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AN INFLUENCE OF TOTAL POLYSACCHARIDE PREPARATIONS ISOLATED FROM PLANTS OF THE GENUS FERULA ON THE STATE OF IMMUNE REACTIONS IN THE BODY OF EXPERIMENTAL ANIMALS

Abstract. The total extractive preparations of polysaccharides isolated from *Ferula kuhistanica* and *F. tenuisecta*, widely distributed in the Central Asian region, when administered to mice once (per os, 250 mg/kg) with simultaneous immunization of animals with ram erythrocytes, have a pronounced activating effect on the process of primary antibody formation, increasing in the spleen, the number of antibody-forming cells. At the same time, an increase in the cellularity of the central and peripheral organs of immunity was also observed, and the content of erythrocytes and leukocytes increased in the blood. Both studied polysaccharide preparations slightly stimulated changes in the delayed-type hypersensitivity reaction, increased the number of phagocytic peritoneal macrophages, increasing their absorption activity.

The polysaccharide preparation from *F.kuhistanica* in its effect on the immune reactivity of the organism was superior to the effect of the polysaccharide preparation from *F. tenuisecta*, as well as the corresponding effect of the reference preparations: immunal and plantaglucid. The polysaccharide preparation from *F.tenuisecta* had a similar effect to immunal and outperformed the effect of plactaglucid.

Keywords: *Ferula kuhistanica, Ferula tenuisecta*, total polysaccharide preparations, immunostimulating effect.

Currently, plant polysaccharides are widely studied biologically; they are quite common plant metabolites that play an important role in their life activity [1; 2]. Polysaccharides have various types of pharmacological activity, and therefore some of them are used in medicine and are used in the production of various drugs [3; 4]. The extremely wide range of biological activity of polysaccharides suggests that they also have immunostimulatory properties, as evidenced by some literary sources [5]. In this regard, the aim of this work was to study two new polysaccharide complexes isolated from *Ferula kuhistanica* and *F. tenuisecta* on some indicators of the functional state of immunity, as well as the

formation of antibody-forming cells in the spleen, delayed-type hypersensitivity reaction and phagocytosis. All experiments were carried out in comparison with known herbal preparations containing active polysaccharides, immunal (which is an active immunomodulating agent [6]) and plantaglucid [7].

Materials and research methods

The influence of total extractive preparations of polysaccharides, isolated from widely distributed in Central Asian region: plants of *Ferula kuhistanica* and *F. tenuisecta* [8] on the primary immune response we studied in male mice (18–20 g), immunized intraperitoneally with ram erythrocytes (2×10^7 cells per mouse). We have defined that the magnitude of the immune response was assessed in the 5^{th} day by the number of antibody-forming cells in the spleen after antigen injection [9]. Total polysaccharide preparations were administered through a special probe into the stomach simultaneously with immunization. In these experiments, the number of cells in the central (thymus, bone marrow – the femur was used) and peripheral (spleen, mesenteric lymph nodes) organs of immunity were counted in experimental animals using a Goryaev camera. We counted the number of erythrocytes and leukocytes in peripheral blood. The delayed-type hypersensitivity reaction was reproduced as described in the literature [10]. Mice were sensitized by intravenous administration of ram erythrocytes (10^6 cells per mouse). To test the reaction after 4 days from the day of sensitization, the animals were subcutaneously injected into the pad of the left hind paw with a permissive dose of antigen (10^8 ram erythrocytes) in 25 μl of a sterile 0.9% NaCl solution. The same amount of solvent was injected into the pad of the right hind paw (control). The severity of the delayed-type hypersensitivity reaction was assessed 24 hours after the administration of the resolving dose of the antigen by the difference in the degree of swelling of the paws of mice in the experimental and control groups using an MK-0.25 micrometer. As in the first case, the preparations were administered simultaneously with the antigen.

To study the effect of the studied polysaccharide preparations on the functional activity of peritoneal macrophages, they were removed by washing the abdominal cavity of mice with 3 ml of agent 199 with heparin; it was washed twice with chilled water, and brought to the required cell concentration. Researches of the functional activity of phagocytes were carried out using standard latex microspheres with a diameter of 1.2 μm [11; 12]. The percentage of activity of phagocytic cells of the peritoneal exudate and the phagocytic index corresponding to the number of latex particles absorbed by one phagocyte were calculated. In this case, the drug was administered a day before the experiment.

The researched polysaccharide preparations from *F.kuhistanica* and *F. tenuisecta* were used at a dose of 250 ml/kg, reference preparations: immunal and plantaglucid were administered in a similar regimen at doses of 50 and 500 mg/kg, respectively.

Removal of animals from the experiment, it was done by cervical dislocation under light ether anesthesia. The results of the experiments were subjected to statistical processing using Student's t-criteria.

Results and discussion. The research results show that the total polysaccharide preparations, isolated from *F.kuhistanica* and *F. tenuisecta*, when injected into the stomach of mice immunized with sheep erythrocytes, they quite effectively activate the process of primary antibody formation, by increasing the number of antibody-forming cells secreting IgM in the spleen in the experiments. This can also be seen when calculating the number of antibody-forming cells per entire spleen and per 1 million splenocytes, the level of which also had a clear upward trend. The total preparation of polysaccharides from *F. kuhistanica* showed a more pronounced effect in this respect than the polysaccharide preparation from *F. tenuisecta*, and its effect also significantly exceeded the corresponding effect of the reference preparations: immunal and plantaglocid. The sum of polysaccharides from *F. tenuisecta* had an immunostimulatory effect at the

level of immunal and exceeded the effect of plantaglucid (Table 1).

The researched polysaccharide preparations, like immunnal and plantaglucid, but in varying degrees of severity, along with the stimulation of humoral immunity, had a slight activating effect on the cellular link of immunity, which could indicate an increase in the functional activity of T-lymphocytes. This was indicated by changes in the severity of the delayed-type hypersensitivity reaction, which was manifested by an increase in swelling of the paws of mice, respectively, by 20.6%($p < < 0.05$), 17.2%($p < 0.05$), 16.8%($p < 0.05$). 0.05) and 14.6% ($p > 0.05$).

The presented figure shows data on the effect of the researched polysaccharides and known drugs containing polysaccharides on the absorption function of peritoneal macrophages, one of the most ancient cells responsible for nonspecific resistance of the organism.

Table 1.– The reaction of the immune system of mice to immunization with sheep erythrocytes with the simultaneous administration of total polysaccharide preparations from *Ferula kuhistanica*, *F. tenuisecta*, compared with immunal and plantaglucid (M ± m, n ± 6)

Conditions of experiment	Number of nucleated cells in the spleen, × 10^6	Number of antibody-forming cells		Number of thymus cells, × 10^6	Number of bone marrow cells, × 10^6	The number of cells of the mesenteric lymph nodes × 10^6
		on the spleen	per 1 million splenocytes			
Intact mice (control)	155.7 ± 6.8	4236 ± 254	27.3 ± 1.6	30.3 ± 2.7	11.2 ± 0.9	17.6 ± 0.8
Polysaccharides from *F. kuhistanica*	220.7 ± 10.1[1,2,3,4]	8200 ± 441[1,2,3,4]	37.7 ± 3.2[1]	52.6 ± 2.1[1,2,3,4]	19.7 ± 1.4[1,2,3,4]	34.8 ± 1.6[1,2,3,4]
Polysaccharides from *F. tenuisecta*	180.2 ± 4.8[1]	6618 ± 386[1,4]	36.7 ± 2.6[1]	44.8 ± 1.8[1,4]	15.8 ± 0.2[1,4]	27.6 ± 2.4[1]
Immunal	177.3 ± 7.3	6403 ± 646[1]	36.3 ± 3.8	42.7 ± 2.1[1]	15.5 ± 0.4[1]	28.8 ± 2.0[1]
plantaglucid	160.2 ± 3.8	5218 ± 292[1]	32.6 ± 2.4	38.2 ± 1.6[1]	14.0 ± 0.2[1]	22.5 ± 1.8[1]

Note: [1] – Significantly in relation to the corresponding indicators in the control; [2] – significantly between the corresponding indicators in groups of animals treated with polysaccharides from Ferula kuhistanica and F. tenuisecta; [3] – significantly between the corresponding indicators in the groups of animals treated with polysaccharides from F. kuhistanica and immunal; [4] – significantly between the corresponding indicators in the group of animals treated with polysaccharides from F. kuhistanica, as well as polysaccharides from F. tenuisecta and plantaglucid (significance level accepted at $p < 0.05$)

Under the influence of polysaccharide complexes from *F. kuhistanica* and *F. tenuisecta*, a noticeable increase in the number of cells capturing latex particles was noted, as well as an increase in the phagocytic index, which indicates an intensification of the phagocytosis process under their influence. Immunal and especially plantaglucid acted noticeably weaker in this regard. Of the other revealed facts of the primary assessment of the immunobiological properties of the total polysaccharides of preparations from *F. kuhistanica* and *F. tenuisecta*, it should be noted that they increase the total cellularity of the central and peripheral organs of immunity under their influence, as well as the stimulation of erythropoiesis and leukopoiesis (in the latter case), which is also more pronounced, than under the influence of reference – preparations (especially plantaglucid) (Table 1.2).

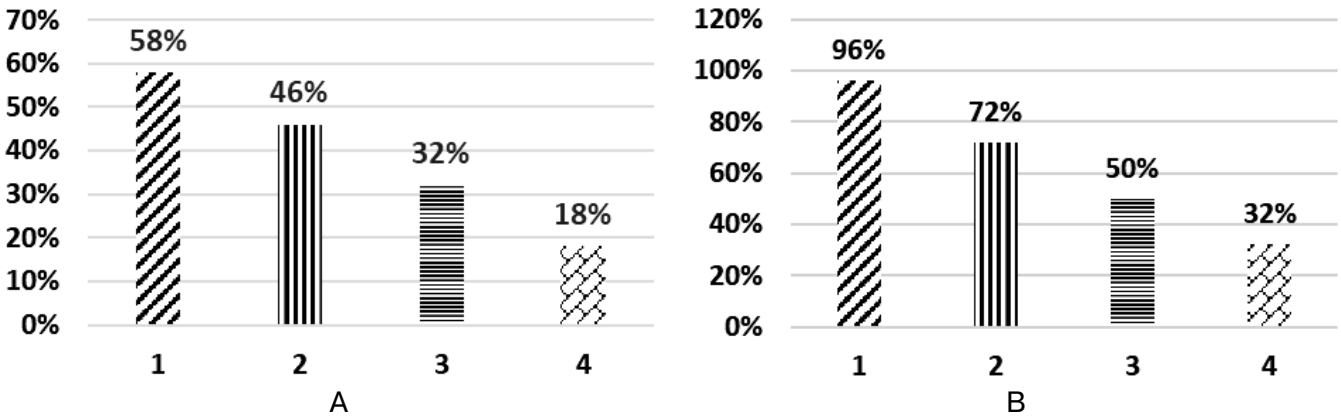

Figure. The influence of total polysaccharide preparations from *Ferula kuhistanica* (1), *F. tenuisecta* (2) and drug preparations: immunal (3) and plantaglucid (4) on the functional activity of peritoneal macrophages. Along the y-axis: A – actively phagocytic cells,%; B – phagocytic index,% (in relation to the corresponding indicators in intact animals). Along the x-axis – the tested agents

Thus, the total polysaccharide-containing preparations isolated from *F. kuhistanica* and *F. tenuisecta* are clearly immunoactive agents that they are superior (or not inferior) in their activity to known drugs containing polysaccharides.

Table 2.– Effects of total polysaccharide preparations from *Ferula kuhistanica* and *F. tenuisecta* compared with immunal and plantaglucid on the content of erythrocytes and leukocytes in the blood of mice, on the 5th day after their immunization with ram erythrocytes (M ± m, n ± 6)

Conditions of experiment	**Erythrocytes x 10^9/мл**	**Leukocytes × 10^6/мл**
Intact animals (control)	7.2 ± 0.5	8.0 ± 0.4
Polysaccharides from *F. kuhistanica*	8.7 ± 0.3^{1}	$7.2 \pm 0.3^{1.2.4}$
Polysaccharides from *F. tenuisecta*	8.3 ± 0.2	$9.8 \pm 0.3^{1.4}$
Immunal	9.0 ± 0.4^{1}	10.0 ± 0.6^{1}
Plantaglucid	7.8 ± 0.4	8.8 ± 0.2

Note: The designations are the same as for Table 1

The obtained data open the prospect of using polysaccharide preparations from *Ferula kuhistanica* and *F. tenuisecta* as new and quite effective immunomodulating agents.

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