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COMPREHENSIVE TOXIC-BIOLOGICAL ASSESSMENT OF CONIFEROUS EXTRACT

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Abstract

The presented work is devoted to a comprehensive assessment of the biological effect of the coniferous extract in dilutions with a concentration of 0.5 g / l and 1.0 g / l on the body of healthy white rats. The features of the physicochemical composition of the coniferous extract and the presence of a bactericidal effect concerning samples of the test culture of Escherichia coli (strain O55K59) were determined. When conducting studies on animals, the toxic impact of coniferous extract in both dilutions was not established. The work results demonstrated structural and functional changes in the internal organs of rats, changes in metabolic parameters, and the state of peripheral blood. Conclusion - both samples have biological activity, the degree of which depends on the concentration of the coniferous extract. Coniferous extract with a concentration of 0.5 g / l has a more gentle effect on the organism of the tested animals.

Keywords: coniferous extract, bactericidal action, toxicity, biological activity, structural and functional state of internal organs.

Introduction

Medicinal plants have long attracted the attention of researchers as carriers of biologically active substances (BAS). As you know, biologically active substances in plants are in optimal proportions, created in the course of evolution during the interaction of their organism with the environment. Herbal preparations contain a complex of biologically active substances and microelements, and therefore have a multifaceted healing effect on the body. In this case, biologically active substances in the body easily penetrate tissues and act at the level of intracellular metabolism [1].

Modern medicine uses, according to various sources, from 300 (in 2012 in European countries) and more than 2000 (China and the countries of the East in 2015) species of medicinal plants (more than 700 herbal remedies have been developed), veterinary medicine uses more than 150 species (of which 300 - 350 phytopreparations). In folk medicine, a much larger number of medicinal plants is used [2, 3, 4, 5]. Currently, the number of plant species in the world, which are used as medicinal, is estimated from 350,000 to 500,000 [6].

Recent years' data demonstrate that the use of natural plant extracts, namely extracts of green coniferous trees, both in the form of natural medicinal products and medicines, is cost-effective and readily available [3, 7].

The needles contain a lot of vitamin C (240 to 355), beta-carotene. One kg of needles contains 150-310 mg of carotene, 345-365 mg of vitamin E (one of the most potent antioxidants), and vitamin A [8]. The needles contain arginine, proline, phenylalanine, valine, threonine, tryptophan, and other essential amino acids [9, 10]. Also, pine extracts (PE) contain up to 1.3% of essential oils, which include up to 40% of penin, limonene, bornyl acetate, borneol, cadinene; in addition, tannins and bitter substances (up to 5%), flavonoids (acylated glycoses of diquercetin, catechin), coumarin, anthocyanins, polyphenols, trace elements (manganese, iron, copper, boron, zinc, molybdenum) and a large amount of ascorbic acid (up to 0,3%), vitamins K, E, carotene, etc. [11-16]. These data indicate that extracts from coniferous trees have biological activity and medicinal properties. This statement concerns mainly the internal use of PE. It should be mentioned that when applied externally (in balneotherapy), natural healing resources (which include not only various mineral waters, brines, peloids, clays, ozokerites, but also coniferous extracts) are also highly effective [17 - 24].

To date, there is much less data on assessing the effect of biologically active compounds and substances that make up PE on the body of experimental animals with external (transdermal) use, in contrast to internal use.

Based on the above, the study aimed to determine the bactericidal effect, to study the safety and the degree of biological activity of the coniferous extract when applied externally in white rats.

Methods

The 1st stage of the investigation of natural (undiluted) coniferous extract (CE) was determined by its physico-chemical characteristics and microbiological studies.

Physico-chemical studies of the CE were analyzed according to the following indicators: pH determination and the proportion of dry substances. During the determination of physico-chemical characteristics of the extract, we used methods regulated by the Passport of the Ukrainian State Standardization Center and the relevant scientific and technical documentation. [25, 26].

Determination of the bactericidal effect of the CE was performed against a test culture of Escherichia coli (strain $O_{55}K_{59}$). Evaluation of the bactericidal effect of the extract was carried out as follows: the samples were contaminated with Escherichia coli (strain $O_{55}K_{59}$) at concentrations of 10^1 - 10^3 in four parallel tests of each concentration series. [27]. Test-culture die-off was recorded on the 10th day (from the moment of contamination of the samples understudy). The number of positive samples in each concentration series was counted, and the bactericidal level was estimated using the formula:

$$B=\frac{\sum A_{1,2\ldots n}}{T},\quad A=\frac{n_x \cdot \lg CFU}{n_o}\cdot 100\ \%$$

Where:

B - bactericidal index, %;

$A_{1,2...n}$ - the score reflecting the number of samples with a positive reaction and the concentration index of the test culture, in one series of samples;

$\sum A_{1,2...n}$ - the total score reflecting the number of samples with a positive reaction in all series;

n_x - number of samples with positive reaction;

n_o - initial number of samples in the series;

t - the period of registration of the test culture die-off, days;

lg CFU - index of the level of concentration of the contaminating dose of the test culture.

When the bactericidal index (B) acquires a value of 2.5 to 10.0%, the sample is evaluated as weakly, from 15 to 30% - moderately, from 37.5 to 60% - significantly bactericidal.

The second stage of the work was to perform experimental studies to determine the optimal concentration of CE for its practical use. Since it is impossible to use CE in its natural form, two dilutions were calculated: 100 g per 200 L (concentration 0.5 g/l) and 200 g per 200 L (concentration 1.0 g/l) of drinking water.

Experimental studies were performed on 30 white clinically healthy male Wistar rats with body weights from 180.0 to 210.0 g. Experimental studies were conducted following the rules established by the Directive of the European Parliament and the Council (2010/63 / EU), by order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 of March 1, 2012 «On Approval of the Procedure for conducting scientific experiments, experiments on animals by scientific institutions» and methodical recommendations [28, 29, 30]. During the experiment, the animals were in the experimental biological clinic (vivarium) of the State Institution «Ukrainian Research Institute of Medical Rehabilitation and Resort Therapy of the Ministry of Health of Ukraine», Odesa in the conditions of free access to food and water. The animals were kept in standard laboratory conditions: photoperiod - light / darkness 12:12; air temperature - 22 ± 2 ° C; humidity - 55 ± 10%.

The way of penetration of CE constituents into the body is skin-resorptive. For this purpose, the rats were located in a particular device, in separate cells. The animals' tails were immersed in test tubes with the investigated substance by 2/3 of their length, which is 5 - 6% of the body surface. The temperature of the test substance was maintained at 38-40 °C. Daily exposure lasted for 2 hours, and the course was six treatments at one-day intervals.

The animals were divided into three groups of 10 animals in each. Group 1 consisted of clinically healthy (intact) rats used as a control group. Group 2 consisted of rats treated with CE in the form of an aqueous solution with a concentration of 0.5 g/l (100 g CE per 200 l of water). Group 3 consisted of rats, treated with CXE procedures with a concentration of 1.0 g/l (200 g XU per 200 l of water).

The influence of CE on the animals' organism was assessed using the study of the functional state of the kidneys. There were determined indices of urine formation processes: glomerular filtration rate (GFR), percentage of tubular reabsorption; a volume of daily diuresis; renal excretion function was estimated by daily creatinine, and urea excretion, and ion-regulating function - by daily excretion of K, Na, and chloride ions. The pH reaction of daily urine was determined. Animals were placed in separate cameras to collect daily urine at the end of the course of CE therapy.

At the end of the course, the animals were withdrawn from the experiment by decapitation under ether anesthesia. Two pieces of internal organs (stomach, liver, heart, and kidneys) of volume 1 sm3 were taken during an autopsy. The first piece was fixed for 24 hours in 4% paraformaldehyde solution, passed through alcohols of increasing concentration and poured into celloidin. Histological sections of 7-9 μm thickness were made from the obtained blocks stained with hematoxylin-eosin. The obtained preparations were investigated using a light microscope to determine kidney changes. Microscopic studies of structural changes in the stomach, heart, liver, and kidneys were performed on the obtained preparations. The second slice was frozen with dry carbon dioxide (-70 °C), histochemical reactions were performed on the prepared cryostat sections to determine succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) activity according to Lojda Z [31]. The activity

of enzymes was assessed in conventional units of optical density (units). SDH activity in tissues of the studied organs in intact animals of the 1st group was - (7.00 ± 0.29) conventional units, the activity of LDH was - (6.00 ± 0.32) conventional units.

Hematological studies studied the body's response to the effects of procedures with CE, which was evaluated by changes in the general indices of peripheral blood (the number of leukocytes, the ratio of blood formula elements) and immunological reactivity - the number of total T-lymphocytes, the amount of circulating immune complexes (CIC) (natural antibodies hemolysins and heterophile agglutinins). The presence of heterophilic antibodies is an indicator of the level of immunological maturity of the body and the normal functioning of the immune system [32].

Biochemical methods were used to determine the activity of re-aminating enzymes - alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in blood serum and the content of total protein and its fractions (albumin, α1, α2-, β-, γ-globulin). Content of total bilirubin and its fractions, urea and creatinine, and seromucoid level were determined. To assess the condition of lipid peroxidation/antioxidant system (LPO/ADS), we determined malondialdehyde (MDA) content and catalase activity.

The used methods are given in the manual and approved by order of the Ministry of Health of Ukraine [33].

All data were processed using the statistical package Statistica 10.0 (Statsoft/Dell, Tulsa, OK, USA). The descriptive statistics of the data in tables include mean ± standard error of the mean (SEM) or mean ± standard deviation. Significance was assessed by using the one-way ANOVA followed by a t-test. Values were considered statistically significant when the P-value was less than 0.05.

Results

Physicochemical studies of natural CE in the form of water extract from coniferous greens showed its compliance with technical requirements "Natural Coniferous Extract, Technical Specifications of Ukraine [34]. Table 1 shows the main physical and chemical characteristics of CE.

Microbiological studies established the presence of the bactericidal effect of the natural CE. The results of these studies are presented in Table 2

During in vitro studies with a test-culture of Escherichia coli (strain $O_{55}K_{59}$), the infecting dose was 10^1, 10^2, 10^3 CC/ml, the presence of bactericidal action of natural CE, defined as significantly bactericidal (bactericidal index B is 60 %), was established.

The study results of the effect of external course application of ECH on the functional state of the kidneys of intact rats are given in Table 3.

In the 2nd group rats, there was a slight but reliable increase of glomerular filtration rate (GFR) - by 18%. Still, stimulation of tubular reabsorption in the form of a significant rise of fluid reabsorption in renal tubules resulted in a 28% decrease of daily diuresis volume. In 3rd group rats, a peculiarity of the identified changes in the process of urine formation is the preservation of GFR at the level of the control group 1 and more minor increase in the percentage of tubular reabsorption, which also results in a 27% decrease in the volume of daily diuresis. A tendency to a reduce in urea excretion was found in 2nd group rats and a reliable decrease in 3rd group rats by 22%, which in both cases was caused by an increase in tubular reabsorption. In both groups, slight oxidation of daily urine was detected, as evidenced by the decrease in the concentration of hydrogen ions in the 1st group by 9% and 12% in the 3rd group.

The study of the ion-regulating function of the kidneys found almost the same decrease in the excretion of Na ions - in the 2nd group by 27%, and in the 3rd group - by 21%. The excretion of Na ions in the 2nd group increases by 33%, in the 3rd group - by 122%. The excretion of chloride ions in the second group remains at the level of the first control group, while in the third group, their excretion increases by 57%.

Consequently, the application of CE in both dilutions has a single-directional but different strength, affecting the functional condition of the kidneys of the test rats in groups 2 and 3.

Table 4 shows the data of the metabolic indices of the rats that received a course of procedures with two dilutions of CE. In the rats of groups 2 and

3, there was a reliable decrease in the content of total bilirubin due to a reduction in its fractions - direct and indirect blood bilirubin. At the same time, a more significant decrease in the content of total bilirubin and its fractions was observed in group 2 rats. Also, in both groups, we found almost the same reliable decrease in the activity of ALT and AST enzymes and a reliable decrease in the value of the Ritis index, i.e., the creatinine content in the blood of rats of both groups were not changed, as evidenced by the absence of reliable changes in comparison with the control group. But the content of urea in 3rd group rats increased by 25%. In 2nd group rats, it did not have any difference from the control value, which may be associated with a reliable decrease in urea excretion with daily urine. In the 2nd group rats, the total protein content in the blood did not change. Still, changes in the ratio of its fractions were observed: the content of α-1 globulin decreased reliably by 53%, and the content of α-2 globulin increased reliably by 43%. 3rd group rats showed a slight but reliable increase in total protein content - by 10% and a more significant increase in β-globulin content - by 24% and γ-globulin content - by 59%.

3rd group rats retained the balance in the LPO/ANS system (MDA content and catalase activity are at the level of the indicators of 1st group of intact animals. And in the 2nd group rats, there are signs of changes in the balance in the LPO/ANS system. The content of MDA tends to increase, and the catalase activity tends to decrease. In the study of seromucoids, neither in the 2nd group nor in the 3rd group reliable changes compared to the 1st group were determined, indicating the absence of signs of inflammation or development of pathological processes.

Data about the effect of CE in two dilutions on the state of peripheral blood and individual indicators of the immune system are presented in Table 5.

The peripheral blood reaction in the 2nd group of rats was characterized by redistribution of the form elements - neutrophils reliably increased by 67%, and lymphocytes decreased by 12%. Group 2 rats demonstrated some more remarkable changes - neutrophils reliably increased by 94%, and lymphocytes decreased by 16%. At the same time, the number of acidophils increased by 33% in the 2nd group; in the 3rd group, it increased by 60%.

The reaction of the immune system parameters in the 2nd group of rats was determined by the tendency to decrease the number of total T-lymphocytes (an indicator of the activity of the cellular component of the immune response). The CIC content did not differ from the relevant indicator of the control group, and the content of heterophilic antibodies exceeded the control data by 73%. In the 3rd group of rats, the total T-lymphocytes didn't differ from the control group data; the CIC content slightly and significantly increased - by 9% (which is a negative fact and indicates the signs of the immune system humoral link stress). At the same time, the content of heterophilic antibodies increased significantly - by 113%. It should be noted that the increase in the percentage of acidophils (the human analog of eosinophils) in both groups indicates the presence of signs of sensitization of the body and a significant increase in the content of heterophilic antibodies indicates activation of nonspecific immune defense. Thus, CE in the dilution of 1.0 g/l (3rd group of rats) causes a more significant response from the peripheral blood and immune system indices.

External application of CE in the 2nd group rats caused the following changes in the target organs. Stomach - submucosa is dense, fibers are short, closely packed. Glands are ordinary tubular in shape. In the outlet channels, the goblet cells are increased in size; they have much mucus. The activity of SDH is (7.0 ± 0.23) conventional units, LDH activity is (7.0 ± 0.13) conventional units.

The liver - the lobular structure was not changed. Hepatocytes were collected in beams with the cytoplasm of homogeneous basophilic color. Hepatocyte nuclei are small, dark. The interstitial spaces are enlarged. Activity of SDH - (7.0 ± 0.16) conventional units, activity of LDH - (6.0 ± 0.27) conventional units.

Heart - layer-by-layer and bundle organization of myocardium and appearance of cardiomyocytes are without obvious changes. SDH activity - (7.0 ± 0.29) conventional units, LDH activity - (7.0 ± 0.14) conventional units.

Kidneys - structural organization of nephron and its components are without obvious changes. Only vacuoles in the glomerular endothelium were

observed. Activity of SDH - (7,0 ± 0,41) conventional units, activity of LDH - (7,0 ± 0,33) conventional units.

Consequently, external application of the coniferous extract at a dilution of 0.1 g/dm3 (group 2 of rats) causes an increase in gastric secretion and the activity of anaerobic glycolysis enzymes.

External application of coniferous extract in a dilution of 200 g per 200 L caused the following changes in target organs of healthy animals. Stomach - submucosal plate was without obvious changes. Glands of usual tubular shape, the cytoplasm of epitheliocytes was edematous, goblet cells of discharge ducts were enlarged. Activity of SDH - (7.0 ± 0.31) conventional units, activity of LDH - (6.0 ± 0.19) conventional units.

The liver - the lobular structure was saved, the blood vessels were moderately full. The interlobular spaces were slotted. Hepatocytes are densely packed in beams; the cytoplasm is homogeneous, nuclei are of medium size. Activity of SDH - (7.0 ± 0.21) conventional units, activity of LDH - (5.0 ± 0.10) conventional units.

Heart - stratified and bundled organization of myocardium without obvious changes. Cardiomyocytes of usual appearance. Activity of SDH - (7.0 ± 0.14) conventional units, activity of LDH - (6.0 ± 0.27) conventional units.

Kidneys - structural organization of nephron and its components without obvious changes. The epithelium of the tubules was edematous. Activity of SDH - (7,0 ± 0,23) conventional units, activity of LDH - (6,0 ± 0,43) conventional units.

Thus, the application of coniferous extract in a dilution of 0.2 g/l (group 3 of rats) established edema of epitheliocytes of glands of gastric mucosa and epitheliocytes of kidney tubules.

Conclusions

1. The studies confirmed the conformity of organoleptic and physicochemical parameters of natural pine extract to the requirements of technical specifications of Ukraine - TU U 20.4-00991545-001:2015.

2. The presence of the bactericidal effect of the natural coniferous extract was determined as significantly bactericidal.

3. The established fluctuations of metabolic parameters in clinically healthy animals under the influence of both solutions of the coniferous extract did not get beyond the physiological norm; toxic phenomena in the study of the structural and functional state of the organs and systems of the body were not determined. Both solutions of the coniferous extract have a one-directional effect on the structural and functional state of the studied organs and systems of the body of experimental animals. This manifests itself in influence on water-electrolyte metabolism (intensification of tubular reabsorption and increased excretion of sodium ions and chloride ions), impact the processes of re-amination in the liver, and stimulating effect on bile excretion processes; activation of peripheral blood state and cellular and humoral component of an immune response.

When exposed to a coniferous extract with a higher concentration, signs of tension in some body systems were established in rats of the 3rd group:

- reduction of urea excretion with daily urine with an increase of its concentration in the blood;
- significant increase in the excretion of sodium ions and chloride ions
- significant changes in the indicators characterizing the state of the peripheral blood and the immune system;
- hyperhydration of tissues of the gastrointestinal tract organs.

Prospects for further research. Based on the data obtained, it is planned to conduct further studies of the coniferous extract (depending on its concentration) under conditions of reproducing various pathological models in rats.

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The authors declare that there are no conflicts of interest.

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Table 1. Physical and chemical properties of coniferous extract

Charasteristics name	In fact	Criteria by TS of Ukraine 20.4-00991545-001:2015
Appearance	A homogeneous thick liquid	A homogeneous thick liquid. A small amount of sludge is allowed.
Color	Braun-red	From braun to braun-red
Smell	Specific, coniferous	Specific, coniferous
Density, g/sm^3 with temperature 20 ^{0}C	1,24	from 1,22 to 1,25
Water ion concentration index, units pH	4,15	From 4,0 to 7,0
Mass fraction of solids, %	52,0	From 50,0 to 54,0

Table 2. Calculation of the bactericidal index of coniferous extract

Investigated samples	Concentration of test culture, colony forming units (CFU)/cm^3	Score considering the weighting coefficient		Bactericidal index on the 10th day, B, %
		Amount	Score considering the weighting coefficient	
Natural coniferous extract	10^1	+ + + +	100	60
	10^2	+ + + +	200	
	10^3	+ + + +	300	

Table 3. Effects of coniferous extract solutions during external application on the functional condition of the rat kidneys

Indicators	1st control group	2nd group	%	3rd group	%
	($M_1 \pm m_1$)	($M_2 \pm m_2$)		($M_3 \pm m_3$)	
Daily diuresis, ml/dm^2 body surface	1,18 ± 0,13	0,73 ± 0,002*	62	0,75 ± 0,008**	63
Glomerular filtration rate, ml/(dm^2·min)	0,11 ± 0,008	0,13 ± 0,001*	118	0,10 ± 0,001	91
Channel reabsorption, percent to filtration, %	99,26 ± 0,06	99,59± 0,003*	0,33	99,47± 0,002**	0,21
Creatinine excretion, mmol	0,011±0,0001	0,012 ± 0,0001	109	0,010 ± 0,0001	91
Urea excretion, mmol	0,64 ± 0,07	0,57 ± 0,11	89	0,50 ± 0,007*	78
pH daily urine, units. pH	7,67 ± 0,28	6,98 ± 0,02*	91	6,76 ± 0,02**	88
Daily excretion of K ions, mmol	0,19 ± 0,02	0,14 ± 0,002	73	0,15 ± 0,002**	79
Daily excretion of Na ions, mmol	0,09 ± 0,01	0,12 ± 0,002*	133	0,20 ± 0,004**	222
Daily excretion of Cl ions, mmol	0,28 ± 0,06	0,27 ± 0,001	96	0,44 ± 0,004**	157

Notes: ($M_1 \pm m_1$), ($M_2 \pm m_2$), and ($M_3 \pm m_3$) are arithmetic mean with error rates;
* - reliable changes ($p<0.05$) were calculated in comparison between ($M_1 \pm m_1$) and ($M_2 \pm m_2$);
** - reliable changes ($p<0,05$) were calculated in comparison between ($M_1 \pm m_1$) and ($M_3 \pm m_3$); Data of the 1st control group of animals were taken as 100%.

Table 4. Impact of coniferous extract solutions on metabolic parameters of rats

Indicators	1 control group	2 group	%	3 group	%
	($M_1 \pm m_1$)	($M_2 \pm m_2$)		($M_3 \pm m_3$)	
Bilirubin, mkmol/l					
In general	8,44 ± 0,28	4,39 ± 0,34*	52	5,09 ± 0,37**	60
direct	3,06 ± 0,18	1,84 ± 0,09*	60	2,17 ± 0,24**	71
indirect	5,38 ± 0,15	2,55 ± 0,28*	47	2,93 ± 0,21**	54
ALT, U/L	113,31 ± 2,13	79,52 ± 3,58*	70	73,82 ± 3,09**	65
AcT, U/L	289,64 ± 12,12	154,23± 7,56*	53	154,24 ± 9,22**	53
Ritis index	2,56 ± 0,11	1,95 ± 0,11*	76	2,09 ± 0,10**	81
Creatinin, mkmol/l	47,80 ± 0,63	51,82 ±1,26	108	44,86 ± 1,65	89
urea, mmol/l	2,80 ± 0,20	2,87 ± 0,35	102	3,52 ± 0,14**	125
POL (MDA), nmol/(min·mg)	5,94 ± 0,21	6,81 ± 0,25	114	5,77 ± 0,33	97
AOS (Catalase), %	76,7 ± 1,52	72,12 ± 1,64	94	77,37 ± 0,36	101
General protein, g/l	68,70 ± 2,74	68,40 ± 2,50	99	75,60 ± 2,46**	110
Albumin, g/l	25,80 ± 1,88	25,19 ± 0,98	97	27,05 ± 1,93	105
α-1 Globulin, g/l	8,28 ± 1,86	3,87 ± 0,84*	47	6,93 ± 1,58	83
α-2 Globulin, g/l	10,70 ± 2,20	15,38 ± 0,93*	143	9,19 ± 3,41	86
β- Globulin, g/l	11,80 ± 1,01	10,31 ± 1,59	87	14,62 ± 0,42**	124
γ- Globulin, g/l	11,10 ± 0,73	13,15 ± 1,46	119	17,72 ± 1,53**	159
Seromucoids	0,204 ± 0,009	0,206 ± 0,010	102	0,221 ± 0,011	105

Notes: ($M_1 \pm m_1$), ($M_2 \pm m_2$), and ($M_3 \pm m_3$) are arithmetic mean with error rates;
* - reliable changes ($p<0.05$) were calculated in comparison between ($M_1 \pm m_1$) and ($M_2 \pm m_2$);
** - reliable changes ($p<0,05$) were calculated in comparison between ($M_1 \pm m_1$) and ($M_3 \pm m_3$); Data of the 1st control group of animals were taken as 100%.

Table 5. Effects of coniferous extract solutions on the peripheral blood and immune system when applied externally

Indicators	1 control group	2 group	%	3 group	%
	($M_1 \pm m_1$)	($M_2 \pm m_2$)		($M_3 \pm m_3$)	
Leukocytes, 10^9/l	5,50 ± 0,20	6,10 ± 0,40	111	5,40 ± 0,50	98
Neutrophils, %	12,79 ± 0,64	21,40 ±0,93*	167	24,80 ±0,86**	194
Acidophiles, %	2,25 ± 0,23	3,00 ± 0,14*	133	3,60 ± 0,24**	160
Monocytes, %	3,72 ± 0,21	3,60 ± 0,24	97	3,20 ± 0,20	86
Lymphocytes, %	81,20 ± 0,8	72,00 ±0,63*	88	68,40 ±0,75**	84
Generak T-Lymphocytes, %	47,20 ± 0,60	43,00 ±1,50	91	46,20 ± 0,90	98
Heterophilic antibodies, ум. од.	6,00 ± 0,80	10,40 ±2,40*	173	12,80 ±1,96**	213
CIC, mg/ml	5,70 ± 0,20	5,92 ± 0,18	104	6,25 ± 0,15**	109

Notes: ($M_1 \pm m_1$), ($M_2 \pm m_2$), and ($M_3 \pm m_3$) are arithmetic mean with error rates;
* - reliable changes ($p<0.05$) were calculated in comparison between ($M_1 \pm m_1$) and ($M_2 \pm m_2$);
** - reliable changes ($p<0,05$) were calculated in comparison between ($M_1 \pm m_1$) and ($M_3 \pm m_3$); Data of the 1st control group of animals were taken as 100%.