УДК 577.27:615.275.4:547.992.2-032.34
https://doi.org/10.20538/1682-0363-2025-4-95-103

Effects of Peat Humic Acids on Phagocytic Activity of Innate Immunity Cells and Humoral Immune Response

Trofimova E.S.[1, 2], Zykova M.V.[2], Ligacheva A.A.[1], Selivanova N.S.[1, 2], Danilets M.G.[1], Karnaukhova E.A.[1], Sherstoboev E.Yu.[1], Belousov M.V.[2]

[1] *E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center, Russian Academy of Sciences*
3 Lenin Ave., 634028 Tomsk, Russian Federation

[2] *Siberian State Medical University*
2 Moskovsky trakt, 634050 Tomsk, Russian Federation

ABSTRACT

Aim. To study the effect of a course administration of peat humic acids from bogs of the Tomsk Region on the phagocytic activity of peritoneal macrophages and blood neutrophils in mice, and the thymus-dependent humoral immune response induced by the administration of sheep erythrocytes.

Materials and methods. The following immunological methods were used: study of the phagocytic activity of peritoneal macrophages and blood neutrophils of mice, determination of the number of antibody-forming cells (AFC) in the spleen of mice, measurement of the serum level of antibodies to sheep erythrocytes by the hemagglutination assay after the course administration of three samples of peat humic acids. The experiment involved 70 C57Bl/6J mice (females), aged 7–8 weeks.

Results. It was found that the course administration of all studied samples of humic acids resulted in an increase in the number of phagocytes and the intensity of particle engulfment. Two out of three samples enhanced the humoral immune response induced by the administration of sheep erythrocytes, which was manifested by an increase in the number of AFCs in the spleen and the hemagglutination assay titer in the blood serum.

Conclusion. The samples of humic acids that influence the phagocytic function of macrophages and neutrophils and enhance the humoral immune response may serve as a basis for the development of new therapeutic agents for the treatment of immunodeficiency states.

Keywords: humic acids, phagocytosis, peritoneal macrophages, neutrophils, humoral immune response, antibody-forming cells, hemagglutinins

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

Source of financing. The study was funded by the state assignment of the Ministry of Health of the Russian Federation No. 056-00071-22-02.

Conformity with the principles of ethics. The study was approved by the Bioethics Committee at E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk NRMC, Russian Academy of Sciences (Minutes No. 227012024 dated April 1, 2024).

For citation: Trofimova E.S., Zykova M.V., Ligacheva A.A., Selivanova N.S., Danilets M.G., Karnaukhova E.A., Sherstoboev E.Yu., Belousov M.V. Effects of peat humic acids on phagocytic activity of innate immunity cells and humoral immune response. *Bulletin of Siberian Medicine.* 2025;24(4):95–103. https://doi.org/10.20538/1682-0363-2025-4-95-103.

✉ *Trofimova Evgenia S.,* evs.trofimova@yandex.ru

Влияние гуминовых кислот торфа на фагоцитарную активность клеток врожденного иммунитета и гуморальный иммунный ответ

Трофимова Е.С.[1, 2], Зыкова М.В.[2], Лигачева А.А.[1], Селиванова Н.С.[1, 2], Данилец М.Г.[1], Карнаухова Е.А.[1], Шерстобоев Е.Ю.[1], Белоусов М.В.[2]

[1] *Научно-исследовательский институт фармакологии и регенеративной медицины им. Е.Д. Гольдберга (НИИФиРМ им. Е.Д. Гольдберга), Томский национальный исследовательский медицинский центр Российской академии наук (Томский НИМЦ)*
Россия, 634028, г. Томск, пр. Ленина, 3

[2] *Сибирский государственный медицинский университет (СибГМУ)*
Россия, 634050, г. Томск, Московский тракт, 2

РЕЗЮМЕ

Цель. Изучение влияния курсового введения гуминовых кислот торфа болот Томской области на фагоцитарную активность перитонеальных макрофагов и нейтрофилов крови мышей и тимусзависимый гуморальный иммунный ответ, вызванный введением эритроцитов барана.

Материалы и методы. Использовались следующие иммунологические методы: изучение фагоцитарной активности перитонеальных макрофагов и нейтрофилов крови мышей, определение количества антителообразующих клеток (АОК) в селезенках мышей, измерение уровня антител к эритроцитам барана в сыворотке крови мышей с помощью реакции гемагглютинации после курсового введения образцов торфяных гуминовых кислот. В эксперименте использовали 70 самок мышей линии C57BL/6J в возрасте 7–8 нед.

Результаты. Курсовое введение всех исследуемых образцов гуминовых кислот приводило к увеличению числа фагоцитов и интенсивности поглощения ими частиц. Два из трех образцов усиливали гуморальный иммунный ответ, индуцированный введением эритроцитов барана, который проявлялся увеличением числа АОК в селезенках и титра гемагглютининов в сыворотке крови.

Заключение. Образцы гуминовых кислот, влияющие на фагоцитарную функцию макрофагов и нейтрофилов и усиливающие гуморальный иммунный ответ, могут послужить основой для разработки новых терапевтических средств для лечения иммунодефицитных состояний.

Ключевые слова: гуминовые кислоты, фагоцитоз, перитонеальные макрофаги, нейтрофилы, гуморальный иммунный ответ, антителообразующие клетки, гемагглютинины

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Работа выполнена при финансовой поддержке государственного задания Министерства здравоохранения РФ № 056-00071-22-02.

Соответствие принципам этики. Исследование одобрено биоэтическим комитетом НИИФиРМ им. Е.Д. Гольдберга Томского НИМЦ (протокол № 227012024 от 01.02.2024).

Для цитирования: Трофимова Е.С., Зыкова М.В., Лигачева А.А., Селиванова Н.С., Данилец М.Г., Карнаухова Е.А., Шерстобоев Е.Ю., Белоусов М.В. Влияние гуминовых кислот торфа на фагоцитарную активность клеток врожденного иммунитета и гуморальный иммунный ответ. *Бюллетень сибирской медицины.* 2025;24(4):95–103. https://doi.org/10.20538/1682-0363-2025-4-95-103.

INTRODUCTION

Phagocytosis is the process by which eukaryotic cells, called phagocytes, absorb microorganisms, damaged and destroyed host cells, and foreign particles. Blood monocytes, tissue macrophages, microglia cells, osteoclasts, dendritic cells, and neutrophilic leukocytes can absorb foreign agents. Phagocytes ensure the functioning of innate immunity and represent the body's first line of defense when interacting with infectious agents. Neutrophils and macrophages are typical phagocytes that can destroy both extracellular and intracellular microorganisms. These cells produce cytokines and growth factors that regulate the development and resolution of inflammation. They also stimulate the activation and proliferation of lymphocytes and other immunocompetent cells, which are necessary for an effective adaptive immune response [1, 2].

In addition, an essential condition for activating acquired immunity is presenting the antigen to cells of adaptive immunity, which is carried out by phagocytes due to their ability to phagocytose and present foreign molecular patterns to T lymphocytes [3]. Thus, phagocytosis is a link between innate and acquired immune responses. It plays a crucial role when the innate immune system is ineffective at eliminating infectious agents.

The humoral immune response is part of the adaptive immune system. It is based on the induction of antigen-specific B lymphocytes, which results in the production and secretion of antigen-specific antibodies. Humoral immune responses can be divided into T-cell-dependent (thymus-dependent) and T-cell-independent (thymus-independent) responses. In thymus-independent reactions, the production of antibodies occurs immediately after the activation of B lymphocytes by antigens. The thymus-dependent humoral immune response is triggered when B lymphocytes are activated by T helper cells, which is preceded by antigen presentation by dendritic cells and macrophages to T lymphocytes [4]. Sheep red blood cells (SRBC), a well-known T-cell antigen, are considered as the gold standard for the formation of a thymus-dependent humoral immune response [5].

Currently, a significant number of means for correcting immune system disorders are known. However, most of them are products of chemical synthesis with a wide range of side effects. Among the immunomodulatory drugs of natural origin, only echinacea extract, drugs based on bacterial lysates, and immunomodulators of endogenous origin are known, which at the present time are usually represented by recombinant analogs [6]. A topical area of experimental medicine is the study of natural compounds, such as humic substances extracted from peat and brown coal, which are formed during the humification of biological remains of plants and animals [7]. The main component of humic substances is humic acids (HAs), which are high-molecular-weight heteropolymers that exhibit similar chemical and biological properties, but their exact molecular structure has not been determined [8]. HAs are widely used in agriculture and veterinary medicine and demonstrate numerous biological effects in experiments, namely wound healing, adaptogenic, hepatoprotective effects, as well as anti-inflammatory, antibacterial, and antioxidant activity [9, 10]. Studies have confirmed the effect of HAs on immune processes, including regulation of cytokine production with proinflammatory (TNFα, IFNγ, IL-1β, IL-12, IL-6, IL-2) and anti-inflammatory (IL-10, IL-4) properties, polarization of macrophages, and intracellular signaling processes [11–14]. Their involvement in the regulation of both humoral and cellular immunity has also been shown [15–17].

Considering the above, studying the influence of peat HAs from bogs located in the Tomsk region on such aspects of the immune system as phagocytic reactions of innate immune cells (macrophages and neutrophils) and indicators of the adaptive humoral immune response is of particular interest. Thus, the aim of this study was to investigate the effects of the course application of peat HAs on the phagocytic activity of peritoneal macrophages and peripheral blood neutrophils of laboratory mice, as well as the thymus-dependent humoral immune response induced by immunization of animals with sheep erythrocytes.

MATERIALS AND METHODS

Obtaining Humic Acids

The objects of the study were HAs isolated from peat by mass-exchange extraction. The extraction of humic substances from peat was carried out at room temperature for 8 hours using a sodium pyrophosphate solution (0.1 mol / l, ratio 1:100). HAs were precipitated from the samples by adding hydrochloric acid to pH = 1–2, then they were centrifuged, washed with water to pH = 7.0, and dried. Three types of high-moor peat obtained from the Vasyuganskoye peat deposit of the Tomsk region were used as raw materials.

The HAsphagn sample was extracted from the sphagnum peat (sampled at a depth of 20–70 cm) with a degree of decomposition of plant residues (R) of 5–10% and an ash content (A) of 2.8%. The HApine-cott sample was obtained from the pine-cotton peat (sampled at a depth of 10–50 cm, R = 30–35%, A = 7.3%), and the HAmagellan sample was isolated from the magellanic peat (sampled at a depth of 100–120 cm, R = 10–15%, A = 2.7%).

Chemical Description of Humic Acids

The elemental composition of each HA sample was studied, and its molecular weight was determined (Mw – weight average molecular weight, Mn – number average molecular weight, Mp – peak molecular weight). The elemental composition (carbon, hydrogen, and nitrogen content) was determined using a C,H,N analyzer (Carlo Erba Strumentazione 1106, Milan, Italy) and expressed as the atomic fractions of each element. The oxygen content was determined by differences. For the HAsphagn sample: C 41.7 ± 0.2%, H 40.1 ± 0.2%, N 1.9 ± 0.01%, O 16.3 ± 0.1%. For the HApine-cott sample: C 38.6 ± 0.5%, H 42.5 ± 0.4%, N 1.40 ± 0.02%, O 17.6 ± 0.2%. For the HAmagellan sample: C 38.4 ± 0.5%, H 42.47 ± 0.4%, N 2.2 ± 0.03%, O 16.9 ± 0.2%. For the HAsphagn sample: Mw = 39.7 kDa, Mn = 7.7 kDa, and Mp = 17.5 kDa. For the HApine-cott sample: Mw = 22.8 kDa, Mn = 6.1 kDa, and Mp = 11.8 kDa. For the HAmagellan sample: Mw = 18.8 kDa, Mn = 4.9 kDa. and Mp = 9.6 kDa.

Experimental Animal Groups and Substance Administration Schemes. Female C57Bl/6J mice (n = 70) aged 7–8 weeks purchased from the Department of Experimental Biological Models of the E. D. Goldberg Research Institute of Physiology and Pharmacology were used in this study (approval of the Bioethics Committee, Minutes No. 227012024 dated February 01, 2024). The HA course lasted 10 days and involved daily intraperitoneal injections. The HA preparations were prepared at a dose of 1 mg / kg of animal body weight (0.9% sodium chloride solution was used as a solvent) and administered in a volume of 100 µl. The control group received 100 µl of normal saline intraperitoneally, the comparison group received 100 µl of glucosaminylmuramyl dipeptide (Lycopid, Skopinfarm, Russia) at a dose of 2 mg / kg for 10 days. The dosing regimen and the scheme for using the studied compounds had been previously optimized in a series of preparatory experiments.

To induce a thymus-dependent humoral immune response, sheep red blood cells (SRBC) were administered intraperitoneally to animals at a dose of 5×10^6 cells on day 5 of administration of the test substances. One day after the end of the course, the number of antibody-forming cells (AFCs) in the spleen of the animals was assessed, and blood was collected to obtain serum and determine the titers of specific antibodies to SRBC (hemagglutinins) [18].

Study of the Phagocytic Capacity of Peripheral Blood Neutrophils. Phagocytosis of peripheral blood neutrophils was stimulated by adding latex particles to them. For this purpose, heparin (500 U / ml) in a volume of 3 µl was mixed with 10 µl of a mouse peripheral blood taken from the tail vein 24 h after the last HA injection. This mixture was then placed in the wells of a round-bottom plate along with 10 µl of latex particles ($60–80 \times 10^3$ / µl) and incubated for 30 minutes at 37 °C on a shaker. Next, the plate was centrifuged for 5 min (1,000 rpm), 10 µl of the supernatant was removed, the sediment was resuspended and used to prepare smears on glass slides. The smears were fixed with May – Grünwald stain and then stained with azure II-eosin. Microscopic analysis of the stained smears was performed to determine the phagocytic index (percentage of neutrophils that phagocytized latex particles) and the phagocytic number (average number of latex particles absorbed by one neutrophil) [18].

Study of the Phagocytic Activity of Peritoneal Macrophages

The phagocytic activity of peritoneal macrophages was stimulated using a 0.05% ink solution, which was administered to mice intraperitoneally at a volume of 2 ml one day after the final administration of HAs. Ten minutes later, the abdominal cavity was washed with normal saline. The cell suspension was precipitated twice by centrifugation, then the total number of peritoneal exudate cells and the number of macrophages that had absorbed the ink were counted. Then the cell suspension was precipitated again, the supernatant was discarded, and the sediment was removed with distilled water. The optical density of the resulting solution was measured spectrophotometrically (λ = 620 nm). The optical density values reflected the volume of ink absorbed by macrophages. Based on the data, the phagocytic index (percentage of macrophages that captured ink particles) and the phagocytic number (the average amount of ink absorbed by one macrophage) were calculated [18].

Determination of the Number of Antibody-Producing Cells in Mouse Spleens. The spleens extracted from mice were homogenized together with a 0.9% sodium chloride solution, the resulting homogenate was filtered through a mesh, and the number of cells in the suspension was determined. In a water bath (50 °C), 900 µl of agarose solution containing 0.7% agar (Difco, USA) in medium 199 (Sigma, USA) was mixed with 200 µl of 20% SRBC suspension, 200 µl splenocytes suspension, and 100 µl of complement (Microgen, Russia). The resulting mixture was poured into Goryaev chambers and incubated in a humid atmosphere at 37 °C for 2 hours. The number of hemolysis zones formed in the erythrocyte monolayer, which corresponded to the number of AFCs, was counted using a light microscope [18].

Determination of the Level of Antibodies to Sheep Erythrocytes (Hemagglutinins) in the Blood Serum of Mice

The serum was inactivated at 56 °C for 30 min. After that, the samples were sequentially diluted in an immunological round-bottom plate, using a volume of 25 μl and a 1:2 dilution step. Then, 25 μl of a 1% SRBC suspension was added to each dilution, and the mixture was incubated at 37 °C for 2 hours The maximum serum dilution at which antigen agglutination was visually observed was considered as the hemagglutinin titer and expressed in logarithmic form of T to the base 2 ($\log_2$T) [18].

Statistical analysis of the data was performed using the Statistica 10 (StatSoft) for Windows. Due to the small sample size, the nonparametric Kruskal – Wallis test was used to assess the statistical significance of differences in quantitative variables of three or more groups. The parameters under study were described using the median (*Me*) and the interquartile range (Q_1; Q_3). Differences were considered statistically significant at $p < 0.05$.

RESULTS

The following results were obtained after the course administration of HAs and the comparison drug Lycopid to mice. All the HA samples and Lycopid had a stimulating effect on the phagocytic capacity of neutrophils. The phagocytic index (the percentage of neutrophils that absorbed latex) increased significantly in all groups that received HAs and Lycopid, the effect of Lycopid was the most pronounced (Table 1). Samples HAsphagn and HAmagellan increased the phagocytic index to a lesser extent.

Table 1

Indicators of Phagocytic Capacity of Peripheral Blood Neutrophils of C57Bl/6J Mice after a Course of Treatment with Peat Humic Acid Preparations, *Me* (Q_1; Q_3)

Observation group, dose, number of animals (*n*)	Phagocytic index	Phagocytic number
1. Control (*n* = 7)	9.00 (6.00; 10.00)	2.17 (1.50; 2.50)
2. Lycopid, 2 mg / kg (*n* = 7)	23.00 (19.00; 28.00); 1–2 $p = 0.001$	2.17 (1.74; 2.80)
3. HAsphagn, 1 mg / kg (*n* = 7)	14.00 (12.00; 15.00); 1–3 $p = 0.02$; 2–3 $p = 0.001$	5.21 (4.14; 7.00); 1–3 $p = 0.001$; 2–3 $p = 0.001$
4. HApine-cott, 1 mg / kg (*n* = 7)	18.00 (18.00; 23.00); 1–4 $p = 0.001$	2.00 (1.72; 3.22)
5. HAmagellan, 1 mg / kg (*n* = 7)	16.00 (13.00; 18.00); 1–5 $p = 0.009$; 2–5 $p = 0.002$	3.69 (3.41; 4.38); 1–5 $p = 0.002$; 2–5 $p = 0.01$

In contrast to Lycopid and HApine-cott, HAsphagn and HAmagellan samples significantly increased the phagocytic number of neutrophils (the number of latex particles per cell). The HApine-cott sample did not affect the phagocytic number, but significantly increased the phagocytic index, approaching Lycopid in efficiency.

It was also found that the administration of Lycopid and HA preparations increased cell population in the peritoneal fluid of the experimental mice, as well as the number of phagocytic cells in the peritoneal exudate (Table 2).

It was also shown that the use of HAs *in vivo* resulted in an increase in the relative number of macrophages that engulfed ink (phagocytic index) in both the comparison drug and experimental groups (Table 3).

Table 2

Quantitative Composition of Peritoneal Exudate Cells of C57Bl/6J Mice after a Course of Treatment with Peat Humic Acid Preparations, *Me* (Q_1; Q_3)

Observation group, dose, number of animals (*n*)	Number of cells in peritoneal exudate, $\times 10^6$	Number of phagocytic cells in peritoneal exudate, $\times 10^6$
1. Control (*n* = 7)	0.78 (0.58; 1.33)	0.20 (0.13; 0.30)
2. Lycopid, 2 mg / kg (*n* = 7)	2.05 (2.03; 2.85); 1–2 $p = 0.002$	0.68 (0.60; 0.90); 1–2 $p = 0.002$
3. HAsphagn, 1 mg / kg (*n* = 7)	3.88 (3.63; 4.18); 1–3 $p = 0.001$; 2–3 $p = 0.005$	1.03 (0.93; 1.30); 1–3 $p = 0.001$; 2–3 $p = 0.008$
4. HApine-cott, 1 mg / kg (*n* = 7)	3.70 (3.43; 4.38); 1–4 $p = 0.001$; 2–4 $p = 0.009$	1.18 (0.98; 1.28); 1–4 $p = 0.001$; 2–4 $p = 0.01$
5. HAmagellan, 1 mg / kg (*n* = 7)	4.93(4.83; 7.00); 1–5 $p = 0.001$; 2–5 $p = 0.001$	1.73 (1.48; 2.13); 1–5 $p = 0.001$; 2–5 $p = 0.001$

All experimental groups showed an increase in the amount of ink absorbed by peritoneal exudate cells compared to control values. However, in all study groups, a significant decrease in the average amount of ink absorbed by one macrophage (phagocytic number) was observed compared to the controls. We associate this decrease with a significant increase in the number of phagocytic cells in the peritoneal exudate.

During the study, we also investigated the parameters of the thymus-dependent humoral immune response induced by the administration of SRBCs to mice receiving a course of HAs. The number of AFCs in the spleens of the mice and the level of antibodies to SRBC (hemagglutinins) in the blood serum were assessed. In the experimental group of animals that received a course of the HAsphagn sample, a decrease in the number of AFCs was recorded relative to the control group and the group that received Lycopid. The level of antibodies produced to SRBC (hemagglutinin titer) also decreased in this group of mice (Table 4).

Table 3

Indicators of Phagocytic Capacity of Peritoneal Macrophages of C57Bl/6J Mice after a Course of Treatment with Peat Humic Acid Preparations, *Me* (Q_1; Q_3)

Observation group, dose, number of animals (*n*)	Phagocytic index	The amount of ink in the cells of peritoneal exudate, OD	Phagocytic number, $\times 10^{-6}$
1. Control (*n* = 7)	22.58 (20.00; 24.53)	0.17 (0.13; 0.21)	0.91 (0.85; 1.08)
2. Lycopid, 2 mg / kg (*n* = 7)	29.63 (27.22; 33.33) 1–2 *p* = 0.02	0.42 (0.39; 0.47) 1–2 *p* = 0.001	0.59 (0.50; 0.65) 1–2 *p* = 0.001
3. HAsphagn, 1 mg / kg (*n* = 7)	27.56 (24.34; 30.10) 1–3 *p* = 0.008	0.47 (0.43; 0.51) 1–3 *p* = 0.001	0.46 (0.40; 0.48) 1–3 *p* = 0.001; 2–3 *p* = 0.04
4. HApine-cott, 1 mg / kg (*n* = 7)	31.45 (26.86; 37.23) 1–4 *p* = 0.006	0.43 (0.39; 0.48) 1–4 *p* = 0.001	0.36 (0.34; 0.39) 1–4 *p* = 0.001; 2–4 *p* = 0.002
5. HAmagellan, 1 mg / kg (*n* = 7)	30.57 (30.00; 31.51) 1–5 *p* = 0.01	0.55 (0.49; 0.64) 1–5 *p* = 0.001; 2–5 *p* = 0.005	0.33 (0.30; 0.37) 1–5 *p* = 0.001; 2–5 *p* = 0.001

Table 4

Indicators of the Humoral Thymus-Dependent Immune Response of C57Bl/6J Mice after a Course of Treatment with Peat Humic Acid Preparations, *Me* (Q_1; Q_3)

Observation group, dose, number of animals (*n*)	Number of antibody-forming cells, $\times 10^3$ / spleen	Hemagglutinin titer, $\log_2 T$
1. Control (*n* = 7)	24.29 (16.43; 31.43)	4.00 (3.00; 4.50)
2. Lycopid, 2 mg / kg (*n* = 7)	70.00 (58.57; 82.86); 1–2 *p* = 0.001	4.00 (3.00; 4.50)
3. HAsphagn, 1 mg / kg (*n* = 7)	5.71 (4.29; 8.57); 1–3 *p* = 0.002; 2–3 *p* = 0.004	2.00 (1.50; 2.50); 1–3 *p* = 0.02; 2–3 *p* = 0.01
4. HApine-cott, 1 mg / kg (*n* = 7)	98.57 (82.86; 150.00); 1–4 *p* = 0.001; 2–4 *p* = 0.002	6.00 (4.50; 7.00); 1–4 *p* = 0.01; 2–4 *p* = 0.03
5. HAmagellan, 1 mg / kg (*n* = 7)	91.43 (62.86; 120.71); 1–5 *p* = 0.001; 2–5 *p* = 0.001	600 (5.50; 7.00); 1–5 *p* = 0.001; 2–5 *p* = 0.005

In the group of mice that were administered the HApine-cott sample, an increase in the number of AFCs in the spleens and the titer of antibodies produced by them was observed. The same trend was observed in the animals that received the HAmagellan sample. Administration of the comparison drug Lycopid to the mice increased the number of AFCs in the spleens, but did not affect their production of hemagglutinins.

DISCUSSION

Natural products, such as mumiyo, peat, and sapropel, rich in humic substances, are widely used in traditional medicine. In addition to the experimentally proven adaptogenic, antioxidant, hepatoprotective, wound-healing, antibacterial, and anti-inflammatory properties [9, 10], it has been found that HAs can influence the immune system. D. Mudroňová et al. [16] demonstrated that a food supplement containing HAs stimulated the phagocytic activity of peripheral blood phagocytes of broiler chickens and also increased the proportion of CD4+ and decreased the proportion of CD8+ lymphocytes. R. Habibian et al. [19] obtained results using the Farmagulator Dry preparation containing HAs and administered to rats ad libitum. These results showed a dose-dependent enhancement of the humoral immune response to the B. melitensis vaccine, as well as an increase in the phagocytosis of yeast particles by rat blood mononuclear leukocytes. Another example is the work by A.V. Vucskits et al. [20], who showed that adding humic and fulvic acids extracted from brown coal to the diet of rats leads to an increase in humoral immunity, manifested in an increase in the titer of antibodies to ovalbumin. Similar observations were made in a broiler chicken model [21], where the use of a feed supplement containing HAs or a mixture of HAs with organic acids increased antibody titers against infectious bronchitis and Newcastle disease viruses, without affecting blood biochemical parameters.

In our work, we studied the effect of HAs isolated by sodium pyrophosphate extraction from three different types of peat from the Tomsk region bogs (the Vasyuganskoye peat deposit) on the phagocytic activity of peripheral blood neutrophils and peritoneal macrophages of laboratory mice, as well as indicators of the thymus-dependent humoral immune response. HA preparations were administered to animals for 10 days. The comparison group received glucosaminylmuramyl dipeptide (Lycopid), an analog of bacterial peptidoglycan that stimulates innate and acquired immunity, including the bactericidal function of phagocytes and the humoral immune response [22].

Impaired ability of innate immune effector cells – neutrophils and macrophages – to absorb pathogens can lead to uncontrolled spread of infection, accumulation of cellular debris, chronic inflammation, and disruptions in the formation of the adaptive immune response [3]. Thus, phagocytosis plays a key role in suppressing inflammatory processes and restoring homeostasis in the body. Compounds that can increase the phagocytic activity of immune cells are therefore considered to be promising immunomodulatory drugs.

The results of the study demonstrated that the course application of peat HAs and Lycopid in mice stimulated the phagocytic activity of peripheral blood neutrophilic leukocytes – an increase in neutrophils that had absorbed latex (phagocytic index) was observed in blood samples of animals that received a course of all the tested HA preparations. It should be noted that the effect of the HApine-cott sample was comparable to that of Lycopid (Table 1), and the introduction of the HAsphagn and HAmagellan samples to the animals resulted in a higher average number of particles absorbed by one cell (phagocytic number), compared to the group receiving Lycopid. In addition, the course administration of the samples caused an increase in the total number of peritoneal exudate cells, including phagocytic elements (Table 2), which was reflected in an increase in the phagocytic index of peritoneal macrophages (Table 3).

It should be noted that the total number of peritoneal exudate cells and the number of phagocytes in the groups receiving HAs exceeded the corresponding indicators for the Lycopid group. We also observed an increase in the amount of ink absorbed by peritoneal phagocytes in all experimental groups compared to the control group receiving normal saline.

During phagocytosis of foreign agents, antigen-presenting cells present foreign molecular structures to lymphocytes. This process activates the mechanisms of adaptive immunity. This study investigated the effect of a course of peat HA application on the indicators of the humoral immune response formed in animals following immunization with SRBC. The study showed that the introduction of the HAsphagn sample suppressed the activity of AFCs in the spleen and reduced the level of hemagglutinins in the blood serum of experimental animals compared to the control group (Table 4). By contrast, the use of the HApine-cott and HAmagellan compounds stimulated the growth of these parameters in mice. Moreover, after a course of HApine-cott and HAmagellan, the number of AFCs and the antibody titer (hemagglutinins) were higher than those recorded for Lycopid. The HApine-cott and HAmagellan samples demonstrated immunostimulatory activity, manifested in the enhancement of the phagocytic function of peripheral blood neutrophils and peritoneal macrophages in mice, as well as in the activation of the humoral immune response to sheep erythrocytes. According to the results of the studies, their effectiveness was comparable to that of the reference drug Lycopid and in some cases even exceeded its performance.

CONCLUSION

Our experiments revealed that course therapy with all tested peat humic acid preparations stimulated the proliferation of phagocytic cells and increased the effectiveness of their interaction with foreign particles. Moreover, the use of HApine-cott and HAmagellan preparations enhanced the humoral immune response caused by the introduction of sheep erythrocytes into experimental animals. This was manifested in an increase in the number of antibody-forming cells in the spleen tissues and an increase in the hemagglutinin titer in the blood serum. Thus, the HApine-cott and HAmagellan samples, isolated from the bogs of the Tomsk region and capable of stimulating the phagocytic activity of macrophages and neutrophils, as well as enhancing the effectiveness of the humoral immune response, represent a promising basis for the creation of innovative drugs of natural origin that can be used in treating immunodeficiency-related diseases.

REFERENCES

1. Prame Kumar K., Nicholls A.J., Wong C.H.Y. Partners in crime: neutrophils and monocytes/macrophages in inflammation and disease. *Cell Tissue Res.* 2018;371(3):551–565. DOI: 10.1007/s00441-017-2753-2.2.

2. Fu Y.Lю, Harrison R.E. Microbial phagocytic receptors and their potential involvement in cytokine induction in macrophages. *Front. Immunol.* 2021;12:662063. DOI: 10.3389/fimmu.2021.662063
3. Uribe-Querol E., Rosales C. Phagocytosis: our current understanding of a universal biological process. *Front. Immunol.* 2020;11:1066. DOI: 10.3389/fimmu.2020.01066.
4. Kojima F., Frolov A., Matnani R., Woodward J.G., Crofford L.J. Reduced T cell-dependent humoral immune response in microsomal prostaglandin E synthase-1 null mice is mediated by nonhematopoietic cells. *J. Immunol.* 2013;191(10):4979–4988. DOI: 10.4049/jimmunol.1301942.
5. Ladics G.S. Primary immune response to sheep red blood cells (SRBC) as the conventional T-cell dependent antibody response (TDAR) test. J. Immunotoxicol. 2007;4(2):149–152. DOI: 10.1080/15476910701337357.
6. Khaitov R.M. Immunotherapy; edited by R.M. Khaitov, R.I. Ataullakhanov, A.E. Shulzhenko. M: GEOTAR-Media, 2020:768. (In Russ.).
7. Kleber M., Lehmann J. Humic substances extracted by alkali are invalid proxies for the dynamics and functions of organic matter in terrestrial and aquatic ecosystems. *J. Environ. Qual.* 2019;48(2):207–216. DOI: 10.2134/jeq2019.01.0036.
8. Ampong K., Thilakaranthna M.S., Gorim L.Y. Understanding the role of humic acids on crop performance and soil health. *Front. Agron.* 2022;4:2022. DOI: 10.3389/fagro.2022.848621.
9. Zykova M.V., Bratishko K.A., Buyko E.E., Azarkina L.A., Ivanov V.V., Mihalyov D.A. et alCoal-derived humic substances: insight into chemical structure parameters and biomedical properties. *Molecules*. 2024;29(7):1530. DOI: 10.3390/molecules29071530.
10. Vašková J., Stupák M., Vidová Ugurbaş M., Žatko D., Vaško L. Therapeutic efficiency of humic acids in intoxications. *Life (Basel).* 2023;13(4):971. DOI: 10.3390/life13040971.
11. Şehitoğlu M.H., Öztopuz Ö., Karaboğa İ., Ovalı M.A., Uzun M. Humic acid has protective effect on gastric ulcer by alleviating inflammation in rats. *Cytol. Genet.* 2022;56:84–97. DOI: 10.3103/S0095452722010091.
12. Verrillo M., Parisi M., Savy D., Caiazzo G., Di Caprio R., Luciano M.A., Cacciapuoti S., Fabbrocini G., Piccolo A. Antiflammatory activity and potential dermatological applications of characterized humic acids from a lignite and a green compost. *Sci Rep.* 2022;12(1):2152. DOI: 10.1038/s41598-022-06251-2.
13. Trofimova E.S., Zykova M.V., Danilets M.G., Ligacheva A.A., Sherstoboev E.Y., Tsupko A.V. et al. Immunomodulating properties of humic acids extracted from Oligotrophic sphagnum magellanicum peat. *Bull. Exp. Biol. Med.* 2021;170(4):461–465. DOI: 10.1007/s10517-021-05088-5.
14. Trofimova E.S., Zykova M.V., Ligacheva A.A., Danilets M.G., Sherstoboev E.Yu., Selivanova N.S. et al. The role of intracellular signaling molecules in macrophage activation by peat humic acids. *Bull Exp Biol Med.* 2023;175(3):353–357 DOI: 10.1007/s10517-023-05866-3.
15. Ahfeethah F., Elazomi A., Kammon A. Effect of humic acid and probiotics on immunity of broiler chickens. *Open Vet. J.* 2023;13(7):839–845. DOI: 10.5455/OVJ.2023.v13.i7.5.
16. Mudroňová D., Karaffová V., Pešulová T., Koščová J., Maruščáková I.C., Bartkovský M. et al. The effect of humic substances on gut microbiota and immune response of broilers. *Food Agric. Immunol.* 2020;31(1):137–149. DOI: 10.1080/09540105.2019.1707780.
17. Bujňák L., Hreško Šamudovská A., Mudroňová D., Naď P., Marcinčák S., Maskaľová I., Harčárová M., Karaffová V., Bartkovský M. The Effect of dietary humic substances on cellular immunity and blood characteristics in piglets. *Agriculture*. 2023;13(3):636. DOI: 10.3390/agriculture13030636.
18. Guidelines for Conducting Preclinical Studies of Drugs. Part One; edited by A.N. Mironov. M: Grif i K, 2013:944. (In Russ.).
19. Habibian R., Morshedi A., Delirezh N. Effect of humic acid on humoral immune response and phagocytosis. *Global Veterinaria.* 2010;4(2):135–139.
20. Vucskits A.V., Hullár I., Bersényi A., Andrásofszky E., Kulcsár M., Szabó J. Effect of fulvic and humic acids on performance, immune response and thyroid function in rats. *J. Anim. Physiol. Anim. Nutr. (Berl).* 2010;94(6):721–728. DOI: 10.1111/j.1439-0396.2010.01023.x.
21. Akaichi A., Jebali A., Abbes O., Haj Taieb S., Feki M., Kaboudi K. et al. Effect of humic acid and organic acids, alone or in combination, on blood biochemical constituents and humoral immune response in broiler chickens. *Livest. Sci.* 2022;258:104880. DOI: 10.1016/j.livsci.2022.104880.
22. Nesmeyanov V.A. Mechanism of Action and Clinical Efficacy of the Immunomodulator Glucosaminylmuramyl Dipeptide (Lycopid). *Clinical Medicine.* 1997;75(3):11–15. (In Russ.).

Author Contribution

Sherstoboev E.Yu. and Danilets M.G. developed the concept and design of the experiments. Zykova M.V. derived the samples and standardized them. Trofimova E.S., Ligacheva A.A., Selivanova N.S., Karnaukhova E.A. assessed the biological activity of the studied substances using immunological methods. Danilets M.G., Ligacheva A.A., Karnaukhova E.A. analyzed and interpreted the data. Trofimova E.S., Zykova M.V. participated in drafting of the article and critical revision of the manuscript for important intellectual content. Sherstoboev E.Yu. and Belousov M.V. approved the manuscript for publication.

Author Information

Trofimova Evgenia S. – Dr. Sci. (Med.), Senior Researcher, E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk; Associate Professor, Division of Pathophysiology, Siberian State Medical University, Tomsk, evs.trofimova@yandex.ru, https://orcid.org/0000-0002-5367-715X

Zykova Maria V. – Dr. Sci. (Pharmaceut.), Associate Professor, Head of the Chemistry Division, Siberian State Medical University, Tomsk, huminolog@mail.ru, https://orcid.org/0000-0002-1973-8983

Ligacheva Anastasia A. – Cand. Sci. (Biology), Research Officer, E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, vittelli@mail.ru, https://orcid.org/0000-0002-3337-1516

Selivanova Natalia S. – Research Assistant, E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk; Laboratory Assistant, Siberian State Medical University, Tomsk, selivan.ns@gmail.com, https://orcid.org/0009-0006-6218-3051

Danilets Marina G. – Dr. Sci. (Biology), Principal Researcher, E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, danilets_mg@pharmso.ru, https://orcid.org/0000-0001-7862-4778

Karnaukhova Elizaveta A. – Postgraduate Student, E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, eak_eak@mail.ru, https://orcid.org/0009-0003-5312-8462

Sherstoboev Evgeny Yu. – Dr. Sci. (Med.), Professor, Principal Researcher, Head of the Immunopharmacology Department, E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, sherstoboev_eu@pharmso.ru, https://orcid.org/0000-0002-6178-5329

Belousov Mikhail V. – Dr. Sci. (Pharmaceut.), Head of the Pharmaceutical Analysis Division, Siberian State Medical University, Tomsk, mvb63@mail.ru, https://orcid.org/0000-0002-2153-7945

(✉) **Trofimova Evgenia S.,** evs.trofimova@yandex.ru

Received on April 25, 2025;
approved after peer review on June 02, 2025;
accepted on June 19, 2025