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SATUREJA MONTANA L. ESSENTIAL OIL VARIOUS DOSAGES EFFECT ON THE MAIN RATS' BIOLOGICAL FEATURES

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ABSTRACT

An application of natural antioxidants remains the focus of research groups. The effect of *Satureja montana* L. essential oil in various doses on the main biological characteristics of Wistar rats was the main aim of the study. The intensification of protein metabolism in the blood plasma of rats on the background of the use of *Satureja montana* L. essential oil was noted. Total protein increases by 17.9 – 19.7%, and albumin by 27.6% in rats of the experimental group received the essential oil at a dose of 0.6 ml per kg of feed. A significant increase in the AST level in control group rats to 207.3 $U.L^{-1}$ was revealed. Its one to a certain extent indicates the hepatoprotective effect of mountain savory oil and a decrease in inflammatory processes in the organs of the gastrointestinal tract in the conditions of cell maintenance of rats of the experimental groups. Gastric epithelium thickness of rats of both experimental groups was lower than the control animals. But it did not bear any signs of atrophy. The difference of this indicator in comparison with control was 12.75 μm ($p \leq 0.05$) in the second group and it was reliable. The number of chief stomach cells increases in animals of the experimental groups, which may indicate a greater enzymatic activity. An increased dose of mountain savory oil contributes to the formation of more damage to hepatocytes on the periphery of the liver lobule. Thus, the relationship between liver enzymes and the state of peripheral hepatocytes was noted.

Keywords: *Satureja montana* L.; essential oil; rats; live weight; plasma; stomach; liver

INTRODUCTION

The interest in natural-origin antioxidants due to the significant content of biologically active substances increased in recent years all over the world. The study of the effect of natural antioxidants on rats is widely practiced because of a simple and understandable model for research **(Nurmawati et al., 2021; Widyastuti et al., 2020; Fauza et al., 2019)**.

Essential oils (EO) are one of these antioxidants. EO has antiseptic, antibacterial, antiparasitic, antifungal, insecticidal, anti-inflammatory, and regenerating properties. It exerts a defined effect on the body, regardless of the different ways of application. The effect is usually intensified also with prolonged use **(Krishan and Narang, 2014)**.

Piper guineense EO effect investigated in mice by intraperitoneal application on the central nervous system (CNS). Sedative, anticonvulsant, and hypothermic effects were detected **(Oyemitan et al., 2015)**. A pronounced anxiolytic, antidepressant, and antioxidant effect in the study of *Ferulago angulata* essential oil in rats was recorded with inhalation use **(Bagci et al., 2016)**. The antidepressant EO effect of the *Asarum heterotropoides* was studied **(Park et al., 2015)**.

The anxiolytic effect of vetiveria essential oil, when inhaled for 7 minutes to reduce the symptom of anxiety on Wistar line male rats, was studied. 2.5% is the most effective concentration was found **(Saiyudthong et al., 2015)**. The anxiolytic and antidepressant effects of inhaled basil (*Ocimum sanctum* L.) and holy basil (*Ocimum basilicum* L.) EOs on the background of experimental Alzheimer's disease in rats have been proven **(Gradinariu et al., 2015)**. Elsholtzia ciliate (*Elsholtzia ciliate*), angelica (*Angelicae gigantis*), and clove (*Eugenia caryophyllata*) essential oils in the complex therapy of withdrawal syndrome can be used **(Choi et al., 2013)**.

Cypress obtuse (*Chamaecyparis obtusa*) essential oil at a concentration of 7.0 mg/l of air on male mice of the ICR line was studied. An anxiolytic effect was recorded **(Bae et al., 2012)**. *Toona ciliata* M. Roem essential oil antidepressant effect on mice in doses of 10, 20, 40, and 80 $mg.kg^{-1}$ was administered creating. Clove tree

(*Syzygium aromaticum*) EO with the intragastric influence of 200 mg.kg^{-1} dosage was established **(Castro et al., 2015)**.

The neuroprotective effect of *Lavandula angustifolia* EO on the background of an experimental stroke in Swiss line male mice at a dose of 0.3 – 2 mg.kg^{-1} intraperitoneally was proved **(Vakili et al., 2014)**.

Lavandula angustifolia essential oil at the background of insomnia reduces anxiety **(Chioca et al., 2013)**. Similar results were obtained with intraperitoneal administration at a dose of 0.7 mg.kg^{-1} of male Wistar rats in the study of *Lavandula angustifolia ssp.* and *Lavandula hybrida Rev. (Lamiaceae)* essential oils **(Hritcu et al., 2012)**. The use of these essential oils significantly reduced the level of anxiety and inhibition, showed an antidepressant effect, reduced the level of nitric oxide and malondialdehyde in brain tissues. Lavender officinalis (*Lavandula officinalis*) essential oil in the conditions of insomnia to rats a sedative effect was rendered **(Takahashi et al., 2014)**.

The effect of perilla essential oil (*Perilla frutescens*) by intragastric administration in rats was studied **(Yi et al., 2013)**. The psychophysiological effect occurs only with prolonged use at least 3 weeks. The effect of lemongrass (*Cymbopogon citratus*) essential oil on Swiss line male mice per intragastric introduction in dosage 0.5 – 1.0 g.kg^{-1}: sedative, anxiolytic, anticonvulsant, anticonvulsive effects were recorded **(Campélo et al., 2011)**.

The effect of chamomile essential oil (*Matricaria chamomilla* L.) in mice that received chamomile EO in 5000, 2500, and 1250 ppm concentrations was studied **(Fabian et al., 2011)**. Chamomile essential oil can improve some parameters of inflammatory models, depending on the concentration used. The essential oil of clove (*Calamintha officinalis*) has antifungal and antimicrobial activity against gram-positive bacteria was founded **(Monforte et al., 2011)**.

The effectiveness of the use of mountain savory has already been studied by us in poultry farming **(Pashtetsky et al., 2020)**. Mountain savory (*Satureja Montana* L.) is a perennial semi-shrub with lignified shoots at the base of the root, belonging to the family of Clear-cut flowers (*Lamiaceae* L), has a sharp, spicy aroma, with a slight burning pepper taste. The biological value of S. montana is due to the complex of biologically active substances, among which the leading position is occupied by volatile compounds. Of the proven therapeutic properties, mountain savory essential oil has anti fungicidal, antibacterial, antiviral, immuno-strengthening, hepatoprotective, analgesic, tonic effects **(Mihajilov-Krstev et al., 2014)**. Mountain savory essential oil on nine phytopathogenic fungi of the genus Fusarium has an effect that suppresses their growth **(Rahimian and Eisvand, 2016)**.

Carvacrol and thymol are the main components of the essential oil. Their mechanism of action is the destruction of the cytoplasmic membrane, which increases its permeability and depolarizes its potential. **(Allaoua et al., 2018)**. The effect of thymol on the smooth muscles of the trachea, the ciliary apparatus of the respiratory tract and the ileum in rats and found that thymol has a dose-dependent antispasmodic property and increases the rate of contraction of the mucous membrane due to the movement of cilia was founded **(Orchardand and van Vuuren, 2017)**.

Essential oils in the Republic of Crimea occupy a significant part in the agrarian-and-industrial complex of the region. In addition to the perfumery, cosmetics, and pharmaceutical areas, the use of essential oils in animal husbandry is little studied, so the construction of an antioxidant model for the use of essential oils is very necessary **(Ostapchuk et al., 2020)**. It is also reported about its effective use in complex therapy in the treatment of combined bacterial and fungal infections, including COVID-19 **(Postnikova et al., 2021)**.

Based on the analyzed sources, the use of essential oils as natural antioxidants continues to be the focus of scientists ' attention. Since essential oils provide a wide range of effects on a living organism, the purpose of these studies is the effect of mountain savory (*Satureja montana* L.) essential oil in various dosages on the growth and basic biological features of rats.

Scientific hypothesis

Research work hypothesis hinged on the assumptions that the phenolic substances contained in mountain savory oil have an antioxidant effect on the body of rats. As an object of the study has been selected *Satureja montana* L. essential oil due to the high content of valuable phenolic substances. According to obtained results the improvement of indicators of the main rats' biological features under the influence of plant phenols, has been vindicated.

MATERIAL AND METHODS

The experimental study was conducted jointly by the staff of the Research Institute of Agriculture of Crimea and the Medical Academy named after S. I. Georgievsky of V.I. Vernadsky Crimean Federal University.

Animals and Biological Material

Wistar line adult male rats aged 5 – 6 months with 180 g average weight. The animals were weighed daily. After removing the animals from the experiment, samples of the liver and stomach were taken.

Chemical

Mountain savory essential oil is obtained by steam distillation. In the laboratory of selection of essential oil crops on the chromatograph. The composition of mountain savory essential oil includes the following plant phenols: a-pinene, camphene, b-pinene, β-myrcene, a-terpinene, limonene, eucalyptol, g-terpinene, p-cymene, cis-linalool oxide, sabinene hydrate, camphor, linalool, linalyl acetate, caryophyllene, caryophyllene oxide, thymol, and carvacrol. The highest content was of the following components: carvacrol (49.88%), p-cymene (15.76%), y-terpinene (15.28%), a-pinene (2.52%), a-terpinene (2.07%) and thymol (0.23%).

The biochemical composition of animal blood plasma was determined according to the following indicators: total protein (TP, g.L^{-1}), albumin (ALB, g.L^{-1}), glucose (GLUC, mmol.L^{-1}), alanine aminotransferase (ALT, U.L^{-1}), aspartate transaminase (AST, U.L^{-1}), alkaline phosphatase (ALP, U.L^{-1}), creatinine (CREA, μmol.L^{-1}), urea (UREA, mmol.L^{-1}), bilirubin (BILT, μmol.L^{-1}). Whole blood was also separated to study the shaped elements of the blood.

Laboratory Methods

After three weeks, the animals were removed from the experiment under ether anesthesia, the stomach and liver were separated, fixed in 10% buffered formalin, and subjected to dehydration and paraffin impregnation on a histology processor. Thin sections with a thickness of 4 microns were made from the obtained paraffin blocks, stained with Hematoxylin and Eosin, and viewed in a light field under a microscope. The images obtained in the *ImajeG* program, after appropriate calibration on the TS-M1 scale of 0.01 mm/100 div stage micrometer, measured the height of the gastric epithelium, the depth of the own glands, the thickness of the gastric mucosa, the number of epithelial cells in the epithelium (per 1 villus), the relative number of the chief and parietal epithelium cells in glands of the stomach. In the photo of liver preparations, the number of binucleated cells, the relative areas of sinuses and hepatocytes in the central and peripheral parts of the hepatic lobule were counted, and the nuclear-cytoplasmic ratio of hepatocytes was calculated.

Instruments

Chromatek-Crystal 5000.2 chromatography device on a capillary quartz column with a fixed phase CR-WAXms (polyethylene glycol salt-and-gel matrix). Capillary quartz column with a length of 60 mm with an internal diameter of 0.32 mm, fixed phase CR-WAXms (polyethylene glycol salt-and-gel matrix). The thermostat temperature is 80 °C, lasting 6 minutes. The next step is programming with a speed of 2.1 °C per min. up to 220 °C. Evaporator temperature: 260 °C. Detector temperature: 240 °C. Column inlet pressure: 100 kPa flow division: 1/50. Sample volume: 0.5×10^{-3} cm^3.

Biochemical Vitalab Flexor E automatic analyzer.

Hematology Biobase analyzer.

LOGOS microwave histology processor.

DM 2000 Leica microscope with Plan 10× and Plan 40× lenses and a DFC295 camera.

Description of the Experiment

The number of heads in each group was 5. The animals were kept in standard vivarium conditions. The rats have divided into 3 groups: the control group received the main diet (MD) (1st group). The 2nd group was experimental: animals received mountain savory (*Satureja montana* L.) essential oil at the rate of 0.3 mg per group daily on the background of MD. The 3rd group was experimental too: animals received essential oil at the rate of 0.6 mg per group daily on the background of MD. The experiment duration was 20 days. The animals obtained the main diet in *ad libitum* conditions and 12-hour daylight hours were. The MD included dry food and fresh vegetables and herbs.

Sample preparation: The blood was collected from the sublingual plexus under weak ether anesthesia into EDTA tubes. The blood was centrifuged at 3000 rpm for 5 minutes to separate the plasma. The stomach and liver were sent for pathomorphological studies.

Number of samples analyzed: 15 heads

Number of repeated analyses: 15

Number of experiment replication: There is no experiment replication in our study

Statistical Analysis.

The obtained data were processed by methods of descriptive statistics with the determination of the normality of the distribution by the Shapiro-Wilk method, calculation of the arithmetic mean, confidence interval, and mean statistic error. Nonparametric statistics are the determination of differences between groups by multiple comparisons of several groups by the Kruskal-Wallis method was used. All data were analyzed statistically using Excel for Windows. Significant differences were considered at $p \leq 0.05$.

RESULTS AND DISCUSSION

The body weight increasing in both experimental groups of rats mountain savory essential oil using contributed. Live weight increasing in the middle and at the end of the experiment was most expressed. The results in Table 1 were stated.

Rats' live weight of the second group on the 10th day of the experiment has no significant advantages in comparison with the control group ones. A significant difference on the 20th day of the experiment was 3.4% ($p \leq 0.05$).

The 3rd group rats have a significant tendency of the live weight during the entire period of the experiment over the control ones. A significant difference was on the 10th day by 6.8% ($p \leq 0.05$), and on the 20th day by 10.5% ($p \leq 0.05$).

Live weight dynamics of all individuals in the experiment are stated in Figure 1.

Thus, the conducted studies have established that the tested essential oil has a growth-stimulating effect on rats. However, live weight data is not enough to fully characterize the development of animals at the background of essential oil use.

Blood plasma biochemistry data on the 21st day of the experiment were obtained. These results are stated in Table 2.

AST increment of the control group animals to 207.3 $U.L^{-1}$ ($p < 0.001$) was significant. One of the main reasons for transaminase enzyme activity level increasing in the animals of the control group probably can be assumed to be the presence of non-critical destructive processes in hepatocytes. This one, probably, is they tend to develop at the standard content of rats in cells. Bilirubin increasing tendency in animals of the control group was noted. The amount of bilirubin decreasing in the 3rd group may be associated with a decrease of this enzyme in the liver. Thus, a mountain savory essential oil hepatoprotective effect on the rats' body to a certain extent.

AST dynamics concentration during the experiment to the studied groups of rats was studied and shown in Figure 2. Unstable variation of this indicator in the animals of the control group was noted. In the animals of the second group, the tendency to decrease from 154.9 $U.L^{-1}$ on the 10th day of the experiment to 135.3 $U.L^{-1}$ on the 20th day was noted. This indicator fluctuation in the animals of the 3rd group during the experiment was practically absent.

The urease level of the experimental groups increased by 2.0 – 1.9 times ($p < 0.001$) on the 10th day. It indicates an increased urease role in the process of amino acid synthesis by bacteria, which are intestinal microbiota nitrogen source forming. That effect in the animals of the experimental groups from the 10th day at the level of 12.5 – 13.2 to 12.6 – 12.2 $mmol.L^{-1}$ was observed (Figure 3).

Table 1 Rats' live weight dynamics in the experiment, g.

Statistical indicator	Day of experiment		
	1st	10th	20th
		1st group	
X±mx	179.0 ±1.2	190.5 ±2.5	208.3 ±3.9
σ	2.7	5.5	8.8
Cv, %	1.5	2.9	4.2
		2nd group	
X±mx	180.0 ±1.1	197.0 ±2.7	223.2 ±6.2*
σ	2.6	6.1	13.8
Cv, %	1.4	3.1	6.2
		3rd group	
X±mx	178.0 ±1.2	203.5 ±3.8*	230.2 ±9.6*
σ	2.7	8.4	21.5
Cv, %	1.5	4.1	9.4

Note: Here in table level of statistical significance: * – $p \leq 0.05$.

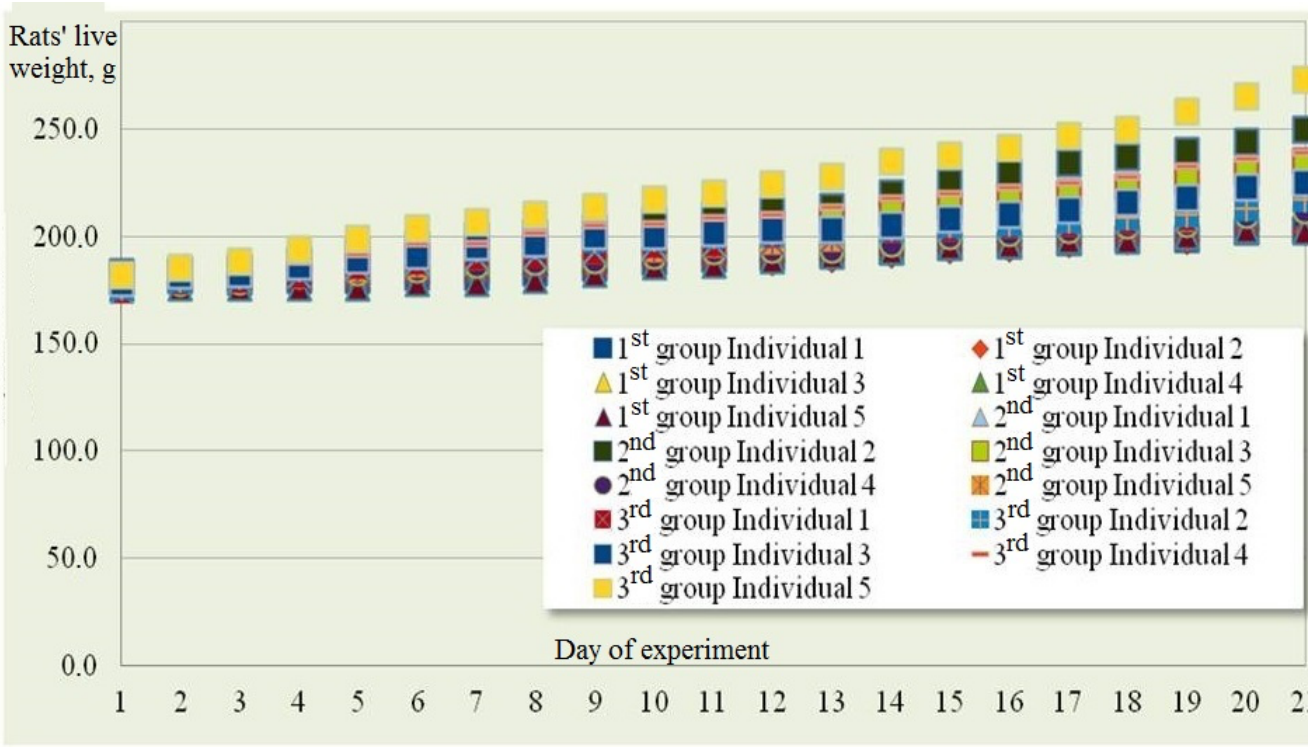

Figure 1 Rats' live weight dynamics of all individuals in the experiment, g.

Table 2 Rats' blood plasma biochemical features on the 21st day of experiment (n = 3).

Biochemistry indicator	1st group		2nd group		3rd group	
	X±mx	Cv, %	X±mx	Cv, %	X±mx	Cv, %
ALT	59.3 ±1.3	3.7	56.1 ±2.4	7.5	64.5 ±4.3	11.4
AST	207.3 ±7.8***	6.5	135.3 ±4.0	5.1	134.2 ±2.7	3.5
BILT	2.4 ±0.3	20.7	2.5 ±0.2	11.0	2.0 ±0.2	14.0
UREA	6.3 ±0.3	8.5	12.6 ±0.4***	5.5	12.2 ±0.3***	3.9
ALP	323.7 ±3.3	1.8	326.7 ±10.9	5.8	322.7 ±18.0	9.6
CREA	46.7 ±2.1	7.9	41.3 ±3.0	12.5	45.2 ±2.1	7.9
GLUC	8.9 ±0.2	4.5	9.4 ±0.3	5.5	9.5 ±0.3	5.3
TP	67.0 ±1.2	3.0	80.2 ±1.7***	3.7	79.0 ±1.1***	2.5
ALB	29.3 ±0.2	1.3	37.4 ±1.5***	6.8	31.3 ±1.4	8.0

Note: Here in table level of statistical significance: * – $p \leq 0.05$; ** – $p \leq 0.01$; *** – $p \leq 0.001$.

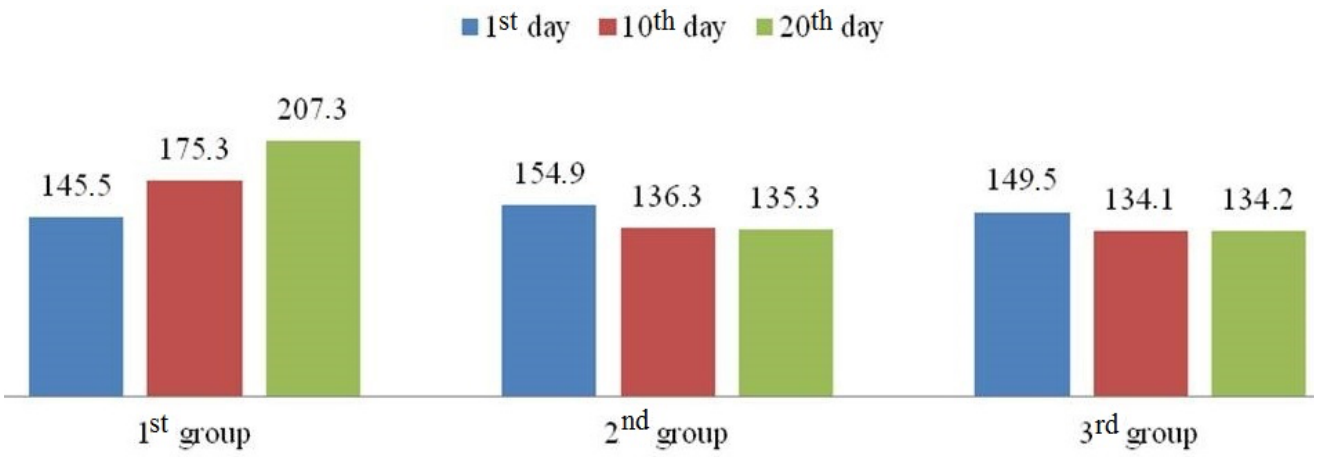

Figure 2 AST dynamics concentration during the experiment, $U.L^{-1}$.

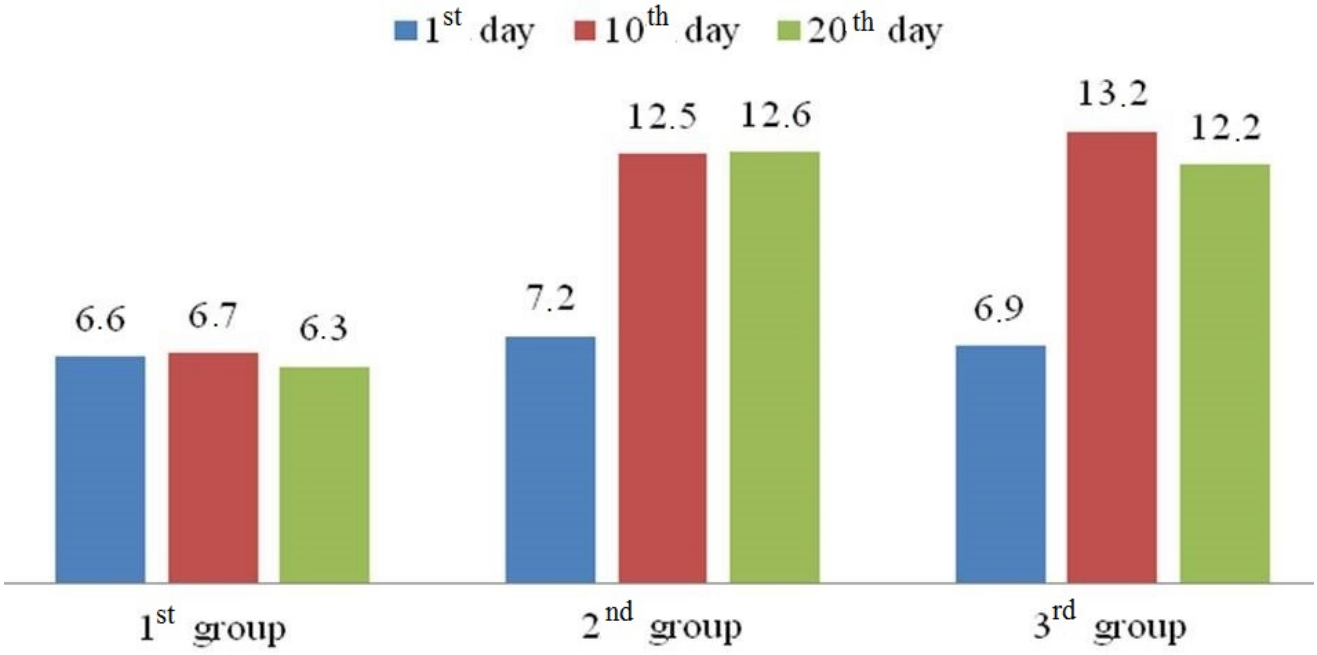

Figure 3 Urease dynamics concentration during the experiment, $mmol.L^{-1}$.

Total protein content in blood plasma in the control group slightly fluctuates. A decrease in the protein content in the blood plasma also indicates a downgrade of kidney function. In our studies, there was an increase in total protein by 19.7% in the 2nd group and by 17.9% in the 3rd group. Albumin increasing observed in animals only in second group 27.6%; in 3rd group, this increase is tendentious, which indicates the feasibility suppression of inflammatory processes in the gastrointestinal tract in rats. Figure 4 shows the total protein dynamics concentration in the blood plasma of rats during the experiment, and the albumin dynamic is in Figure 5.

The positive total protein dynamics concentration in the rats' blood plasma of both experimental groups was noted. This indicator varies in the range of 80.2 – 80.3 $g.L^{-1}$ on the 10th day of the experiment. Including in animals of the second group, this concentration occurs due to albumin, the content of which in the plasma increases during the experiment from 30.0 to 37.4 $g.L^{-1}$. In the animals of the 3rd group, the albumin content varies slightly during the experiment, ranging from 31.0 to 31.7 $g.L^{-1}$. In the control group rats, this indicator takes values from 30.0 $g.L^{-1}$ at the beginning of the experiment, with a tendency to decrease at the end of the experiment to 29.3 $g.L^{-1}$.

Glucose concentration is the most important nutrient base for the vast majority of nervous cells, especially brain tissue. Half of the energy consumed by the body is released from glucose. Hypoglycemia is often associated with a decrease in insulin blood level **(Salman et al., 2017)**. Thus, the observed decreased glucose concentration in rats of the control group is a consequence of the presence of possible inflammatory processes in the internal organs but is not critical because of the unreliable difference in indicators between the study groups (Figure 6).

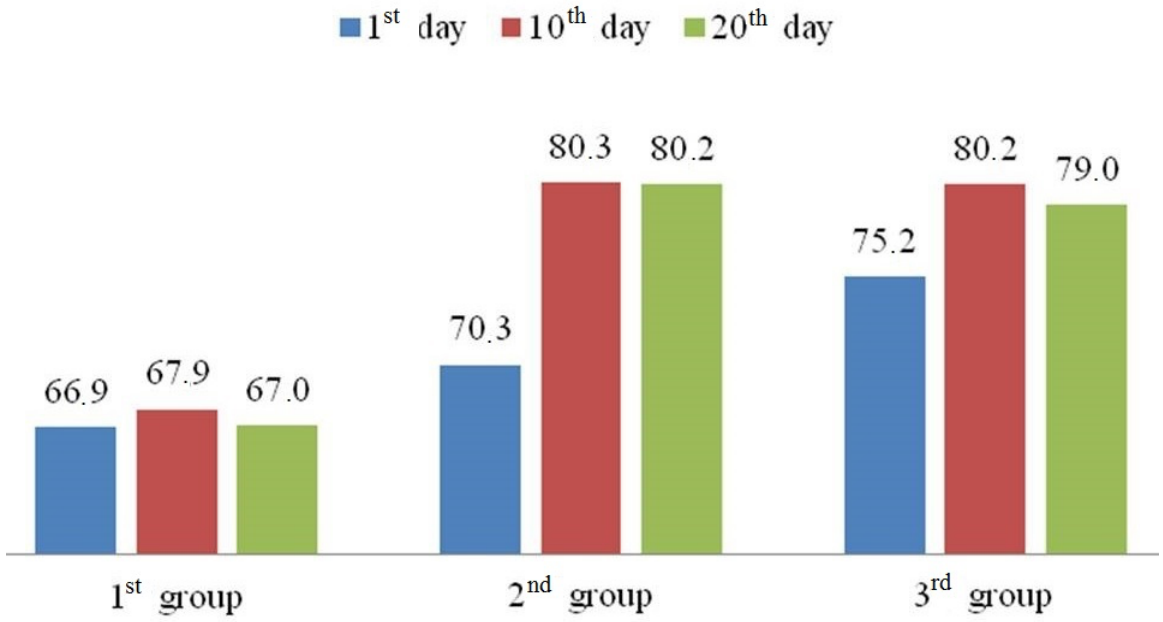

Figure 4 Total Protein dynamics concentration, g.L^{-1}.

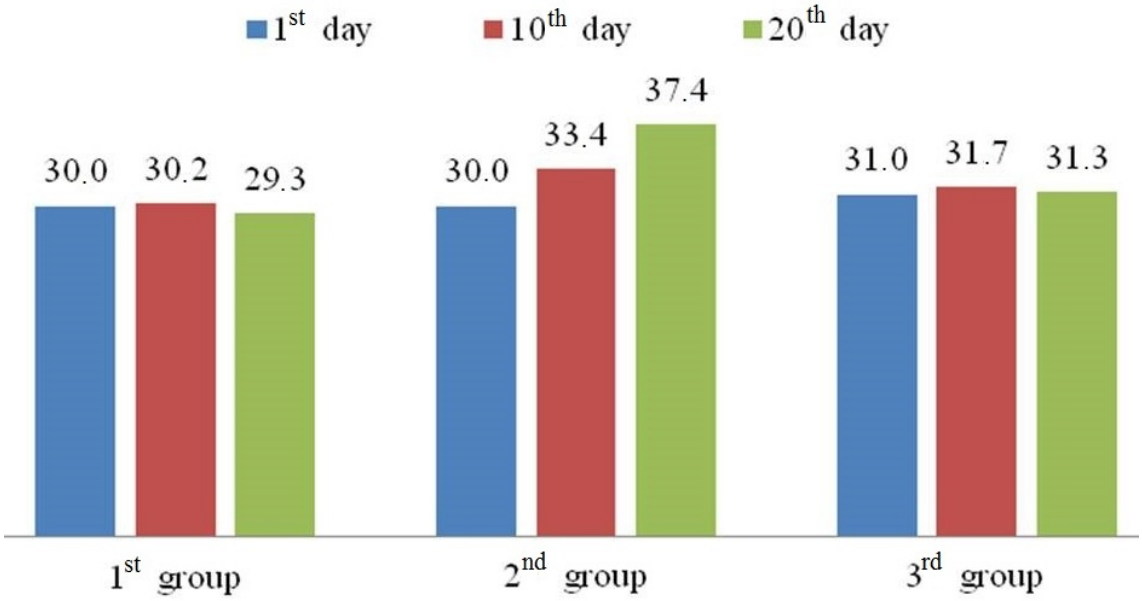

Figure 5 Albumin dynamics concentration, g.L^{-1}.

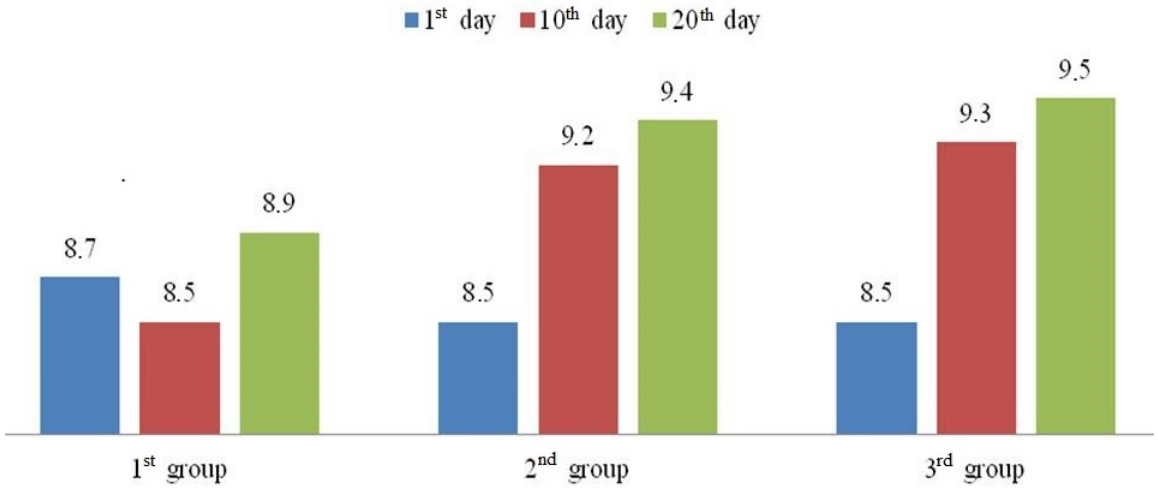

Figure 6 Glucose dynamics concentration, mmol.L^{-1}.

RBCs are an important component of the blood, which is involved in the saturation of tissues with oxygen, and WBCs are an indicator of the physiological norm, and their dynamics indicate the development of inflammatory and other pathological processes. An increase in blood cells was observed: RBCs by 0.44 cells × 10^{12} per liter in animals of the 2nd group, and the content of WBCs practically did not change in all the studied groups (Table 3).

However, the WBCs' composition in the experimental groups was mainly due to neutrophils responsible for the phagocytic activity, and monocytes in the second group were formed (Table 4).

Non-specific immune response provides by neutrophils and monocytes. Such a leukocyte formula contributes to an increased level of protection against pathogenic environmental influences and provides a high level of protection in lung diseases of a coldlike nature **(Luo et al., 2014)**. Regular consumption of small doses (about 0.3 mcg per day) of essential oil with food or drinking water increased the life expectancy of AKR high-cancer line mice by 30% and reduced the incidence of leukemia **(Oyemitan et al., 2015)**.

The systematic intake of small doses of oregano essential oil by healthy BALB/c line mice increased their average life expectancy by 120 days or 17%. At the same time, it was found that the intake of oil throughout life did not cause toxic effects, did not affect the body weight, the size of the immune-competent organs, and the blood formula **(Saiyudthong et al., 2015)**.

The gastric mucosa of rats has a covering epithelium, between the folds of which the mouths of simple tubular glands open. The mucosal layer is underlain by its own muscle plate of the mucosa, formed by smooth myocytes organized parallel to the longitudinal axis of the organ, as well as the connective tissue base.

The main function of the gastric epithelium is protective. Its thickness is normally about 150 μm in rats and begins to increase when exposed to pathogenic factors, both physical and chemical. It is worth noting that in all groups of animals that consumed oil, the thickness of the integumentary epithelium was slightly lower than the control values, while it did not bear any signs of atrophy (Figure 7).

The depth of the glands also became slightly smaller, which generally affected the overall thickness of the mucous layer (Table 5). These changes are most pronounced in the 2nd group. In general, the thickness of the mucous layer remained within the normal range for this age group of animals.

The number of epithelial cells in the integumentary epithelium, main and lining cells in the glands of the fundal part of the stomach in the control group also agree with the literature data **(Yuldashev et al., 2014)**.

At the same time, in the groups that received mountain savory essential oil, the number of cells decreased slightly, which is more pronounced in the group with ginger (Table 5). However, these differences are not reliable. The number of chief cells, on the contrary, increases, which may indicate a greater enzymatic activity and, consequently, an improvement in the functional characteristics of the stomach is approximately the same in all groups that received oil. Changes in the number of parietal cells are reversed and more pronounced in groups with a single dose of any of the oils.

Table 3 Rats' blood hematology (n = 3).

Biometrics indicator	1st group	2nd group	3rd group
	RBCs count (×10^6 Cells per mm^3)		
X±m_X	6.67 ±0.06	7.24 ±0.21	7.11 ±0.28
C_v, %	1.69	5.00	6.90
	WBCs count (× 10^9 Cells per mm^3)		
X±m_X	10.18 ±0.6	9.62 ±0.51	10.13 ±0.46
C_v, %	11.80	9.13	7.79

Table 4 Rats' blood leukocyte formula, %.

Rats' group	Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophils
1st	40.7 ±1.4	51.7 ±1.7	2.3 ±0.3	5.0 ±0.4	0.3 ±0.3
2nd	42.0 ±1.2	49.7 ±0.6	2.7 ±0.5	5.7 ±0.3	0.0 ±0.0
3rd	42.7 ±1.4	49.3 ±1.3	3.0 ±0.4	4.7 ±0.6	0.3 ±0.3

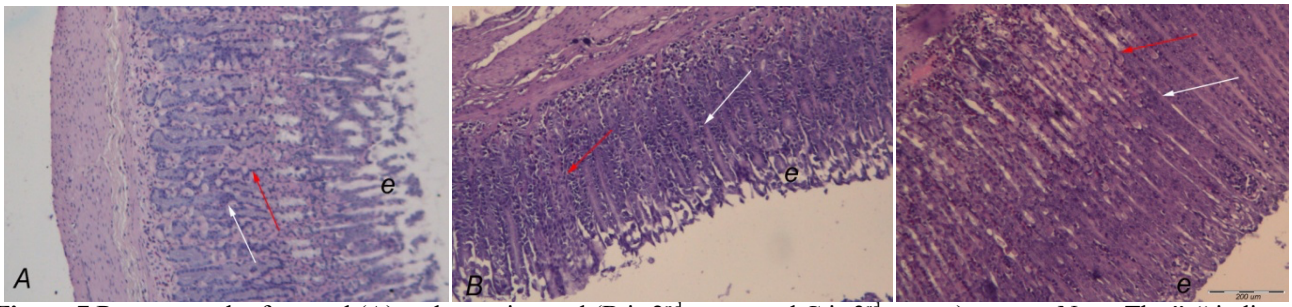

Figure 7 Rats stomach of control (A) and experimental (B is 2nd group and C is 3rd group) groups. Note: The "e" indicates the integumentary epithelium. Red arrows show the lining cells of the gastric glands, and white arrow is the main ones. Hematoxylin and Eosin stained. The lens is 40×.

Table 5 Rats' stomach morphometric characteristics, μm.

Rats' group	Epithelial height	Glands thickness	Stomach wall mucous layer thickness	Superficial epithelial cells number	Chief exocrine cells number	Parietal exocrine cells number
1st	152.64 ±5.54	479.70 ±10.92	632.34 ±12.03	21.2 ±7.6	34.2 ±8.014	51.4 ±10.14
2nd	139.89 ±3.18*	290.33 ±24.24*	380.23 ±25.96*	21.00 ±0.52	53.0 ±1.0	29.00 ±1.0*
3rd	124.50 ±3.66	470.78 ±11.36	595.28 ±13.92	20.00 ±0.84	54.4 ±5.54	36.3 ±6.64

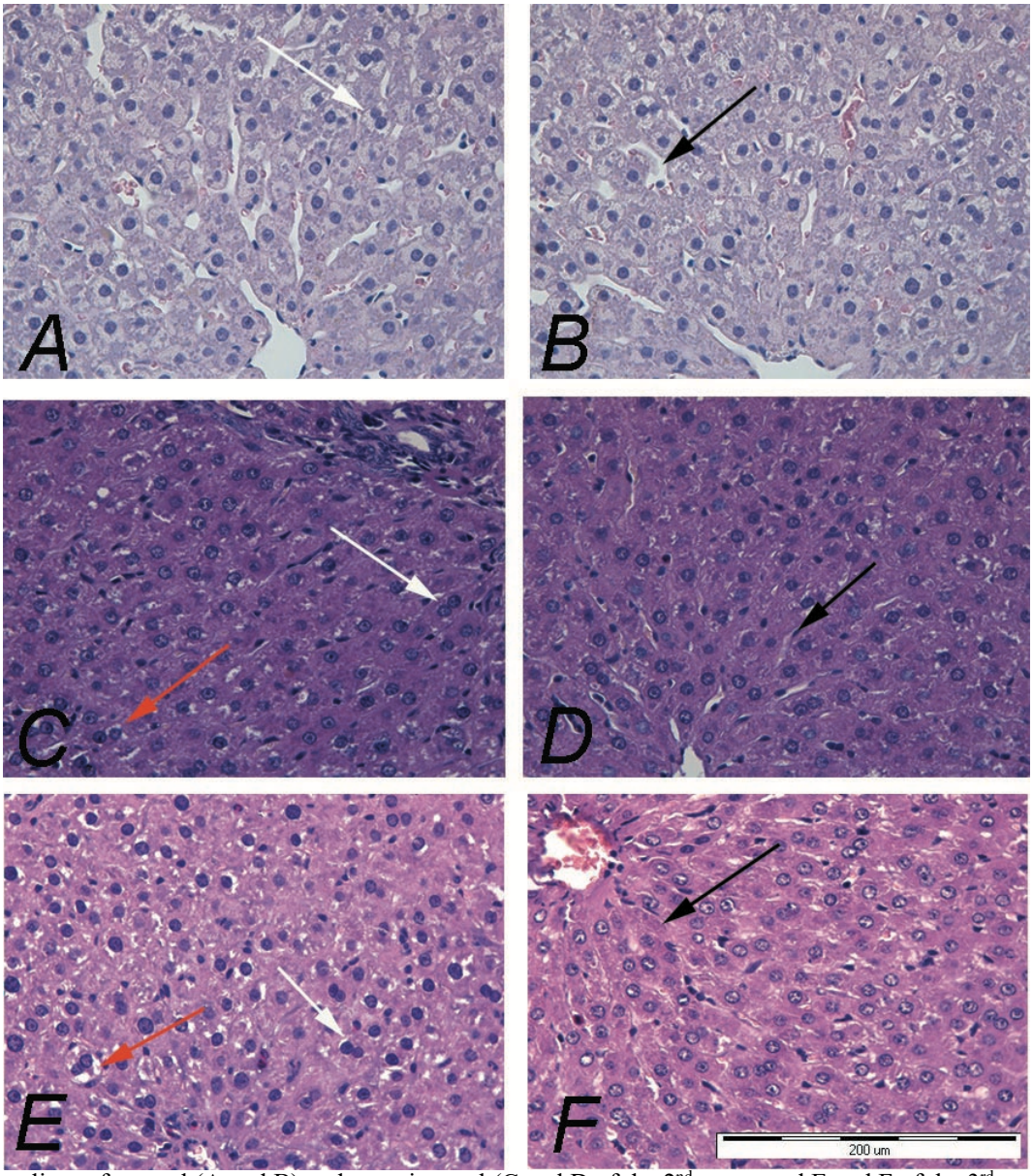

Figure 8 Rats liver of control (A and B) and experimental (C and D of the 2nd group and E and F of the 3rd group) groups. Note: In the left column, the peripheral part of the hepatic lobule, in the right – the central one. The black arrows indicate the sinuses of the hepatic lobule. The white arrows represent binucleated hepatocytes. Fatty cell dystrophy was marked by red arrows. Hematoxylin and Eosin stained. The lens is 40×.

Table 6 Rats' liver morphometric characteristics in the central (c) and peripheral (p) parts of the classical liver lobes, μm.

Rats' group		Binuclear cells number	Parenchyma percentage	Stroma percentage	Cell area	Core area	Nuclear and Cytoplasmic Ratio
1st	c	6.60 ±0.98	81.20 ±1.32	18.80 ±1.32	300.35 ±9.98	54.19 ±2.30	0.23 ±0.01
	p	7.80 ±0.97	79.80 ±2.48	20.20 ±2.48	338.15 ±11.04	54.35 ±2.54	0.17 ±0.01
2nd	c	8.20 ±2.31	87.60 ±1.16	12.40 ±1.16	360.47 ±13.82	55.72 ±2.91	0.19 ±0.01
	p	5.40 ±1.70	85.80 ±1.69	14.20 ±1.69	355.98 ±11.52	59.19 ±2.89	0.20 ±0.01
3rd	c	5.60 ±0.93	82.80 ±1.93	17.20 ±1.93	283.07 ±10.18**	49.57 ±2.74	0.22 ±0.01
	p	8.40 ±1.50	89.40 ±0.60	10.60 ±0.60	320.34 ±12.14	51.90 ±2.36	0.20 ±0.01

The liver of the rats of all groups retained a typical structure. The lobules of the liver consisted of beams of hepatocytes, between which sinusoids and blood capillaries were located. The use of oils as a top dressing led to a narrowing of the sinus-capillary network, they began to look less transparent on histopreparations, which may indicate the saturation of the sinuses with fats. Hepatocytes of the liver of rats of the experimental groups differed from the control group by the presence of fat inclusions in the cytoplasm in the form of rounded drops (Figure 2, Table 6).

There is some regularity in the morphometric indicators, depending on the site of the hepatic lobule. Thus, the number of binuclear cells in the 3rd experimental group is greater on the periphery of the lobule, which corresponds to a greater degree of damage to hepatocytes in this area, the first to meet the blood entering the lobule. The same region showed a greater narrowing of the capillary network compared to the central regions of the lobules.

CONCLUSIONS

The intensification of protein metabolism in the rats' blood plasma on the background of the use of mountain savory essential oil was noted. Total protein increases by 17.9 – 19.7%, and albumin by 27.6% in rats treated with essential oil at a dose of 0.6 mL per kg of feed. A significant increase in the level of aspartate aminotransferase in animals of the control group to 207.3 $U.L^{-1}$ was revealed, which to a certain extent indicates the hepatoprotective effect of mountain savory oil and a decrease in inflammatory processes in the organs of the gastrointestinal tract in the conditions of cell maintenance of rats of the experimental groups. Gastric epithelium thickness of experimental groups rats was lower than the control animals, while it did not bear any signs of atrophy. The difference of this indicator in comparison with control was 12.75 μm ($p \leq 0.05$) in the second group and it was reliable. The number of chief stomach cells increases in animals of the experimental groups, which may indicate a greater enzymatic activity. An increased dose of mountain savory oil contributes to the formation of more damage to hepatocytes on the periphery of the liver lobule.

Thus, the relationship between liver enzymes and the state of peripheral hepatocytes was noted.

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Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

Wistar line adult male rats in the experiment were used. The experimental work in accordance with the Declaration on the Humane Treatment of Animals was carried out. Animals of all groups from the experiment by decapitation under ether anesthesia were removed.

REFERENCES

Allaoua, M., Etiene, P., Noirot, V., Carayon, J.-L., Téné, N., Bonnafé, E., Trelhou, M. 2018. Pharmacokinetic and antimicrobial activity of a new carvacrol-based product against a human pathogen, *Campylobacter jejuni*. *Journal of Applied Microbiology,* vol. 125, no. 4, p. 1162-1174. https://doi.org/10.1111/jam.13915.

Bae, D., Seol, H., Yoon, H.-O., Na, J.-R., Oh, K., Choi, Ch. Y., Le, D.-W., Jun, W., Lee, K. W., Lee, J., Hwang, K., Lee, Y.-H., Kim, S. 2012. Inhaled essential oil from Chamaecyparis obtuse ameliorates the impairments of cognitive function induced by injection of beta-amyloid in rats. *Pharmaceutical Biology,* vol. 50, no. 7, p. 900-910. https://doi.org/10.3109/13880209.2011.642886

Bagci, E., Aydin, E., Mihasan, M., Maniu, C., Hritcu, L. 2016. Anxiolytic and antidepressant-like effects of *Ferulago angulata* essential oil in the scopolamine rat model of Alzheimer's disease. *Flavour And Fragrance Journal*, vol. 31, no. 1, p. 70-80. https://doi.org/10.1002/ffj.3289

Campélo, L. M. L., Gonçalves, F. C. M., Feitosa, Ch. M., de Freitas, R. M. 2011. Antioxidant activity of Citrus limon essential oil in mouse hippocampus. *Pharmaceutical Biology*, vol. 49, no. 7. p. 709-715. https://doi.org/10.3109/13880209.2010.541924.

Castro, M. R., Victoria, F. N., Oliveira, D. H., Jacob, R. G., Savegnango, L., Alves, D. 2015. Essential oil of *Psidium cattleianum* leaves: Antioxidant and antifungal activity. *Pharmaceutical Biology*, vol. 53, no. 2, p. 242-50. https://doi.org/10.3109/13880209.2014.914231

Chioca, L. R., Ferro, M. M., Baretta, I. P., Oliveira, S. M., Silva, C. R., Ferreira, J., Losso, E. M., Andreatini, R. 2013. Anxiolytic-like effect of lavender essential oil inhalation in mice: Participation of serotonergic but not GABA(A)/benzodiazepine neurotransmission. *Journal of Ethnopharmacology*, vol. 147, no. 2. p. 412-418. https://doi.org/10.1016/j.jep.2013.03.028

Choi, M.-S., Choi, B.-sub., Kim, S. H., Pak, S. Ch., Jang, Ch. H., Chin, Y.-W., Kim, Y.-M., Kim, D.-I., Jeon S., Koo, B.-S. 2015. Essential Oils from the Medicinal Herbs Upregulate Dopamine Transporter in Rat Pheochromocytoma Cells. *Journal Of Medicinal Food*, vol. 18, no. 10, p. 1112-20. https://doi.org/10.1089/jmf.2015.3475

Fabian, D., Juhás, Š., Bukovská, A., Bujňáková, D., Grešáková, Ľ., Koppel, J. 2011. Anti-inflammatory effects of chamomile essential oil in mice. *Slovak Journal of Animal Science*, vol. 44, no. 3, p. 111-116. Available at http://www.cvzv.sk/slju/11_3/Fabian-SJAS-3-2011.pdf.

Fauza, A., Al-Baarri, A. N., Djamiatun, K. 2019. Potency of Okra flour (Abelmoschus esculentus) in improving adiponectin level and total antioxidant capacity of high fat diet streptozotocin rat model. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 13, no. 1, p. 644-650. https://doi.org/10.5219/1136

Krishan, G., Narang, A. 2014. Use of essential oils in poultry nutrition: A new approach. *Journal of Advanced Veterinary and Animal Research,* vol. 1, no. 4, p. 156-162. https://doi.org/10.5455/javar.2014.a36

Gradinariu, V., Cioanca, O., Hritcu, L., Trifan, A., Gille, E., Hancianu, M. 2015. Comparative efficacy of *Ocimum sanctum L.* and *Ocimum basilicum L.* essential oils against amyloid beta (1-42)-induced anxiety and depression in laboratory rats. *Phytochemistry Reviews*, vol. 14, no. 4, p. 567-575. https://doi.org/10.1007/s11101-014-9389-6

Hritcu, L., Cioanca, O., Hancianu, M. 2012. Effects of lavender oil inhalation on improving scopolamine-induced spatial memory impairment in laboratory rats. *Phytomedicine*,

vol. 19, no. 6, p. 529-534. https://doi.org/10.1016/j.phymed.2012.02.002

Luo, B., Shi, H., Wang, L., Shi, Y., Wang, Ch., Yang, J., Wan, Y., Niu, J. 2014. Rat Lung Response to PM2.5 Exposure under Different Cold Stresses *International Journal Environmental Research Public Health*, vol. 11, p. 12915-12926. https://doi.org/10.3390/ijerph111212915

Mihajilov-Krstev, T., Jovanović, B., Jović, J., Ilić, B., Miladinović, D., Matejić, J., Rajković, J., Dorđević, L., Cvetković, V., Zlatković, B. 2014. Antimicrobial, antioxidative, and insect repellent effects of *Artemisia absinthium* essential oil. *Planta Med*, vol. 80, no. 18, p. 1698-705. https://doi.org/10.1055/s-0034-1383182

Monforte, M. T., Tzakou, O., Nostro, A., Zimbalatti, V., Galati, E. M. 2011. Composition and Biological Activities of *Calamintha officinalis* Moench Essential Oil. *Journal of Medicinal Food*, vol. 14, no. 3, p. 297-303. https://doi.org/10.1089/jmf.2009.0191

Nurmawati, L., Sulchan, M., Fatimah-Muis, S., Djagat Purnomo, H., Djamiatun, K., Ardiaria, M., Karlowee, V. 2021. The effect of single clove Black garlic on the hemostasis status and lipid profile in male Sprague Dawley rats with non-alcoholic fatty liver disease. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 15, no. 1, p. 387-395. https://doi.org/10.5219/1516

Orchardand, A., van Vuuren, S. 2017. Commercial Essential Oils as Potential Antimicrobials to Treat Skin Diseases. *Evidence-Based Complementary and Alternative Medicine*, vol. 2017, p. 92. https://doi.org/10.1155/2017/4517971

Ostapchuk, P., Kuevda, T. A., Yemelyanov, S. A., Kashirina, A. N., Yermolaeva, V. M., Zyablitskaya, Yu. E., Makalish, P. T., Ostapova, S. V. 2020. Influence of Satureja Montana essential oil on biological indicators formation of broiler chickens. *Proceedings of Nizhnevolzhskiy agrouniversity complex: science and higher vocational education*, vol. 2, no. 58, p. 251-265. https://doi.org/10.32786/2071-9485-2020-02-25 (In Russian).

Oyemitan, I. A., Olayera, O. A., Alabi, A., Abass, L. A., Elusiyan, C. A., Oyedeji, A. O., Akanmu, M. A. 2015. Psychoneuropharmacological activities and chemical composition of essential oil of fresh fruits of *Piper guineense* (Piperaceae) in mice. *Journal Of Ethnopharmacology* vol. 166, p. 240-249. https://doi.org/10.1016/j.jep.2015.03.004

Park, H.-J., Lim, E-J., Zhao, R. J., Oh, S. R., Jung, J. W., Ahn, E-M., Lee, E. S., Koo, J. S., Kim, H. Y., Chang, S., Shim, H. S., Kim, K. J., Gwak, Y. S., Yang. Ch. H. 2015. Effect of the fragrance inhalation of essential oil from Asarum heterotropoides on depression-like behaviors in mice. *BMC complementary and alternative medicine*, vol. 15, no. 1, p. 571-578. https://doi.org/10.1186/s12906-015-0571-1

Pashtetsky, V., Ostapchuk, P., Postnikova, O., Zubochenko, D., Kuevda, T. 2020. Effect of *Satureja montana* essential oil on the bactericidal activity of broiler chickens blood serum. *E3S Web of Conferences*, vol. 224, p. 04023. https://doi.org/10.1051/e3sconf/202022404023

Postnikova, O. N., Shevkoplyas, L. A., Kuevda, T. A. 2021. Influence of Satureja montana esential oil on the biofilms of opportunistic microorganisns isolated from patients with COVID-19. *Problems in medical mycology,* vol. 23, no. 2, p. 130. Available at https://mycology.szgmu.ru/images/files/2021/2_2021__Журнал_со_сборником_тезисов.pdf.

Rahimian, F., Eisvand, H. R. 2016. Effects of savory essential oil on germination parameters of Fusarium infected seeds of wheat (*Triticum aestivum* L.). *Iranian Journal of Plant Physiology*, vol. 6, no. 3, p. 1745-1750.

Saiyudthong, S., Pongmayteegul, S., Marsden, C. A., Phansuwan-Pujito, P. 2015. Anxiety-like behaviour and c-fos expression in rats that inhaled vetiver essential oil. *Natural Product Reserch*, vol. 29, no. 22, p. 2141-2144. https://doi.org/10.1080/14786419.2014.992342

Salman, M. T., Ajayi, P. O., Alagbonsi, A. I., Olatunji, L. A. 2017. Artesunate-Induced Hemolysis and Hypoglycemia in Rats: Gender Implication and Role of Antioxidant Enzymes. *Journal of Applied Hematology*, vol. 8, no. 1, p. 23-32. https://doi.org/10.4103/joah.joah_37_16

Takahashi, M., Yamanaka, A., Asanuma, Ch., Asano, H., Satou, T., Koike, K. 2014. Anxiolytic-like Effect of Inhalation of Essential Oil from Lavandula officinalis: Investigation of Changes in 5-HT Turnover and Involvement of Olfactory Stimulation. *Natural Product Communications*, vol. 9, no. 7, p. 1023-1026. https://doi.org/10.1177/1934578X1400900735

Vakili, A., Sharifat, S., Akhavan, M. M., Bandegi, A. R. 2014. Effect of lavender oil (*Lauandula angustifolia*) on cerebral edema and its possible mechanisms in an experimental model of stroke. *Brain Research*, vol. 1548, p. 56-62. https://doi.org/10.1016/j.brainres.2013.12.019

Widyastuti, N., Anjani, G., Almira, V. G., Putri, S. E., Pratiwi, A. R. 2020. Effects of the administration of brewed Robusta coffee leaves on total antioxidant status in rats with high-fat, high-fructose diet-induced metabolic syndrome. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 14, no. 1, p. 258-263. https://doi.org/10.5219/1350

Yi, L.-T., Li, J., Geng, D., Liu, B.-B., Fu, Y., Tu, J.-Q., Liu, Y., Weng, L.-J. 2013. Essential oil of Perilla frutescens-induced change in hippocampal expression of brain-derived neurotrophic factor in chronic unpredictable mild stress in mice. *Journal of Ethnopharmacology*, vol. 147, no. 1, p. 245-53. https://doi.org/10.1016/j.jep.2013.03.015

Yuldashev, A. Yu., Yuldasheva, S. Z., Rakhmatova, M. Kh., Nishanova A. A., Tarinova, M. V. 2014. Morphologic characteristics of fundal part of stomach's mucosa formation of rats at the postnatal ontogenesis. *World of Medicine and Biology*, vol. 4, no. 47-2, p. 214-219. Available at https://womab.com.ua/en/smb-2014-04-2/478.

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