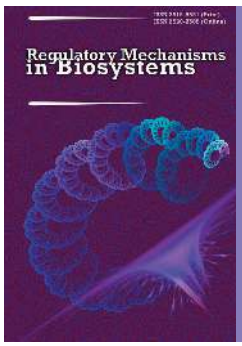


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# Hematological parameters and protein metabolism in the blood of pregnant rats under the effect of vanadium citrate

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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 prohemolized 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 in 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 $B_{12}$ (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

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 fermentopathies, impairment of reproduction, growth and development of the organism. In the body, vanadium compounds act as cofactors that modulate enzyme activity, 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-glycine (Levina et al., 2015). Vanadium simulates some effects of insulin in adipocytes due to staurosporin-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 ferum. In particular, hemoglobin is a protein that binds the cytoplasmic ions of vanadyl (Cantley & Aisen, 1979). According to the literature, vanadium is associated with transferrin and albumin in the incubation serum at low concentrations (Sánchez et al., 2011). The ability of transferrin and albumin to coordinate $VO_2^+$-ion (Gunasinghe & Kim, 2018) is known. In the form of vanadate, decavanadate-ion also interacts with proteins such as actin, myosin, and $Ca^{2+}$-ATPase, thus affecting mitochondrial functions, including modification of certain cellular antioxidant markers (Aureliano et al., 2014). The effect of methavanadate on erythrocytes is due to the interaction with proteins located on the outer part of the membrane and may still involve other minor lipid components. In addition, partially unsaturated lipids can interact differently with fully saturated chains in model systems (Suwalsky et al., 2013).

Therefore, the purpose of our research was to clarify the effect of the vanadium citrate organic compound on the total protein content and its fraction, as well as the activity of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AP), urea and creatinine in the blood plasma of pregnant female rats, the resistance of erythrocytes to the acid hemolytic, the age population distribution of erythrocytes and hematological parameters in general.

## Material and methods

All manipulations with animals were carried out in accordance with the "European convention for the protection of vertebrate animals used for research and scientific purposes" (Strasbourg, 1986) and "General ethical principles of animal experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001). Protocol of the Bioethics Committee meeting at the Institute of Animal Biology No. 70 dated July 11, 2018.

The studies were performed on white female laboratory rats with the bodyweight of 140–160 g, which were divided into five groups: Group I – non-pregnant females, II – pregnant females which consumed pure water without additives, rats in groups III, IV, V consumed the solution of vanadium citrate at the concentrations of 0.03, 0.125 and 0.50 μg V/mL, respectively, in the period of mating and pregnancy. The rats were kept under vivarium conditions with a standard diet for laboratory animals. Water (in groups I and II) and the solution of vanadium citrate (in groups III, IV, V) were provided in the volume of 20 mL per animal. The study material was blood plasma of rats, in which the content of total protein was determined after the Lowry method (Vlizlo et al., 2012) and its spectrum by the method of vertical electrophoresis (Table 1) (Vlizlo et al., 2012), the activity of ASAT, ALAT, AP, the content of urea and creatinine were determined using Humalyzer 2000 biochemical analyzer (Germany), applying the kits produced by "Biola". Acid erythrograms were determined after Terskov and Gitelzon in the whole blood (Terskov & Gitelzon, 1959), hematological parameters were determined on a hematology analyzer (Orphèe Mythic 18, Switzerland), and blood samples were put in tubes (Terumo Europe N. V., Belgium) containing the anticoagulant EDTA-$K_3$. Age population distribution of erythrocytes was carried out in a sucrose gradient.

Polyacrylamide gel electrophoresis are given in Table 1. Materials and reagents: acrylamide (AA); methylene bisacrylamide (MBA); persulfate ammonium (PSA); tetramethylenediamine (TEMED) (standard, ready to use); HCl concentrated; glycine; tris; glycerin; bromophenol blue; amido black 10B; distilled water. Sample preparation: dilute 0.10 mL of blood plasma (serum) with electrode buffer in a ratio of 1:10–12 and add a drop of pre-prepared phenol blue solution. Soluble tissue proteins: grind 0.10 g of tissue with 0.10 $cm^3$ of electrode buffer or saline and add a drop of pre-prepared phenol blue solution. Preparation of phenol blue solution – add a drop of bromophenol blue to a drop of glycerin. Preparation of electrode buffer (1 $dm^3$) – add HCl concentrated (0.60 $cm^3$) to glycine (2.88 g) and make it up to 1 $dm^3$ with distilled water.

**Table 1**
Preparation of gel for electrophoresis

| Component | 7.5% separating gel, $cm^3$ | Concentrating gel, $cm^3$ |
|---|---|---|
| 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 $cm^3$) – add MBA (0.35 g) to AA (9.65 g) and make it up to 25 $cm^3$ with distilled water.1.5 M tris (25 $cm^3$) – add HCl concentrated (0.60 $cm^3$) to tris (4.54 g) make it up to 25 $cm^3$ 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 $cm^3$ 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 $cm^3$ 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 reachesd 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 $cm^3$). 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 (Gitelzon & 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

0.700–0.750 at a wavelength of 630 nm. The studies were performed in a thermostatted 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 hemolyzing 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 resistance was expressed as an erythrogram, a curve of the relationship between 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 |
| 26 | 13 | 50 |
| 22 | 11 | 50 |
| 18 | 9 | 50 |
| 14 | 7 | 50 |
| 10 | 5 | 50 |
| 6 | 3 | 50 |

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 as 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 | Albumins, % | 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 + 0.03μgV/mL | 55.26 ± 1.59$^{*}$ | 26.61 ± 0.32 | 21.80 ± 0.32$^{*}$ | 27.44 ± 0.49$^{\#}$ | 24.16 ± 0.57$^{*\#}$ |
| IV | Pregnant + 0.125 μgV/mL | 61.20 ± 3.53 | 23.90 ± 1.71 | 23.88 ± 0.99 | 28.24 ± 0.53$^{*}$ | 24.50 ± 0.34$^{*\#}$ |
| V | Pregnant + 0.50 μgV/mL | 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 | Alanine 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$^{*}$ | 509.4 ± 17.7$^{*}$ |
| III | Pregnant + 0.03 μgV/mL | 56.61 ± 0.87 | 0.288 ± 0.017$^{***\#}$ | 146.80 ± 0.66$^{***\#}$ | 74.93 ± 0.32$^{*}$ | 460.2 ± 20.3$^{*\#}$ |
| IV | Pregnant + 0.125 μgV/mL | 54.63 ± 2.30 | 0.229 ± 0.002$^{\#}$ | 163.20 ± 1.03$^{***\#\#\#}$ | 83.50 ± 2.43$^{\#}$ | 394.2 ± 39.8$^{*\#}$ |
| V | Pregnant + 0.50 μgV/mL | 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.

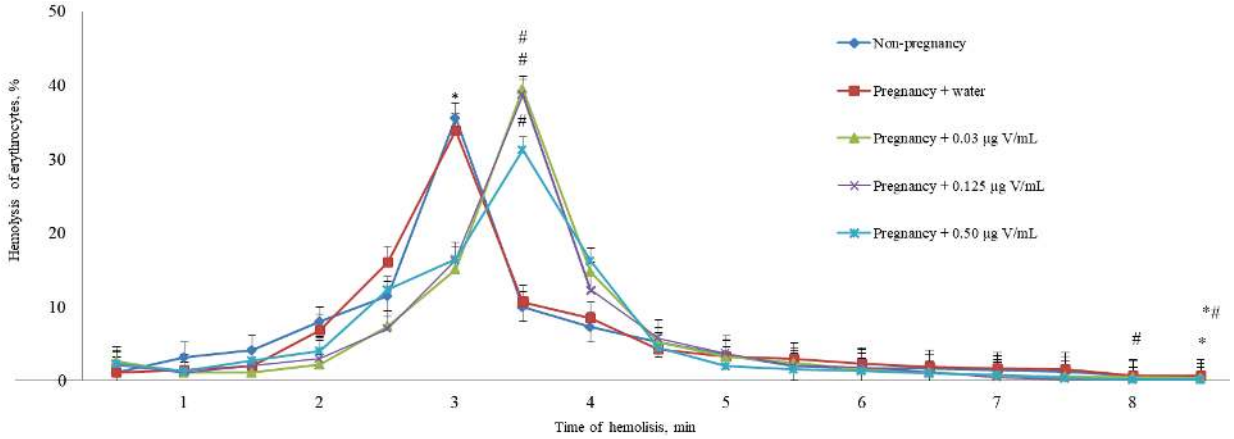


**Fig. 1.** Acid erythrograms (after Gitelzon) in the blood of pregnant female rats under the effect of vanadium citrate (x ± SD, n = 7)

**Table 5**
Acid erythrograms (after Gitelzon) in the blood of pregnant female rats under the effect of vanadium citrate (x ± SD, n = 7)

| Number of group | Animal group | Maximum hemolysis, min | Hemolysis time, min | Maximum hemolysis, % |
|---|---|---|---|---|
| I | Non-pregnant | 3.10 ± 0.10 | 8.80 ± 0.12 | 35.55 ± 1.83 |
| II | Pregnant | 3.00 ± 0.12 | 8.40 ± 0.10* | 33.89 ± 0.45* |
| III | Pregnant + 0.030 μgV/mL | 3.40 ± 0.40# | 8.40 ± 0.44 | 39.47 ± 2.28# |
| IV | Pregnant + 0.125 μgV/mL | 3.50 ± 0.21*# | 8.10 ± 0.14*# | 38.74 ± 3.42# |
| V | Pregnant + 0.500 μgV/mL | 3.60 ± 0.13*# | 8.30 ± 0.36*# | 31.30 ± 3.70# |

*Note*: see Table 3.

In pregnant animals of group II, there was a decrease 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).

**Table 6**
The age population distribution of erythrocytes in the density gradient of sucrose under the action of vanadium citrate (x ± SD, n = 7)

| Number of group | Animal groups | Percentage of erythrocyte populations | | |
|---|---|---|---|---|
| | | old, % | mature, % | young, % |
| I | Non-pregnant | 17.52 ± 0.34 | 68.17 ± 3.56 | 14.30 ± 0.87 |
| II | Pregnant | 6.90 ± 0.16*** | 64.31 ± 2.67* | 28.85 ± 2.10*** |
| III | Pregnant + 0.03 μgV/mL | 8.70 ± 0.25***# | 65.36 ± 3.37*# | 25.96 ± 1.70***# |
| IV | Pregnant + 0.125 μgV/mL | 8.42 ± 0.20**# | 66.62 ± 2.13# | 24.04 ± 1.60***# |
| V | Pregnant + 0.500 μgV/mL | 9.50 ± 0.21***# | 56.65 ±2.23***# | 28.81 ± 1.52***# |

*Note*: see Table 3.

Under the effect of vanadium citrate, there was a slight increase in the content of old and mature erythrocytes, but a slight decrease in the content of young erythrocytes in almost all groups fed with vanadium citrate solution, as compared with the pregnant animals in group II. The content of young erythrocytes in animals fed with vanadium citrate solutions 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 group II of pregnant animals. The concentration of hemoglobin 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 8). The value of thrombocrit in the rats of group II increased by 88.8%, as compared with 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 content decreased, 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 7**
The quantity of erythrocytes and their functional properties in the peripheral blood of pregnant rats under the effect of vanadium citrate (x ± SD, n = 7)

| Number of group | Animal groups | Erythrocytes, $1\times10^{12}$/L | Hemoglobin, g/L | Hematocrit, % | Mean corpuscular volume, $\mu m^3$ | Mean corpuscular hemoglobin in absolute quantities, pg | Mean corpuscular hemoglobin concentration, mg×mol/L |
|---|---|---|---|---|---|---|---|
| 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 μgV/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 μgV/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 μgV/mL | 6.15 ± 0.28*# | 147.0 ± 4.6*# | 0.32 ± 0.017 | 52.00 ± 3.64 | 23.93 ± 2.00 | 460.75 ± 3.31# |

*Note*: see Table 3.

**Table 8**
Platelet content and platelet indices of peripheral blood of pregnant rats under the action of vanadium citrate (x ± SD, n = 7)

| Number of group | Animal groups | Platelets, $10^9$/L | Mean platelet volume, fL | Thrombocrit, % | Relative platelet distribution width by volume, % |
|---|---|---|---|---|---|
| I | Non-pregnant | 253.00 ± 4.01 | 6.00 ± 0.44 | 0.152 ± 0.010 | 19.25 ± 1.40 |
| II | Pregnant | 289.20 ± 4.50$^{***}$ | 6.70 ± 0.30 | 0.286 ± 0.014$^{***}$ | 15.29 ± 0.73$^{*}$ |
| III | Pregnant + 0.03 μgV/mL | 215.00 ± 6.78$^{***\#\#\#}$ | 6.40 ± 0.46 | 0.206 ± 0.015$^{*\#\#\#}$ | 12.15 ± 0.89$^{**\#}$ |
| IV | Pregnant + 0.125 μgV/mL | 179.50 ± 5.18$^{***\#\#\#}$ | 6.33 ± 0.22 | 0.161 ± 0.008$^{\#\#\#}$ | 15.56 ± 0.78$^{\#}$ |
| V | Pregnant + 0.50 μgV/mL | 227.50 ± 4.52$^{*\#\#\#}$ | 6.51 ± 0.28 | 0.226 ± 0.007$^{**\#}$ | 16.62 ± 1.42$^{\#}$ |

*Note*: see Table 3.

**Table 9**
The concentration of leukocytes and their fractions in the peripheral blood of the pregnant rats under the effect of vanadium citrate (x ± SD, n = 7)

| Number of group | Animal groups | Leukocytes, $1\times10^9$/L | Lymphocytes, $1\times10^9$/L | Monocytes, $1\times10^9$/L | Granulocytes, $1\times10^9$/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 μgV/mL | 15.20 ± 0.25$^{***\#}$ | 9.75 ± 0.26$^{***\#}$ | 2.15 ± 0.16 | 3.30 ± 0.24$^{\#}$ |
| IV | Pregnant + 0.125 μgV/mL | 16.45 ± 0.57$^{\#}$ | 11.25 ± 0.21$^{***\#\#\#}$ | 2.35 ± 0.26 | 1.85 ± 0.20$^{\#\#\#}$ |
| V | Pregnant + 0.50 μgV/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 content of granulocytes 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). Synthesis of albumin, which is the most osmotically active plasma protein, is carried out in the liver. It is involved into the transport of free fatty acids, bile acids, bilirubin, calcium, hormones and drugs (Alberghina et al., 2010). According to the literature, reduced albumin concentration during pregnancy is a common phenomenon and may be due to an increase in blood plasma volume in females during pregnancy, α-fetoprotein growth, as well as hormonal changes, namely, progesterone and estradiol concentration increase (Dai et al., 2017). The studies showed an increase in the mean levels of β-globulins in pregnant females of group II, as compared to non-pregnant rats, which may be due to their physiological state since the β-fraction contains such important protein-like components as haemopexin, transferrin, ferritin, C-reactive protein, as well as some immunoglobulins IgA and IgM, located between the β2 and γ sites (Gloria et al., 2018). It is known that α-globulins are synthesized in the liver and any deviations in their synthesis are signals of the state of this organ (Alberghina et al., 2010; Gloria et al., 2018). The γ-fraction includes all types of immunoglobulins produced by the lymphoid tissue in response to antigenic stimulation. In the females of group II, there were no changes in the α- and γ-globulins fraction, which indicates the normal course of pregnancy. The tendency to increase in albumin levels in the animals which consume vanadium citrate at the concentration of 0.03 μg V/mL is probably due to the stabilizing properties of vanadium at this concentration, affecting the synthesizing liver function (Mohammadi & Yazdanparast, 2010). Stimulation of the body immune system by the vanadium compound is caused by the γ-globulins level growth at concentrations 0.125–0.50 μg V/mL. The fraction 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 urea 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 the 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-hydrolyzase. 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 osteoblasts differentiation (Haenlein & Anke, 2011; Crans et al., 2018).

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). 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. 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 (Dudok 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 erythropoiesis 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 erythropoietin 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 erythropoietin 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 (Lymans'ka 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 (Jwa 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 (Szklanna 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 immunoregulatory 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 neutrophilic granulocytes in healthy pregnancy may be a part of the mother's immune suppression, which is important for fetal protection (Lampé 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 (Elghetany & 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 (Tsiclauri, 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 is 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 $10^{-4}$ M $NaVO_3$, the formation of granulocytes decreased by about 70%, while under the influence of $10^{-7}$ M vanadate, 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 prohemolized 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.

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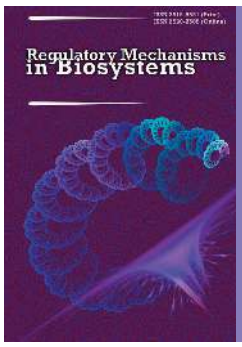


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# Features of modern winter wheat varieties in terms of winter hardiness components under conditions of Ukrainian Forest-Steppe

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In recent years, there has been a significant change in climatic conditions affecting the cultivation and yield of winter wheat. Therefore, the creation of wheat 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 characters enabling plants to overwinter. To a large extent, winter hardiness is determined by gene systems that control vernalization requirement duration, photoperiod reaction, and frost resistance. The research is aimed at determining the features of modern winter wheat varieties developed at the V. M. Remeslo Myronivka 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 bread wheat varieties Estafeta myronivska, Hratsiia myronivska, MIP Assol, and Balada myronivska were studied. They also were crossed on incomplete diallele scheme with three near-isogenic lines derived from Erythrospermum 604 with different alleles of Vrd genes 1) Vrd1Vrd1vrd2vrd2, 2) vrd1vrd1Vrd2Vrd2, and 3) vrd1vrd1vrd2vrd2. It was established that vernalization requirement duration in the varieties Estafeta myronivska and Balada myronivska was short whereas in the varieties Hratsiia 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, Hratsiia 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.

## Introduction

In recent years, there has been a significant change in climatic conditions affecting the cultivation and yield of winter wheat (Kristensen et al., 2011; Blyzniuk et al., 2019). Therefore, the creation of varieties with increased adaptive potential is one of the priority tasks of winter wheat breeding today (Rybas et al., 2018). Winter hardiness is one of the main components of the general adaptive potential of winter wheat (Sandve et al., 2010). Genetic systems associated with winter hardiness include the genes determining plant response to vernalization (Vrn), photoperiod sensitivity (Ppd) and frost resistance (Fr) (Sutka, 2001; McIntosh et al., 2013; Kiss, 2014).

Wheat photoperiod sensitivity is plant response to daylength expressed in the delay of heading date in photoperiod sensitive genotypes when daylength is shortening. The genes Ppd-A1, Ppd-B1, Ppd-D1 which control photoperiod sensitivity are located on the chromosomes 2A, 2B, 2D, respectively (Law et al., 1978; Worland et al., 1998). Variation in photoperiod sensitivity is a factor that leads to variability of adaptability and productivity of winter wheat varieties. Low responsiveness to the daylength reduction in the majority of varieties is due to the presence of the dominant allele Ppd-D1a in their genotype (Fayt et al., 2014). Varieties with dominant allele of the Ppd-D1 gene were less tolerant to low temperatures as compared to plants with an alternative recessive allele (Toptikov & Chebotar, 2019).

Vernalization is prolonged exposure to low temperatures which induces the transition of winter crops from the vegetative to the generative stage of development (Deng et al., 2015). The combination of different dominant alleles of Vrn loci affects the heading date and, accordingly, duration of the whole growing season (Stelmakh, 1993). It has been shown that the mechanism for determining sensitivity to vernalization and heading date is based on mutations in the Vrn gene loci which correct the dependence of the transition to heading stage on the vernalization factor and transmit the gene from recessive into dominant (Distelfeld et al., 2009). More prolonged vernalization requirement leads to slower development in the initial stages of organogenesis, so the transition to formation of differentiated growth point and primordia of reproductive organs in such genotypes occurs much later (Prasil et al., 2004). The reduction of the vernalization duration may lead to an earlier transition of the plant from dormancy in spring and winter during thaws, which causes the reduction of winter hardiness and frost resistance. Sometimes it has significant negative influences on the grain yield (Fayt, 2003; Koemel et al., 2004). The winter type of development is manifested when the three major Vrn

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; Yoshiba 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; Emtseva 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 Fr1 and Fr2 are localized on chromosomes 5A and 5D, respectively (Sutka, 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 (Bakuma, 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., 2018; Kawakita et al., 2020; Royo et al., 2020).

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 hardiness 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, Hratsiia 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 Hratsiia 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 |
|---|---|
| Estafeta myronivska (var. lutescens) | *Myronivska 64* [Myronivska yuvileina (Lutescens106/ Bezostaya 4) / KM 66-10-1-79] / *Lutescens 50713* {Myronivska 27 [Lutescens 6915 (Prybii / Myronivska yuvileina) / Lutescens 6538)] / Nike} |
| Hratsiia myronivska (var. erythrospermum) | *Erythrospermum 52422* [Erythrospermum 9736 (Narino 59 / Veneda)] / *Erythrospermum 52687* from Erythrospermum 10071 {Erythrospermum 5226 [WRH* k-43822 / Lutescens 2274 (Lutescens 106 / Bezostaya 4 // Bezostaya 4)] / Lutescens 6075} / Gama // Donskaya intensivnaya |
| MIP Assol (var. lutescens) | *Sakwa / Myronivska 65* {Myronivska 61 [Illichivka (Bezostaya 4 / Myronivska 808) / Hadm. 6508-74)] / Myronivska 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 [Illichivka (Bezostaya 4 / Myronivska 808) / Hadm. 6508-74] / Lutescens 20051 {Myronivska 61 [Illichivka (Bezostaya 4 / Myronivska 808) / Hadm. 6508-74]} / NS 954 / Kavkaz // Rezo / Lutescens 8133 [Siete Cerros 66 / Myronivska yuvileina (Bezostaya 4 / Myronivska 808)] |
| Balada myronivska (var. erythrospermum) | *Donskaya polukarlikovaya* / *Estet* (Illichivka / SK-2542, CZE // CIMMYT-151) / Erythrospermum 10071 {Erythrospermum 5226 [WRH k-43822 / Lutescens 2274 (Lutescens 106/ Bezostaya 4 // Bezostaya 4)] / Lutescens 6075} // Erythrospermum 53321 [Lutescens 9950 (Illichivka / SK-2542 // CIMMYT-151)] / Erythrospermum 10071 {Erythrospermum 5226 [WRH k-43822 / Lutescens 2274 (Lutescens 106 / Bezostaya 4 // Bezostaya 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 Erythrospermum 604 Vrd1Vrd1vrd2vrd2, Erythrospermum 604 vrd1vrd1Vrd2Vrd2, Erythrospermum 604 vrd1vrd1vrd2vrd2 created at the Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation of NAAS (Fayt, 2006).

*a*

*b*

*c*

*d*

**Table 2**
Days to heading under the natural and shortened photoperiod in new varieties of winter wheat (x ± SE, n = 30)

| Variety | 2016 | | 2017 | | 2018 | |
|---|---|---|---|---|---|---|
| | natural photoperiod | shortened photoperiod | natural photoperiod | shortened photoperiod | natural photoperiod | shortened photoperiod |
| Estafeta myronivska | 51.0 ± 0.5[a] | 58.4 ± 1.0[a] | 55.0 ± 0.2[a] | 61.5 ± 0.3[a] | 59.5 ± 1.3[a] | 68.7 ± 1.7[a] |
| Hratsiia myronivska | 48.8 ± 0.4[b] | 55.9 ± 1.1[b] | 51.9 ± 0.2[b] | 58.7 ± 0.6[b] | 53.0 ± 1.3[b] | 63.1 ± 1.9[b] |
| MIP Assol | 51.8 ± 0.4[a] | 57.5 ± 0.4[ab] | 55.5 ± 0.3[a] | 65.3 ± 0.4[c] | 73.2 ± 0.9[c] | 77.9 ± 1.5[c] |
| Balada myronivska | 49.8 ± 0.4[ab] | 55.7 ± 0.6[b] | 54.3 ± 0.3[a] | 59.0 ± 0.7[b] | 56.2 ± 1.2[ab] | 69.8 ± 2.8[a] |

*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 new winter wheat varieties (x ± SE, n = 90)

| Genotype of near-isogenic line | 2016 | | 2017 | | 2018 | | Vernalization requirement |
|---|---|---|---|---|---|---|---|
| | vernalization requirement | days to heading | vernalization requirement | days to heading | vernalization requirement | days to heading | |
| *Vrd1Vrd1vrd2vrd2* | 30 | 65.3 ± 1.8[a] | 30 | 57.4 ± 2.6[a] | 40 | 55.9 ± 0.9[a] | short duration (31–40 days) |
| *vrd1vrd1Vrd2Vrd2* | 40 | 72.4 ± 4.1[b] | 30 | 75.4 ± 3.0[b] | 40 | 74.8 ± 2.0[b] | |
| *vrd1vrd1vrd2vrd2* | 50 | 70.5 ± 2.5[ab] | 50 | 72.3 ± 2.6[b] | 50 | 68.7 ± 5.2[b] | 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 | 2016 | | 2017 | | 2018 | | Vernalization requirement |
|---|---|---|---|---|---|---|---|
| | vernalization requirement | days to heading | vernalization requirement | days to heading | vernalization requirement | days to heading | |
| *Vrd1Vrd1vrd2vrd2* | 30 | 65.3 ± 1.8[a] | 30 | 57.4 ± 2.6[a] | 40 | 59.4 ± 0.9[a] | short duration (31–40 days) |
| *vrd1vrd1Vrd2Vrd2* | 40 | 72.4 ± 4.1[b] | 30 | 75.4 ± 3.0[b] | 40 | 74.8 ± 2.0[b] | |
| *vrd1vrd1vrd2vrd2* | 50 | 70.5 ± 2.5[ab] | 50 | 72.3 ± 2.6[b] | 50 | 68.7 ± 5.2[b] | medium duration (41–50 days) |

*Note*: see Table 2.

**Table 5**
The segregation ratio in $F_2$ population for "heading occurrence: no heading occurrence" after vernalization duration 40 and 30 days

| Days of vernalization | Variety | *vrd1vrd1vrd2vrd2* | | | *Vrd1Vrd1vrd2vrd2* | | | *vrd1vrd1Vrd2Vrd2* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | fact | theoretical | $\chi^2$ | fact | theoretical | $\chi^2$ | fact | theoretical | $\chi^2$ |
| 30 | Estafeta myronivska | 136:132 | 3:1* | 147.14 | 124:14 | 15:1 | 3.57 | 111:25 | 3:1 | 3.18 |
| | Hratsiia myronivska | 13:122 | 1:15 | 2.63 | 118:28 | 13:3 | 0.02 | 13:122 | 1:15 | 2.63 |
| | MIP Assol | 0:143 | – | – | 161:27 | 13:3 | 2.38 | 22:107 | 3:13 | 0.24 |
| | Balada myronivska | 3:113 | 1:15 | 2.66 | 109:18 | 13:3 | 1.74 | 4:136 | 1:15 | 2.75 |
| 40 | Estafeta myronivska | 113:32 | 3:1 | 0.66 | 146:16 | 15:1 | 3.63 | 85:8 | 15:1 | 0.88 |
| | Hratsiia myronivska | 140:36 | 3:1 | 1.94 | 198:35 | 13:3 | 2.13 | 130:11 | 15:1 | 0.58 |
| | MIP Assol | 97:24 | 3:1 | 1.72 | 213:48 | 13:3 | 0.02 | 119:9 | 15:1 | 0.13 |
| | Balada myronivska | 105:25 | 3:1 | 2.31 | 108:6 | 15:1 | 0.19 | 137:23 | 13:3 | 2.01 |

*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 Hratsiia myronivska and MIP Assol, as the highest percentage of heading plants was observed in this variant of the experiment (Table 3).

For the varieties Estafeta myronivska and Balada myronivska a high percentage of heading plants was found in the variant with 40 days vernalization. In 2017, plants of the variety Estafeta myronivska had the highest percentage of heading in the variant with vernalization during 50 days (100%). However, after 40 days of vernalization duration, heading plants were observed at the level of 70.8%, which makes it possible to consider this particular period to be necessary for transition of plants to generative state. The same period is necessary for plants of wheat varieties Hratsiia myronivska and MIP Assol. In the variety Balada myronivska, the higher percentage of heading plants was observed in both variants, therefore, for this variety, the vernalization requirement was 30 days. Vernalization requirement for the variety Hratsiia 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 Hratsiia 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 Hratsiia 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 Erythrospermum 604 Vrd1Vrd1vrd2vrd2, Erythrospermum 604 vrd1vrd1Vrd2Vrd2, and Erythrospermum 604 vrd1vrd1vrd2vrd2. In 2016 (Table 4), the lines Erythrospermum 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 Erythrospermum 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 Erythrospermum 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/Hratsiia myronivska, Vrd1Vrd1vrd2vrd2/MIP Assol and Vrd1Vrd1vrd2vrd2/Balada myronivska was 13:3, 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 vrd1vrd1Vrd2 Vrd2/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 Hratsiia 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 13:3, 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 Hratsiia 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 the percentage of live plants after freezing at minus 18 °C (99 ± 1.2), and for the temperature minus 20 °C – percentage of live plants was at its level (59 ± 5.6). In the variety MIP Assol 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 of 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 Experimental and Breeding Station, the Ppd-D1a allele determines insensitivity to the photoperiod and promotes an earlier heading date. Only the variety Lehenda bilotserkivska 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-D1 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[a] | 58.0 ± 5.5[a] |
| Estafeta myronivska | 99.0 ± 1.2[b] | 59.0 ± 5.6[a] |
| Hratsiia myronivska | 79.0 ± 4.5[a] | 50.0 ± 5.5[a] |
| MIP Assol | 91.0 ± 3.2[a] | 70.0 ± 5.2[b] |
| Balada myronivska | 82.0 ± 4.4[a] | 53.0 ± 5.6[a] |

*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 nd. a. V. Y. Yuriev of NAAS, the majority of genotypes had the dominant allele Vrd3 (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 & Avksentieva, 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 vrd1vrd1 vrd2vrd/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, 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 tubing 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

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" 13:3 in the varieties Hratsiia 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, 2006b). Comparing the results of hybridological analysis obtained (Table 5) with the theoretically expected segregation ratio, we assume that in the varieties Estafeta myronivska, Hratsiia 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; Bulavka et al., 2018; Cherenkov et al., 2018; Grabovets, 2019). It is 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, Hratsiia 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 hardiness. 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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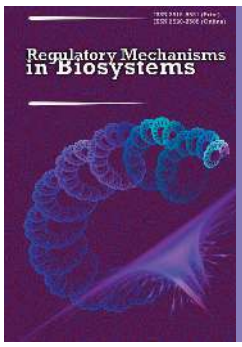


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# *Staphylococcus aureus* and *S. epidermidis* in biological systems of hospital environment: Antibiotic resistance patterns in regions of Ukraine

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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* bacteria 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 antibiotics sensitivity in *S. epidermidis* strains. Comparison of antibiotic 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, cephalosporins, 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 lincozamids, 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 cephalosporins, 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 passports of microorganism resistance in the regions and various health-care settings, as well as 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; Voidazan 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 antiseptic 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% (Mermel, 2017). In the etiological structure of infection complications associated with peripheral and central veins catheterization, the *S. aureus* strains and coagulazonegative staphylococci, including *S. epidermidis*, prevail (Zhang et al., 2016; Guembe 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 antibacterial 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; Brusselaers 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 expediency of estimating territorial differences in staphylococci antibacterial 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 antibacterial resistance of *S. aureus* in Kharkiv and Poltava regions respectively were carried out in 2013–2019 and 15,589 tests to determine antibiotic resistance in *S. epidermidis* strains in Kharkiv region were carried out in 2013–2015 (Table 1).

**Table 1**
Number of tests carried out for detecting resistance to various antibiotics in staphylococci isolates in health-care settings at bacteriological laboratories in Kharkiv and Poltava regions in 2013–2019

| Antibiotics group or antibiotic | *S. aureus* (2013–2019) | | *S. epidermidis* (2013–2015) |
|---|---|---|---|
| | Kharkiv region | Poltava region | Kharkiv region |
| Penicilins | 28,904 | 15,676 | 3,579 |
| Cephalosporins | 17,359 | 5,340 | 2,818 |
| Carbapenems | 3,202 | 3,038 | 396 |
| Aztreonam | 0 | 0 | 11 |
| Aminoglycosides | 21,144 | 10,762 | 1,782 |
| Macrolides | 7,642 | 10,092 | 590 |
| Lincozamides | 10,156 | 8,792 | 772 |
| Tetracyclines | 3,534 | 9,934 | 596 |
| Vancomycin | 7,870 | 7,120 | 939 |
| Rifampicin | 5,606 | 908 | 237 |
| Fluoroquinolones | 33,619 | 15,771 | 3,297 |
| Linezolid | 11,210 | 8,097 | 544 |
| Co-trimaxazol | 14 | 1,025 | 0 |
| Chloramphenicol | 755 | 1,355 | 28 |
| Phosphomicin | 0 | 293 | 0 |
| Fusidic 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/clavulanat), cephalosporins (cefazolin, cefalotin, cephalexin, cefuroxime, cefoperazon, cefotaxime, ceftriaxone, ceftazidime, cefixime, cefepim, cefpirom, cefoperazon/sulbactam), carbapenems (imipenem, meropenem, imipenem/cilastatin), aminoglycosides (kanamycin, gentamycin, tobramycin, netilmycin, amikacin), macrolides (erythromycin, clarithromycin, azithromycin, spiramycin), lincozamides (clindamycin, lincomycin), tetracyclines (tetracycline, doxycycline, tigecycline), glycopeptides (vancomycin), an antituberculosis medicine rifampicin, fluoroquinolones (ciprofloxacin, ofloxacin, cefloxacin, norfloxacin, lomefloxacin, levofloxacin, moxifloxacin, gatifloxacin), oxazolidions (linezolid), sulphanilamides (co-trimoxazol), amphenicols (chloramphenicol) and others (phosphosycin, 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, and 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/clavulanat), cephalosporins (cefazolin, cefalotin, cephalexin, cephaklor, cefuroxime, cefoperazon, cefotaxime, ceftriaxone, ceftazidime, cefixime, ceftibuten, cefepim), carbapenems (imipenem, meropenem), monobacts (aztreonam), aminogly-

cosides (gentamycin, tobramycin, amikacin), macrolides (erythromycin, clarithromycin, azithromycin), lincozamides (clindamycin, lincomycin), tetracyclines (tetracycline, doxycycline), glycopeptides (vancomycin), an antituberculosis medicine rifampicin, fluoroquinolones (ciprofloxacin, ofloxacin, pefloxacin, norfloxacin, lomefloxacin, levofloxacin), oxazolidions (linezolid), sulphanilamides (co-trimoxazol), amphenicols (chloramphenicol). In some years, the resistance of *S. epidermidis* isolates was tested only for one antibiotic within a certain class: in 2013 – for imipenem, aztreonam, tetracycline; in 2014 – for erythromycin, tetracycline; in 2015 – for amikacin, clindamycin and doxycycline.

By analogy with *S. aureus*, to increase data validity, we calculated the mean proportion of *S. epidermidis* isolates resistant to antibiotics of the following groups: penicillins, cephalosporins, carbapenems, aminoglycosides, macrolides, lyncozamides, and fluoroquinolones.

We performed statistical analysis using the Epi Info™ for Windows (Version 7.2). For estimation of the standard deviation of proportions we calculated the standard error (SE). Comparison of *S. aureus* strains' antibiotic resistance in Kharkiv and Poltava regions was performed using the Pearson Chi-square test ($\chi^2$) and Fisher's exact test. The level of significance was set at 5% ($P < 0.05$).

**Results**

In Kharkiv region, the largest proportion of *S. aureus* strains resistant to the penicillin group drugs was identified in 2013 (62.7%), and the smallest proportion – in 2016 (10.4%). In Poltava region, as well as in Kharkiv region, the largest proportion of *S. aureus* isolates resistant to penicillin antibiotics was identified in 2013 (30.1%). The smallest proportion of *S. aureus* strains resistant to the penicillin group drugs in Poltava region was identified in 2015 (16.7%). During the whole period of the study (except 2016), the proportion of *S. aureus* strains resistant to penicillins was statistically significantly higher in Kharkiv region than in Poltava region (Fig. 1a). On the whole, in 2013–2019 the proportion of *S. aureus* strains resistant to the penicillin drugs group in Kharkiv region was 2.3 times statistically significantly higher than in Poltava region (50.8% / n = 14,687 of 28,904 versus 21.8% / n = 3,412 of 15,676; $\chi^2 = 3,555.9$; $P < 0.001$).

The comparative analysis of *S. aureus* resistance to medications of the cephalosporin group has demonstrated in dynamics that the largest proportion of resistant *S. aureus* strains in both regions was identified in 2015 (39.9% in Kharkiv region; 9.5% in Poltava region), while the smallest proportion was identified in 2016 (4.0% in Kharkiv region; 6.4% in Poltava region). It should be noted that in 2015 and in 2017 the proportion of *S. aureus* strains resistant to cephalosporins was statistically significantly higher in Kharkiv region than in Poltava region. Nevertheless, in 2016 the proportion of *S. aureus* isolates resistant to cephalosporin antibiotics was statistically significantly higher in Poltava region (Fig. 1b). On the whole, in 2015–2017, the proportion of *S. aureus* strains resistant to the cephalosporin medication group was 1.7 times statistically significantly higher in Kharkiv region compared with Poltava region (14.7% / n = 1,590 of 10,815 versus 8.9% / n = 473 of 5,340; $\chi^2 = 109.6$; $P < 0.001$).

The analysis of resistance in *S. aureus* isolates to carbapenems demonstrates that the largest proportion of resistant strains in Kharkiv region was identified in 2017 (42.0%), while in Poltava region – in 2015 (10.0%). The smallest proportion of *S. aureus* strains resistant to carbapenems was identified in Kharkiv region in 2013 (9.0%), and in Poltava region – in 2016 (1.7%). Statistically significant differences between the proportion of *S. aureus* strains resistant to carbapenems in Kharkiv and Poltava regions were not found in 2015 (Fig. 1c). Nevertheless, during the period from 2015 to 2017 in general, the proportion of *S. aureus* strains resistant to carbapenem drugs was statistically significantly higher by 3.7 times in Kharkiv region (26.6% / n = 337 of 1,268 versus 7.1% / n = 215 of 3,038; $\chi^2 = 304.4$; $P < 0.001$).

When identifying the resistance in *S. aureus* isolates to aminoglycosides, the largest proportion of resistant strains in both regions was identified in 2013 (32.9% in Kharkiv region; 22.3% in Poltava region) The smallest proportion of resistant isolates in Kharkiv area was identified in 2016 (2.6%), while in Poltava region – in 2014 (6.4%). During the whole period of study (except 2016), the proportion of *S. aureus* isolates resistant to aminoglycosides was statistically significantly higher in Kharkiv region (Fig. 1d). Overall, in 2013–2019, the proportion of *S. aureus* strains resistant to medications of the aminoglycoside group was 1.2 times statistically significantly higher in Kharkiv region compared with Poltava region (16.0% / n = 3,377 of 21,144 versus 13.2% / n = 1,424 of 10,762; $\chi^2 = 41.9$; $P < 0.001$).

The largest proportion of *S. aureus* isolates resistant to macrolides in Kharkiv region was identified in 2017 (55.4%), and in Poltava region – in 2019 (42.0%). The smallest proportion of *S. aureus* isolates resistant to macrolides in Kharkiv region was identified in 2013 (6.5%), and 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. 2a). 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 lincozamids 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 lincozamids group antibiotics. The lowest resistance to lincozamids in Poltava region was in *S. aureus* strains identified in 2015 (10.0%). Statistically significant differences in proportion of *S. aureus* strains resistant to lincozamids 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 lincozamids 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 = 1995 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.

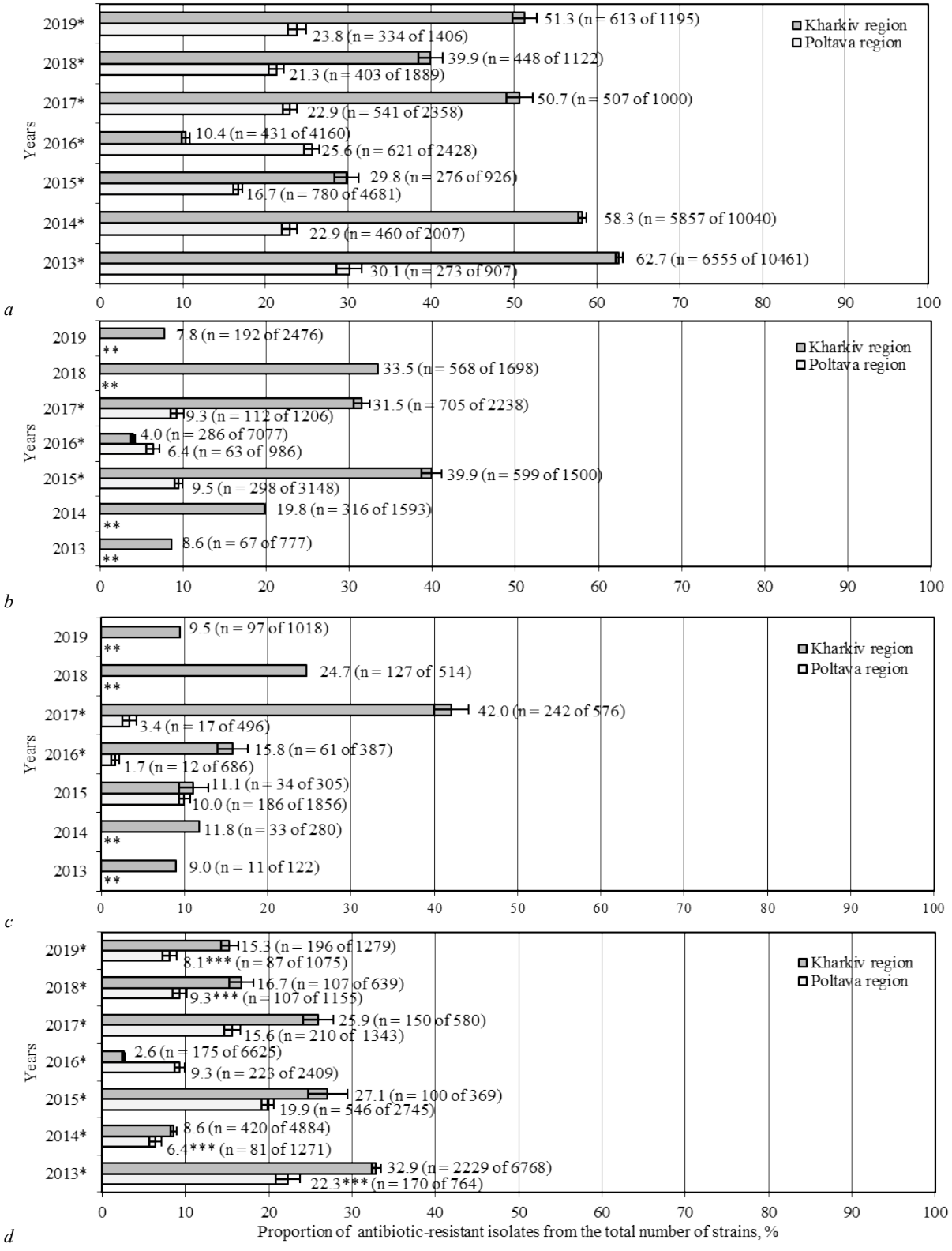


**Fig. 1.** Proportion (x ± SE, %) of penicillin antibiotics-resistant (*a*), cephalosporin antibiotics-resistant (*b*), carbapenem antibiotics-resistant (*c*), aminoglycoside antibiotics-resistant (*d*) strains of *S. aureus* in Kharkiv and Poltava regions during 2013–2019: * – the differences are statistically significant at $P < 0.01$, ** – antibiotic resistance was not studied in Poltava region that year, *** – there are available data on resistance to only one amiglycoside antibiotic (gentamycin)

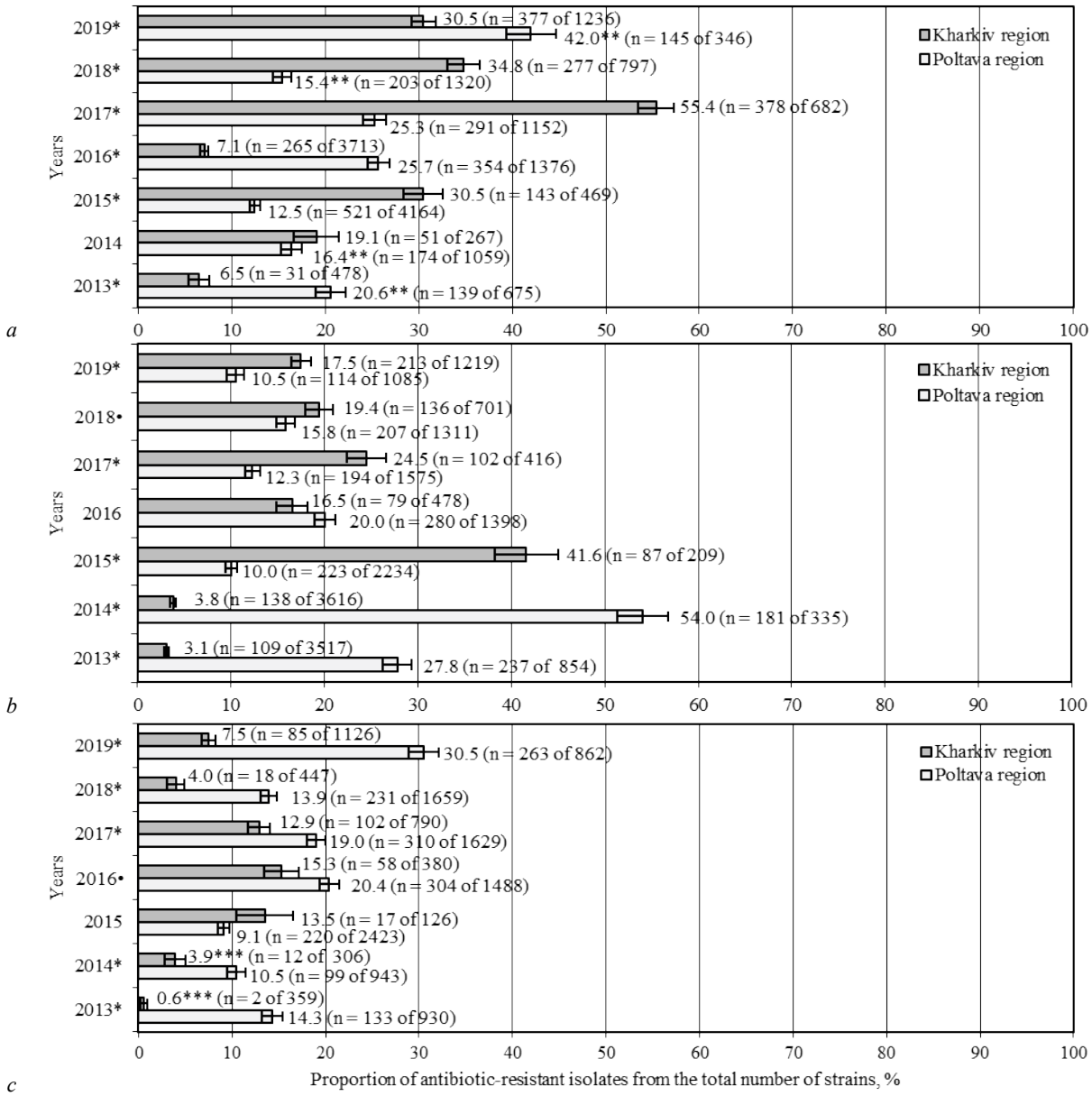


**Fig. 2.** Proportion (x ± SE, %) of macrolide antibiotics-resistant (*a*), lincozamid antibiotics-resistant (*b*), tetracycline antibiotics-resistant (*c*), strains of *S. aureus* in Kharkiv and Poltava regions during 2013–2019: * – the differences are statistically significant at $P < 0.01$, • – the differences are statistically significant at $P < 0.05$, ** – there are available data on resistance to only one macrolide antibiotic (erythromycin), *** – there are available data on resistance to only one tetracycline antibiotic (tetracycline)

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 lincozamid-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, amino-

glycosides, and macrolides were identified more often than in Poltava region. In Poltava region, strains resistant to lincozamids, 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 *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 *S. aureus* can make antibiotics of this group totally inefficient rather soon.

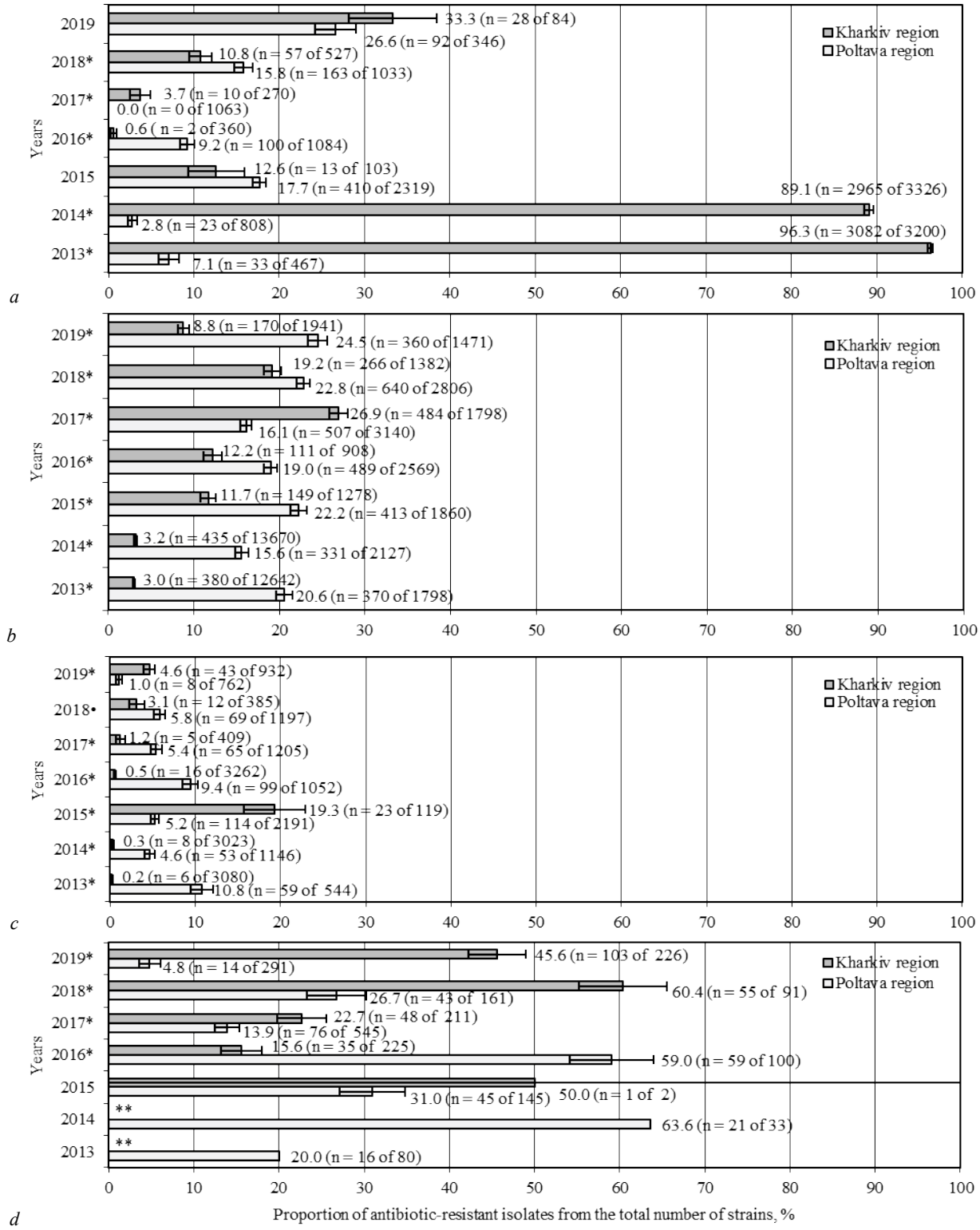


**Fig. 3.** Proportion (x ± SE, %) of vancomycin-resistant (*a*), fluoroquinolones-resistant (*b*), linezolid-resistant (*c*), chloramphenicol-resistant (*d*) strains of *S. aureus* in Kharkiv and Poltava regions during 2013–2019: * – the differences are statistically significant at $P < 0.01$, • – the differences are statistically significant at $P < 0.05$, ** – antibiotic resistance was not studied in Kharkiv region in this year

We noted that in Poltava region, the proportion of *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 *S. aureus* to these groups of medications and to apply them in treatment protocols with care.

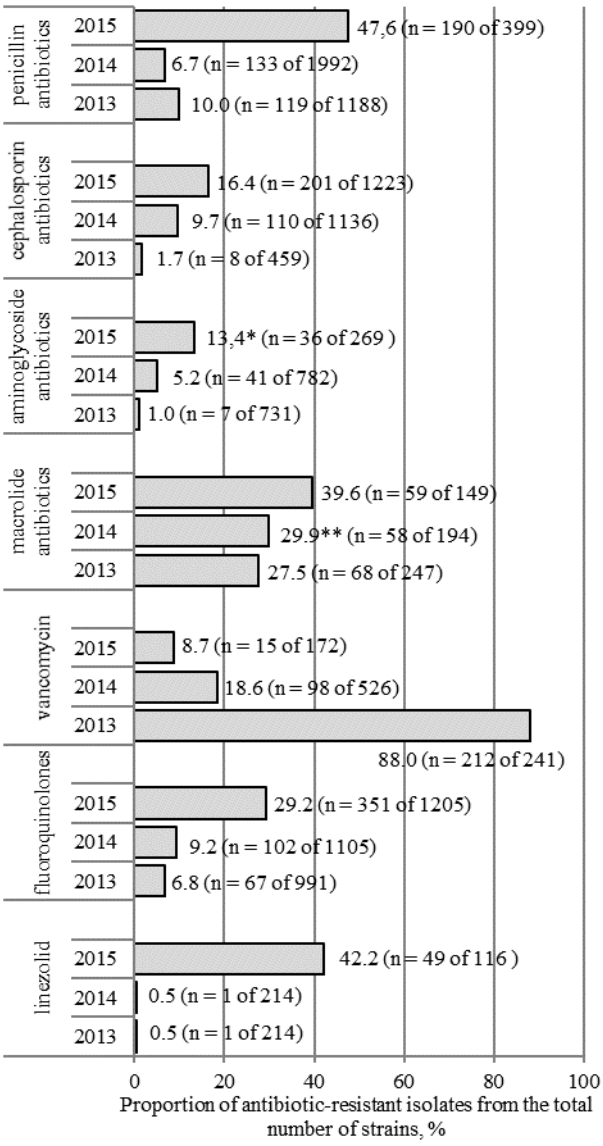


**Fig. 4.** Proportion (%) of antibiotic-resistant strains of *S. epidermidis* in Kharkiv region during 2013–2015: * – there are available data on resistance to only one amiglycoside antibiotic (amikacin), ** – there are available data on resistance to only one macrolide antibiotic (erythromycin)

Hauschild et al. (2008) reported that 38.1% of *S. aureus* isolates in their study were resistant to at least one antibiotic of the aminoglycoside group. In the present study, the aminoglycoside resistance in both regions was generally much lower. In comparison with the work by Liu et al. (2017), the authors identified a high resistance in *S. aureus* isolates to the macrolides group. Also, the authors have identified a generally lower resistance in *S. aureus* isolates to the tetracycline group medications than Ullah et al. (2013).

It is considered that resistance in *S. aureus* to fluoroquinolones is formed as a result of treating illnesses caused by other agents when the skin of the hands and mucous coverings of a person receiving medications of this group are colonized by *S. aureus*. In that case, *S. aureus* is influenced by sub-therapeutic concentrations of drugs, which may cause mutations (Lowy, 2003). In the our study, fluoroquinolone-resistant *S. aureus* strains were identified in some years in a quarter of cases. Therefore, departing from the resistance formation mechanism, prescribing of any antibacterial medication should be duly substantiated.

In comparison with the study by Rağbetli et al. (2016), we have identified a high resistance to linezolid (0% of resistant strains according to their data versus 19.3% of resistant strains identified in 2015 in Kharkiv region in our study). Linezolid is an antibiotic which is efficient against 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-trimaxazol 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 lincozamid 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 (Sabaté Brescó 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 antibiotics 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 naphycillin, penicillin (100% each), to cephalotin (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, cefazolin, 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 glycopeptide antibiotics 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-trimaxazol. 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 antibiotics 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 fit into standard procedures and ensure an individual approach to every patient within personalized healthcare.

The territorial differences in antibiotic resistance of *S. aureus* isolates disclosed in the present study attest to the expediency in maintaining microbiological monitoring both on the regional level and the institutional level. Considering the growing resistance in *S. epidermidis* isolates to penicillins, cephalosporins, aminoglycosides, macrolides, and fluoroquinolones identified in Kharkiv region, obligatory assessment of sensitivity of this genus of microorganisms to antibacterial medication should be conducted on a regular basis at healthcare settings of both Kharkiv region and other districts and regions. Forming the unified national data network on microorganism resistance in regions will become the foundation for developing strategies on infection control and prevention of cases of infection associated with rendering of health-care aid, including prevention of catheter-related bloodstream infections. Considering the available data on resistance in hospital strains of microorganisms, it is necessary to develop new and improve the acting local protocols of patients' antibiotic treatment. Rational and scientifically substantiated application of antibiotic medications in medical practices will enable efficient prevention of formation of microorganism-resistant strains, which will raise the quality of health-care aid rendered to the population, decrease the number of complications, and diminish economic losses caused by infections associated with resistant microorganisms.

## Conclusion

We identified peculiarities in antibiotic resistance patterns in regions of Ukraine. In total, the proportion of *S. aureus* strains resistant to penicillins, cephalosporins, 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 lincozamids, tetracycline antibiotics, and fluoroquinolones in Poltava region was higher in terms of statistical validity than in Kharkiv region. Assessment of antibiotic resistance in *S. epidermidis* isolates in Kharkiv region showed increase in resistance to the most antibiotics (penicillins, cephalosporins, aminoglycosides, macrolides, and fluoroquinolones).

Further studies are needed to create of passports of microorganism resistance in the regions and medical institutions, as well as to create a unified national database network on microorganism resistance using modern methodologies of determining the phenotypes and genotypes of microorganisms.

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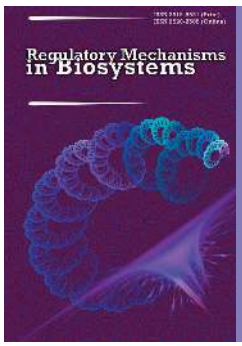


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# Influence of *Lavandula angustifolia*, *Melissa officinalis* and *Vitex angus-castus* on the organism of rats fed with excessive fat-containing diet

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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 poses to the organism is limited and contradictive. Obesity and overeating are some of the commonest health issues around the world, and people are increasingly consuming workability-enhancing preparations as a simple and fast method of weight control. The plant-based preparations are considered less harmful than the synthetic chemical ones. *Lavandula angustifolia* Mill., *Melissa officinalis* L. and *Vitex angus-castus* L. are broadly used as food additives and medicinal plants, despite the fact that their complex physiological assessment on model animals in the conditions of obesity has not yet been performed. We carried out a 30-day experiment on white male rats. All the animals were given high-fat diet, and the experimental animals, in addition to this diet, received 5% crumbled dry herbs of *L. angustifolia*, *M. officinalis* or *V. angus-castus*. Taking into account the overall amount of consumed food, the mean daily gain in body weight; at the end of the experiment, we determined the index of the weight of the internal organs, biochemical and morphological blood parameters. At the beginning and the end of the experiment, the rats were examined for motor and orienting activities, and emotional status. Rats on high-fat diet gained up to 112% body weight by the end of the experiment, while rats that had received *V. angus-castus* gained up to 119%, *M. officinalis* – 135%, *L. angustifolia* – 139%, compared with the initial body weight. Addition of medicinal plants to the diet led to increase in average daily weight increment, significantly and reliably after consuming lavender and lemon balm, less significantly and unreliably after eating *Vitex*. *L. angustifolia* and *M. officinalis* reduced the relative brain weight, and ingestion of *L. angustifolia* and *M. officinalis* caused notable decrease in the relative mass of the thymus (down to 58% and 47% of the relative weight of thymus in animals of the control group respectively). Also, these plants decreased the motor and orienting activities of the rats by the end of the experiment. As for the biochemical parameters of blood, the activity of alkaline phosphatase significantly increased to 406% following consumption of *Melissa*, to 350% after consuming lavender, and to 406% after *Vitex*, compared to the control group. Furthermore, all the groups were observed to have increased AST and ALT activities. Intake of lavender led to increases in cholesterol (to 125%) and LDL cholesterol (to 228%), whereas the groups that consumed lemon balm were observed to have decreases in urea nitrogen (to 79%), totalbilirubin (to 63%) and triglycerides (to 63%). Addition of *Vitex* led to increase in the index of aterogenecity 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 compliations, 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 cholyterol levels, they also may cause deleterious side effect. Thus, treating metabolic diseases requires seeking new agents for development of novel preparations (Heghes et al., 2020). Against the backround 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; Bukvicki et al., 2020). Most often, for those purposes, the treatment involves plants of Lamiaceae family (Michel et al., 2020). Zvezdina et al. (2020) have made a review of 71 species from 30 genera of Lamiaceae family, and

drew the conclusion that the immense potential of plants of this family is still unexplored. These valuable medicinal plants could help in development of neurotropic preparations. Biologically active substances of Lamiaceae plants comprise phenolic compounds, chiefly phenolcarboxylic and cinnamic acids and their derivatives, flavonoids, including flavones, isoflavones, flavanols, flavanones, flavanones, 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* L. is a perennial herbaceous plant of Lamiaceae family. It can reach 150 cm in height. Its is considered to have been originated from the territory spanning from the Eastern Mediterranean to Iran, Central Asia, the Black Sea and Western Asia, and also North Africa. Currently, this plant is cultivated ubiquously for its essential oil. Content of essential oil ranges 0.02–0.20%, only seldom reaching 0.80%. Content of essential oil in the herbs is 0.06–0.13%, and 0.39–0.44% in the leaves. Leaves and young shoots of lemon balm that had been cut before blooming are used as spices in the European and American quisines. Fresh and dried, they are added to salads, cheese, soups, meat, fish dishes, mushrooms, tea, vinegar, liquors, salting of cucumbers and tomatos (Shakeri et al., 2016). Furthermore, essential oil of *Melissa*, similarly to other species of plants considered in this article, has for a long time been used to eliminate or scare off storage pest insects (Martynov et al., 2019a, 2019b). The essential oil`s most distinctive constituents are monoterpens citral (geranial + neral), geraniol, nerol, citronellol, citronellal. Essential oil of lemon balm also contains linalool, geranilacetate, 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 geranial, 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 phenylpropanoids, including rosmarinic acid as the most distinctive ones (Noguchi-Shinohara et al., 2015). Phenylpropanoids are also represented by ethyl oil of rosmarinic acid, caffeic acid, chlogenic acid, para-coumaric acid, ferulic acid and sinapinic acid. Using the method of liquid chromatography, we determined that content of rosmarinic acid in the leaves of lemon balm accounts for 0.54–1.79%. Among the phenol substances, antioxidant activity was exhibited by flavonoids apigenin, cosmosiin, luteolin, cynaroside, and also ramno-citrin (7-metoxy kaempferol) and iso- quercetin (3- quercetin glucoside), rhamnazin (3,7 dimetoxycampheroll). Moreover, the plant contains phenolcarbonic 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. Broad spectrum of therapeutic effect of the plant-based preparations is related to the content of various substances: sedative effect was described for citronellal, and spasmolytic – for geraniol and citronellol. Phenylpropanoids (rosmarinic, caffeic, chlorogenic and other hydroxycinnamic acids) are responsible for anti-viral, immune-modulling, antihistamine, antioxidant and antimicrobial properties (Bounihi et al., 2013; Seol et al., 2013; Joukar et al., 2014; Joukar & Asadipour, 2015; Hamza et al., 2016; Jeung et al., 2016; Safaeian et al., 2016; Shakeri et al., 2016; Sedighi et al., 2019). Furthermore, the plant has for a long time been broadly used as sedative preparation and so as to optimize the irritation of the nervous system (Bayat et al., 2012; de Cássiada Silveirae Sá et al., 2013; Feliú-Hemmelmann et al., 2013; Akbarzadeh et al., 2015; Ozarowski et al., 2016; Naderi Dastjerdi et al., 2019). There are reports about antihyperglicemia effect of *Melissa* essential oil in laboratory experiment (Hasanein & Riahi, 2015), positive effect from regulation of visceral adipose-tissue function in the conditions of nonalcoholic fatty liver diseases caused by high-fat diet (Kim et al., 2017). Park et al. (2015) indicate that angiogenesis inhibitor ALS-L1023 of *M. officinalis* helps to decrease the adipose tissue mass. *Melissa* speeds up the rates of restoration of traumas of the spinal marrow (Hosseini, 2020). Traditional Persian Medicine recommends rosmarinic acid-containing lemon balm as a dietary component for improving memory, treating cognitive disorders and, due to its anti-amyloidogenic and other properties, for consumption when having Alzheimer's disease (Iranshahy & Javadi, 2019). Its flavour is generated by geraniol (3–40%), neral (3–35%), geranial (4–85%), (E)-caryophyllene (0–14%), and citronellal (1–44%) (Setzer, 2009). Lemon balm has been traditionally used as tonic, antispasmodic, carminative, diaphoretic, surgical dressing for wounds, sedative-hypnotic preparation that strengthens the memory, and relieves of stress-induced headaches, though modern pharmacology evalueates its efficacy against mild to moderate Alzheimer's diseases, migraines and rheumatism, antitumel and antioxidant activities (Moradkhani et al., 2010).

*M. officinalis* is widely used not only in the traditional medicine of the Old World, but in the folk medicine of the Americas as well. For instance, in the state of Rio Grande do Sul (South Brazil), this plant is used (Gross et al., 2019) against symptoms related to the central nervous system (*M. officinalis* was included in 77.7% of ethnobotanical that cited 94 species of plants). Records of practices involving *M. officinalis* in traditional medicine are reported mostly for European countries, Mediterranean region and Middle East countries (Shakeri et al., 2016).

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 mechanism 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 inhibition of the microtubule affinity-regulating kinase MARK4, and (III) a potent inhibition of aldehyde dehydrogenase isoform ALDH1A3, related to cancer stem cell proliferation and chemoresistance (Bailly, 2020). Unfortuanately, 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 Laminiaceae family, is broadly used as culinary herbs: *Ocimum basilicum* L. (basil), *Ocimum tenuiflorum* L. (holy basil), *M. officinalis* L. (lemon balm), *Salvia rosmarinus* Spenn. (rosemary), *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 contains rosmarinic acid in the vacuoles, separately from oxidase enzymes. In case of plant's trauma, oxidases influence the rosmarinic acid, and the phenol hydroxyl group of rosmarinic acid become oxidized to orto-chinons. They bind with proteins of bacteria, fungi or herbivorous animal, thus inactivating them (Häusler et al., 1993). Rosmarinic acid of Lamiaceae plants exerted inhibition of 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 Shakeri et al. (2016) describes the botanical characterization, traditional uses, phytochemistry, pharmacological activities, pharmacokinetics and toxicity of *M. officinalis*, and discusses blanks in the data and perspectives of surveying this plant.

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

*Lavandula angustifolia* Mill. is a perennial shrub of Lamiaceae family. Height of cultivated plants reaches 100–200 cm, and the plants in the nature grow up to 50–70 cm. The leaves are opposite, elongated-linear, with bent margins, 2–6 cm in length, grey-green from the indumentum. All the parts of the plant contain essential oil: leaves – up to 0.4%, the stems – to 0.2%, inflorescences – 3.5–4.5%. The main constituents of the essential oil (30–60%) are complex ethers of L-linalool alcohol and acids (acetic, butyric, valeric, and caproic acids). Furthermore, it was found to

contain cineol, geraniol, borneol (Karabagias et al., 2019). The gas chromatography revealed the shares of the main components to equal as follows: linalyl acetate (25–46%), linalool (20–45%), terpinen-4-ol (1.2–6.0%), lavendulyl acetate (> 1.0%), 1,8-cineole (< 2.5%), 3-octanone (< 2.5%), camphor (< 1.2%), limonene (< 1.0%), and alpha-terpineol (< 2.0%) (Koriem, 2021). Flowers and oil of lavender are used as a culinary spice. It is particularly popular in Spanish, French and Italian cuisines. Sedative effect of lavender during neurasthenia and heightened pulse is 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, heart-vascular diseases, kidney-stone disease and pyelonephritis, for medical baths during joint inflammation, for would-healing, durig skin diseases and neuralgias, bruises and paralyses (Ziaee et al., 2015; Sadeghzadeh et al., 2017; Samarth 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 Laminiaceae family, lavender is a good nectar-bearer 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-moduling 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; Oskouie 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 (Ayaz et al., 2017). Also, this oil was reported to have neuroprotective effects (Ayaz et al., 2017). Lavender and lavandin essential oils prepared by stem distillation are usually composed of terpenes (e.g. linalool and linalyl acetate) and terpenoids (e.g. 1,8-cineole), responsible for their distinctive flavour and biological and therapeutic properties (Lesage-Meessen et al., 2015). Extract from *L. angustifolia* exhibited positive influence of motor dysfunction on the model of SCI bruise and contributed to the morphological improvement, having therapeutic potential for treatment of spinal cord damage (Kaka et al., 2019). Traditional medical practices all around the globe prescribe essential oils to treat a number of health issues (Raut &Karuppayil, 2014). The study by Todorov et al. (2014) discussed phytochemical composition of the essential oil concerning the influence on particular stages of viral life. The composition of the essential oil of the plant varies depending on various climates, as well as light, nutrients, temperature, and cultural practice genotype and oter factors. Nonethless, the major components are always citral (geranial and neral), citronellal, geraniol (Moradkhani et al., 2010).

Herbal essential oils are being more and more often used in pharmacology, medicine and food processing (Greff et al., 2020). Lemon balm and lavender are among the top ten most broadly applied medicinal and aromatic plants (Greff et al., 2020). Well-substantiated is the wound-healing property of essential oil of lavender (Samuelson et al., 2020). It significantly enhanced would healing and heightened the expression level of collagen, as well as activity of proteins taking part in tissue remodeling in wounds (Samuelson et al., 2020). Through studies of essential oil, it is possible to identify new bioactive compounds and find formulae of new preparations to treat cardiovascular diseases such as arterial hypertension, angina pectoris, heart failure, and myocardial infarction (Saljoughian et al., 2018). Over the recent five years, in the USA, health care-expenses related to cardiovascular diseases have increased 50%, accounting for 350 billion dollars (Bojic et al., 2019). In the recent decade, the number of research on antiaggregatory effect of polyphenol increased two-fold. Bojic et al. (2019) reviewed the antiaggregatory effects of most abundant polyphenols and flavonoids and plyphenols-rich plants (for example *L. angustifolia* and *M. officinalis*) on platelet aggregation, association of chemical composition and antioxidant properties with the observed biological effect, and possible clinical significance of the results they published. Cardiovascular diseases are among the most damaging health issues nowadays. Strokes and heart attacks often cause death, and another threat is posed by development of thrombus. Therefore, the therapy pays great attention to the level of primary hemostasis, first of all the clot formation, using acetylsalicyc acid and clopidogrel treatment. Use of plants rich in polyphenols to prevent thrombus development is relevant, as indicated by the two-fold increase in research on this issue over the last decade (Bojic et al., 2019).

*Vitex agnus-castus* L. is a tree-like shrub of the Lamiaceae family of up to 800 cm height. The plant is grey from pubulent dense adjascent hairs with 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 iridoidglycoside (agnuside, aucubin), flavonoids (casticin, vitexin, isovitaxin, orientin, isoorientin), p-hydroxybenzoicacid, alkaloids, tannins, essential oil (Stojković etal., 2011). The essential oil from leaves contains 1,8-cineole, trans-beta-farnesene, alpha-pinene, trans-beta-caryophyllene, andterpinen-4-ol. The oil from leaves of *V. agnus-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%), limonene (4.8%), alpha-terpineol (3.8%), sclarene (3.3%), alpha-terpinyl acetate (3.1%), p-cymene (3.0%) (Stojković et al., 2011). 1,8-cineole and alpha-pinene exerted notable antimicrobial potency as well (Stojković et al., 2011). The oil, particularly such from white flowering plants, is surveyed for its potential antibacterial effects (Stojković et al., 2011). Extract from *V. agnus-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 anticancer agent (Kikuchi et al., 2014). Vitex was observed to induce dose- and time-dependent decrease in cell viability following the induction of apoptosis and G(2)/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. agnus-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, pregnatone, prefemin, 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. agnus-castus* may lead to complications during

pregnancy. In the studies by Maleki-Saghooni et al. (2018), *V. agnus-castus* and *M. officinalis* mitigated symptoms of premenstrual syndrome (PMS).

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

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

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

## Materials and methods

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

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

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

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

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

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

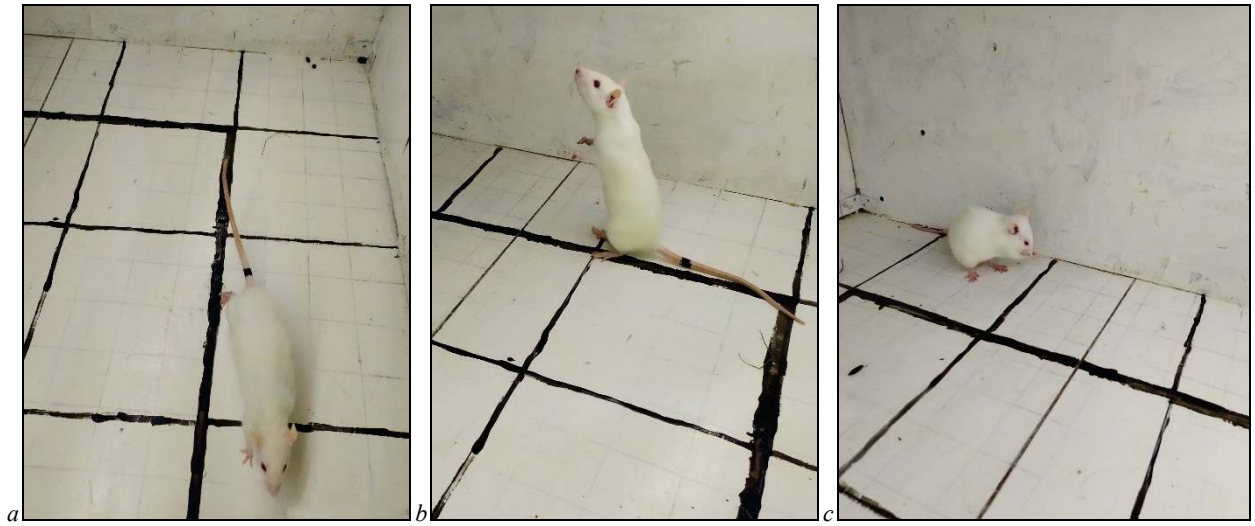


**Fig. 1.** Behaviour of rats in the "open field" test: *a* – crossing of peripheral squares, *b* – central stance, *c* – act of grooming

Blood samples taken during te euthanasia were then used for biochemical and morphological assays. Biochemical parameters were determined using Miura automated analyzer (I.S.E. Srl, Italy) and a set of High-Technology reagents (USA), PZCormay S.A. (Poland) и Spinreact S.A. (Spain). The erythrocytes and leukocytes in stabilized blood were counted using automated BC-2800Vet analyzer (Mindray, China).For the leukogam, 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. Stems of *L. angustifolia* caused almost even gain in body weight of the animals to 139.2% by the 30th days as compared with the initial weight for this group. Male rats that consumed shoots of *M. officinalis*, to the 30th days of the experiment, had the weight of 134.5% of their individual initial weight (Fig. 2b). Shoots of *V. angus-castus* caused slower weight gain than lavender and lemon balm – to118.8% of the body weight at the beginning of the experiment (Fig. 2c).

Shoots of *L. angustifolia* and *M. officinalis* significantly decreased the food intake to 83.0% and 84.1% compared with the control respectively (Table 1). Water intake in the experimental groups was no different from the control.

At the same time, adding shoots of *L. angustifolia* and *M. officinalis* caused reliable (more than 2.5-fold) increase in the mean daily body weight gain (Table 1): instead of 700 mg/day, the animals gained 1,943 and 2,024 mg/day, respectively. Herbs of *V. angus-castus* led to less notable and statistically insignificant increase (up to 138.1% of the control group) in daily body weight increment of the animals compared with the control group. We observed no significant changes in the belly volume caused by the three tested species of plants. Under the influence of addition of the shoots of *L. angustifolia* and *M. officinalis* to the diet, we seen significant decrease in the relative weight of the brain (Table 2), being the result of increase in the weight of the rest organs of the rats. When consuming *L. angustifolia* and *M. officinalis*, the relative weight of the thymus significantly decreased (to 58.3% and 46.7% of the relative mass of the thymus in animals of the control group, respectively). Intake of the shoots of *V. angus-castus* led to statistically significant increase in the relative weight of the spleen (Table 2).

High-fat diet in the experiment led to malfunctioned lipid metabolism (Table 3), which manifested in almost two-fold elevation of the level of triglycerides compared with the reference values, while the level of HDL cholesterol was lowered, and LDL cholesterol and the level of cholesterol was in the norm. Also, consumption of large amount of fat caused increase in the glucose level, whereas during addition of medicinal plants 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, urea, total protein) were within the norm.

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

**Table 1**

Change in the body weight and food consumption of young male rats under the impact of addition of crumbled shoots of *Lavandula angustifolia* Mill., *Melissa officinalis* L. and *Vitex angus-castus* L. to their diet (x ± SD, n = 8, duration of experiment – 30 days)

| Parameter | Control | *L. angustifolia* | *L. angustifolia* compared to the control, % | *M. officinalis* | *M. officinalis* compared to the control, % | *V. angus-castus* | *V. angus-castus* compared to the control, % |
|---|---|---|---|---|---|---|---|
| Consumption of food by animals, g/day | 20.09 | 16.67 | 83.0 | 16.90 | 84.1 | 18.81 | 93.6 |
| Consumption of liquid by animals, g/day | 18.42 | 18.50 | 100.5 | 19.05 | 103.4 | 18.93 | 102.8 |
| Change in body weight, µg/day | 700 ± 271 | 1943 ± 496*** | 277.6 | 2024 ± 393*** | 289.1 | 1171 ± 417 | 167.3 |
| Change in body weight, %/day | 13.6 ± 5.9 | 35.7 ± 8.0*** | 261.7 | 36.7 ± 7.9*** | 269.3 | 18.8 ± 6.5 | 138.1 |
| Объем живота, см | 14.0 ± 0.5 | 14.0 ± 0.9 | 99.9 | 13.8 ± 0.4 | 98.9 | 14.8 ± 1.0 | 106.0 |

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

**Table 2**

Changes in relative mass of the organs (%) of male rats under the influence of addition of crumbled shoots of *Lavandula angustifolia* Mill., *Melissa officinalis* L. and *Vitex angus-castus* L. to their diet (x ± SD, n = 8, duration of experiment – 30 days)

| Organ | Control | *L. angustifolia* | *L. angustifolia* compared to the control, % | *M. officinalis* | *M. officinalis* compared to the control, % | *V. angus-castus* | *V. angus-castus* compared to the control, % |
|---|---|---|---|---|---|---|---|
| Heart | 0.352 ± 0.023 | 0.362 ± 0.036 | 103.0 | 0.330 ± 0.025 | 93.7 | 0.352 ± 0.033 | 100.2 |
| Liver | 4.08 ± 0.17 | 4.32 ± 0.36 | 105.8 | 3.87 ± 0.36 | 94.8 | 3.71 ± 0.26 | 91.0 |
| Lungs | 0.979 ± 0.169 | 0.880 ± 0.190 | 89.9 | 0.872 ± 0.138 | 89.2 | 0.967 ± 0.365 | 98.8 |
| Brain | 0.867 ± 0.052 | 0.700 ± 0.073*** | 80.8 | 0.738 ± 0.075* | 85.2 | 0.698 ± 0.142 | 80.5 |
| Thymus | 0.285 ± 0.046 | 0.166 ± 0.077** | 58.3 | 0.133 ± 0.040*** | 46.7 | 0.221 ± 0.043 | 77.6 |
| Spleen | 0.370 ± 0.036 | 0.473 ± 0.159 | 128.0 | 0.412 ± 0.150 | 111.5 | 0.470±0.070* | 127.0 |
| Stomach | 0.699 ± 0.060 | 0.769 ± 0.211 | 110.0 | 0.764 ± 0.206 | 109.4 | 0.847 ± 0.266 | 121.3 |
| Small intestine | 2.58± 0.52 | 2.16 ± 0.25 | 83.8 | 2.20 ± 0.25 | 85.0 | 2.44 ± 0.32 | 94.7 |
| Cecum | 0.509 ± 0.176 | 0.457 ± 0.099 | 89.9 | 0.422 ± 0.077 | 82.9 | 0.509 ± 0.262 | 100.1 |
| Large intestine | 0.374± 0.085 | 0.397 ± 0.121 | 106.2 | 0.400 ± 0.082 | 107.0 | 0.431 ± 0.089 | 115.4 |
| Rectum | 0.398 ± 0.073 | 0.307 ± 0.077 | 77.2 | 0.203 ± 0.104** | 51.0 | 0.366 ± 0.063 | 92.0 |
| Right kidney | 0.358 ± 0.031 | 0.321 ± 0.027 | 89.6 | 0.317 ± 0.031 | 88.4 | 0.310 ± 0.026 | 86.4 |
| Left kidney | 0.372 ± 0.040 | 0.319 ± 0.022 | 85.7 | 0.321 ± 0.039 | 86.4 | 0.308 ± 0.030 | 82.8 |

*Note*: see Table 1.

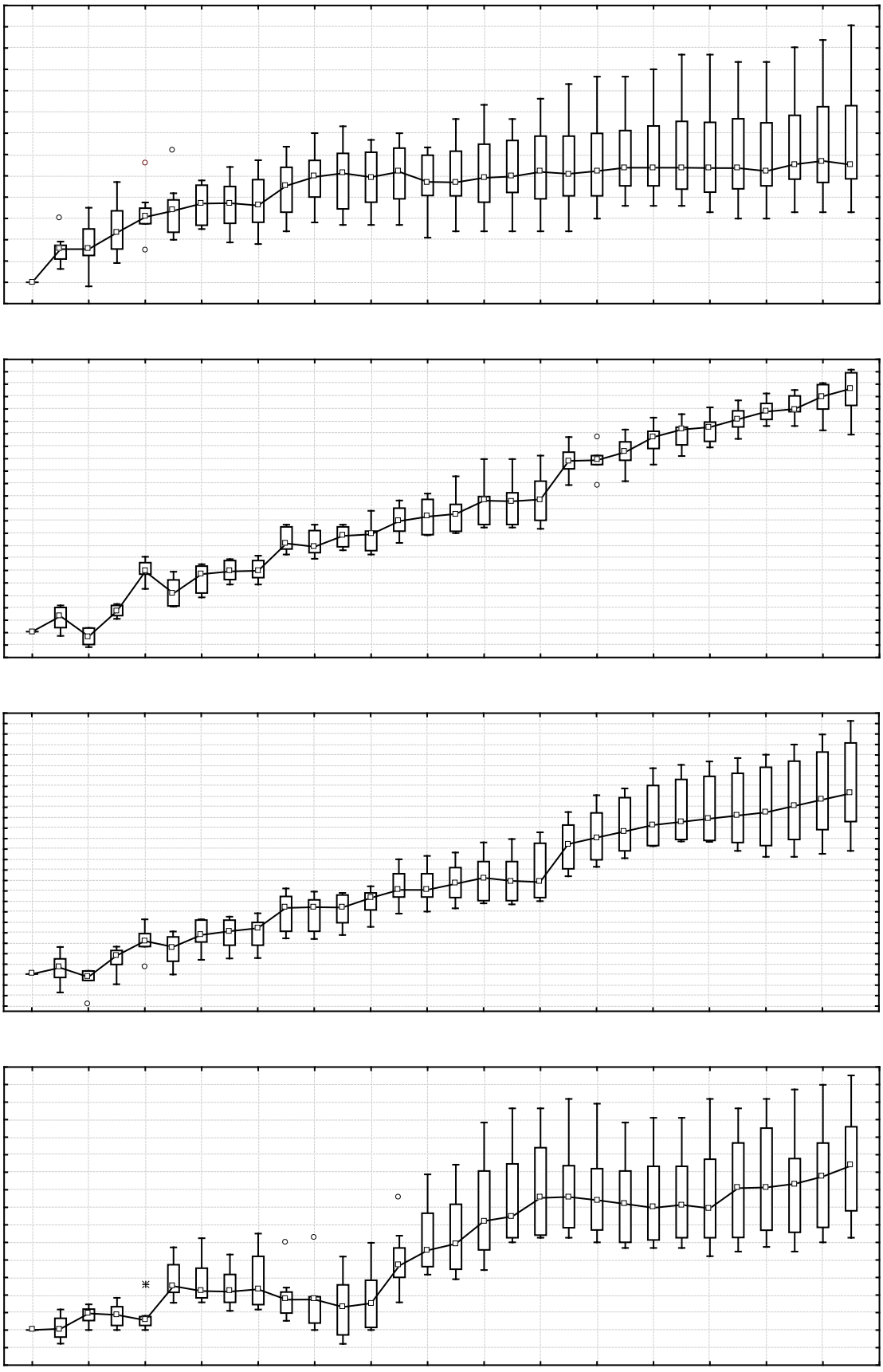


**Fig. 2.** Changes in the body weight of the rats in the control variant of the experiment (*a*) and when adding grined seeds of *Lavandula angustifolia* Mill. (*b*), *Melissa officinalis* L. (*c*) and *Vitex angus-castus* L. (*d*) into the diet: on the abscissa axis – 24 h of the experiment, on the ordinate axis – body weight of the animals (% of the initial wody weight before the experiment, considered 100% for each experimental animal); small square – median, upper and lower borders of the square – 75% and 25% of quartiles, the upper line – minimum and maximum values, circles – emissions; n = 8

Analysis of protein metabolism revealed that high-fat diet did not increase the concentration of the total protein. Addition of vitex and lemon balm to high-fat diet elevated the level of total protein beyond the limits of the normal values, while lavender has not. At the same time, in all groups, we observed slight increase in globuline fraction, especially noted at addition of *Vitex*.

High-fat diet did not significantly affect the morphological composition of blood of the experimental animals. Almost all the parameters were within the reference values. Exception was the level of monocytes in blood, which in all the groups was 1.5–2.0 times above the normal parameters. Intake of dry shoots of *L. angustifolia* and *M. officinalis* contributed to significant increase in the concentration of leukocytes in blood (to 165.4% and199.9% 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* snd *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* | *L. angustifolia* compared to the control, % | *M. officinalis* | *M. officinalis* compared to the control, % | *V. angus-castus* | *V. angus-castus* compared to the control, % |
|---|---|---|---|---|---|---|---|
| Total protein, g/L | 77.0 ± 4.9 | 76.0 ± 4.0 | 98.7 | 80.0 ± 3.7 | 103.9 | 86.4 ± 4.7* | 112.2 |
| Albumins, g/L | 39.6 ± 2.6 | 38.7 ± 2.5 | 97.8 | 41.3 ± 2.2 | 104.3 | 44.0 ± 1.7 | 111.2 |
| Globulins, g/L | 37.4 ± 3.9 | 37.3 ± 3.3 | 99.6 | 38.7 ± 3.3 | 103.4 | 42.4 ± 3.8 | 113.4 |
| Protein coefficient, U | 1.10 ± 0.15 | 1.06 ± 0.12 | 96.1 | 1.09 ± 0.14 | 98.7 | 1.04 ± 0.09 | 94.8 |
| Urea, mmol/L | 6.84 ± 1.02 | 6.00 ± 0.74 | 87.7 | 5.40 ± 0.58 | 78.9 | 5.13 ± 1.48 | 74.9 |
| Urea nitrogen, mg/100 g | 13.1 ± 2.0 | 11.5 ± 1.4 | 87.7 | 10.3 ± 1.1* | 79.0 | 10.6 ± 1.4 | 81.3 |
| Creatinine, μmol/L | 63.0 ± 4.4 | 61.0 ± 7.4 | 96.8 | 74.9 ± 12.8 | 118.8 | 67.0 ± 11.5 | 106.3 |
| Aspartate aminotransferase (AST), U/L | 186 ± 61 | 160 ± 48 | 85.7 | 182 ± 33 | 97.8 | 191 ± 39 | 102.3 |
| Alanine aminotransferase (ALT), U/L | 131 ± 41 | 129 ± 39 | 98.5 | 111 ± 18 | 85.2 | 179 ± 46 | 136.8 |
| De Ritis ratio (AST/ALT), U | 1.63 ± 0.78 | 1.37 ± 0.45 | 84.2 | 1.64 ± 0.30 | 100.9 | 0.94 ± 0.43 | 57.9 |
| Alkaline phosphatase, U/L | 129 ± 64 | 451 ± 94*** | 349.7 | 601 ± 149*** | 465.7 | 524 ± 143*** | 406.2 |
| Total bilirubin, μmol/L | 6.1 ± 1.7 | 4.1 ± 2.8 | 67.3 | 3.8 ± 1.4* | 63.3 | 5.7 ± 1.5 | 93.6 |
| Glucose, mmol/L | 7.39 ± 1.04 | 6.36 ± 0.63 | 86.1 | 6.40 ± 0.55 | 86.7 | 5.97 ± 0.58* | 80.9 |
| Totalcalcium, mmol/L | 2.53 ± 0.09 | 2.51 ± 0.11 | 99.4 | 2.59 ± 0.14 | 102.3 | 2.59 ± 0.16 | 102.3 |
| Non-organic phosphorus, mmol/L | 3.07 ± 0.58 | 3.67 ± 0.40 | 119.5 | 3.46 ± 0.29 | 112.6 | 3.13 ± 0.57 | 101.9 |
| RatioofCa/P | 0.843 ± 0.129 | 0.686 ± 0.099 | 81.4 | 0.743 ± 0.090 | 88.1 | 0.857 ± 0.192 | 101.7 |
| Gamma-glutamyl transpeptidase (GGT), units/L | 9.3 ± 2.6 | 9.1 ± 4.4 | 98.5 | 6.7 ± 0.7 | 72.3 | 6.4 ± 1.2 | 69.2 |
| Cholesterol, mmol/L | 1.27 ± 0.13 | 1.59 ± 0.20* | 124.7 | 1.43 ± 0.18 | 112.4 | 1.43 ± 0.07 | 112.4 |
| Tryglicerides, mmol/L | 2.13 ± 0.55 | 1.36 ± 0.38 | 63.8 | 1.34 ± 0.31* | 63.1 | 1.20 ± 0.38** | 56.4 |
| High-dense lipoprotein cholesterol (HDL cholesterol),mmol/L | 0.65 ± 0.13 | 0.66 ± 0.19 | 101.5 | 0.80 ± 0.44 | 122.6 | 0.34 ± 0.24* | 51.9 |
| Low-dense lipoprotein cholesterol (LDL cholesterol), mmol/L | 0.52 ± 0.29 | 1.18 ± 0.08*** | 227.7 | 0.51 ± 0.11 | 98.0 | 0.95 ± 0.40 | 184.3 |
| C-reactive protein, mg/L | 12.5 ± 5.4 | 13.2 ± 5.2 | 105.7 | 10.2 ± 1.4 | 81.7 | 12.6 ± 3.0 | 100.3 |
| Aterogenecity index, units | 1.04 ± 0.45 | 1.85 ± 1.41 | 177.1 | 1.30 ± 0.91 | 124.5 | 5.57 ± 3.32*** | 524.0 |

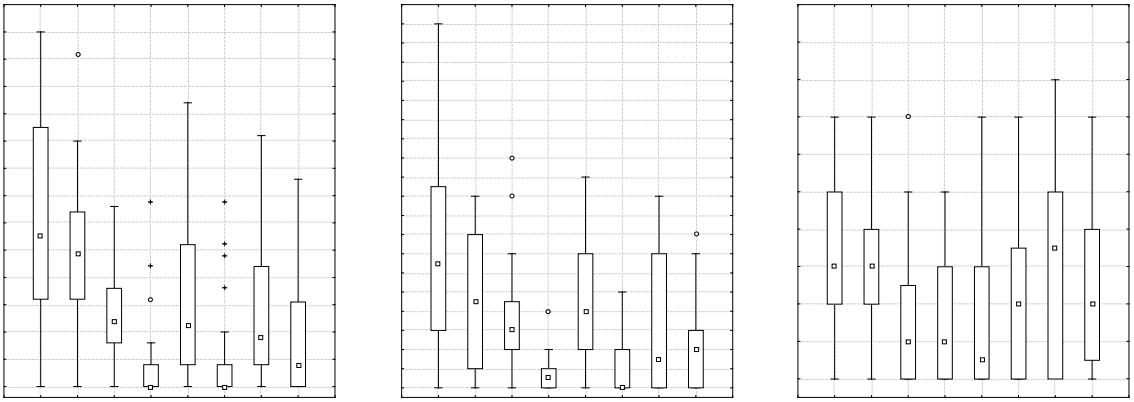
*Note*: see Table 1.

**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* | *L. angustifolia* compared to the control, % | *M. officinalis* | *M. officinalis* compared to the control, % | *V. angus-castus* | *V. angus-castus* compared to the control, % |
|---|---|---|---|---|---|---|---|
| Hemoglobin, g/L | 126.8 ± 7.0 | 119.3 ± 7.1 | 94.1 | 130.4 ± 6.9 | 102.8 | 126.7 ± 11.8 | 99.9 |
| Hematocrit, % | 40.5 ± 2.7 | 38.6 ± 2.4 | 95.3 | 42.0 ± 1.9 | 103.6 | 40.3 ± 4.0 | 99.5 |
| Erythrocytes, $10^{12}$/L | 6.93 ± 0.29 | 7.13 ± 0.30 | 102.8 | 7.03 ± 0.91 | 101.3 | 7.32 ± 0.67 | 105.6 |
| Erythrocyte sedimentation rate (ESR), mm/h | 1.17 ± 0.37 | 1.33 ± 0.47 | 114.3 | 1.00 ± 0.00 | 85.7 | 1.00 ± 0.00 | 85.7 |
| Thrombocytes, $10^9$/L | 339 ± 66 | 351 ± 87 | 103.7 | 336 ± 66 | 99.3 | 284 ± 72 | 83.7 |
| Leukocytes, $10^9$/L | 8.6 ± 1.6 | 14.2 ± 2.3*** | 165.4 | 17.1 ± 5.9*** | 199.9 | 11.3 ± 6.1 | 131.6 |
| Leukocytic formula | | | | | | | |
| Basophils, % | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | 0.0 |
| Eosinophils, % | 1.50 ± 0.76 | 1.17 ± 0.37 | 77.8 | 1.57 ± 0.73 | 104.8 | 0.86 ± 0.35 | 57.1 |
| Mielocits, % | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | 0.0 |
| Neutrophils, %: | | | | | | | |
| – young | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | 0.0 |
| –band | 1.17 ± 0.69 | 1.17 ± 1.07 | 100.0 | 0.71 ± 0.45 | 61.2 | 0.29 ± 0.45* | 24.5 |
| – with segmented nuclei | 23.0 ± 8.2 | 22.3 ± 5.1 | 97.1 | 25.6 ± 6.4 | 111.2 | 21.0 ± 4.4 | 91.3 |
| Lymphocytes, % | 68.8 ± 8.6 | 67.2 ± 6.3 | 97.6 | 65.9 ± 6.9 | 95.7 | 72.1 ± 3.6 | 104.8 |
| Monocytes, % | 5.5 ± 1.3 | 8.2 ± 3.3 | 148.5 | 6.3 ± 2.3 | 114.3 | 5.6 ± 1.7 | 101.3 |

*Note*: see Table 1.



*a* *b* *c*

**Fig. 3.** Changes in the motor activity (*a*), orienting activity (*b*) and emotional status (*c*) of male rats that in addition to their diet received 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 grined shoots of the plants (in parentheses there are indicated day after the experiment: beginning – 1–4th or the end – 26–30th days), on ordinate axis – absolute number of markers of this type of behavior during 120 seconds of the experiment: for the motor activity – the number of attended squares of the "open field", for the orienting activity – number of stances, for the emotional status – number of acts of grooming, defecation and urination; small square – median, the upper and lower line of the rectangle – 75% and 25% quartiles, the upper line – minimum and maximum values, circles – emissions; different letters within each figure indicate significant differences between the groups (P< 0.05) according to the results of Tukey test

**Table 5**

Changes in the behaviouristic characteristics of the three groups of rats during 120 seconds of the experiment when crumbled shoots of *Lavandula angustifolia* Mill., *Melissa officinais* 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 | $28.13 \pm 18.04^{a}$ | $24.33 \pm 14.45^{a}$ | $13.64 \pm 8.59^{ab}$ | $3.71 \pm 7.96^{b}$ | $16.11 \pm 14.29^{ab}$ | $4.57 \pm 9.32^{b}$ | $13.54 \pm 12.78^{ab}$ | $8.96 \pm 11.30^{ab}$ |
| Number of attended central squares | $1.00 \pm 2.341$ | $0.292 \pm 1.042$ | $0.00 \pm 0.00$ | $0.00 \pm 0.00$ | $0.821 \pm 1.887$ | $0.000 \pm 0.000$ | $0.107 \pm 0.416$ | $0.000 \pm 0.000$ |
| Number of stances in peripheral squares | $5.58 \pm 4.53^{a}$ | $3.79 \pm 3.13^{ab}$ | $3.04 \pm 2.13^{ab}$ | $0.79 \pm 1.03^{b}$ | $3.75 \pm 2.74^{ab}$ | $1.00 \pm 1.44^{b}$ | $2.93 \pm 3.33^{ab}$ | $1.86 \pm 2.21^{ab}$ |
| Number of stances in the central squares | 1.292±1.429 | 0.708±0.999 | $0.607 \pm 0.994$ | $0.036 \pm 0.189$ | $0.750 \pm 1.602$ | $0.000 \pm 0.000$ | $0.222 \pm 0.641$ | $0.000 \pm 0.000$ |
| Number of acts of groomin | 0.583±0.830 | 0.583±0.929 | $1.000 \pm 1.122$ | $0.536 \pm 0.744$ | $1.071 \pm 1.464$ | $0.643 \pm 1.224$ | $0.393 \pm 0.994$ | $0.393 \pm 0.786$ |
| Number of fecal boli | 2.250±2.027 | 2.375±1.555 | $0.536 \pm 1.071$ | $0.964 \pm 1.478$ | $0.571 \pm 1.317$ | $1.679 \pm 2.056$ | $2.679 \pm 2.568$ | $2.179 \pm 2.195$ |
| Number of urinations | 0.333±0.482 | 0.375±0.495 | $0.107 \pm 0.416$ | $0.036 \pm 0.189$ | $0.036 \pm 0.189$ | $0.036 \pm 0.189$ | $0.000 \pm 0.000$ | $0.000 \pm 0.000$ |

*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 indicated 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 contravercial. 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 overating 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 (Milevskaya 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.

Valable 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 metallo proteinase-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 (Lude et al., 2016).

High content of fat in diet is considered to inevitably cause increase in the parameters of lipid metabolism such as total cholesterol, level of triglycerides, content and proportion of lypoproteins of different density, which are expressed by such a parameter as aterogenecity index. In our experiments, in rats, the consumption of the diet with heightened content of fat during 30 days caused no elevation of the level of total cholesterol, which remained within the reference values. As known, the parameters of lipid metabolism in rats were lower than such in human due to production of specific bile acids – α- and β-muricholic acids, absent in humans (Thomas et al., 1984). Bile acids in particular are those considered responsible for fast removal of cholesterol from the rats' 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 Swiader et al. (2019) analyzed the literature data on the chemical composition of *M. officinalis* and the possibilities of using it in medicine and food. Heshmati et al. (2020) indicated the relationship between consumption of *M. officinalis* and decreased total cholesterol and reduced systolic blood pressure. Intake of *M. officinalis* was not observed to be related to statistically significant changes in triglycerides, low-density lipoprotein, diastolic blood pressure, high sensitivity c-reactive protein levels, fasting blood sugar, HbA1c, insulin or high-density lipoprotein levels. No serious side effects were reported. According to the study by Heshmati et al. (2020), *M. officinalis* is safe beneficial supplement. In our experiment, addition of lemon balm to high-fat diet of rats reduced the intensity of increase in the level of triglycerides and HDL cholesterol compared with the control, and at the same time the indicator of total cholesterol, LDL cholesterol, did not change significantly.

In a 21-day experiment on rats that received high-fat diet and various doses of extract of melissa, Zarei et al. (2014) observed significant decrease in the activity of hepatic enzymes. In our experiment, by the 30th day, the rats fed with fat diet were seen to AST, ALT and alkaline phosphatase exceeding the reference values, indicating damaged cellular membranes of hepatocytes. Adition 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 and non-scientific articles, but clinical trial of ethanol extract from *M. officinalis* was only made so far toward Alzheimer disease (Mahboubi, 2019). Action mechanisms of *M. officinalis* comprise inhibitory effects against beta-amyloid, reactive oxygen species, and acetylcholine esterase.

*M. officinalis* can mitigate psychological symptoms in the patients undergoing operation (Shabanian et al., 2019). Use of medicinal herbs before and after surgery alleviates anxiety, depression, aggressive and impulsive behavior, stress, delirium and cognitive dysfunction. Trials on children with attention deficit hyperactivity disorder (ADHD) who consumed *M. officinalis* preparations showed low evidence for their effectiveness (Anheyer et al., 2017); but no concrete recommendations could be made while there is still a lack of sufficient numbers.

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

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

*M. officinalis* and *L. angustifolia* are commonly considered to take generally calming effect. Experimental pharmacology using *L. angustifolia* includes anesthetic, anticonvulsant, sedative, anti-inflammatory, antimicrobial, antispasmodic, antispasmodic, central nervous system depressant effects; clinical pharmacology includes anxiolytic, analgesic, and cardiovascular effects (Koriem, 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 (Fismer & Pilkington, 2012). Some studies have shown the effiency 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 vitex 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. agnus-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. agnus-castus* displayed anti-anxiety activity and may be used to treat anxiety (Mehlhorn, 2016). Interaction between phytoestrogens from *V. agnus-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 (Bilan 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 seen broader range of antibacterial activity in *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 ivanovi*, *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* (Wedl, 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 nema-

todes of *Strongyloides* genus, specific various species of model animals and human.

## Conclusion

Against the background of high-fat diet, lemon balm and lavender manifested similar influences. Addition of these plants to the diet led to significant decrease in food intake, and at the same time the intensity of weight gain was greater than in the animals that consumed high-fat diet supplemented by vitex. 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 vitex. Also, lemon balm and lavender, by the end of the experiment, led to significant decrease in the relative weights of the brain and the thymus.

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

Additional of medicinal plants to high-fat diet led to impaired activity of blood enzymes – alkaline phosphatase, AST and ALT; increase in triglycerides, LDL cholesterol against the background of decrease in HDL cholesterol and normal value of total cholysterol. Also, all the groups were characteristic of monocytosis, more notable at addition of lavender and lemon balm to the diet, which also caused leukocytosis compared with the control group and addition of vitex.

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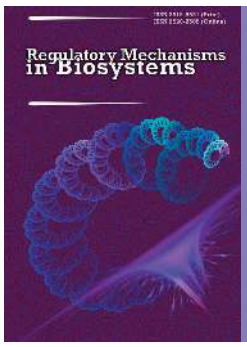

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