the duration of the stages of development of winter bread wheat in isogenic and substituted lines with different alleles of Vrn1 genes suggests that growing season duration depends mainly on the duration of period “tillering-the first node” (Pankova & Kosner, 2004; Emteeva et al., 2013). Stelmakh et al. (2005) reported the identification of three vernalization requirement duration genes of winter wheat, designated by authors as Vrd1, Vrd2, and Vrd3. The Vrd1 gene is located on chromosome 4A, Vrd2 is on chromosome 5D. It was found that presence of dominant gene Vrd1 reduces the vernalization requirement duration to 20–35 days, depending on the photoperiod sensitivity of the variety, and Vrd2 does to 40–45 days (Balashova et al., 2006).

Genotypes with recessive alleles of two Vrd genes (vrd1vrd1vrd2vrd2) require at least 50–60 days of vernalization for transition from the vegetative to the generative stage of development. A third gene (Vrd3) is also thought to be present, which determines the duration of vernalization up to 40 days and is located on one of the chromosomes 1A, 6A, or 4B (Fayt et al., 2007). Other scientists suggest that vernalization requirement duration is determined by changes in a locus of the Vrn-A1 gene (Yan et al., 2015) or Vrn-B1 (Guedira et al., 2013). It has also been suggested that the trait vernalization requirement duration in winter wheat may be controlled by the TaVrn-A1 gene at the protein level (Li et al., 2013). The study of the effects of genes controlling the vernalization duration (Vrd) on agronomic traits in isogenic lines of winter wheat shows that the dominant alleles of the genes Vrd1 and Vrd2 cause reduction in plant height as well as the shortening of the period to heading as compared to carriers of only recessive alleles of the gene vrd1vrd2 (Fayt, 2007).

Frost tolerance is the ability of plants to withstand negative temperatures during wintering (Sutton et al., 2009). Genes associated with freezing tolerance of winter wheat Frl and Fr2 are localized on chromosomes 5A and 5D, respectively (Satka, 2001). It is assumed that the presence of the Vrn-D1 and Fr-D1 genes in the wheat genotype not only determines the level of freezing tolerance, but also plant resistance to snow mold (Francia et al., 2007; Erath et al., 2017).

Given the above, to characterize modern genotypes of winter wheat on genetic systems that determine the processes of vernalization, photoperiod sensitivity, frost resistance and their impact on growth, development and general adaptive potential is relevant (Bakurina, 2016; Fayt et al., 2017; Jones et al., 2017). It was found that under various agroclimatic conditions there are different combinations of vernalization requirement and photoperiod sensitivity in winter wheat genotypes, which leads to increased adaptive potential, in particular, frost resistance (Whittal et al., 2015; Jones et al., 2017). It was found that under various agroclimatic conditions there are different combinations of vernalization requirement and photoperiod sensitivity in winter wheat genotypes, which leads to increased adaptive potential, in particular, frost resistance (Whittal et al., 2015).

The aim of the research was to identify the features of modern varieties of winter wheat developed at the V. M. Remeslo Myronivka Institute of Wheat of the National Academy of Agrarian Sciences of Ukraine (MIW) by vernalization requirement, photoperiod reaction and frost resistance as components of winter hardness and adaptive potential in the Central part of Ukrainian Forest-Steppe.

Materials and methods

The research was conducted in 2016–2019 at the MIW. We used new Myronivka winter wheat varieties (Estafeta myronivska, Hrotsia myronivska, MIP Assol and Balada myronivska) which are included in the State Register of Plant Varieties Suitable for Dissemination in Ukraine since 2018. When analyzing the pedigrees of these varieties, it was established that the varieties Estafeta myronivska and Hrotsia myronivska were created on the basis of crossing local varieties and lines with each other while in creating the varieties Balada myronivska and MIP Assol, collection samples of different ecological origin from Hungary and Russia were used (Table 1).

Table 1 Genealogical characteristics of the studied winter wheat varieties

| Variety, biological variety | Genealogy |
|-----------------------------|-----------|
| Estafetia myronivska (var. lutescens) | Myronivska 64 [Myronivska yuvileina (Lutescens106/ Bezosta taya 4) / KM 66-10-1-79] / Lutescens 50713 [Myronivska 27 (Lutescens 6915 (Prybii / Myronivska yuvileina) / Lutescens 6538)] / Nike |
| Hrotsia myronivska (var. lutescens) | Eorythrospermum 52422 [Eorythrospermum 9736 (Narior 59 / Vondka) / Eorythrospermum 5267 from Eorythrospermum 10071 [Eorythrospermum 5226 [WRH k-43822 / Lutescens 2274 (Lutescens 106 / Bezosta taya 4) / Bezosta taya 4]] / Lutescens 6075] / Gunia |
| MIP Assol (var. lutescens) | Salsa Myronivska 65 [Myronivska 61 (Illichka (Bezosta taya 4) / Eorythrospermum 27612)] / Myronivska 808 / Hadm. 6508-74] / Lutescens 27 [Lutescens 6915 (Prybii / Myronivska yuvileina) / Lutescens 6538 (Hadm. 6508-74)] / Lutescens 52948 [Lutescens 2060 from Myronivska 27 (Lutescens 6915 (Prybii / Myronivska yuvileina) / Lutescens 6538)] / Myronivska 61 (Illichka (Be zosta taya 4) / Myronivska 808) / Hadm. 6508-74] / Lutescens 20051 [Myronivska 61 (Illichka (Bezosta taya 4) / Myronivska 808) / Hadm. 6508-74] / NS 954 / Kavkaz / Rezo / Lutescens 8133 [Nie Coros 66 / Myronivska yuvileina (Bezosta taya 4) / Myronivska 808] |
| Balada myronivska (var. lutescens) | CIMMYT-151) / Eorythrospermum 10071 [Eorythrospermum 5226 [WRH k-43822 / Lutescens 2274 (Lutescens 106 / Bezosta taya 4) / Bezosta taya 4]] / Lutescens 6075] / Eorythrospermum 53321 [Lutescens 9950 (Illichka / SK-2542 / CIMMYT-151)] / Eorythrospermum 10071 [Eorythrospermum 5226 [WRH k-43822 / Lutescens 2274 (Lutescens 106 / Bezosta taya 4) / Bezosta taya 4]] / Lutescens 6075] |

Note: *WRH—wheat-rye hybrid.

To determine photoperiod sensitivity of the winter wheat varieties two variants of the experiment were laid: in the first the plants were grown under natural daylight; in the second they were under artificially shortened daylight (12 hours). Before sowing, the germinated seeds were artificially vernalized for 60 days (at 0…+1 °C). Using a special marker, germinated seeds of each variety were planted 20 pcs in each of two vegetative pots per variant of the experiment. Then the pots were placed in an open area. To shorten day length, the plants in the pots were covered with black boxes (Fig. 1a). The date of heading occurrence for individual plants was marked with labels (Fig. 1b). According to photoperiod sensitivity, wheat varieties were divided into three groups: high-, medium- and low sensitive. In our experiment, the first group included varieties that responded to the daylight reduction with significant heading delay of 10–13 days, the second group with delay of 6–9 days, and the third group with less than 6 days.

To determine vernalization requirement duration, 100 seeds of each variety were watered and placed for germination in a thermostat at the temperature of +19…+20 °C for one day. To go through vernalization, the seedlings were placed in the LVN-200G chamber at the temperature of 0…+1 °C for different periods (50, 40, and 30 days). The vernalized seedlings were planted in spring at a time when the level of long-term air temperature avoids additional vernalization of experimental samples in the field (for the research period it was on April 14–18). Previously, the field was divided into strips of width 1 m and tracks between strips of 50 cm. The seedlings were planted on two rows for each variant of the experiment, about 50 seeds per row. The plants were counted in early August using the envelope method. The duration of the vernalization period was considered to be sufficient if the most plants of the variety reached heading.

As testers that allow to one establish differences in wheat plant development at early stages of organogenesis we used near-isogenic by genes Vrd winter wheat lines Eorythrospermum 604 VrdIvrd1vrd2vrd2, Eory throspermum 604 vrd1vrd1vrd2vrd2, Eorythrospermum 604 vrd1vrd1vrd2 created at the Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation of NAAS (Fayt, 2006).
Days to heading under the natural and shortened photoperiod in new varieties of winter wheat (x ± SE, n = 30)

| Variety                  | natural photoperiod | shortened photoperiod | natural photoperiod | shortened photoperiod |
|--------------------------|---------------------|-----------------------|---------------------|-----------------------|
|                          | 2016                | 2017                  | 2018                | 2018                  |
| Estafeta myronivska      | 51.0 ± 0.5*         | 58.4 ± 1.0           | 55.0 ± 0.3*         | 61.5 ± 0.3*           |
| Hratsia myronivska       | 48.8 ± 0.4b         | 55.9 ± 1.1b          | 51.9 ± 0.2b         | 58.7 ± 0.6b           |
| MIP Assol                | 51.8 ± 0.4b         | 57.5 ± 0.4b          | 55.5 ± 0.3b         | 65.3 ± 0.4b           |
| Balada myronivska        | 49.8 ± 0.4b         | 55.7 ± 0.6b          | 54.3 ± 0.3b         | 59.0 ± 0.7b           |

Note: different letters indicate values which reliably differed one from another within one column of the table according to the results of comparison using the ANOVA with Bonferroni correction.

Table 3
Vernalization requirement duration and days to heading in winter wheat near-isogenic lines (x ± SE, n = 90)

| Genotype of near-isogenic line | Vernalization requirement | days to heading | Vernalization requirement | days to heading | Vernalization requirement | days to heading | Vernalization requirement | days to heading | Vernalization requirement | days to heading |
|--------------------------------|--------------------------|----------------|--------------------------|----------------|--------------------------|----------------|--------------------------|----------------|--------------------------|----------------|
| Vrd1Vrd1vrd2vrd2              | 30                       | 65.3 ± 1.8     | 30                       | 57.4 ± 2.6     | 40                       | 55.9 ± 0.9     | short duration            | (31–40 days)   |
| vrd1vrd1Vrd2vrd2              | 40                       | 72.4 ± 4.1b    | 30                       | 75.4 ± 3.0b    | 40                       | 74.8 ± 2.0b    | medium duration           | (41–50 days)   |
| vrd1vrd1vrd2vrd2              | 50                       | 70.5 ± 2.5b    | 50                       | 72.3 ± 2.6b    | 50                       | 68.7 ± 5.2b    | medium duration           | (41–50 days)   |

Note: see Table 2.

Table 4
Vernalization requirement duration and days to heading in winter wheat near-isogenic lines (x ± SE, n = 90)

| Genotype of near-isogenic line | Vernalization requirement | days to heading | Vernalization requirement | days to heading | Vernalization requirement | days to heading | Vernalization requirement | days to heading |
|--------------------------------|--------------------------|----------------|--------------------------|----------------|--------------------------|----------------|--------------------------|----------------|
| Vrd1Vrd1vrd2vrd2              | 30                       | 65.3 ± 1.8     | 30                       | 57.4 ± 2.6     | 40                       | 59.4 ± 0.9     | short duration            | (31–40 days)   |
| vrd1vrd1Vrd2vrd2              | 40                       | 72.4 ± 4.1b    | 30                       | 75.4 ± 3.0b    | 40                       | 74.8 ± 2.0b    | medium duration           | (41–50 days)   |
| vrd1vrd1vrd2vrd2              | 50                       | 70.5 ± 2.5b    | 50                       | 72.3 ± 2.6b    | 50                       | 68.7 ± 5.2b    | medium duration           | (41–50 days)   |

Note: see Table 2.

Table 5
The segregation ratio in F2 population for “heading occurrence: no heading occurrence” after vernalization duration 40 and 30 days

| Days of vernalization | Variety                  | Vrd1Vrd1vrd2vrd2       | Vrd1Vrd1vrd2vrd2       | Vrd1vrd1Vrd2vrd2      |
|-----------------------|--------------------------|------------------------|------------------------|-----------------------|
| 30                    | Estafeta myronivska       | 3:1*                   | 3:1*                   | 13:3                 |
|                       | Hratsia myronivska        | 3:1                    | 3:1                    | 13:3                 |
|                       | MIP Assol                | 3:1                    | 3:1                    | 13:3                 |
|                       | Balada myronivska         | 3:1                    | 3:1                    | 13:3                 |
| 40                    | Estafeta myronivska       | 3:1                    | 3:1                    | 13:3                 |
|                       | Hratsia myronivska        | 3:1                    | 3:1                    | 13:3                 |
|                       | MIP Assol                | 3:1                    | 3:1                    | 13:3                 |
|                       | Balada myronivska         | 3:1                    | 3:1                    | 13:3                 |

Note: * – does not correspond to the theoretical segregation ratio; \( \chi^2 \) < 3.84 at the P = 0.05.

Vernalization requirement. In 2016, duration 50 days was considered sufficient vernalization period for the winter wheat varieties Hratsia myronivska and MIP Assol, as the highest percentage of heading plants was observed in this variant with 40 days vernalization (41–50 days). In 2017, plants of the variety Estafeta myronivska had the highest percentage of heading plants observed in both variants, therefore, for this variety, the vernalization requirement was 30 days. Vernalization requirement for the variety Hratsia myronivska in 2018 was 50 days, because for this vernalization duration heading plants were observed at the level of 70%. Whenever seedlings of this variety were vernalized during 40 and 30 days, the heading occurred only in 47.4% and 41.2% of plants, respectively. In the variety MIP Assol 91.7% of plants were heading at 50 days of vernalization and 65.0% at 40 days. After 30 days of vernalization duration there was a low percentage of heading plants, so we consider 40 days of vernalization to be sufficient for transition of plants of this variety to the generative state. The varieties Balada myronivska and Estafeta myronivska required 40 days of vernalization too. They differ from the previous ones in that in the variant with 30 days vernalization most of the plants of these varieties remained at the tillering phase. The heading dynamic of the varieties under study indicated that the average time to heading varied over the years from 55.9 to 77.9 days. The highest range of variation of this trait among the varieties was observed in 2018, and the lowest was in 2016. The shortest time to heading at the established duration of vernalization of 40 days in 2016 was noted in the varieties Estafeta myronivska and Balada myronivska (66.3 ± 0.26 and 65.1 ± 0.50 days). In 2017 and 2018 the shortest time to heading (59.8 and 59.0 days, respectively) was observed in the variety Hratsia myronivska. In varieties with short vernalization requirement duration, this period was, on average, 31–40 days, with medium vernalization requirement duration 41–50 days, and with a long-term ~51–60 days. The results of the research show that the varieties MIP Assol and Hratsia myronivska require medium vernalization duration (41–50 days), whereas the varieties Estafeta myronivska and Balada myronivska require short vernalization duration (31–40 days). No varieties with long-term vernalization duration were revealed in our research.
Vernalization duration requirement under environmental conditions of the Ukrainian Forest-Steppe was determined also in winter wheat near-isogenic lines Erythroneuronum 604 Vrd1vrd1vrd2vrd2, Erythronium 604 vrd1vrd1vrd2vrd2, and Erythronium 604 vrd1vrd1vrd2vrd2. In 2016 (Table 4), the lines Erythronium 604 with dominant allele of Vrd1 or Vrd2 genes required 30 and 40 days of vernalization duration, because in 70.0 and 63.0% of plants heading occurred. The line vrd1vrd1vrd2vrd2 required 50 days of vernalization duration, because in variants 40 and 30 days of vernalization most plants remained at the tillering stage. In 2017, the line Vrd1vrd1vrd2vrd2, with dominant allele of the Vrd1 gene, at the vernalization of seeds during 50, 40, and 30 days demonstrated heading in 100% of plants. The same level was noted in the line vrd1vrd1vrd2vrd2, which means vernalization duration 30 days was sufficient for it. Heading occurrence for the line vrd1vrd1vrd2vrd2 after 40 days of vernalization duration was noted only in 47% plants, after 30 days no heading was noted. After 50 days of vernalization duration, heading occurrence was observed in all plants. In 2018, the lines Erythronium 604 with dominant alleles of Vrd1 or Vrd2 genes required 40 days of vernalization duration, and then heading occurred in 70.6 and 64.0% of plants, respectively.

For the near-isogenic line Erythronium 604 vrd1vrd1vrd2vrd2 being a carrier of recessive allele of these genes, vernalization requirement duration was 50 days, because for shorter vernalization duration (30 and 40 days) no heading was noted. As indicated by heading dynamic for three years, heading time averaged 59.5 days in the line Vrd1vrd1vrd2vrd2, 74.2 days in the line vrd1vrd1vrd2vrd2 and 70.5 days in the line vrd1vrd1vrd2vrd2. The variation in heading dynamic of the tester lines among the years is explained by differing weather conditions over the years of the research. Since, weather in April and May in 2017 was cooler as compared to these months in 2018, so more plants did not reach heading.

Hybridological analysis. The actual segregation ratio for “heading occurrence: no heading occurrence” in all cross combinations with recessive tester of gene Vrd corresponded to the theoretical 3:1 (Table 5). The segregation ratio in populations Vrd1Vrd1vrd2vrd2/Estafeta myronivska and vrd1vrd1vrd2vrd2/Estafeta myronivska corresponded to 15:1. The segregation ratio in combinations Vrd1vrd1vrd2vrd2/Hratia myronivska, Vrd1vrd1vrd2vrd2/MIP Assol and Vrd1vrd1vrd2vrd2/Estafeta myronivska was 15:1, and in the population created with these varieties and tester line vrd1vrd1vrd2vrd2 it was 15:1. The segregation ratio in combinations Vrd1vrd1vrd2vrd2/Balada myronivska and vrd1vrd1vrd2vrd2/Balada myronivska was 15:1 and 13:3, respectively.

After 30 days vernalization in the population vrd1vrd1vrd2vrd2/Estafeta myronivska the fact segregation ratio for “heading occurrence: no heading occurrence” was 136:132, which did not correspond to the theoretical ratio 3:1. In combinations vrd1vrd1vrd2vrd2/Estafeta myronivska and vrd1vrd1vrd2vrd2/Estafeta myronivska this ratio was 15:1 and 3:1 respectively. In the combinations of the varieties Hratia myronivska and Balada myronivska with the tester of recessive genes (vrd1vrd1vrd2vrd2) the fact segregation corresponded to the theoretical ratio 1:15 as well as with tester of dominant gene Vrd2 to the ratio 13:3. In the combination vrd1vrd1vrd2vrd2/MIP Assol even for 90 days segregation was not observed. The segregation ratio with the tester Vrd1vrd1vrd2vrd2 was 15:3, and with the tester vrd1vrd1vrd2vrd2 it was 3:13.

Frost resistance. On average for 2017–2019, the percentage of viable plants of the variety Myronivska 808 after freezing at temperature minus 18 °C and minus 20 °C was 87% and 58%, respectively, freezing tolerance at the level of the standard variety (according to Fisher’s test) at both freezing temperatures was observed in the varieties Hratia myronivska (79 ± 4.5, 50 ± 5.5) and Balada myronivska (82 ± 4.4, 53 ± 5.6, Table 6). The variety Estafeta myronivska significantly exceeded the standard for percentage of live plants at the freezing temperature minus 18 °C was of the level of the standard (91%), and at the minus 20 °C significantly exceeds the standard.

Discussion

It has been established that in the conditions of the south of Ukraine the Ppd-D1a allele significantly shortens the duration time to heading, reduces plant height, reduces the spike and stem length, number of spikes and fertile spikelets per spike, spike density; increases grain number per spike and grain weight per main spike, grain weight of secondary stems, 1000 kernel weight (Bakuma et al., 2018). The genotype Ppd-A1b Ppd-B1b Ppd-D1a is prevalent in the varieties bred at Bila Tserkva Experiment and Breeding Station, the Ppd-D1a allele determines insensitivity to the photoperiod and promotes an earlier heading date. Only the variety Lebenda blotsoderivka is a carrier of the recessive allele Ppd-D1b and has a later heading date (Filimonov et al., 2018). In the Ppd-1 gene system of the varieties bred at the Institute of Irrigated Agriculture of NAAS the dominant Ppd-D1a allele there were identified and recessive b alleles were identified in the Ppd-A1 and Ppd-B1 loci (Bakuma et al., 2019). According to the results of our research, we assume that the new varieties of winter bread wheat of breeding at the MIW do not have the dominant allele of the Ppd-D1a gene, because varieties insensitive to the photoperiod were not detected among them.

Table 6
The percentage of surviving plants of winter wheat varieties after freezing (%), x ± SE, n=80, 2017–2019

| Variety                  | Temperature of freezing |
|-------------------------|-------------------------|
|                          | -18 °C                  | -20 °C                  |
| Myronivska 808, standard | 87.0 ± 3.8°             | 58.0 ± 5.5°             |
| Estafeta myronivska     | 90.0 ± 1.2°             | 59.0 ± 5.6°             |
| Hratia myronivska       | 79.0 ± 4.5°             | 50.0 ± 5.3°             |
| MIP Assol               | 91.0 ± 3.2°             | 70.0 ± 5.8°             |
| Balada myronivska       | 82.0 ± 4.4°             | 53.0 ± 5.6°             |

Note: different letters indicate values which reliably differed one from another within one column of the table according to the results of comparison using the Fisher’s criterion.

Vernalization requirement in wheat can be considered as an adaptive mechanism that provides delay in transition to the reproductive stage of development during winter (Muterko et al., 2015). Analysis of the assortment of winter wheat of Ukrainian breeding showed that among the genotypes created at the Plant Breeding and Genetics Institute – National Centre of Seed and Cultivar Investigation of NAAS the majority of wheat genotypes possessed dominant allele of the Vrd1 gene (54.5%) (Fayt, 2012). Among the varieties of the Plant Production Institute n.d. a. V. Y. Yurev of NAAS, the majority of genotypes had the dominant allele Vrd1 (36.4%) or the three recessive vrd genes (36.4%). At the same time, the possibility of modifying the vernalization duration requirement by Ppd genes is not excluded (Stelmakh et al., 2019; Zubrich & Avskeniieva, 2019). It was noted that introduction into the modern enhanced in productivity wheat gene pool of genetic material relating to the rates of initial development typical for varieties of past generations would improve the adaptive properties of future breeding material (Stelmakh & Fayt, 2015).

The segregation in F2 population for heading occurrence resulting from hybridological analysis (Fayt, 2006b, 2012) was not observed if the genotype of the variety and the tester coincided. When crossing a variety that is assumed to have dominant gene Vrd1, Vrd2 or Vrd3 with a recessive tester after 40 days of vernalization, the segregation ratio is 3:1. If in the genotype of the variety there are two dominant genes of vernalization requirement at the same time, the segregation ratio is 15:1. The segregation ratios of 3:1 and 15:1 indicate a difference in genes in the variety and tester studied. It was determined that the vernalization requirement duration of Vrd gene testers in the Right-bank Forest-Steppe of Ukraine was 30–50 days. So, to reveal differences among genotypes in this agroclimatic zone 30 and 40 days are sufficient terms of vernalization.

In our case, after 30 days vernalization in the population vrd1vrd1vrd2vrd2/Erythroneuronum 604/Erythronium 604/Erythronium 604 the fact segregation ratio for “heading occurrence: no heading occurrence” was 136:132, which did not correspond to the theoretical ratio 3:1, which could have been caused by weather conditions, at the same time it indicates the difference between the variety studied and the tester. In the combination vrd1vrd1vrd2vrd2/MIP Assol on the date of the last accounting 20 plants remained at tubbing stage, and a significant number (123 plants) remained at the tillering stage. The obtained results of plant accounting in this combination indicate the significant influence of weather conditions on plant development, because at 40 days of vernalization many of the plants reached heading, and the average time

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to heading was 80 days. Taking this into account, we can assume that the plants of this hybrid combination could reach heading after 93–94 days with corresponding theoretical segregation ratio of 1:3.

Theoretical segregation at 40 days of vernalization in the combinations with testers Vrd1Vrd1vrd2vrd2 and vrd1vrd1Vrd2vrd2 in the ratio “heading occurrence: no heading occurrence” 13:5 in the varieties Hratsia myronivska, MIP Assol and Balada myronivska is probably caused by different photoperiod sensitivity. The influence of weather conditions on the segregation pattern in hybrid populations has also been noted in earlier publications (Fayt, 2000b). Comparing the results of hybridological analysis obtained (Table 5) with the theoretically expected segregation ratio, we assume that in the varieties Estafeta myronivska, Hratsia myronivska, MIP Assol and Balada myronivska the vernalization requirement duration is controlled by a gene (genes) other than Vrd1 and Vrd2.

Despite climate change, development of winter wheat varieties with increased frost tolerance is still one of the main tasks of scientific institutions not only in Ukraine but also abroad (Riabchun, 2012; Lytvynenko, 2016). Increased frost tolerance is still one of the main tasks of scientific institutions in the Forest-Steppe of Ukraine and abroad (Riabchun, 2012; Lytvynenko, 2016). Comparing the results of hybridological analysis obtained (Table 5) with the theoretically expected segregation ratio, we assume that in the varieties Estafeta myronivska, Hratsia myronivska, MIP Assol and Balada myronivska the vernalization requirement duration is controlled by a gene (genes) other than Vrd1 and Vrd2.

We established that the level of frost tolerance in winter wheat varieties bred by institutions in the Forest-Steppe of Ukraine varies from 74.5% to 90.8% (Golyk et al., 2017). In our research we have found that newly developed winter wheat varieties at MIW with increased productive and adaptive potential in the Forest-Steppe of Ukraine are characterized by medium photoperiod sensitivity as well as medium and short vernalization requirement duration. Moreover, frost tolerance of these varieties is at the level and above the variety Myronivska 808, which is highly tolerant to low temperatures.

Conclusion

It was determined that winter wheat varieties Estafeta myronivska, Hratsia myronivska, MIP Assol and Balada myronivska developed at MIW in recent years with increased productive and adaptive potential in the conditions of the Central part of the Ukrainian Forest-Steppe are characterized with medium photoperiod sensitivity, and medium or short vernalization requirement duration. We didn’t establish the presence of Vrd1 and Vrd2 genes in these varieties. At the same time, the genetically determined frost tolerance of the varieties studied is at the level and above the variety Myronivska 808, which is highly tolerant to low temperatures. Our results indicate the possibility to recombine in the genotype different levels of manifestation of these traits by selection and to develop varieties with their optimal combination for certain ecological conditions. This is of great practical importance in farming, as the increase in the share of sown areas of crops harvested late (corn, sunflower, etc.) in the Central part of the Forest-Steppe of Ukraine in recent years has caused a shift in winter wheat sowing dates to later. Under such conditions, the varieties mentioned above can undergo sufficient hardening, meet their vernalization requirement and form a high level of winter hardness. The relatively medium level of photoperiod sensitivity allows the vegetation to be restored a little earlier in spring and winter reserves of moisture to be used more effectively.

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Berezhna, A. V., Tertyshnyi, V. O., Makarova, V. I., & Chumachenko, T. O. (2021). Staphylococcus aureus and S. epidermidis in biological systems of hospital environment: Antibiotic resistance patterns in regions of Ukraine. *Regulatory Mechanisms in Biosystems, 12*(1), 160–168. doi: 10.15421/022124

**Staphylococcus aureus** and **S. epidermidis** are ubiquitous and often circulate in the biological systems of the hospital environment. Staphylococci have developed antibiotic resistance mechanisms resulting in a significant medical and economic burden to the healthcare system. The goal of our research was to conduct a comparative analysis of resistance to antibiotics in **S. aureus** and **S. epidermidis** isolates found in surgical hospitals in Kharkiv and Poltava regions. In 2013 through 2019, 151,015 and 98,754 tests were made by disc-diffusion method to identify the sensitivity in the **S. aureus** strains to antibiotics in Kharkiv and Poltava regions respectively. In 2013–2015, 15,589 tests were made in Kharkiv region to identify resistance of the **S. aureus** strains in Kharkiv and Poltava regions was performed using the Pearson Chi-square test ($\chi^2$) and Fisher’s exact test. The proportion of **S. aureus** strains resistant to penicillins, cephapsporins, carbapenems, aminoglycosides, and macrolides was higher in Kharkiv region in terms of statistical validity than in Poltava region. Overall, the proportion of **S. aureus** strains resistant to lincomazid, tetracycline antibiotics, and fluoroquinolones in Poltava region was higher in terms of statistical validity than in Kharkiv region. An analysis of resistance of **S. aureus** strains to linezolid demonstrated that in Poltava region the proportion of resistant microorganisms was higher in terms of statistical validity in 2013–2014 and in 2016–2018. In Kharkiv region, in 2013 and in 2014, 96.3% and 89.1% of isolated strains of **S. aureus** respectively, were resistant to vancomycin. In 2019, more than a quarter of the located isolates (26.6%) in Poltava region were resistant to this antibiotic. The analysis of the dynamic of resistance in **S. epidermidis** isolates demonstrated that in 2015 nearly half of the isolates located in Kharkiv region were insensitive to penicillin antibiotics. Between 2013 and 2015, the spread of resistance to cephapsporins, aminoglycosides, macrolides, and fluoroquinolones among the **S. epidermidis** isolates noticeably increased. When **S. epidermidis** resistance to vancomycin was analyzed, a decrease in the proportion of resistant strains from 88.0% in 2013 to 8.7% in 2015 was noted. A promising direction for further research is the creation of a unified national database network on microorganism resistance using modern methodologies for determining the phenotypes and genotypes of microorganisms.

**Keywords:** healthcare-associated infections; catheter-related bloodstream infections; biofilms; infection control; bacteremia; genomic variability.

**Introduction**

Bacteria of the *Staphylococcus* genus, especially *S. aureus* are among most frequently encountered infectious agents associated with rendering medical aid (Canadian Nosocomial Infection Surveillance Program, 2020; Voidazar et al., 2020). This is partially due to the fact that staphylococci colonize the mucous membranes and skin of humans. The nose is considered the most frequent localization of *S. aureus* (Wertheim et al., 2005; Brown et al., 2014). Extranasal localizations of *S. aureus* include the skin, crotch area, armpits, and the gastrointestinal tract. It should be noted that a nasal *S. aureus* carrier is prone to be a source of extranasal transmission. For instance, 90% of *S. aureus* nasal carriers usually have the skin on their hands contaminated as well (Wertheim et al., 2005). Another potentially dangerous hospital infection pathogen is *S. epidermidis*. Microorganisms of this genus are referred to resident microflora of the human external surface and are usually normal representatives of the microbiocenosis of every healthy person’s skin, which is also a significant link in the pathogenesis of infections associated with rendering of medical aid (Hellmark et al., 2013). Previously published researches point to the existing problem of incidence of methicillin-resistant genotypes of *S. aureus* and *S. epidermidis* in healthcare workers at hospitals (Du et al., 2013; Widerström et al., 2016; Sharma et al., 2019). In cases of violation of aseptic and antisepic rules and improper observance of hand hygiene, microorganisms that are on medical personnel’s skin are transmitted to patients, medical devices, equipment, and other objects of the medical environment. When invasive manipulations like catheterization of vessels are conducted, microorganisms may pass from medical personnel’s hands to the surface of a vessel catheter and be the cause of development of catheter-related bloodstream infections (Cherifi et al., 2014).

It is general knowledge that patients in hospitals have at least one peripheral intravenous catheter installed in 30% to 80% of cases (Zhang et al., 2016; Aghdassi et al., 2019). The patients, who need a transfusion of massive amounts of liquid, total parenteral feeding, hemodialysis, or for other reasons, have central venous catheters installed (Smith & Nolan, 2013). Considering the broad application of vessel appliances (including peripheral intravenous catheters) in medical practice, infectious complications associated with vessels’ catheterization account for a large share of infections associated with health care provision. The incidence of blood-
stream infections connected with application of peripheral intravenous catheters varies from 0.0% to 2.2%, amounting on average to 0.18%, with the incidence of nosocomial catheter-related bloodstream infections due to peripheral venous catheters reaching 6.2% to 60.0% (Mermed, 2017).

In the etiological structure of infection complications associated with peripheral and central veins catheterization, the Staphylococcus aureus strains and coagulase-negative staphylococci, including S. epidermidis, prevail (Zhang et al., 2016; Guenbe et al., 2017; Nguyen et al., 2017; Mandolfo et al., 2019; Tatsuno et al., 2019).

Due to the ability of staphylococci to produce biofilms, the infections caused by them are more difficult to treat and may develop into chronic forms. The biofilm forms of bacteria possess a considerably higher resistance to antibiotics than the plankton forms (Wu et al., 2003; Costerton et al., 2005; Kaplan, 2011). S. aureus strains producing biofilms possess a higher resistance level to most medications, which is prognostically an unfavourable factor in such patients’ treatment (Manandhar et al., 2018).

Under conditions of growth in microorganism resistance to medications, of grave concern are bacteremia cases caused by antibiotic-resistant strains of S. aureus and S. epidermidis. It is established that bacteremia caused by methicillin-resistant strains of S. aureus is more often associated with health care provision, in particular with application of central venous catheters. In such cases, apart from considerable economic losses, the life prognosis for patients is unfavourable. The mortality in a 28-day-period from methicillin-resistant S. aureus bacteria is 1.6 times higher than from bacteremia caused by S. aureus strains producing penicillinase (Jokinen et al., 2017).

Identifying microorganism resistance and determining the mechanisms influencing their resistance to antibiotics and their ability to produce biofilm forms, as well as an increase in their virulence and pathogenicity should be one of priority directions in the healthcare system of any state. Due to staphylococci’s (especially S. aureus) possessing a high degree of adaptability and genomic variability (Deurenberg et al., 2007; Deurenberg & Stobberingh, 2008; Lindsay, 2010; Conlan et al., 2012), it is necessary to maintain a permanent monitoring of their resistance.

In 2018, the results of a wide-scale multicentered epidemiology research project on determining antibiotic-resistance (Survey of Antibiotic Resistance, SOAR) concerning Ukraine and Slovakia were published. On the example of non-hospital respiratory infections agents Streptococcus pneumoniae and Haemophilus influenzae, territorial differences in the resistance level of the located isolates in the two neighbouring countries were found (Torumkuney et al., 2018). Overall, other studies on microorganism resistance to antibiotics, currently conducted in Ukraine, are narrowly specialized and do not encompass the problem in general.

It should be noted that there are differences in antibiotic resistance in organisms circulating in different departments of the same healthcare setting. For instance, the strains found in intensive care patients are often more resistant and may possess multiple resistance to antibiotics (Kollef & Fraser, 2001; Brusseleers et al., 2011). It was also noted that in the countries with lower income levels, the burden of antibiotic resistance in intensive care units is much heavier than in the countries with high incomes (Saharman et al., 2021). Everything mentioned above points to the necessity of estimating territorial differences in staphylococci antibiotic resistance in the regions of Ukraine. Therefore, the goal of this study is to perform a comparative analysis of antibiotic resistance in S. aureus and S. epidermidis isolates identified in surgical hospitals of two neighbouring areas: the Kharkiv and the Poltava regions.

Materials and methods

The study included clinical specimens collected from surgical patients by bacteriological laboratories in health-care settings of Kharkiv and Poltava regions in 2013–2019. S. aureus and S. epidermidis strains were isolated and identified according to standard methods. Antibacterial resistance in staphylococci was determined using the disc-diffusion method on Mueller-Hinton agar according to Clinical and Laboratory Standards Institute Guidelines (Bauer et al., 1966; Clinical Laboratory Standards Institute, 2014). In total, 151,015 and 98,754 tests to determine antibiotic resistance of S. aureus in Kharkiv and Poltava regions respectively were carried out in 2013–2019 and 15,589 tests to determine antibiotic resistance of S. epidermidis strains in Kharkiv region were carried out in 2013–2015 (Table 1).

| Antibiotics group or antibiotic | S. aureus (2013–2019) | S. epidermidis (2013–2015) |
|---------------------------------|-----------------------|--------------------------|
|                                 | Kharkiv region        | Poltava region           | Kharkiv region |
| Penicillin                      | 28,904                | 15,676                   | 5,579         |
| Cephalosporins                  | 17,359                | 5,340                    | 2,818         |
| Carbenicillin                   | 5,302                 | 3,038                    | 396           |
| Aztreonam                       | 15                    | 0                        | 11            |
| Aminoglycosides                 | 21,144                | 10,762                   | 1,782         |
| Macroildes                      | 7,642                 | 10,902                   | 590           |
| Linzolazidose                   | 10,156                | 8,792                    | 772           |
| Tetracyclines                   | 3,534                 | 9,934                    | 596           |
| Vancomycin                      | 7,870                 | 7,120                    | 939           |
| Rifampicine                     | 5,606                 | 908                      | 237           |
| Fluoroquinolones                | 33,619                | 15,771                   | 3,297         |
| Linzolidose                     | 11,219                | 8,097                    | 544           |
| Cotrimoxazol                    | 14                    | 1,025                    | 0             |
| Chloramphenicol                 | 755                   | 1,355                    | 28            |
| Phosphomicin                    | 0                     | 293                      | 0             |
| Fusid acid                      | 0                     | 220                      | 0             |
| Nitrofuran derivatives          | 0                     | 331                      | 0             |
| Total                           | 151,015               | 98,754                   | 15,589        |

For assessment of the resistance of S. aureus strains, the tested antibiotics included penicillins (penicillin, benzylpenicillin, ampicillin, amoxicillin, oxacillin, carbenicillin, ampicillin/sulbactam, amoxicillin/clavulanic acid, cephalosporins (cefazolin, cefalotin, cephalexin, cephaklor, cefuroxime, cefoperazon, cefoxitine, ceftriaxone, cefaluzidine, cefixime, cefepim, cefipim, cefoperazon/sulbactam), carbapenems (imipenem, meropenem, imipenem/cilastatin), aminoglycosides (kanamycin, gentamycin, tobramycin, netilmycin, amikacin), macrolides (erythromycin, clarithromycin, azithromycin, spiramycin), linezolidates (clindamycin, lincomycin), tetracyclines (tetracycline, doxycycline, tigecycline), glycopeptides (vancomycin), an antituberculosis medicine rifampicin, fluoroquinolones (ciprofloxacin, ofloxacin, cefoxitin, norfloxacin, lomefloxacin, levofloxacin, moxifloxacin, gatifloxacin), oxazolidinones (linezolid), sulphanilamides (co-trimoxazol, amphenicols (chloramphenicol) and others (phosphonycin, fusidic acid, nitrofuran derivatives).

Depending on the year and the region, the study to estimate resistance in the S. aureus strains had its peculiarities. In particular, in 2013, 2014, 2018, 2019, no tests were made in Poltava region to estimate resistance to cephalosporin group antibiotics and carbapenems in S. aureus isolates. In 2013, 2014, 2018, 2019, resistance in S. aureus isolates was studied in relation to only one antibiotic of the aminoglycoside group, gentamicin, and to only one antibiotic of the macrolide group, erythromycin in Poltava region. In 2013 and 2014, the resistance in S. aureus isolates was studied in relation to only one antibiotic of the tetracycline group, tetracycline in Kharkiv region. Resistance in S. aureus isolates to co-trimoxazol in Kharkiv region was assessed only in 2015 and 2018. Resistance of S. aureus isolates to chloramphenicol in Kharkiv region was not assessed in 2013 and 2014. Resistance of S. aureus isolates to phosphomycin, fusidic acid, nitrofuran derivatives was estimated only in Poltava region (in 2014, 2015, and 2017 – to phosphomycin; in 2016–2018 – to fusidic acid; in 2015, 2016, and 2018 – to nitrofuran derivatives).

To increase data validity, we calculated the average proportion of S. aureus isolates resistant to antibiotics groups (penicillins, cephalosporins, etc.) rather than to individual medications (with the exception of vancomycin, rifampicin, co-trimoxazol, chloramphenicol, phosphomycin, fusidic acid as the sole representatives of their antibiotic groups).

For estimation of resistance of S. epidermidis strains, the tested antibiotics included penicillins (penicillin, ampicillin, amoxicillin, oxacillin, carbenicillin, ampicillin/sulbactam, amoxicillin/clavulanate), cephalosporins (cefazolin, cefalotin, cephalexin, cefaluzidine, cefixime, cefepim, cefipim, cefoperazon/sulbactam), carbapenems (imipenem, meropenem, imipenem/cilastatin), aminoglycosides (kanamycin, gentamycin, tobramycin, netilmycin, amikacin), macrolides (erythromycin, clarithromycin, azithromycin, spiramycin), linezolidates (clindamycin, lincomycin), tetracyclines (tetracycline, doxycycline, tigecycline), glycopeptides (vancomycin), an antituberculosis medicine rifampicin, fluoroquinolones (ciprofloxacin, ofloxacin, cefoxitin, norfloxacin, lomefloxacin, levofloxacin, moxifloxacin, gatifloxacin, oxazolidinones (linezolid), sulphanilamides (co-trimoxazol), amphenicols (chloramphenicol) and others (phosphonycin, fusidic acid, nitrofuran derivatives).
cosides (gentamicin, tobramycin, amikacin), macrolides (erythromycin, clarithromycin, azithromycin), lincomycins (clindamycin, lincomycin), tetracyclines (tetracycline, doxycycline), glycopeptides (vancomycin), an antituberculosis medicine rifampicin, fluoroquinolones (ciprofloxacin, ofloxacin, pefloxacin, norfloxacin, lomefloxacin, levofloxacin), azalides (linzolid), sulphonamides (co-trimoxazol), amphenicols (chloramphenicol).

In some years, the resistance of \( S.\ aureus \) strains identified in 2013 (62.7%), and the smallest proportion was identified in 2016 (10.4%). In Poltava region, the largest proportion of \( S.\ aureus \) strains resistant to penicillin group drugs was identified in 2017 (42.0%), while in Poltava region – in 2015 (12.5%). It was found that the proportion of \( S.\ aureus \) isolates resistant to macrolides in 2013, 2016, and 2019 was statistically significantly higher in Poltava region. In other years (except 2014) statistically significant differences were found to be in favour of a higher resistance of \( S.\ aureus \) isolates in Kharkiv region (Fig. 1a). Generally, during the period from 2013 to 2019, the proportion of \( S.\ aureus \) strains resistant to the macrolides group medications was slightly higher (1.1 times) in Kharkiv region (19.9% / n = 1,522 of 7,642 versus 18.1% / n = 1,827 of 10,092; \( \chi^2 = 9.3; P = 0.002 \)).

In Kharkiv region, the largest proportion of \( S.\ aureus \) strains resistant to lincomazids was identified in 2015, and the smallest – in 2013 (41.6% and 3.1% respectively). In Poltava region more than half of the identified \( S.\ aureus \) strains in 2014 (54.0%) were resistant to lincomazids group antibiotics. The lowest resistance to lincomazids in Poltava region was in \( S.\ aureus \) strains identified in 2015 (10.0%). Statistically significant differences in proportion of \( S.\ aureus \) strains resistant to lincomazids in the compared regions were found in 2013–2015 and in 2017–2019 (Fig. 2b). On the whole, during the analyzed period 1.9 times more \( S.\ aureus \) isolates resistant to lincomazids were identified in Poltava region than in Kharkiv region (16.3% / n = 1,436 of 8,792 versus 8.5% / n = 864 of 10,156; \( \chi^2 = 270.6; P < 0.001 \)).

The proportion of \( S.\ aureus \) isolates resistant to the tetracycline medications group in Kharkiv region varied within 0.6% in 2013 to 15.3% in 2016. The proportion of tetracycline resistant \( S.\ aureus \) strains in Poltava region reached its maximum in 2019, amounting to 30.5%. The smallest proportion of the resistant isolates in Poltava region was identified in 2015 (9.1%). The statistically significant differences between the proportion of tetracycline-resistant \( S.\ aureus \) isolates in the compared regions were identified in 2013, 2014, and 2016-2019 (Fig. 2c). On the whole, during the studied period, the proportion of \( S.\ aureus \) strains resistant to tetracycline antibiotics was 1.9 times higher in Poltava region (15.7% / n = 1,560 of 9,934 versus 8.3% / n = 294 of 3,534; \( \chi^2 = 119.7; P < 0.001 \)).

In Kharkiv region in 2013 and 2014, 96.3% and 89.1% isolated \( S.\ aureus \) strains respectively were resistant to vancomycin. The smallest amount of \( S.\ aureus \) strains resistant to vancomycin in Kharkiv region was identified in 2016 (0.6%). In Poltava region, of the 1063 \( S.\ aureus \) isolates studied in 2017, none was resistant to vancomycin. Nevertheless, more than the quarter of the identified isolates in 2019 (26.6%) in Poltava region were resistant to this antibiotic. In 2013, 2014 and 2017, the proportion of \( S.\ aureus \) isolates resistant to vancomycin was statistically significantly higher (P < 0.001) in Kharkiv region (Fig. 3a).

Using Fisher’s exact test, we found statistically significant differences in proportion of rifampicin resistant isolates of \( S.\ aureus \) in the compared regions in 2013, 2014 and 2016 (P < 0.05). The proportion of rifampicin-resistant isolates of \( S.\ aureus \) in the Poltava region was higher. It should be noted that during the whole studied period, the proportion of rifampicin-resistant \( S.\ aureus \) isolates in Kharkiv region did not exceed 7.8% (n = 19 of 244 in 2017), and in Poltava region it did not exceed 11.6% (n = 11 of 95 in 2016).

Comparative analysis by the regions of resistance of \( S.\ aureus \) isolates to fluoroquinolones medications showed that in Poltava region the proportion of resistant strains prevailed over that of Kharkiv region during the whole period of observation except 2017 (Fig. 3b). In Poltava region, the proportion of the resistant strains varied between 15.6% in 2014 and 24.5% in 2019. In Kharkiv region, the proportion of the resistant isolates was between 3.0% in 2013 and 26.9% in 2017. Overall, during the studied period, the proportion of fluoroquinolone resistant \( S.\ aureus \) strains was 3.3 times higher in Poltava region (19.7% / n = 3,110 of 15,771 versus 5.9% / n = 1,995 of 3,3619; \( \chi^2 = 2201.3; P < 0.001 \)).
The analysis of S. aureus strains resistant to linezolid has demonstrated that in Poltava region the proportion of resistant microorganisms was statistically significantly higher in 2013–2014 and 2016–2018 (Fig. 3c). Notably, in Kharkiv region linezolid-resistant strains accounted for 19.3% of antibiotic resistant strains identified in 2015, which exceeded by 8.5% the maximum proportion of linezolid-resistant strains of S. aureus identified in Poltava region. Due to the small number of co-trimoxazol sensitivity tests (n = 14) of S. aureus made in Kharkiv region, it is impossible to interpret correctly the negative results obtained. Nevertheless, more tests were made in Poltava region, and the proportion of resistant isolates varied there between 3.4% (n = 9 of 268) in 2019 and 43.7% (n = 52 of 119) in 2016.

Fig. 1. Proportion (x ± SE, %) of penicillin antibiotics-resistant (a), cephalosporin antibiotics-resistant (b), carbapenem antibiotics-resistant (c), aminoglycoside antibiotics-resistant (d) strains of S. aureus in Kharkiv and Poltava regions during 2013–2019: * – the differences are statistically significant at P < 0.01, ** – antibiotic resistance was not studied in Poltava region that year, *** – there are available data on resistance to only one aminoglycoside antibiotic (gentamycin).
The largest proportion of *S. aureus* isolates resistant to chloramphenicol was identified in Kharkiv region in 2018 (60.4%), and in Poltava region – in 2014 (63.6%). In 2016-2019, the differences in both regions were statistically significant (Fig. 3d). During the period of 2014–2015 and in 2017, in Poltava region 9.2% (n = 27 of 293) of *S. aureus* isolates were found to be resistant to phosphomycin. 8.2% (n = 18 of 220) of *S. aureus* strains were found to be resistant to fusidic acid in Poltava region in 2016–2018. Also, in 2015–2016 and in 2018 in Poltava region, 41.4% (n = 137 of 331) of *S. aureus* isolates were identified as resistant to nitrofuran derivatives. The analysis of *S. epidermidis* isolates’ resistance has demonstrated that in Kharkiv region in 2015, nearly half (47.6%) of the isolates were found to be resistant to the penicillin antibiotics (Fig. 4).

Notably, from 2012 to 2015 among the *S. epidermidis* isolates there was a growth in the prevalence of resistance to cephalosporins, aminoglycosides, macrolides, and fluoroquinolones. When determining the carbapenems resistance in 2013, 3.1% (n = 1 of 32) of *S. epidermidis* were found to be resistant to imipenem. In 2014 and 2015, the proportion of the carbapenems-resistant strains was considerably larger, 15.5% (n = 25 of 161) and 16.3% (n = 33 of 203) respectively. In 2013, sensitivity in 11 strains of *S. epidermidis* to aztreonam was determined. In three cases, the isolates were resistant. In 2013 and 2014, relatively few linezolid-resistant isolates were identified: 1.7% (n = 8 of 470) and 5.5% (n = 16 of 289) respectively. In 2015, sensitivity to clindamycin was identified in 13 *S. epidermidis* strains. In four cases, the isolates were resistant. The proportion of tetracycline-resistant isolates in 2013 comprised 4.2% (n = 9 of 213), in 2014 – 4.9% (n = 11 of 223). When analyzing *S. epidermidis* resistance to vancomycin, a decrease in the proportion of resistant strains from 88.0% in 2013 to 8.7% in 2015 was noted. The resistance of *S. epidermidis* isolates to rifampicin was studied only in 2013 and 2014. The proportion of the resistant strains was 3.7% (n = 8 of 214) and 8.7% (n = 2 of 23) respectively. In 2015, the proportion of linezolid-resistant strains grew sharply. In 2015, sensitivity to chloramphenicol was identified in 28 *S. epidermidis* strains. In 24 cases (85.7%), the isolates were resistant.

**Discussion**

The study has confirmed the existence of differences in the prevalence of *S. aureus* isolates resistant to various groups of antibacterial medications in Kharkiv and Poltava regions. In Kharkiv region, strains resistant to medications of penicillin group, cephalosporins, carbapenems, aminoglycosides, macrolides, and fluoroquinolones. When determining the carbapenems resistance in 2013, 3.1% (n = 1 of 32) of *S. epidermidis* were found to be resistant to imipenem. In 2014 and 2015, the proportion of the carbapenems-resistant strains was considerably larger, 15.5% (n = 25 of 161) and 16.3% (n = 33 of 203) respectively. In 2013, sensitivity in 11 strains of *S. epidermidis* to aztreonam was determined. In three cases, the isolates were resistant. In 2013 and 2014, relatively few linezolid-resistant isolates were identified: 1.7% (n = 8 of 470) and 5.5% (n = 16 of 289) respectively. In 2015, sensitivity to clindamycin was identified in 13 *S. epidermidis* strains. In four cases, the isolates were resistant. The proportion of tetracycline-resistant isolates in 2013 comprised 4.2% (n = 9 of 213), in 2014 – 4.9% (n = 11 of 223). When analyzing *S. epidermidis* resistance to vancomycin, a decrease in the proportion of resistant strains from 88.0% in 2013 to 8.7% in 2015 was noted. The resistance of *S. epidermidis* isolates to rifampicin was studied only in 2013 and 2014. The proportion of the resistant strains was 3.7% (n = 8 of 214) and 8.7% (n = 2 of 23) respectively. In 2015, the proportion of linezolid-resistant strains grew sharply. In 2015, sensitivity to chloramphenicol was identified in 28 *S. epidermidis* strains. In 24 cases (85.7%), the isolates were resistant.
glycosides, and macrolides were identified more often than in Poltava region. In Poltava region, strains resistant to linezolid, tetracycline antibiotics, and fluoroquinolones were identified more often than in Kharkiv region. This can be ascribed to the regional peculiarities in antibiotics consumption by the population and in medical practices. A high occurrence of \textit{S. aureus} isolates resistant to natural and some synthetic and semisynthetic penicillin antibiotics (penicillin, ampicillin) is confirmed by other authors' studies (Deyno et al., 2017; Yılmaz & Aslantaş, 2017). Inhibitor-protected penicillins still remain quite efficient, although the high adaptability of \textit{S. aureus} can make antibiotics of this group totally inefficient rather soon.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Proportion (x ± SE, \%) of vancomycin-resistant (a), fluoroquinolones-resistant (b), linezolid-resistant (c), chloramphenicol-resistant (d) strains of \textit{S. aureus} in Kharkiv and Poltava regions during 2013–2019: * – the differences are statistically significant at \( P < 0.01 \), ** – antibiotic resistance was not studied in Kharkiv region in this year.}
\end{figure}

We noted that in Poltava region, the proportion of \textit{S. aureus} isolates resistant to cephalosporins and carbapenems is relatively low. The monitoring of resistance to these antibiotics groups in Poltava region is not conducted on a regular basis. In Kharkiv region, the largest proportion of strains resistant to cephalosporins and carbapenems medications amounted to 39.9% and 42.0% respectively. Therefore, Poltava region also needs to monitor on a regular basis the resistance of \textit{S. aureus} to these groups of medications and to apply them in treatment protocols with care.
Linezolid is an antibiotic which is efficient against their data versus 19.3% of resistant strains identified in 2015 in Kharkiv identified a high resistance to linezolid (0% of resistant strains according to gram-positive flora including metycillin-resistant staphylococci (Gu et al., 2012), therefore the results obtained by us in this study call for further study and identifying the causes of such a high resistance.

Similar data (compared with Poltava region) concerning S. aureus resistance to co-trimoxazol were demonstrated by Deyno et al. (2017). Due to the insufficient amount of tests in Kharkiv region, we think that it is necessary to continue studying the sensitivity to co-trimoxazol in Kharkiv region to find out whether there are any territorial differences in S. aureus resistance to this antibiotic.

When analyzing the dynamics of prevalence of resistant S. aureus strains in the regions, we found a broad difference in the proportion of isolates resistant to some antibiotics. For instance, in Kharkiv region, 96.3% of S. aureus isolates were found to be vancomycin-resistant in 2013, while in 2016 – less than 1%. Also in Kharkiv region, 3.1% of strains were found to be resistant to the lincomycin group in 2013, while in 2015 – 41.6%. These results can be probably ascribed to the disproportional amount of cultures sampled in healthcare settings for our study. This indicate that every healthcare setting has unique microbiological profile.

The research by Mohaghegh et al. (2015) has demonstrated efficiency in using chloramphenicol against S. aureus isolates sampled from the patients with suspected bacteremia. In our study, the proportion of chloramphenicol-resistant isolates was high in both regions in certain years. In view of this, we think that the study of S. aureus sensitivity to this medication should be continued by increasing the number of the studied isolates.

As to S. epidermidis, it should be noted that despite its long since proven pathogenicity (Morgunov & Kukharchik, 1986), this microorganism is still underestimated in medical practice as being a cause of a number of infections. At the same time, S. epidermidis possesses properties and mechanisms owing to which it manages to fix itself on the human body and avoid being destroyed by the immune system. Normally, owing to its adhesive properties, S. epidermidis fixes itself to the host’s proteins in the skin, and in cases of damage, wounds, introduction of foreign bodies (prosthetics, vessels catheterization), the infectious agent fixes itself to deeper-laying tissues or to the surface of the implanted appliances (Sadhat Bresco et al., 2017).

The analyses on determining S. epidermidis strains resistance to antibiotics made in Kharkiv region show in the dynamics a rise in the proportion of isolates resistant to most of the antibiotic groups: to penicillins, cephalosporins, aminoglycosides, macrolides, and fluoroquinolones. As early as in 1980, Archer & Tenenbaum in their study on patients surviving heart operations reported a high proportion of S. epidermidis isolates resistant to naphthyllin, penicillin (100% each), to cephalothin (93%), to cephamandol (80%), to streptomycin (67%). Other researchers report the high prevalence of resistance in hospital S. epidermidis strains to many medications: to penicillin, cepfoxolin, tetracycline, erythromycin. Moreover, in the isolated strains simultaneous resistance to more than three groups of antibiotics was observed, and in 17.4% – to seven different groups of antibiotics (Chabi & Momtaz, 2019). Of great concern is the high proportion of vancomycin-resistant S. epidermidis strains isolated in Kharkiv region in 2013. Nunes et al. (2016) state that the resistance in S. epidermidis strains to glycopeptides is influenced by the thickness of the cell’s membrane. Also, the authors report the heterogenic resistance of S. epidermidis to glycopeptides. At the same time, in another study, vancomycin is viewed as the most efficient medicine against S. epidermidis for treating patients with suspected bacteremia (Mohaghegh et al., 2015). Chabi & Momtaz (2019) also reported the high prevalence of S. epidermidis resistance to co-trimoxazol. Because in the present study we did not identify resistance to this medication, it should be accounted for in further studies. Therefore, considering the aforementioned, the study of resistance of S. epidermidis isolates should be obligatory all over the country without limitation to individual regions. Our study demonstrates that in Ukraine there is a need for the introduction of a complex approach to the issue of antibiotic resistance. Epidemiological monitoring of hospital infectious agents should be strengthened at the national level, and in the regions, the scope of conducted bacteriological researches should be broadened with further identification of antibiotic sensitivity in the isolated strains. It is also necessary to identify in the isolated microorganisms the ability to form biofilms and the traits of their biofilm forms. Additional introduction of molecular-genetic methods, identifying hetero-resistance in microorganisms and the study of their subpopulations can help the patients whose treatment does not

### Table 4

Proportion (%) of antibiotic-resistant isolates from the total number of strains, %

| Antimicrobial Group | 2015 | 2014 | 2013 |
|--------------------|------|------|------|
| aminoglycoside antbiotics | 36.0 | 56 (n= 150 of 269) | 29.9 (n= 65 of 227) |
| macrolide antibiotics | 59.6 | 59 (n= 149) | 39.6 (n= 120 of 306) |
| fluoroquinolones | 57.8 | 102 (n= 120 of 205) | 29.2 (n= 351 of 1205) |
| vancomycin | 88.0 (n= 212 of 241) | 102 (n= 120 of 1105) | 9.2 (n= 67 of 961) |
| tetracycline | 6.8 (n= 67 of 961) | 80% (n= 120 of 150) | 42.2 (n= 49 of 116) |

Fig. 4. Proportion (%) of antibiotic-resistant strains of S. epidermidis in Kharkiv region during 2013–2015: * – there are available data on resistance to only one aminoglycoside antibiotic (amikacin), ** – there are available data on resistance to only one macrolide antibiotic (erythromycin).
The study has been performed within the framework of the science-and-research laboratory Center of the MoH of Ukraine for their assistance in conducting this study.

The authors would like to express their sincere gratitude to the SI Khar-...
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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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Lieshchova, M. A., & Brygadyrenko, V. V. (2021). Influence of Lavandula angustifolia, Melissa officinalis and Vitex angus-castus on the organism of rats fed with excessive fat-containing diet. Regulatory Mechanisms in Biosystems, 12(1), 169–180. doi:10.15421/022125

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 unreliable 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%), totalprotein (to 63%) and triglycerides (to 63%). Addition of Vitex led to increase in the index of atherogenicity 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 cholesterol 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 backdrop 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 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 neuroprotective treatments. Biologically active substances of Lamiaceae plants comprise phenolic compounds, chiefly phenolic acids, which are named carboxylic and cinnamic acids and their derivatives, flavonoids, including flavones, isoflavones, flavonols, flavanones, flavones, flavans, flavans 3,4-diols, catechins, biflavonoids and proanthocyanidins (Milevskaya et al., 2019). Rich in biologically active compounds (BAC), species of Lamiaceae family are broadly used in pharmacology.

Melissa officinalis is a perennial herbaceous plant of Lamiaea 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%, 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, liquors, salting of cucumbers and tomatoes (Shakiri 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 (Martyov 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, geranialacetate, 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 proportion 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 phenylnaupropoains, including rosmarinic acid as the most distinctive ones (Nogouchi-Shinohara et al., 2015). Phenylpropoains 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 amounts for 0.54–1.79%. Among the phenol substances, antioxidant activity was exhibited by flavonoids apigenin, cosmostin, luteolin, cynaroside, and also tenuo-citrin (7-etoxy kaemeprol) and iso-queretin (3-queretin glucoside), rhod-nazin (3,7 dimetoxycampheroll). Moreover, the plant contains phenol-carboxic acids: gentisic, salicylic, p-hydroxybenzoic, vanillic, syringic, narzin (3,7 dimetoxycampheroll). Moreover, the plant contains phenol-carboxic acids: gentisic, salicylic, p-hydroxybenzoic, vanillic, syringic, protocatechuic acids and also tannins and coumarins. Out of sterols, the plant was observed to have daucosterol, out of saponins – ursolic acid. Rich in biologically active compounds (BAC), species of Lamiaceae family are broadly used in pharmacology.

Lemon balm is third plant according to the content of antioxidants out of 57 species of spicy and medicinal plants used in the experiment (Sammar et al., 2019). Citral it contains is able to efficiently inhibit cancer cells and induce cell apoptosis (Bailly, 2020). Its complex anticancer mecha-nism involves three actions: (I) the preparation leads to accumulation of reactive oxygen types in cancer cells, thus entailing an oxidative burst and damage to DNA, (II) a colchicine-like inhibition of tubulin polymerization and promotion of microtubule depolymerization, associated with inhibi-tion of the microtubule affinity-regulating kinase MARK4, and (III) a potent inhibition of aldehyde dehydrogenase isofrom ALDH1A3, related to cancer stem cell proliferation and chemoresistance (Bailly, 2020). Unfortunately, the citral’s potential is limited, mostly due to insufficient stability of the drug and its low bioavailability, and low selectivity for cancer cells against non-tumour cells. Nonetheless, citral is promising for development of effective analogues and drug combinations having a reinforced potential to treat tumours (Bailly, 2020).

Rosmarinic acid, present in many plants of Lamiaea family, is broadly used as culinary herbs: Ocimum basilicum L. (basil), Ocimum tenuiflorum L. (holy basil), M. officinalis L. (lemon balm), Salvia rosmarinus Spenn. (rosmary), Origanum majorana L. (marjoram), Salvia officinalis L. (sage), Thymus vulgaris L. (thyme) and Mentha × piperita L. (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 con-tains 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 become oxidized to orto-chi-nons. They bind with proteins of bacteria, fungi or herbivorous animal, thus inactivating them (Häusler et al., 1993). Rosmarinic acid of Lamiaea plants exerted inhibition of chlorine 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 Lamiaea 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, bornol (Karabagias et al., 2019). The gas chromatography revealed the shares of the main components to equal as follows: linyl acetate (25–46%), linolal (20–45%), terpinen-4-ol (1.2–6.0%), lavendalyl acetate (1.0%), 1,8-cineole (2.5%), 3-octalone (2.5%), camphor (1.2%), linalool (1.0%), and alpha-terpineol (<2.0%) (Korrem, 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 achieved through baths. It is also used in dental practice for inhalation treatment of rhinites, laryngitis; it is applied to speed up the wound healing 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 migranes, neurasthenia, stress (Kennedy & Wightman, 2011; Lundstrom et al., 2017; Uritu et al., 2018), rheumatism, cardiovascular diseases, kidney-stone disease and pyelonephritis, for medical baths during joint inflammation, for wound-healing, during skin diseases and neuralgias, bruises and paralyses (Zaee et al., 2015; Sadeghzadeh et al., 2017; Samantha et al., 2017; Xu et al., 2017; Cardia et al., 2018; Boukhatem 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-bear 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 (Dobetsberger & 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; Osokou et al., 2018). Essential oils from L. angustifolia improved cognitive performance and took positive effects on animals and humans suffering neurodegenerative disorders such as Alzheimer disease and dementia (Ayz et al., 2017). Also, this oil was reported to have neuroprotective effects (Ayz et al., 2017). Lavender and lavandin essential oils prepared by steam distillation are usually composed of terpenes (e.g. linalool and linalyl acetate) and terpenoids (e.g. 1,8-cineole), responsible for their distinctive aroma. The leaves are large, opposite, palmate, on long petioles. Has numerous blue flowers. Its range comprises North Africa, Southern Europe, West Asia, Transcaucasia, and Central Asia (Artz, 2007; Brown & Murray, 2012). It has been cultivated in gardens as an ornamental plant since the Middle Ages. The medicinal raw material is leaves, flowers, fruits, branches, and more rarely bark (Ross, 2001). All the parts of the plant contain iridoglycoside (agndasne, aucubin), flavonoids (casticin, vitexin, isovitaxin, orientin, isoorientin), p-hydroxyphenylzaid, alokaidos, tannins, essential oil (Stojkovic et al., 2011). The essential oil from leaves contains 1,8-cineole, trans-beta-farnesene, alpha-pinene, trans-beta-caryophyllene, androsten-4-ol. The oil from leaves of V. angus-castus contains 46 compounds. The major constituents of the leaves are 1,8-cineole (22.0%), trans-beta-farnesene (9.4%), alpha-pinene (9.4%), trans-beta-caryophyllene (8.2%), terpinen-4-ol (7.8%), linalool (4.8%), alpha-terpineol (3.8%), sclarene (3.3%), alpha-terpinyl acetate (3.1%), p-cymene (3.0%) (Stojkovic et al., 2011). 1,8-cineole and alpha-pinene exerted notable antimicrobial potency as well (Stojkovic et al., 2011). The oil, particularly such from white flowering plants, is surveyed for its potential antibacterial effects (Stojkovic et al., 2011). Extract from V. angus-castus exhibited the greatest cytotoxic activity out of 57 medicinal plants tested in the experiment (Sammar et al., 2019). The authors also indicate that powerful cytotoxicity is not related to low concentrations of antioxydants in it, but manifests through other signal pathways. The ripe fruit of V. angus-castus could be a promising antitumor agent (Kikuchi et al., 2014). Vitex was observed to induce dose- and time-dependent decrease in cell viability following the induction of apoptosis and G2/M cell cycle arrest. Clinical applications of Vitex revealed new data on interaction of Vitex with other conventional drugs able to affect intracellular redox status (Kikuchi et al., 2014). Alpha.beta-unsaturated gamma-lactam moiety, 9 alpha-hydroxy-13(14)-labden-16,15-amide (1), together with five known ones, were isolated from the fruits of V. angus-castus (Pal et al., 2013).

Fruits and herbs of V. angus-castus are included in the European pharmacopoeia. The plant is used during insufficient lactation, menstrual period problems, and also as diuretic and irritating preparation. The leaves are added to meat meals, soups, jam and half-smoked sausage, canned fish. During food preservation, vitex is used as a substitute of allspice. In men’s bodybuilding, it is used to control testosterone level. Vitex-based preparations are used in gynecology during premenstrual syndrome accompanied by edemas, poor bleedings or absence of them, anovulatory cycles, period disorders after using birth control preparations, infertility related to hyperprolactinaemia, breast pains (Arzi et al., 2019). For this purpose, the plant is processed to prepare cyclodynon, mastodynon, pregnacone, perfinen, biocycline, and others. Vitex is traditionally recommended medicine against premenstrual stress syndrome, premenstrual dysphoric disorder and other reproductive health issues in women. Nonetheless, despite the fact that it is often recommended in Germany, there are some indications that V. angus-castus may lead to complications during...
were maintained in the room with the temperature of 20–22 °C and relate
cells with steel grid covers, food pit, 4 individuals per a cell. The rats
of experimental groups with 8 animals in each. The rats were kept in polycarbo-
No. 3447-IV as of 21.02.2006 “On protection of animals against abuse”
and the order
Protection of Vertebrate Animals used for Experimental and Other Scien-
(1986, ETS No. 123) and the order
Materials and methods
Selection of animals for the experiment, the study protocols, euthana-
sia of animals were approved by the local ethics committee of the Dnipro
State Agrarian-Economic University. Content, feeding, care for the ani-
als 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 Sci-
cific 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 exper-
imental groups with 8 animals in each. The rats were kept in polycarbo-
ate 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 rela-
tive 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 ani-
imals received water ad libitum.
The diet of all animals had excessive fat content (3,600 kcal/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 com-
plex) 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 experimen-
tal 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 com-
plex, 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 instal-
lation of 1 m² square area divided into 16 squares and limited non-
transparent 20 cm-high wall. The experiment was performed in com-
plete silence with intense light on the field itself. An experimental ani-
imals had been taken from the cage from previously shadowed compart-
ment 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) – orientation 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 as-
essed 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
cut 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 Miura automated analyzer (I.S.E. Srl, Italy) and a set of High-Technology reagents (USA), PZCormay 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 ± 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. The mean daily gain (Table 1): instead of 700 mg/day, the animals gained 1.943 g/day, which caused reliable (more than 2.5-fold) increase in the mean daily body weight gain (Table 1). A statistically insignificant increase (up to 138.1% of the control) caused by the addition of crumbled shoots of Lavandula angustifolia to the diet, 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, uric acid, total protein) were within the norm.

Table 2

| Parameter                  | Control                  | L. angustifolia compared to the control, % | M. officinalis compared to the control, % | V. angus-castus compared to the control, % |
|----------------------------|--------------------------|------------------------------------------|------------------------------------------|-------------------------------------------|
| Consommation of food       | 20.09 ± 0.71             | 1943 ± 406***                            | 2776 ± 2024***                           | 269.1 ± 417***                            |
| Consumption of liquid      | 18.42 ± 1.43             | 1005 ± 289***                            | 1905 ± 103.4***                          | 18.93 ± 102.8***                          |
| Change in body weight, mg/d | 70.2 ± 0.71              | 35.7 ± 0.18***                           | 261.7 ± 367***                           | 103.4 ± 138.1***                          |
| Change in body weight, %/day | 13.6 ± 5.9               | 140.0 ± 0.5                              | 99.9 ± 13.8                              | 106.0 ± 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.
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 globulin 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
Change in biochemical parameters of blood of males of rats under effect of addition of crumbled shoots of Lavandula angustifolia Mill., Melissa officinalis L. and Vitex angus-castus L. (x ± SD, n = 8, duration of experiment – 30 days)

| Parameters            | Control       | 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                               | 80.3 ± 3.1                               | 102.3                                   |
| Albumin, g/L          | 39.6 ± 2.6    | 38.7 ± 2.5                               | 41.3 ± 2.2                               | 104.3                                   |
| Globulins, g/L        | 37.4 ± 3.9    | 37.3 ± 3.3                               | 38.4 ± 3.3                               | 103.4                                   |
| Protein coefficient, U| 1.10 ± 0.15   | 1.06 ± 0.12                              | 1.09 ± 0.14                              | 103.4                                   |
| Urea, mmol/L          | 6.84 ± 1.02   | 6.00 ± 0.74                               | 5.40 ± 0.58                              | 78.8                                    |
| Urea nitrogen, mg/100 g| 13.1 ± 2.0   | 11.5 ± 1.4                               | 10.3 ± 1.1                               | 79.0                                    |
| Creatinine, mmol/L    | 63.0 ± 4.4    | 61.0 ± 7.4                               | 74.9 ± 12.8                              | 118.8                                   |
| Aspartate aminotransferase (AST), U/L | 186.6 ± 61 | 160.6 ± 48                               | 182.3 ± 33                               | 97.8                                    |
| Alanine aminotransferase (ALT), U/L | 131.4 ± 41 | 129.3 ± 39                               | 111.1 ± 18                               | 85.2                                    |
| De Ritis ratio (AST/ALT), U | 1.63 ± 0.78 | 1.37 ± 0.45                              | 1.64 ± 0.30                              | 100.9                                   |
| Alkaline phosphatase, U/L | 129.6 ± 64 | 451.9 ± 94***                           | 601.6 ± 140***                           | 465.7                                    |
| Total bilirubin, µmol/L | 61.1 ± 1.7 | 41.2 ± 2.8                               | 3.8 ± 1.4*                               | 63.3                                    |
| Glucose, mmol/L       | 7.29 ± 1.04   | 6.36 ± 0.63                               | 6.40 ± 0.55                              | 86.7                                    |
| Totalcalcium, mmol/L  | 2.53 ± 0.00   | 2.51 ± 0.11                               | 2.59 ± 0.14                              | 102.3                                   |
| Non-organic phosphorus, mmol/L | 3.07 ± 0.58 | 3.67 ± 0.40                               | 3.46 ± 0.29                              | 112.6                                   |
| Ratio Ca/P            | 0.843 ± 0.129 | 0.686 ± 0.09                             | 0.743 ± 0.09                             | 88.1                                    |
| Gamma-glutamyl transferase (GGT), units/L | 9.3 ± 2.6 | 9.1 ± 4.4                                 | 6.7 ± 0.7                                | 72.3                                    |
| Cholesterol, mmol/L   | 1.27 ± 0.13   | 1.59 ± 0.20*                             | 1.43 ± 0.18                              | 112.4                                   |
| Triglycerides, mmol/L | 2.13 ± 0.55   | 1.36 ± 0.38                               | 1.34 ± 0.31*                             | 63.1                                    |
| High-dense lipoprotein cholesterol (HDL, cholesterol), mmol/L | 0.65 ± 0.13 | 0.66 ± 0.19                              | 0.88 ± 0.44                              | 122.6                                   |
| Low-dense lipoprotein cholesterol (LDL, cholesterol), mmol/L | 0.52 ± 0.29 | 1.18 ± 0.08***                             | 0.51 ± 0.11                              | 98.0                                    |
| C-reactive protein, mg/L | 12.5 ± 5.4 | 13.2 ± 5.2                               | 10.2 ± 1.4                               | 81.7                                    |
| Atherogeneix index, units | 1.04 ± 0.45 | 1.85 ± 1.41                              | 1.71 ± 0.40                              | 124.5                                   |

Note: see Table 1.

Table 4
Change in general analysis of blood and leukogram of male rats under effect of intake of crumbled shoots of Lavandula angustifolia Mill., Melissa officinalis L. and Vitex angus-castus L. (x ± SD, n = 8, duration of experiment – 30 days)

| 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 ± 7.0   | 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                              | 103.6                                   |
| Erythrocytes, 10^6/L       | 6.93 ± 0.29   | 7.13 ± 0.30                              | 7.03 ± 0.9                               | 101.3                                   |
| Erythrocyte sedimentation rate (ESR), mm/h | 1.17 ± 0.37 | 1.33 ± 0.47                              | 1.00 ± 0.00                             | 85.7                                    |
| Platelets, 10^3/µL         | 339 ± 66      | 351 ± 87                                 | 336 ± 66                                | 99.3                                    |
| Leukocytes, 10^6/L         | 8.6 ± 1.6     | 14.2 ± 2.3***                            | 17.1 ± 5.9***                           | 199.9                                   |

Leukocytic formula

| Basophils, %               | 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                             | 104.8                                  |
| Monocytes, %               | 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                                    |
| – young                    | 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                             | 61.2                                    |
| – with segmented nuclei    | 23.0 ± 8.2    | 22.3 ± 5.1                               | 25.6 ± 6.4                              | 111.2                                   |
| Lymphocytes, %             | 68.8 ± 8.6    | 67.2 ± 6.3                               | 65.9 ± 6.9                              | 95.7                                    |
| Monocytes, %               | 5.5 ± 1.3     | 8.2 ± 3.3                                | 6.3 ± 2.3                               | 114.3                                   |

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 *Vitex 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 marks 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 *Vitex 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 ± 8.59b                | 16.11 ± 14.29b               | 4.57 ± 9.32b                | 13.54 ± 12.78b              | 8.96 ± 11.30b                 |
| Number of attended central squares    | 1.00 ± 2.34i        | 0.29 ± 1.04           | 0.00 ± 0.00                  | 0.00 ± 0.00                  | 0.82 ± 1.88                 | 0.00 ± 0.00                  | 0.107 ± 0.416                 | 0.00 ± 0.00                   |
| Number of stances in the central squares | 5.58 ± 4.53i     | 3.70 ± 3.13i         | 3.04 ± 2.13i                 | 0.79 ± 1.03i                 | 3.75 ± 2.74i                | 1.00 ± 1.44i                | 2.93 ± 3.33i                  | 1.86 ± 2.21i                  |
| 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               | 0.00 ± 0.00                  | 0.222 ± 0.641                 | 0.00 ± 0.00                   |
| 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 ± 0.00                  | 0.000 ± 0.00                   |

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

### 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 receipes 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 (geranial and neral), citronellal, geraniol. In the experiments, *M. officinalis* notably decreased body weight (Valizadeh 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 GABA(A) receptors, as well as inhibition of matrix metalloproteinase-2 (Shakeri et al., 2016). Lemon balm is applied during a number of health issues, particularly anxiety and some other disorders of the central nervous system, but substantiation of its effects needs trials in clinical settings (Shakeri et al., 2016). The most frequent clinical effects of application plant food supplements that contained *M. officinalis* were...
neurotoxicity and gastro-intestinal symptoms. The symptoms in most cases were mild (Ludke 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 lipoproteins of different density, which are expressed by such a parameter as atherogeneity 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’ organisms. 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 (Stehbens, 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 Swiaider et al. (2019) analyzed the literature data on the chemical composition of M. officinalis and the possibilities of using it in medicine and food. Heshmari 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 Heshmari 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 membranes 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, antioxidants, 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 studies, 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’ organisms. 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 (Stehbens, 1986).

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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 depressant effects; clinical pharmacology includes anxiolytic, analgesic, and cardiovascular effects (Korien, 2021). In the quantitative synthesis, inhalation 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 (Finner & Pilkington, 2012). Some studies have shown the efficiency of oral lavender supplements, but independent replications are needed to draw conclusions (Perry et al., 2012). In the “open field” method, 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 vinit manifesting calming effect (Mehlhorn, 2016), it exhibited no inhibition of motor and orienting activities in our experiment. 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 combination 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 determines 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 intestine of animals, (Bilam et al., 2019). In our earlier experiments, ethanol extract from M. officinalis powerfully inhibited growth of colonies of bacteria of Salmonella typhimurium, poorly inhibited such of Escherichia coli, Klebsiella pneumonia and Corinebacterium xerosis, and caused no effect on Proteus mirabilis, Listeria monocytogenes and fungus of Candida albicans (Zazharskyi et al., 2019). Similar effects were observed for ethyl extract of the leaves of L. angustifolia: it notably inhibited growth of colonies of Salmonella typhimurium and Klebsiella pneumonia, weakly affected Escherichia coli, Proteus mirabilis and fungus of Candida albicans and inhibited no growth of cells of bacteria of Listeria monocytogenes and Corinebacterium xerosis (Zazharskyi et al., 2019). We saw broader range of antibacterial activity in vitro experiments exhibited by ethyl extract from V. angus-castus that notably inhibited growth of Corinebacterium xerosis, Serratia marcescens, Salmonella typhimurium, Proteus mirabilis; weakly affected growth of colonies of Rhodococcus equi, Pseudomonas aeruginosa, Yersinia enterocolitica, Klebsiella pneumonia, Enterococcus faecalis, Escherichia coli, and took no inhibitory effect on growth of colonies of Enterobacter aerogenes, Listeria ivanovii, L. innocua, L. monocytogenes, Campylobacter jejuni and fungus of Candida albicans (Zazharskyi 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 papillosus (Wied, 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. papillosus, 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 nematodes.
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 vitez. 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 vitez. 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 (hyperproteinaemia as a result of hyperglobulinemia).

Additional of medicinal plants to high-fat diet led to impaired activity of blood enzymes – alkaline phosphatase, AST and ALT; increase in triglycerides, LDL cholesterol against the background 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 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 vitez.

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