Anti-Inflammatory Effect of Nicavet-2500 in Rodent Models of Acute Inflammation

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Abstract: This study examines the influence of the tissue preparation "NICAVET 2500" on an organism of mammals with use of rodent models of acute inflammation. It is established that action of a preparation leads to decrease in ESR. Hematologic and biochemical parameters also testify to anti-inflammatory action of "NICAVET 2500". Results of histological and morphometric research of a spleen and a thymus show processes of proliferation and migration of immunocytes, testifying to activization of immune reactions. In a thymus of rats of experimental group in comparison with control an increase in the dimensions of thymic lobules and also an increase in quantity of Hassal's bodies, testifying to intensification of synthesis of the thymic hormones participating in process of an immunopoesis are revealed. As a result of the use of the preparation an increase of ability of an organism to resist alteration and also essential decrease in a level of development of inflammatory reaction of an organism are observed. The conducted research demonstrates the expressed immunomodulatory action of a preparation "NICAVET 2500" at an experimental model of inflammation.

Keywords: Immunomodulation, inflammation, thymus, spleen.

INTRODUCTION

The significant load with environmental, psychosocial, age-related, drug-associated and other factors leads to change of immune function of human and other mammals in the modern world. This fact causes the necessity of search for biologically active agents capable to support and stimulate immune functions [1-7].

Immunomodulators are meant as the substances having impact on individual mechanisms of the immune response. Thus, "immunomodulation" represents change, strengthening or suppression of parameters of cell-mediated and humoral immunity and non-specific factors of protection. It's important that immunomodulatory effect is generally reversible. Immunosuppression and immunostimulation are the extreme manifestations of immunomodulatory effect of biologically active agents, often associated with the process of inflammation [8-14]. The ability of the body to sustain injury, resist attack by microbial agents, and repair damaged tissue is dependent upon the inflammatory reaction, the immune system response, and tissue repair and wound healing. Although the effects of inflammation are often viewed as undesirable because they are unpleasant and cause discomfort, the process is essentially a beneficial one that allows a person to live with the effects of everyday stress. Without the inflammatory response, wounds would not heal, and minor infections would become overwhelming.

A significant immunostimulating effect is demonstrated by preparations derived from tissues of living organisms. Such biologically active tissue preparations exist in the form of extracts, emulsions, hydrolysates, products of a bacterial fermentation, supernatants [15-21]. Supernatant fluids of tissue extracts, containing biologically active agents with a low-molecular mass (< 10 kD), possess especially high biological potential, high bioavailability and comprehensibility [22].

So, it is shown that inclusion of the phosphate-buffered saline extract of the porcine placenta in the pig's diet considerably raises lymphocyte activation, the percentage of granulocytes and concentrations of interferon-γ in blood serum of animals and increases animal resistance to rotavirus [23]. Application of the low-molecular peptide derived from placenta of goats promotes the immunological function of normal murine macrophages and improves the phagocytic and clearance abilities of immunosuppressed mice [24]

Methanolic extract of marine sponge Xestospongia testudinaria demonstrates a marked anti-inflammatory

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and immunomodulation effects at rodent model of carrageenan-induced edema, showed, particularly, in decrease of level of inflammatory cytokines, decrease in capillary congestion and number of inflammatory cells infiltrate in damaged tissues [25]. Complementary feeding with fish oil emulsion increases total liver and lung macrophage number and phagocytosis in rats, and also has an immunomodulatory effect on lipid-modulated generation of human leukocytes in postoperative trauma [26].

Bovine lactoferrin (bLF) shows anti-infective, anti-cancer, and anti-inflammatory effects. Treating with BLF in influenza virus infected mice reduced the lung consolidation score and the number of infiltrating leukocytes in bronchoalveolar lavage fluid. It is also showed that bovine lactoferrin activates the transcription of important immune-related genes in the small intestine, and such transcriptional activation may promote a systemic immunity of an organism [27].

Embryonic tissues have the most significant potential as source of biostimulating preparations, and the most promising source are avian eggs and developing avian embryos [28-33].

Based on the data described above, we, in turn, assumed potential immunomodulatory and anti-inflammatory effect of a tissue preparation of an embryonic origin "NICAVET 2500", made of embryonic tissues of chickens, on organism of mammals on model of an experimental acute inflammation at subplantar introduction of carrageenan. Characteristics of the substances which are a part of preparation “NICAVET 2500” allowed us to expect its positive influence on immunity and total condition of an organism.

The preparation "NICAVET 2500" is made of natural raw materials of an embryonic origin and contains a wide range of biologically active agents, including organic acids (among them a significant amount of free DNA and RNA), vitamins, enzymes, hormones, macro- and microelements, i.e. the natural components inherent for live cell or tissue. Earlier it was shown by us the certain influence of preparation on morphofunctional condition of a liver of rats and the expressed homeostasis-stabilizing effect at norm and at experimental non-alcoholic steatohepatitis [34].

In essence, "NICAVET 2500" constitutes a tissue preparation with the low-molecular nanostructured activated composition which properties are reached due to a complex of the manipulations directed on activation of biochemical processes in embryonic tissues that allows to increase the level of content of biologically active agents in a substratum, including due to formation of biogenous stimulators. The main feature of technology of this biologically active product is use of method of high pressure homogenization (HPH). The way of production of the preparation "NICAVET 2500" passed patent search and is presented to Federal Service for Intellectual Property (Rospatent), the request No. 2014139637 of 30.09.2014.

The multiplex chemical composition and structural features of this preparation allow to expect its positive influence on mechanisms of regulation of hormonal, metabolic and probably the immune status, and are also able to provide an anti-inflammatory and homeostatic effects.

Basing on above-described data, we conducted research on influence of a preparation "NICAVET 2500" on an organism of mammals at an experimental acute inflammation.

MATHERIALS AND METHODS

Animals

Male Wistar Albino rats of body weights ranging from 170 g to 200 g were used in the study. The rats were group-housed in polypropylene cages with no more than four animals per cage. They were maintained under standard laboratory conditions with natural dark-light cycle and were allowed free access to standard pellet diet and tap water *ad libitum*. All the experiments were carried out using three groups, each containing 20 animals. All the animal experiments were performed according to the compliance with the EC Directive 86/609/EEC and with the Russian law regulating experiments on animals.

Carrageenan-Induced Hind Paw Edema in Rats

Acute inflammation was produced by injecting 0.1 ml of carrageenan (1% in saline) locally into the plantar aponeurosis of the right hind paw of the rats [35,36]. First group served as intact control, where no inflammation was induced. At rats of the control group the carrageenan-induced paw edema was modelled. At animals of experimental group, the carrageenan-induced paw edema was also modelled, but at the same time these rats were subdermally injected with preparation "NICAVET 2500" in a dose of 30 mg per 1 kg of body weight. Injections were made twice, for 14 and in 7 days prior to modeling of an inflammation.

After the end of experiment, animals were sacrificed in carbon dioxide chamber.

Hematological Analysis

Hematological analysis was performed using the hematological analyzer Abacus junior vet (Diatron, Austria). The examined parameters included red blood cells (RBC), hematocrit (HCT), hemoglobin, white blood cells (WBC), lymphocytes, neutrophils, monocytes, eosinophils and basophiles.

The Westergren method was used for the measurement of ESR. The value of ERS was measured in mm/h.

Histopathological Analysis

Thymus and spleen were taken and fixed in 10% formaldehyde. After several treatments for dehydration in alcohol, sections having 5μm thickness were cut. Sections were subjected to stain with hematoxylin and eosin, and then the histopathological analysis was carried.

Morphometric Studies

By means of image analyzer "ImageScope" («Leica Mycrosystems Gmbh», Austria) at hematoxylin and eosin stained sections the area of segments of thymus and area of white pulp of a spleen were defined. A percentage ratio of cortical and medullary substances of a thymus and quantity of Hassal's bodies were also defined [37]. All calculations were carried out on photographs of a histologic specimens made from 10 various fields of vision with calculation of average value.

Statistical Analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 11.0 packed program. Data were presented as mean ± standard. Difference between the control and experimental groups was analyzed using Mann-Whitney U test. $P < 0.05$ was considered statistically significant.

RESULTS

Results of research of hematological parameters of experimental animals are presented in Table **1**. The analysis of results testifies that in 24 hours in group of animals previously injected with a preparation "NICAVET 2500" the parameter of ESR decreased almost twice in comparison with control, that can testify to decrease in inflammatory reaction. It should be noted that the value of this parameter in treated animals does not differ significantly from the indices of intact rats.

In the blood of the rats of the control group there are signs of an acute inflammatory reaction - an increase in the level of leukocytes, granulocytes, as well as in the percentage of LYM, MID, and GRA. There is also an increase in the number of young platelets, which is associated with the action of interleukins.

Table 1: Some Hematological Parameters of Rats

	Intact group (n=20)	Control group (n=20)	Experimental group (n=20)
WBC,10^9/l	6.48±0.61	8.59±1.63*	5.60±0.68
LYM ,10^9/l	4.14±0.12	3.32±0.29	2.50±0.35
MID, 10^9/l	0.81±0.10	1.46±0.22	0.72±0.09
GRA, 10^9/l	1.53±0.21	2.82±0.39*	1.32±0.11
LYM, %	60.36±3.97	46.11±4.56**	54.43±5.12
MID, %	14.20±0.94	19.70±1.12*	15.45±1.05*
GRA, %	25.41±2.82	34.18±5.33**	29.28±2.31
RBC, 10^{12}/l	10.42±1.18	9.19±0.98	9.19±1.16
HGB, g/l (hemoglobin)	158.25±6.81	143.65±8.94	142.71±5.38
HCT, % (hematocrit)	55.07±3.70	51.0±5.80	48.2±5.97
P-LCC	156.68±15.24	109.65±5.88*	134.37±16.09*
P-LCR	31.20±2.61	23.19±1.52**	27,40±2.55
ESR, mm/h	7.58±1.28	4.28±1.03**	6.30±0.93

Hereinafter marked values significantly different from that of the control group (* - p≤0.05, **- p≤0.005, *** - p≤0.0005).

The results of the blood test of the rats of the experimental group testify to the pronounced anti-inflammatory effect of the preparation (Table **1**), and it can be said that it exerts a stimulating effect by activating interleukins (eg., IL-3).

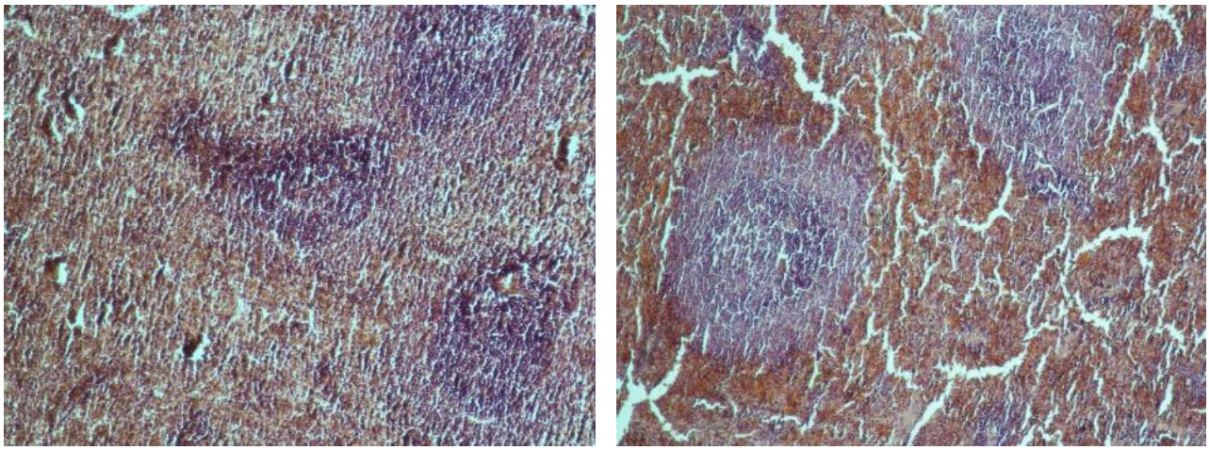
This assumption is confirmed by results of morphological researches.

In a spleen of rats of experimental group in distinction from control a large number of follicles with well-marked light centers and also a large number of leukocytes in a red pulp are revealed.

Results of morphometric researches testify to increase of the area of a white pulp of a spleen from 23.84±5.21% in intact to 26.33±4.31% in control and to 40.80±3.00 at experimental rats (Figure **1**). Besides, we observed an intensive in-filtration of a parenchyma of a spleen with cells of white blood, particularly, macrophages and plasmatic cells, at rats experimental group.

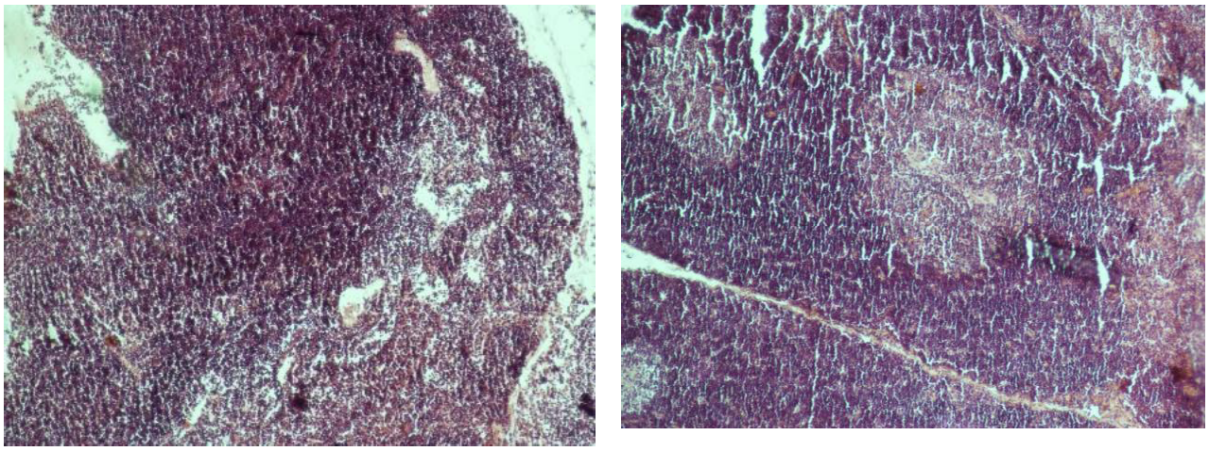
At the same time, we noted an increase of the area of segments of a thymus from 550.80±19.56 μm^2 in organs of intact animals to 582.0±16.10 μm^2 in thymus of rats of control group and to 615.80±20.65 μm^2 in a thymus of rats of experimental group. In a thymus of rats of experimental group, in comparison with control, the arterial hyperemia and lymphoid infiltrates around vessels, and also well-expressed border between cortical and medullary substances are found (Figure **2**).

As a result of morphological researches of a thymus of rats it is established that application of a preparation "NICAVET 2500" at a carrageenan-induced inflammation leads to significant increase in a percentage of cortical substance and increase in a cortical/medullary ratio in a thymus (Table **2**). Besides,



a **b**

Figure 1: Spleen of rats of control (**a**) and experimental (**b**) groups. H&E, × 100.



a **b**

Figure 2: Thymus of rats of control (**a**) and experimental (**b**) groups. H&E, × 100.

Table 2: Morphometric Parameters of Thymus of Rats

	Intact group (n=20)	Control group (n=20)	Experimental group (n=20)
Percentage of cortical substance, %	34.8±2.89	37.60±3.11	41.4±2.75*
Percentage of medullary substance, %	22.2±1.72	23.40±1.89	20.60±1.39
Percentage of cortico-medullary region, %	23.70±1.82	22.51±2.10	18.50±1.22*
Percentage of capsule and trabecules, %	20.34±1.22	17.49±3.22	19.50±1.90
Cortical-medullary ratio	1.59±0.12	1.60±0.10	1.95±0.15**
Count of Hassal's bodies per 1 mm^2	3.52±0.25	4.93±0.29	7.52±0.23***

in a thymus of experimental rats the count of Hassal's bodies per 1 mm^2 increases.

DISCUSSION AND CONCLUSION

As a result of the conducted research it is established that dynamics of inflammatory reaction, which is characterized by change of ESR of an animal, was less expressed in rats of experimental group. Such dynamics testifies that application of the preparation "NICAVET 2500" according to the scheme used by us brings immune system of rats to more balanced state in comparison with control. As a result, there is an increase of resistance of an organism to alteration, and also significant reducing of a level of inflammatory reaction development in an organism.

The considerable decrease in ESR in experimental group also argues for this. Considering almost unaltered quantity of erythrocytes, it clearly testifies for decrease in concentration of inflammatory proteins in blood serum. The above-described hematologic parameters also speak well for the subacute course of inflammatory process in experimental group.

The results of histological and morphometric researches of a spleen and thymus testify to proliferation and migration of immunocytes, which, in turn, are an evidence of activization of immune reactions. The results, consistent with the data of Xi Li *et al*.[38] about the immunomodulating effect of extracts based on embryonic bird tissues, are probably due to the chemical composition of the preparation NIKAVET 2500, in particular the content of some amino acids and low molecular weight peptides, including the carnosine dipeptide, which, according to Li Y.F. *et al*. [39] provides inhibition of apoptosis of the lymphocytes of the spleen and, as a consequence, maintenance of the number of immune cells and immune functions.

In a thymus of rats of experimental group in comparison with control the increase in the dimensions of thymic lobules, and also the increase in quantity of Hassal's bodies, testifying to intensification of synthesis of the thymic hormones, participating in process of an immunopoesis, are revealed. Due to the content in the preparation of amino acids, including arginine and lysine, which, according to Mocchegiani E. *et al*. [40], are capable to increase the synthesis and/or release of thymulin and lead to an increase in the population of peripheral T cells with a more obvious increase in T-helper type.

The conducted research testifies to the expressed immunomodulatory action of the preparation "NICAVET 2500" at an experimental model of acute inflammation.

It is possible to assume that the expressed immunomodulatory effect of the preparation "NICAVET 2500" is based on its ability to correction of the impaired parameters of immune system at all levels: humoral, cell-mediated and nonspecific.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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