

## RESEARCH ARTICLE

# Gender Difference Response of Male and Female Immunodeficiency Rats Treated with Tissue-specific Biomolecules

Liliya V. Fedulova<sup>1</sup>, Alexandr A. Basov<sup>2,3</sup>, Ekaterina R. Vasilevskaya<sup>1</sup> and Stepan S. Dzhimak<sup>1,3\*</sup>

<sup>1</sup>Experimental Clinic - Laboratory of Biologically Active Substances of Animal Origin, V.M. Gorbatov's Federal Research Center for Food Systems of Russian Academy of Sciences, Moscow, Russian Federation; <sup>2</sup>Department of fundamental and clinical biochemistry, Kuban State Medical University, Krasnodar, Russian Federation; <sup>3</sup>Department of Radiophysics and Nanotechnology, Kuban State University, Krasnodar, Russian Federation

**Abstract: Background:** The modern immunology is targeted to the detailed study of various immunopathological conditions at the molecular and cellular level, development of new methods for the prevention, diagnostics and treatment of contagious and non-contagious diseases of humans and animals.

**Methods:** In the present work we took the rats with model of cyclophosphamide-induced immunodeficiency and studied the features of gender impact of the complex extract of immunocompetent organs (thymus, spleen and mesenteric lymph nodes) *Sus scrofa* and its separate fraction with molecular weight less than 30 kDa administered to male and female rats.

**Results and Conclusion:** The impact of gender differences and tissue-specific biomolecules (30 kDa fraction) on hematological parameters (leukocytes, erythrocytes, platelets), functional activity of immune system (IL-2, IL-4, IL-6, complement system, IgG, IgM), biochemical parameters of hepatocytes functioning (activity of ALP and LDG), carbohydrate metabolism (glucose) and lipid metabolism (triglycerides).

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## 1. INTRODUCTION

The most effective way to reduce morbidity, to improve the organism's resistance and to raise the quality of life is to strengthen the general nonspecific immunobiological status [1-3]. At the present time, while genomic and proteomic technologies are developed, the new opportunities have appeared to conduct systemic studies of various immunopathological states at molecular and cellular level in order to develop new methods for its diagnosis and therapy [4, 5]. The newest approach in the sphere of human medicine and veterinary medicine is the development of biological substances that can directly stimulate generation and accumulation of biomolecules to enhance the functional activity of immunocompetent cells [6-8]. There are some certain stimulators of nonspecific immunity of natural origin, including adaptogens, presented either by the preparations of plant origin [9, 10], or substances isolated from animal organs [11].

The above-mentioned agents act by controlling the signaling processes on the molecular-biological (protein-peptide) level, biochemical level and physical & chemical levels (equalization of the chemical potential, decrease of total entropy in processes). These approaches create a whole system that in case of violation of immune homeostasis provides in addition to rapid reaction of innate immunity the reaction that leads to intensification of subsequent polarization of the adaptive immune response with creation of effective immunity in response to the antigen effect [12].

The endogenous immunomodulators (*Endogenous immunoregulatory agents*) are represented mainly by peptides isolated from specialized organs, epithelial tissues, and blood of mainly productive animals, in particular pigs and cattle. Immunomodulators based on immune organs are most widely distributed; the preparations isolated from spleen, thymus and lymph nodes are of particular interest.

Thymus preparations are the native hormonal polypeptides with molecular weight of up to 5 kDa. Its action is targeted to normalize and enhance the functional activity of T-system of immunity and hemopoiesis, enhancement of cellu-

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\*Address correspondence to this author at the Department of Radiophysics and Nanotechnology, Kuban State University, Krasnodar, Russia; Tel +79054083612; E-mail: [jimack@mail.ru](mailto:jimack@mail.ru)

lar immune responses and antibody response, coagulation and anti-coagulation system, neuroendocrine regulation and reparative regeneration of tissues [13, 14]. Immunoregulators of a spleen – are thymus-dependent, thymus-independent and macrophage immune factors: nonspecific serum tetrapeptide-tuftsins found in the Fd-fragment of IgG, which activates the functional properties of macrophages and polymorphonuclear leukocytes; complement components – C3- and C4-fragments (for example, C3b); factor P [15]. These preparations significantly increase the content of T-lymphocytes in peripheral circulation and its functional activity, stimulate STF production, normalize free-radical peroxide oxidation of lipids, stimulate reparative and trophic processes. Also, immunomodulating preparations of animal origin are represented by preparations based on peptide fractions isolated from embryonic tissue; the preparations like these stimulate activity of T-killers and NK cells, activate regeneration processes of blood, including preparations of immunoglobulins (histaglobulins, pentaglobins, *etc.*) and antimicrobial peptides [16].

Also, the recent developments have demonstrated that reduction of deuterium concentration in organism fluids promotes activation of adaptogenic processes at the molecular level [17-21]. As supposed mechanisms of action of low concentrations of deuterium in drinking diet for an organism, the potentiation of nonspecific defense systems is assumed as well as implementation of “isotopic shock” that leads to the activation of energy reserves accumulation in mitochondria [22, 23].

The modern researches are mainly directed to the development of innovative stimulants of innate immunity on the basis of substances of animal origin, including biomolecules isolated from immunocompetent organs of farm animals by solubilization of deuterium depleted solution [24, 25].

The purpose of this research was to study the effect of tissue-specific biomolecules isolated from immunocompetent organs of *Sus scrofa* on the functional activity of immune system of rats with cyclophosphamide-induced immunodeficiency, taking into account the gender-determined specific effects of the obtained protein-peptide complexes.

## 2. MATERIALS AND METHOD

### 2.1. Method of Isolation of Tissue-specific Biomolecules from the Immunocompetent Organs of *Sus Scrofa*

The objects of research were a complex extract of immunocompetent organs (thymus, spleen and mesenteric lymph nodes) of *Sus scrofa* (complex extract) and its fraction with molecular weight less than 30 kDa (fraction less than 30 kDa).

The extracts were obtained using 0.9% sodium chloride based on deuterium depleted water (DDW) as the extractant solution, under an extractant-tissue ratio of 1:5, at a temperature of  $6\pm 2^{\circ}\text{C}$ , time of extraction was 4 hours; the insoluble precipitate was separated by centrifugation at 3500 rpm during 8 minutes on CM-6M centrifuge (ELMI, Latvia). The fraction with molecular weight of less than 30 kD was obtained by the method of step-by-step ultrafiltration in VivaFlow200 device (Vlidisart, Russia) using polysulfone filter modules (Sartorius, Germany) under pressure of 2.5 bar.

### 2.2. Method of DDW Production

DDW was produced by the electrolysis, the residual deuterium content was  $\delta^2\text{H} = -743 \text{ ‰}$ , the electrical conductivity of obtained water was  $2.2 \mu\text{s}$ . For the production of DDW we used the method of electrolysis of distillate in electrolyser, dehumidification of obtained electrolysis gases with its conversion into water and subsequent condensation of water vapors. The water for pouring into in the electrolyser was prepared by system of reverse osmosis and column of mixed-action (cationite-anionite) connected in subsequent cascade chain. Then the distillate was electrolysed by nickel electrodes, the mixture of hydrogen and oxygen obtained at the electrolyser outlet was processed through a system of hydraulic locks and a refrigerator, and gases were converted into water by incineration until high-temperature vapor was obtained which was later condensed in the refrigerator-condenser [26].

### 2.3. Method of Modeling of Rats' Cyclophosphamide-induced Immunodeficiency

To study the development and regulation of rats' immune responses, the cyclophosphamide-induced immunodeficiency (CIID) was modeled. The study was conducted on mature, sexually naive Wistar rats (male and female,  $n = 80$ ) of SPF category.

The animals were delivered from the Scientific and Production Enterprise “Nursery of laboratory animals” of the Branch of Institute of Bioorganic Chemistry of the Russian Academy of Sciences (Moscow region). Before the research the rats underwent adaptation for 5 days during which they were kept in a system of individually ventilated cages (EHRET, Germany) in following microclimate: temperature  $22\pm 3^{\circ}\text{C}$ , humidity  $55\pm 5\%$ , lighting period 6.00 a.m. – 6.00 p.m. The rats received full concentrated premix feed (Russia) and water *ad libitum*.

The experimental animals were divided into 8 groups (each group consisted of 10 rats):

- Group 1 - intact males;
- Group 2 - males exposed to modeling of CIID;
- Group 3 - males, which received complex extract (1,5 ml/kg) for 14 days, then they were exposed to the modeling of CIID;
- Group 4 - males which received fraction 30 kDa (2,0 ml/kg) for 14 days, then they were exposed to modeling of CIID;
- Group 5 - intact females;
- Group 6 - females exposed to modeling of CIID;
- Group 7 - females which received complex extract (1,5 ml/kg) for 14 days, then they were exposed to modeling of CIID;
- Group 8 – females which received a fraction of 30 kDa (2,0 ml/kg) for 14 days, then they were exposed to modeling of CIID;

The animals in groups 3, 4, 7, 8 got intragastrically injected tested samples through animal feeding needles 16x2”W/3 (Popper, USA) in amount of 2 g. of protein per

100 g. of the animal body mass for 14 days. Rats in groups 2, 6 received intragastrically distilled water, the volume was 2.0 ml per 100 g of the animal body mass.

Animals in groups 2, 3, 4, 6, 7, and 8 were exposed to modeling of CIID by triple intraperitoneal injection of "Cyclophosphamide" (Sigma, USA) on the 15<sup>th</sup>, 18<sup>th</sup> and the 21<sup>st</sup> day of the experiment at dose of 7.5 mg. per 100 g. of the animal body mass [11].

On the 25<sup>th</sup> day of experiment the animals were euthanized in an euthanasia chamber (VET tech) in accordance with the Directive 2010/63/EU of the European Parliament and the European Union Council for protection of animals used for scientific purposes. The animals' blood was taken from their right ventricle of the heart.

#### 2.4. Methods for Studying the Functional Activity of the Immune System in Rats

Quantitative analysis of formed blood elements was conducted by automatic veterinary hematological analyzer Abacusjuniorvet 2.7 (Diatron Messtechnik GmbH, Austria): leukocytes (WBC), lymphocytes (LYM), erythrocytes (RBC), hemoglobin (HGB), hematocrit (HCT), platelets (PLT), mean platelet volume (MPV), platelet distribution width (PDW). Cytometric analysis and immunophenotyping were performed on an automatic flow cytofluorimeter GuavaeasyCyte (Merch Millipore, France) using species-specific monoclonal antibodies CD8+, CD4+. The study of cytokine profile, including determination of pro-inflammatory (IL-2, IL6) and anti-inflammatory (IL-4) interleukins, and study of the status of blood serum complement components (C1q, C3, C4, C5) was conducted on immunoenzymometric "sandwich" method on the analyzer Immunochem 2100 (HTI, USA) using sets of species-specific reagents ELISA (rat).

#### 2.5. Methods of Study of Biochemical Indicators

The biochemical parameters of blood serum were analyzed on an automatic biochemical analyzer FC-360 (HTI, USA), using sets of reagents (HTI, USA) in accordance with the attached instructions for biochemical analysis.

#### 2.6. Statistic Methods

Statistical processing of the data was carried out by methods of variation statistics, and evaluation of reliability of the differences in average values (M) between groups was performed using non-parametric U-test (Mann-Whitney test, significant difference was considered for  $P < 0.05$ ). Software "STATISTICA 10.0" was used.

The results were presented as average values (M) and arithmetic mean error ( $\pm m$ ).

### 3. RESULTS

#### 3.1. Hematology Analysis

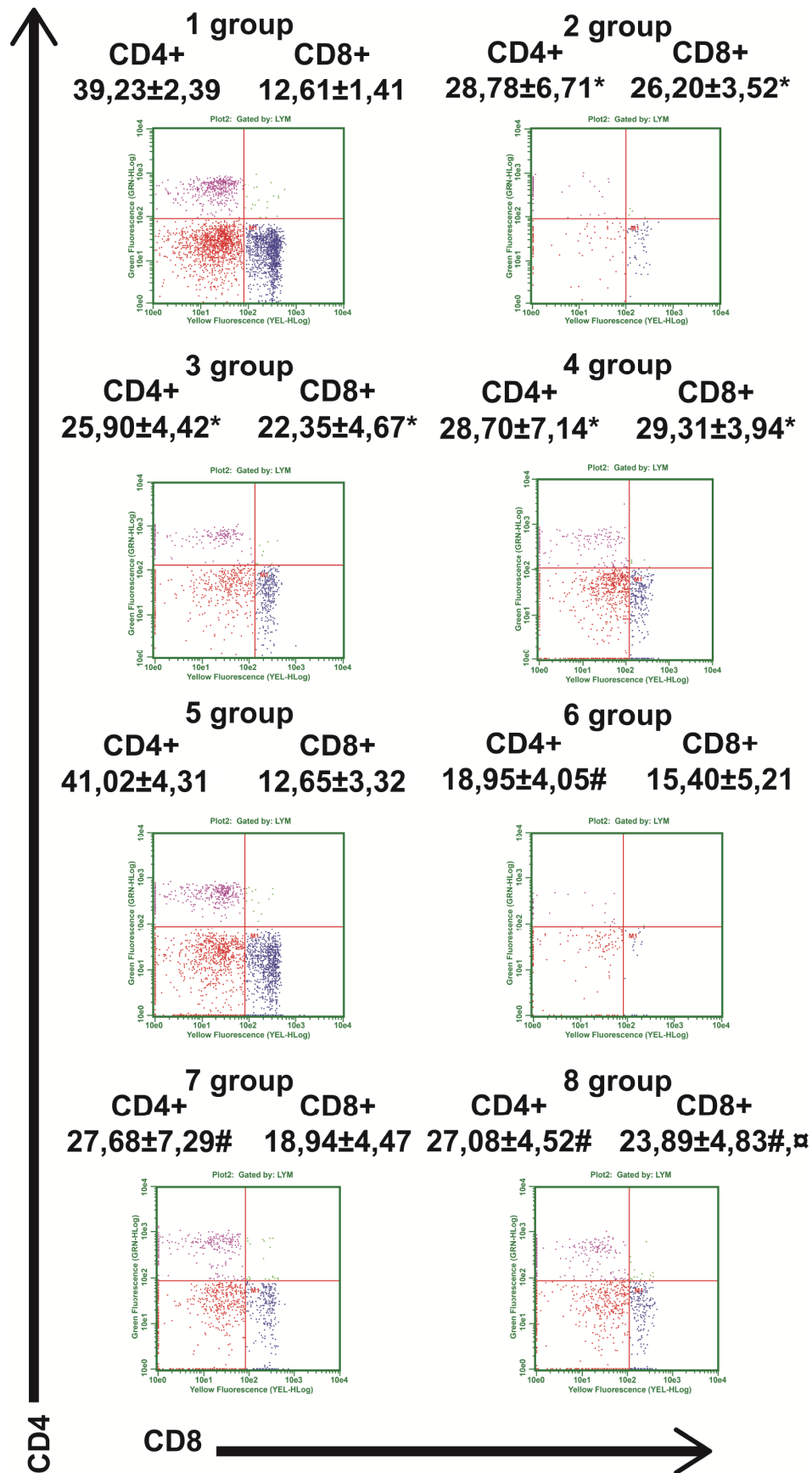
In result of the experiment, the animals of group 2 showed decrease in leukocytes number (by 98.1%), lymphocytes (by 99.7%, refer to Table 1) and pathological change in relative content of monocytes (decrease by 22.6%), granulocytes (increase by 98.5%, Table 2), clusters of CD4+ differentiation (decrease by 26.6%) and CD8+ (increase by 107.9%, Fig. 1) in comparison with animals of group 1; while the animals in group 6 in comparison to group 5 showed decrease in leukocytes number (by 96.4%), lymphocytes (by 99.8%, Table 1) and pathological change in relative content of monocytes (decrease by 25.9%), granulocytes (increase by 80.6%, Table 2), clusters of CD4+ differentiation (decrease by 53.8%, Fig. 1). All those facts in general confirm the development of immunodeficiency among rats in groups 2 and 6, while the severity of immune disorders was observed more in males than in females.

The change in the other formed elements of animals' blood in groups 2 and 6 was less noticeable (Table 1). Reduction of hematocrit among rats of the 2<sup>nd</sup> group in comparison with the 1<sup>st</sup> group was equal to 20.5%; the number of erythrocytes and platelets decreased by 16.4% and 97.6%, hemoglobin content in blood decreased by 20.7%. Reduction of hematocrit among the rats of group 6 in comparison with group 5 was 21.2%, the number of erythrocytes and platelets decreased by 12.6% and 99.4%, hemoglobin content in the blood decreased by 20.4%.

Table 1. Results of rat blood hematological analysis.

Parameters	Groups of Experimental Animals, M $\pm$ m							
	1	2	3	4	5	6	7	8
WBC, 10 <sup>9</sup> /l	7.30 $\pm$ 0.36	0.14 $\pm$ 0.01*	0.59 $\pm$ 0.05*, ^	0.65 $\pm$ 0.05*. ^	4.39 $\pm$ 0.13	0.16 $\pm$ 0.02#	1.32 $\pm$ 0.14#. □	0.58 $\pm$ 0.03#. □
LYM, 10 <sup>9</sup> /l	5.74 $\pm$ 0.29	0.02 $\pm$ 0.01*	0.36 $\pm$ 0.04*, ^	0.44 $\pm$ 0.05*. ^	3.95 $\pm$ 0.13	0.01 $\pm$ 0.01#	0.81 $\pm$ 0.12#. □	0.39 $\pm$ 0.04#. □
RBC, 10 <sup>12</sup> /l	8.16 $\pm$ 0.05	6.82 $\pm$ 0.21*	7.40 $\pm$ 0.07 ^	7.74 $\pm$ 0.06 ^	8.04 $\pm$ 0.05	7.03 $\pm$ 0.13#	7.44 $\pm$ 0.08#	7.21 $\pm$ 0.02#
HGB, g/l	149.08 $\pm$ 0.94	118.17 $\pm$ 3.36*	123.01 $\pm$ 1.15*	138.92 $\pm$ 1.14 ^	146.17 $\pm$ 0.53	116.34 $\pm$ 2.09#	128.83 $\pm$ 1.31#	126.58 $\pm$ 0.27#
HCT, %	42.46 $\pm$ 0.23	33.81 $\pm$ 0.95*	35.76 $\pm$ 0.31*	41.09 $\pm$ 0.33 ^	42.03 $\pm$ 0.15	33.12 $\pm$ 0.61#	37.93 $\pm$ 0.39#	37.21 $\pm$ 0.09#
PLT, 10 <sup>9</sup> /l	714.67 $\pm$ 6.81	17.01 $\pm$ 3.18*	105.75 $\pm$ 9.04*, ^	308.42 $\pm$ 5.27*, ^	832.74 $\pm$ 6.62	5.01 $\pm$ 0.43#	335.58 $\pm$ 29.18#. □	227.92 $\pm$ 3.5#. □
MPV, $\mu$ m <sup>3</sup>	6.55 $\pm$ 0.08	3.29 $\pm$ 0.34*	6.38 $\pm$ 0.21 ^	5.91 $\pm$ 0.32 ^	6.73 $\pm$ 0.13	4.20 $\pm$ 0.36#	7.09 $\pm$ 1.04□	5.95 $\pm$ 0.82□
PDW, %	32.50 $\pm$ 0.14	16.08 $\pm$ 1.69*	31.69 $\pm$ 1.03 ^	30.71 $\pm$ 2.08 ^	32.75 $\pm$ 0.09	22.26 $\pm$ 1.65#	34.68 $\pm$ 1.21□	31.06 $\pm$ 0.97□

\* -  $p < 0.05$  in comparison with group 1, # -  $p < 0.05$  in comparison with group 5, ^ -  $p < 0.05$  in comparison with group 2, □ -  $p < 0.05$  in comparison with group 6.



**Fig. (1).** Results of CD4+ and CD8+ analysis.

\* -  $p < 0.05$  in comparison with group 1, # -  $p < 0.05$  in comparison with group 5, □ -  $p < 0.05$  in comparison with group 6.

The animals in group 3, in comparison with the animals of group 2, showed less expressed development of disorders in immune system parameters: the increase in leukocytes number by 4.2 times, increase in lymphocytes number by 18.0 times (refer to Table 1). Also group 3 has higher relative content of granulocytes (by 110.0%) in comparison with animals of group 2 without significant changes in relative monocytes content (Table 2), CD4+ and CD8+ (refer to Fig. 1). Even more significant positive changes of hematological parameters were noted among animals in group 4 in comparison with animals of group 2. Those rats showed increase in number of leukocytes 4.6 times, lymphocytes 22.0 times (Table 1), lower relative content of granulocytes (by 82.9%), increase in relative content of monocytes (by 118.0%, Table 2) without changes in clusters of differentiation of CD4+ and CD8+, Fig. 1).

Among the groups 3 and 4 in comparison with group 2 we found respectively an increase in number of red blood cells (8.5% and 13.4%) and platelets (6.2 times and 18.1 times). It should be noted that in comparison with group 2, the level of the following hematological parameters in animals of group 3 and 4 also increased: mean platelet volume by 93.9% and 79.6%, platelet distribution width by 97.1% and 91.0% respectively. But increase in hemoglobin (by 17.6%) and hematocrit (by 21.5%) was detected only among the group 4 in comparison to group 2 (Table 1).

In groups 7 and 8 the female rats showed, in comparison with the animals of group 6, the less expressed development of violations in immune system parameters, respectively: increase of leukocytes number by 8.3 times and 3.6 times, lymphocytes number in 81.0 times and 39.0 times (refer to Table 1). Also, a lower relative lymphocyte count (14.7%) was noted in group 7 in comparison with the animals group 6 simultaneously with higher relative content of monocytes (by 175.8%) without significant changes in relative content of granulocytes (Table 2), CD4+ and CD8+ (Fig. 1). At the same time the animals of groups 8 in comparison with group 6 showed lower relative content of granulocytes (by 82.6%), increase in relative content of monocytes (by 140.9%, Table 2) and CD8+ (by 55.1%) without significant changes in relative

content of lymphocytes, clusters of CD4+ differentiation (Fig. 1).

### 3.2. Immunology Analysis

In male rats of group 2 in comparison with animals of group 1, the significant decrease of IL-2 (by 84.9%), IgG (by 54.3%), IgM (by 83.5%), C1q (by 25, 8%), C3 (by 14.1%), C4 (by 45.8%) and C5 (by 89.9%) was observed, while content of IL-4 increased by 46.9%, IL-6 by 80.0%. The male rats of group 3, as in comparison group 2, showed increase ( $p < 0.05$ , Table 3) in content of IL-2 (by 2.2 times) with decrease in C3 content (by 14.6%). Among the rats of group 4, in comparison with group 2, more significant increase was found than in animals of group 3 for the following: IL-2 content 3.0 times, IgM 2.9 times, C1q (1.14 times), C5 (2.6 times), whereas group 4 in comparison with group 2 showed decrease in content of IL-4 (by 35.7%), IL-6 (by 68.7%), C3 (by 15.2%).

Among female rats of group 6, compared with group 5, there was a significant decrease in IL-2 (by 86.1%), IgG (by 58.0%), IgM (by 73.9%), C5 (81.2%), along with increase in the content of IL-6 (by 87.0%). At the same time rats of group 7 in comparison with group 6 showed increase in IL-2 content by 2.9 times, C5 by 3.1 times with simultaneous decrease in IL-6 content by 42.0%, C3 by 20.2%. Changes of the same direction for the most part of immunological parameters were observed in female rats in group 8 in reference to group 6, including increase in content of IL-2 by 2.5 times and C5 by 4.2 times with decrease of IL-6 concentration by 54.6% (refer to Table 3). While the parameters of group 7 differed from group 8 (in comparison with group 6) by decrease in group 8 of IL-4 content by 49.9%.

### 3.3. Biochemistry Analysis

During analysis of the obtained results it was found that concentration of albumin was reduced by 6.6% in male rats group 2 (in comparison group 1, Fig. 2A) and by 10.1% in female rats group 6 (in comparison group 5, Fig. 3A) without significant changes in groups 3, 4, 7, 8. No significant changes

**Table 2. Results of rat blood cytometric analysis.**

Groups of Animals	Relative Content, %		
	Lymphocytes	Granulocytes	Monocytes
1 group	84.59±3.88	1.41±0.34	14.02±1.98
2 group	86.34±7.91	2.80±0.29*	10.86±0.73*
3 group	81.70±3.50	5.91±1.22*, ^	12.39±1.84
4 group	75.79±5.60	0.48±0.19*, ^	23.73±5.70^
5 group	89.00±2.45	1.23±0.41	9.77±0.63
6 group	90.52±3.09	2.24±0.79#	7.24±0.52#
7 group	77.19±3.72#, □	2.85±0.63#	19.97±4.86#, □
8 group	82.17±7.83	0.39±0.17#, □	17.44±3.80#, □

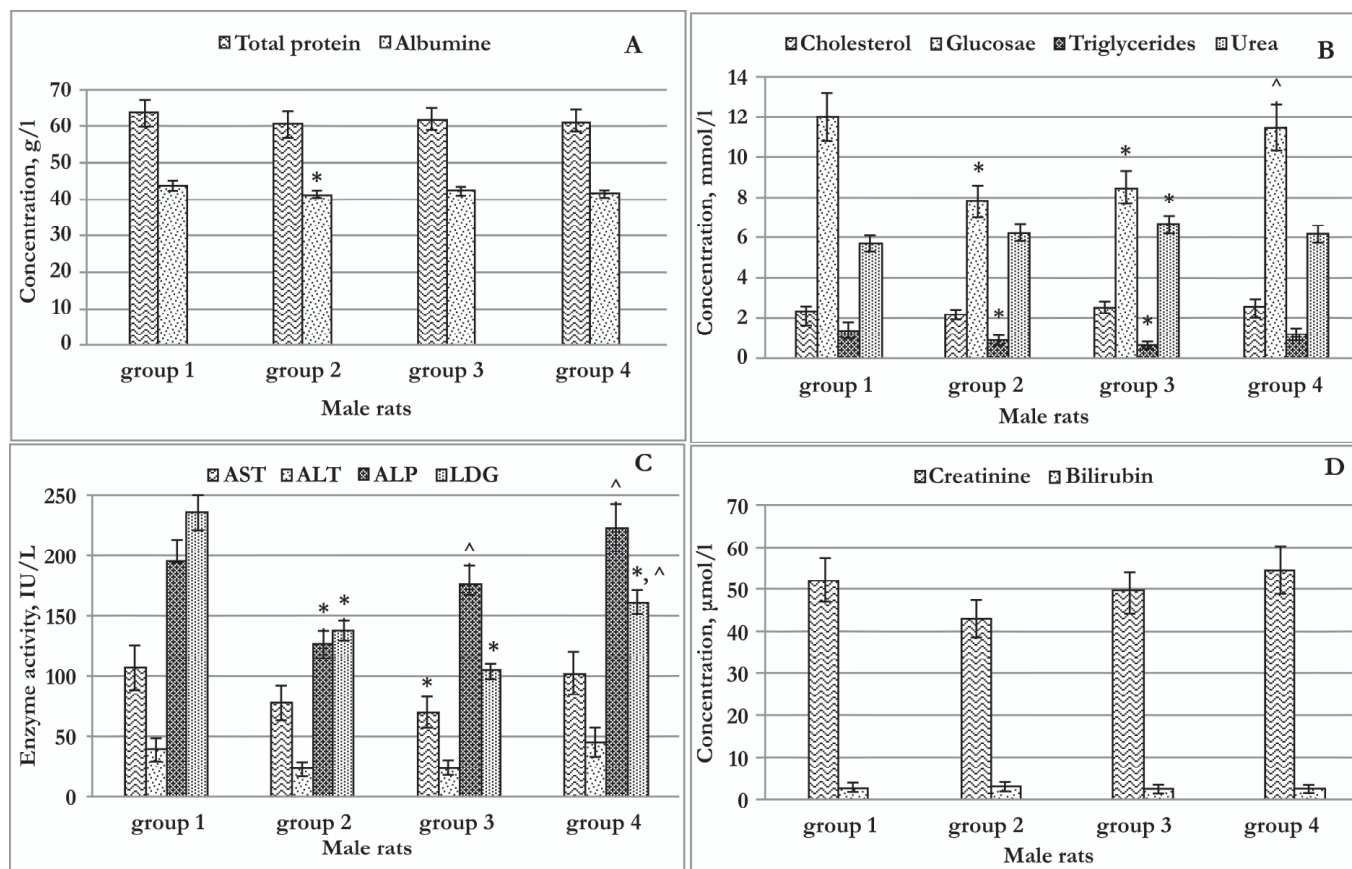
\* -  $p < 0.05$  in comparison with group 1, # -  $p < 0.05$  in comparison with group 5, ^ -  $p < 0.05$  in comparison with group 2, □ -  $p < 0.05$  in comparison with group 6

The female rats in groups 7 and 8, in comparison with group 6, showed increase in platelets number 66.9 times and 45.4 times respectively. At the same time animals of groups 7 and 8, in comparison with group 6, both showed increase in the mean platelet volume (by 68.7% and 41.7%) and the platelet distribution width (by 55.8% and 39.5%).

**Table 3.** Results of rat blood immunoenzymometric analysis.

Parameters	Groups of Experimental Animals, M±m							
	1	2	3	4	5	6	7	8
IL-2, pg/ml	324,60±44,51	48.73±7.45*	104.80±9.56*,^	146.79±27.90*,^	262.13±20.35	36.49±1.82#	107.52±29.84#□	91.75±14.73#□
IL-4, pg/ml	32,97±11,56	48.46±14.78*	44.71±8.12*	31.16±10.64^	37.12±8.15	34.45±9.86	26.71±6.43	17.24±3.14#□
IL-6, pg/ml	15,21±1,24	27.38±1.93*	16.95±8.17	8.57±2.51*,^	10.48±2.42	19.60±1.7#	11.36±2.32□	8.89±2.35□
IgG, ng/ml	3,83±0,68	1.75±0.32*	1.77±0.39*	1.48±0.46*	5.26±1.47	2.21±0.35#	2.64±0.72#	1.58±0.41#
IgM, ng/ml	0,85±0,04	0.14±0.03*	0.23±0.15*	0.41±0.22*,^	0.73±0.18	0.19±0.02 #	0.18±0.03#	0.21±0.09#
C1q, ng/ml	7,36±0,51	5.46±0.82*	6.32±1.01	6.54±1.07^	7.75±1.38	5.89±1.32	6.74±0.85	7.50±1.24
C3, ng/ml	50,64±1,32	43.49±1.81*	37.14±1.65*,^	36.89±1.97*,^	45.01±2.07	40.73±2.78	32.51±2.59#□	41.45±2.79
C4, ng/ml	405,07±29,46	219.42±48.61*	244.29±32.97*	268.60±42.61*	262.43±50.57	198.90±48.06	221.02±35.79	203.30±39.85
C5, ng/ml	52,98±10,94	5.30±1.47*	5.78±1.26*	13.86±1.74*,^	42.64±2.52	8.01±0.19#	24.65±1.53#□	33.28±1.21#□

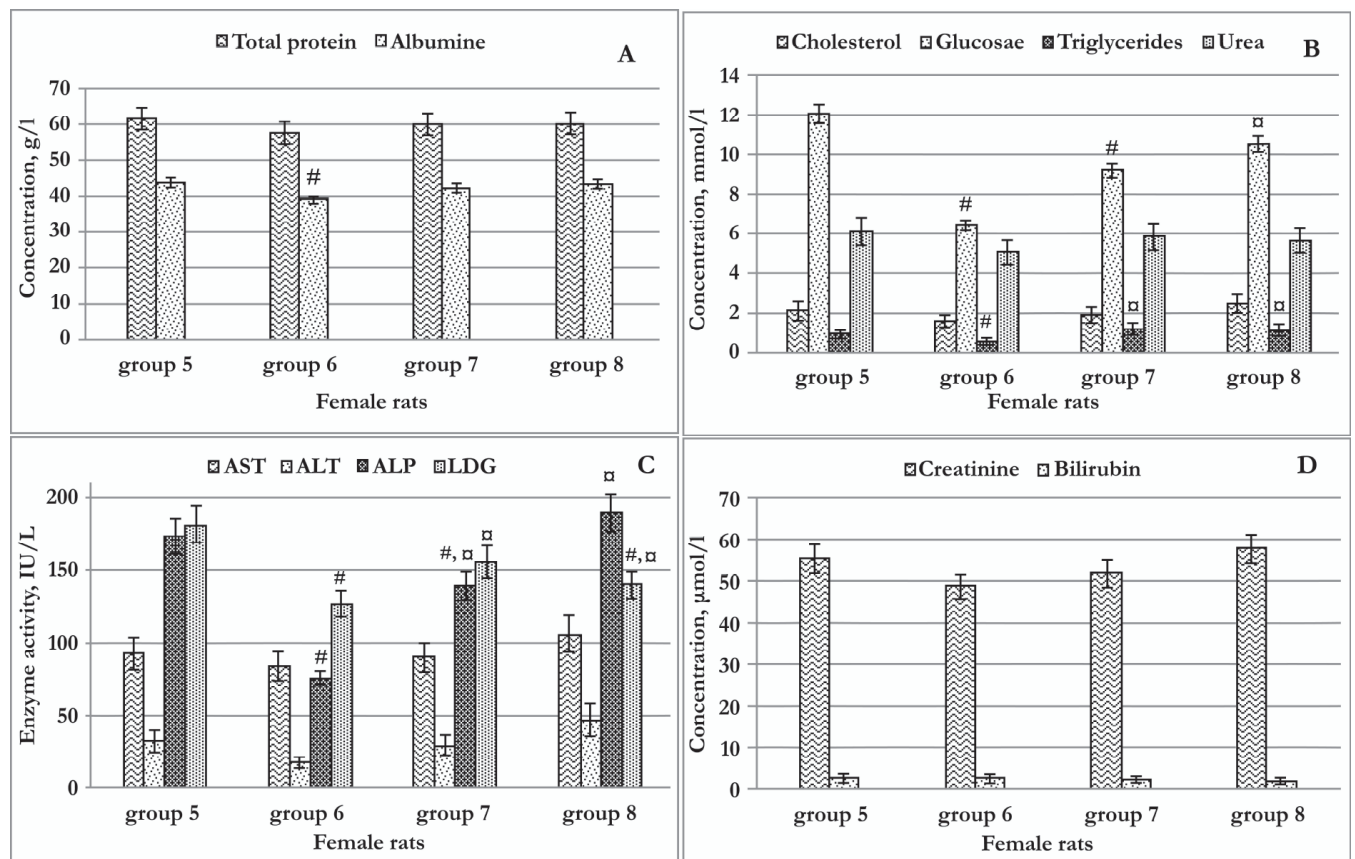
\* -  $p < 0.05$  in comparison with group 1, # -  $p < 0.05$  in comparison with group 5, ^ -  $p < 0.05$  in comparison with group 2, □ -  $p < 0.05$  in comparison with group 6.

**Fig. (2).** Results of biochemistry analysis of rats' blood (male).

\* -  $p < 0.05$  in comparison with group 1, ^ -  $p < 0.05$  in comparison with group 2

in total protein in blood serum were observed in any of the examined group of animals. Also, a significant decrease in glucose levels in male rats in groups 2 and 3, in comparison group 1, by 36.0% and 30.3% respectively, while among the animals of group 4 the glucose index significantly exceeded the same index in group 2 by 47.4% (Fig. 2B).

In female rats of group 6 and 7, in comparison with group 5, decrease in blood serum glucose was found to be 47.1% and 23.9% respectively, while among female rats of group 8 the glucose index significantly exceeded this index in group 6 by 64.1% (Fig. 3B). The violations of lipid metabolism were characterized by decrease in triglycerides concentration in



**Fig. (3).** Results of biochemistry analysis of rats' blood (female).

# -  $p < 0.05$  in comparison with group 5, □ -  $p < 0.05$  in comparison with group 6

groups 2 and 3 in comparison with group 1 by 35.7% and by 57.1% respectively, whereas the triglycerides content in group 4 did not differ significantly from triglycerides content in group 1. There was no difference in the cholesterol content among all the groups of male rats (Fig. 2B). The decrease in triglycerides content was noted only in group 6 by 44.5% in comparison with its content among the animals of group 5 (Fig. 3B). At the same time, in female rats of groups 7 and 8, triglycerides were increased compared with group 6 by 120.8% and 111.3% respectively.

Among male rats of group 2, we found decrease in ALP and LDG activity by 34.8% and 41.5% in comparison with group 1 (Fig. 2C), while among female rats of group 6 we found decrease in ALP and LDG activity in comparison with group 5: 56.8% and 30.0% (Fig. 3C).

In groups 3 and 4, in comparison with group 2, ALP activity increased by 38.6% and 76.4% respectively, whereas LDG activity increased only in group 4 by 17.1% (Fig. 2C). Among female rats of group 7 in comparison with group 6 ALP and LDG activity increased by 84.7% and 22.7%, whereas among the rats of group 8 in comparison with group 6 increase in ALP and LDG activity was 152.1% and 10.2%, respectively (Fig. 3C).

There were no significant changes in AST and ALT activity, in creatinine levels and bilirubin levels among the male rats of the groups 2, 3, 4 (Fig. 2C and 2D), and female rats of the groups 6, 7, 8 (Fig. 3C and 3D).

#### 4. DISCUSSION

The significant decrease in content of leukocytes, lymphocytes, changes in relative content of monocytes, granulocytes, and clusters of CD4+ and CD8+ differentiation confirmed the development of immunodeficiency among rats of groups 2 and 6 after administration of cyclophosphamide. At the same time the more significant decrease in leukocytes content, as well as a more expressed change in relative content of granulocytes and clusters of CD8+ differentiation clearly indicate a higher expression of immune disorders among male rats in comparison with female rats, which has been confirmed in other scientific studies conducted on animals [27] and can be explained by the peculiarities of hormonal regulation, which are significantly different from male and female animals [28, 29]. Rats in groups 2 and 6 showed changes of hematocrit, number of erythrocytes and platelets, and hemoglobin concentrations, which facts indicate greater sensitivity of the leukocyte and platelet links in rats' blood homeostasis in case of CIID modeling in comparison with erythrocyte link, which may be primarily caused to damage of faster dividing cells of bone marrow.

It is necessary to note that changes in content of leukocytes, lymphocytes, relative amount of granulocytes and monocytes described in groups 3, 4, 7 and 8, as well as CD4+ and CD8+ differentiation clusters indicate a greater efficiency of fraction 30 kDa for all links of immune system among male rats, while among the female rats the complex

extract showed more efficiency in reference to the phagocytic link of leukocytes, along that fraction 30 kDa increased the functional activity of the T-cell link (CD8+). Therefore, administration of fraction 30 kDa is useful in viral infection and cell dysplasia, as well as for males as universal immunoregulator and in bacterial infection, while administration of complex extract is possible only for female rats with combined therapy of opportunistic infections. Activation of C5 component of the complement system to greater extent during introduction of fractions 30 kDa may indicate the ability of the obtained tissue-specific biomolecules to increase the rate of chemoattraction and efficiency of phagocytosis of immunocompetent cells in inflammatory lesion, and to accelerate the cytolysis of pathogenic microorganisms.

In the presence of anemia and thrombocytopenia in male rats, it is advisable to use fraction 30 kDa, whereas in female rats in group 7 a high efficiency of complex extract is noted in the thrombocytopenic state.

The most significant cytokine imbalance (IL-2 and IL-6/IL-4) was noted in group 2, while the male rats in group 3 which received complex extract showed an improvement in interleukins ratio but with more significant imbalance in the production of pro-inflammatory cytokines in relation to anti-inflammatory cytokines in comparison with group 4, which reflects higher efficiency of the fraction 30 kDa for chronic diseases of infectious genesis caused by insufficient activity of the regulatory link of the immune system (for example, in the presence of reduced activity of inflammation amplifier [30]). It is also necessary to note the higher efficiency in male rats of fraction 30 kDa in comparison with complex extract to stimulate the effector link of the immune system (IgM), which may be important for the correction of B lymphocytes differentiation disorders, for example primary immunodeficiencies with a predominance of Ig-genesis disorders. Changes in the cytokine spectrum in males and females for an introduction of a fraction of 30 kDa are explained by gender differences in the reactivity of the immune system, described in a few studies [2, 3].

The female rats which received complete extract showed higher increase in intensity of interleukins production and, as a consequence, stimulation of B-lymphocytes with IgG formation. While the female rats which received fraction 30 kDa showed less expressed increase (in comparison with group 7) in production of IL-2, IL-4, IL-6, accompanied by decrease in IgG synthesis and compensatory increase in complement system activity, mainly C3 and C5 components.

It is necessary to note that in contrast to complex extract the use of fraction 30 kDa for hypersensitivity of III type (*i.e.* for diseases accompanied by accumulation of circulating immune complexes (CIC), for example, vasculitis, glomerulonephritis, rheumatoid arthritis), especially among male rats, is contraindicated due to the risk of intensifying the inflammatory reaction in the lesion and aggravating the severity of the autoimmune disease.

## CONCLUSION

In general, the female rats hepatocytes metabolism in comparison with male rats was found more resistant to cyclophosphamide. Decrease of albumin fraction of protein in

male rats (group 2) and female rats (group 5) is associated with disorder of protein-synthetic function of liver mediated by administration of cyclophosphamide, while restoration of protein-synthetic function of liver after administration of complex extract and fraction 30 kDa is combined with the ability of tissue-specific biomolecules to regulate not only cells of the immune system, but also cells of other somatic organs. Reduction of glucose levels in both male and female rats after administration of cytostatic (cyclophosphamide) may be caused by both inhibition of gluconeogenesis enzymes and depletion of glycogen reserves in hepatocytes. At the same time fraction 30 kDa has higher capacity to correct disorders of carbohydrate metabolism in liver cells in comparison with complex extract. Reduction of triglycerides content in the animals' blood exposed to administration of cyclophosphamide can be caused by a glycolysis disorder, accompanied by a deficiency of nicotinamide adenine dinucleotide reduced form (NADN2), which is confirmed by both decrease of glucose level and a decrease in LDG activity. It is necessary to note that both complex extract and fraction 30 kDa are more effective for female rats than for male rats in correcting metabolic and lipid metabolism disorders after administration of cyclophosphamide. The decrease of ALP activity is probably caused by inhibition of bilis formation in a liver after introduction of cytostatic agent, and in contrast to complex extract, the administration of fraction 30 kDa allows improving bilis production in a liver of male rats. During administration of complex extract its higher efficiency to treat disorders of bilis production among male rats in comparison with female rats was noted.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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