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THE *IN VITRO* EFFECT OF THE EXTRACT OF WALNUT PREPARATION ON THE PRE-IMMUNE RESISTANCE INDICES IN PATIENTS WITH CHRONIC TONSILLITIS

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The pre-immune resistance is the most ancient type of defense that appeared before the acquired immunity. Numerous observations showed that immunity (immunological reactivity) begins its fight with infection no earlier than 7-8 days from the moment of the microbe penetration into the body. During this time, microbes do not multiply freely in the body. They are resisted by biological mechanisms of pre-immune protection against infections (integument-tary tissues, vascular reactions, bactericidal and bacteria bindinghumoral components of secretions and serum, phagocytes). Consequently, the cell-carriers of the pre-immune defense against infections and their soluble products recognize the fact of penetration of the pathogen into the body first, long before the immune cells.

The studies performed by many authors (1;2) confirm that there is a unified system of protective factors in the body, including phagocytosis, which plays an important role at the initial stages of body protection from infections. They indicate the presence of a regulatory link between the protective factors of neutrophilic granulocytes and cellular immunity. This is the manifestation of the ontogenesisof immune inflammation with its initial nonspecific phase playing a preparatory and auxiliary role, and the final phase of a specific immune response.

The preparations isolated from various herbs and seedshave great importance for the regulation of altered parameters of pre-immune resistance, especially medicinal herbs, seaweed, fungi of biologically active substances, which have an immunomodulating effect and allow developing pharmacological immunomodulating agents. The creation of such drugs is provided by the fractionation of the isolated substances and the evaluation of the immunomodulating activity of the isolated fractions. Among them the most important and promising for the further development of the problem of immunomodulation are substances with a characterized chemical structure obtained as a result of subsequent resynthesis (3).

Polyphenols (isolated from plants) are one of the types of antioxidants that protect cells of the human body from harmful reactions. In turn, polyphenols are divided into subspecies: (bio) flavonoids (the most numerous group) and phenolic acids. Scientists know several thousands of flavonoids, on which the smell and color of fruits, vegetables, berries and flowers predominantly depend.

The study of the effect of some plant polyphenols on E. coli bacteria showed that quercetin and tannin possessed the greatest antioxidant activity. A correlation was found between the ability of polyphenols to protect bacteria from oxidative stress and the indices characterizing their antioxidant activity *in vitro* (4).

Polyphenols are not only antioxidants, but also prebiotics, i.e. they suppress the growth of the pathogenic microflora of the digestive tract, thereby promoting the vital activity of beneficial bacteria.

The ongoing differentiation and specialization of immunology branchesraise the today`surgent problems - the need to develop a new direction that emerged at the junction of immunology, phytoimmunology and biotechnology -***immunobiotechnology***.

The preparation EN (extract of walnut**)** is of interest, being prepared from walnut, which contains polyphenols up to 20-22%, free amino acids up to 0.92-1.02% (of which essential ones are up to 0.17-0.20%, immunoactive onesbeing up to 0.20 -0.22%), iodine - up to 0.2 mg/l and iron - up to 0.4 mg / l. In addition, the drug has antimicrobial (bactericidal and bacteriostatic) action.

In this regard, we set the goal to investigate the *in vitro* effect of the EN preparation on neutrophil activity at NBT test.

Material and methods. The study included 39 patients with chronic tonsillitis (age 20±1,5) and 116 healthy people (age 22±2,7). The object of study is patients`neutrophils. A standard NBT test was performed (5), a suspension of leukocytes was poured into the control holes, whilea leukocyte suspension and EN preparation diluted 1/128 was poured in the test holes.

Discussion of the results. The analysis of the effect of the drug EN on NBT-test showed that the EN preparation showed a stimulating effect on NBT-test in 23 (60%) of 39 patients, in 10 (25%) of 39 patients the EN preparation had no modulating action and in 6 (15%) patients of 39 the EN preparation showed a suppressive effect on NBT-test. Thus, EN had a stimulating effect on the overwhelming number of patients examined (Table 1).

Table 1.

Analysis of NBT-test indices in subgroups of patients

Index	EN (39 patients)		
	1 (stimulating) 23 (60%) patients	2 (with out modulation) 10 (25%) patients	3 (suppressive) 6 (15%) patients
NBT-test (healthy)	0,12±0,003		
NBT-test (control)	0,09±0,001■	0,12±0,002○	0,14±0,004□
NBT-test (EN)	0,12±0,002	0,12±0,002○	0,11±0,003□

■ - *reliability between 1-2 subgroups,* □ - *reliability between 1-3 subgroups,* ○ - *reliabilitybetween 2-3 subgroups*

The NBT-test in the group of healthy children was 0.12 ± 0.003, which is significantly higher than in the 1 subgroup of patients with the stimulating effect ($p<0.001$) and significantly less than in the 3 subgroup of patients with a suppressive effect ($p< 0.001$). The NBT-test in the 2 subgroup of patients where no modulating effect was found was the same as in the healthy group. The NBT-test in the 1 subgroup, where the stimulating effect was noted, was initially significantly lower than in the 2 and 3 subgroups ($p<0.001$ in both cases), and under the influence of the EN preparation it increased to the level of healthy ones.

The NBT-test in group 3, where the suppressive effect was noted, was initially significantly higher than in the 1 and 2 subgroups ($p<0.001$ in both cases), and under the influence of the EN preparation it decreased to the level not significantly different from the healthy ones. Thus, the EN preparation does not have a modulating effect on the NBT-test parameters close to those of healthy ones. EN has a stimulating effect on the NBT-test whichis lower than in healthy people, and EN has a suppressive effect on the NBT-test which is higher than in healthy ones.

Next, we analyzed the immunity state in the above three subgroups of patients.

The analysis of the leukoformula values among the analyzed three subgroups of patients (Table 2) revealed a shift to the left of the leukoformula among the patients of subgroup 1, where the stimulating effect of EN was revealed. They had a significantly higher content of leukocytes ($p<0.05$ in comparison with subgroup 3), a significantly higher content of segmented neutrophils ($p<0.05$ in comparison with subgroup 2 and $p<0.01$ compared with subgroup 3), significantly higher levels of stab neutrophils ($p<0.05$ compared with subgroup 3), and significantly the lowest lymphocyte counts ($p<0.05$ compared to subgroup 2 and $p<0.01$ compared to subgroup 3). All this suggests a greater degree of suppression of nonspecific resistance in patients of subgroup 1, where the EN preparation had a stimulating effect in comparison with patients of the 3 subgroup, where the drug had a suppressive effect on the NBT-test.

Table 2.

Analysis of leukoformula indices in subgroups of patients

Index	EN (39 patients)		
	1 (stimulating) 23 (60%) patients	2 (without modulation) 10 (25%) patients	3 (suppressive) 6 (15%) patients
Leukocytes	9,6±0,27	8,8±0,34	7,7±0,91□
SegmentedN.	61,8±0,48■	59,5±0,76	58,5±0,97□
Stab N.	2,7±0,30	1,9±0,43	0,7±0,73□
Eosinophils	2,0±0,32	2,1±0,33	2,0±0,80
Basophils	0,4±0,11	0,4±0,17	0,7±0,23
Lymphocytes	27,3±0,62■	30,1±0,99	32,7±1,38□
Monocytes	5,9±0,25	6,0±0,54	5,5±0,79

■ - *reliability between 1-2 subgroups,* □ - *reliability between 1-3 subgroups,* ○ – *reliability between 2-3 subgroups*

Table 3.

Analysis of functional activity indices of T lymphocytes and cell sensitization in subgroups of patients

Index	EN (39 patients)		
	1 (stimulating) 23 (60%) patients	2 (without modulation) 10 (25%) patients	3 (suppressive) 6 (15%) patients
TTBL PHA	62,3±0,47■	66,6±0,70	70,2±1,85□
TTBLstreptococcus	4,9±0,32■	3,5±0,40○	1,9±0,41□
TTBL staphylococcus	3,2±0,22■	2,6±0,14	2,1±0,40□
TTBLpneumococcus	1,6±0,13	1,2±0,12	1,5±0,14

■ - *reliability between 1-2 subgroups,* □ - *reliability between 1-3 subgroups,* ○ - *reliability between 2-3 subgroups*

The analysis of the indices (Table 3) of the functional activity of T lymphocytes and cell sensitization in the subgroups of the patients analyzed showed that the most suppressed functional activity of T lymphocytes was in 1 subgroup of patients, where the stimulating effect of the EN preparation was detected ($p <0.001$ in

comparison with 2 and 3 subgroup). Cellular sensitization to streptococcus antigens was most pronounced in 1 subgroup of patients, where the stimulating effect of the drug EN was detected (p <0.001 in comparison with the 3 subgroup and p <0.05 in comparison with the 2 subgroup). Cellular sensitization to staphylococcus antigens was also most pronounced in 1 subgroup of patients, where the stimulating effect of the EN preparation was found (p <0.05 in comparison with 2 and 3 subgroups). These data are typical for patients with chronic tonsillitis and confirm that in patients of the 1st subgroup, the indices of functional T lymphocyte activity and cell sensitization are most varied in comparison with patients of subgroups 2 and 3.

Analysis of the lymphocyte subpopulations (Table 4) revealed significantly the lowest CD3 lymphocyte count in the 1 subgroup of patients where the EN drug had a stimulating effect (p<0.001 vs 2 subgroup) and significantly the lowest CD16 lymphocyte count (p<0.001 in comparison with the 3 subgroup and p<0.01 between 2 and 3 subgroups). The remaining subpopulations of lymphocytes did not show any significant changes between the subgroups analyzed. This indicates a greater damage to cellular immunity in 1 subgroup of patients (they had the lowest content of T lymphocytes and natural killers).

Table 4.

Analysis of lymphocyte subpopulations indices in subgroups of patients

Index	EN(39 patients)		
	1 (stimulating) 23 (60%) patients	2 (without modulation) 10 (25%) patients	3 (suppressive) 6 (15%) patients
CD3	63,0±0,63■	66,6±0,57	70,2±3,55
CD4	42,3±0,69	41,5±0,57	46,2±2,81
CD8	21,2±0,94	19,6±0,42	23,8±2,58
CD4/CD8	2,1±0,08	2,1±0,06	2,0±0,16
CD20	12,0±0,65	10,1±1,00	11,0±1,52
CD16	12,7±0,40	13,3±0,69○	16,5±0,84□

■ - *reliability between 1-2 subgroups,* □ - *reliability between 1-3 subgroups,* ○ - *reliabilitybetween 2-3 subgroups*

Analysis of the parameters of circulating immune complexes (Table 5) in the subgroups of patients revealed their highest content also in 1 subgroup of patients, and if for PEG 2.5% and PEG4.2% this was only a trend, the content of CIC PEG - 8,0% was significantly the highest (p<0,001 in comparison with the 3 subgroup and p<0,05 in comparison with the 2 subgroup and p<0,05 between the 2 and 3 subgroup of patients). This indicates the highest tonsillogenic toxicity in patients of subgroup 1.

Table 5.

Analysis of circulating immune complexes in subgroups of patients

Index	EN (39 patients)		
	1 (stimulating) 23 (60%) patients	2 (without modulation) 10 (25%) patients	3 (suppressive) 6 (15%) patients
CIC PEG – 2,5%	17,1±2,08	13,8±2,07	14,2±3,95
CIC PEG– 4,2%	36,1±3,54	31,9±4,66	31,5±6,37
CIC PEG– 8,0%	419±26,5■	305±28,9○	163±43,9□

■ - *reliability between 1-2 subgroups,* □ - *reliability between 1-3 subgroups,* ○ - *reliabilitybetween 2-3 subgroups*

Thus, the 1 subgroup of patients, where the stimulating effect of the drug EN on the NBT-test was revealed, was characterized by the highest rates of leukoformula impair, the T cell lymphocyte functional activity and cell sensitization, lymphocyte subpopulations, circulating immune complexes, that is, it was the most burdened.

Conclusion:

- the EN preparation showed a multidirectional effect;
- the EN preparation acted in a stimulating manner on low indices, on high indices - in a suppressive manner, on indices close to normal - did not have any modulating effect;
- the EN preparation can be used to modulate the preimmune resistance indices in patients with chronic tonsillitis.

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