The impact of the *Chimaphila umbellata (L.) W.P.C.Barton* extract on the immune response in animals

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Abstract. With the water-alcohol extraction method was obtained liquid extract from *Chimaphila umbellata (L.) W.P.C. Barton*. Experimental studies on the azathioprine immunosuppression model showed a pronounced opposition to the development of a secondary immunodeficiency state under the influence of 14-day course use of a dealcoholized extract of the aerial part of *Chimaphila umbellata (L.) W.P.C. Barton*. The results of the humoral state assessment and cellular link of the immune response in F1 line laboratory mice (C57Bl/6 × CBA) indicate that the plant extracts:

1. Restores the immune status by the delayed-type hypersensitivity reaction to 85.6%, with 100% in the intact control and 69.9 in the control - azathioprine.
2. Influences the number of antibody-forming cells, significantly increasing their absolute value by 2.36, and the relative - by 1.84 times in comparison with control-azathioprine.

The immunocorrecting activity of umbellata wintergreen *Chimaphila umbellata (L.) W.P.C.Barton* extract is consistent with previously obtained results on the study of antipyretic, analgesic, antimicrobial, and wound healing anti-inflammatory effects.

1. Introduction

The growth of the populations well-being, industrial and agricultural production in recent decades is overshadowed by the high incidence of humans and animals. Among them, are of particular concern calls malignant neoplasms and pathologies associated with viral and bacterial infections. These pathologies are widespread not only in humans, but also in domestic animals, both productive and unproductive. When these diseases occur, an important role is played by the immunodeficiency of the body state. Modern living conditions of humans and domestic animals are not always close to ideal and often lead to acquired immune disorders. The medicine development allows us to assert with confidence that most of the pathological processes occurring in the body can be both a cause and a consequence of a violation of the body's immune status [1]. These disorders are usually divided into primary immunodeficiencies, which are congenital, that is, inherited and appearing shortly after birth, and secondary, which are acquired in nature and are the result of exposure to the body of foreign agents [2; 3].

Secondary immunodeficiencies have a known feature of being subjected to immunocorrection, as a result of which the market for medications is constantly replenished with tools that help restore the body's immune state. Despite the success of the pharmaceutical industry, research remains relevant to minimize the negative consequences of taking medications [4; 5]. If human medicine sets as its priority
the reduction of side effects, then veterinary practice, along with this, also strives to improve the quality of livestock products, which can be significantly reduced under the influence of medications. [6]. In this regard, the research and development of agents possessing immunomodulatory properties from plants growing in Siberia and the Far East are presented of current interest to us.

Umbellata wintergreen (Chimaphila umbellata (L.) W.P.C. Barton) is a representative of the genus Chimaphila of the family Pyrolaceae. In the aerial part of the umbellata wintergreen, phenols and their derivatives - arbutin, homoarbutin, isohomoarbutin, renifolin are contained; tannins; flavonoids; quinones; triterpenoids [7; 8]. It grows in Europe, Japan, North America, Russia (Western and Eastern Siberia, the Far East and Sakhalin). It occurs in the western regions of the Irkutsk region [9].

We determined the humidity and content of extractive substances in the aerial part of the umbellata wintergreen, according to the methods described in the State Pharmacopoeia, 11th edition [10]. For further studies, water-alcohol extraction of this plant was obtained [11].

2. Conditions, materials and methods
The studies were performed on laboratory animals (white laboratory mice, line F1 (C57Bl/6×CBA) with a live weight of 18-20 grams). Laboratory animals were divided into three groups: “control”, “experiment” and “intact control”. The “control” group - laboratory animals that received for 5 days inside every day 50 mg/kg cytotastic azathioprine, which is an immunosuppressant commonly accepted in experimental pharmacology. The "experiment" group - animals with experimental immunosuppression, received inside herbal remedy at a rate of 200 mg/kg daily for 14 days, starting from the end of the immunosuppressant. An “intact control” group of animals was given distilled water in an equivalent volume according to a similar scheme, starting from day 6 from the start of the experiment.

By the standard methodology of the local delayed-type hypersensitivity reaction (HRT), the status of the cellular link of the immune response was evaluated [12]. Hypersensitivity in the body of mice was created in all three groups, 4 days before the end of the experimental therapy, with a 0.1% suspension of sheep erythrocytes in physiological saline with intraperitoneal injection. After 4 days, 50 μl of a 50% suspension of sheep erythrocytes was injected into the plantar aponeurosis of the right hind paw in all laboratory mice. In the opposite hind paw, physiological saline was injected in an equivalent volume. The result of a delayed-type hypersensitivity reaction was evaluated after 24 hours by the difference in the volume of the paws of the right and left hind limbs (calcaneal joint), expressed as a percentage [12].

Counting the number of antibody-forming cells (AFC) by local hemolysis according to A.J. Cunningham [13] allowed us to evaluate the status of the humoral link in the immune response. Local hemolysis was reproduced by intraperitoneal immunization with ram erythrocytes at a dose of 2×10^6 cells/gol. The degree of the immune response was interpreted on the 5th day after immunization by the number of antibody-forming cells in the spleen and by 10^6 nucleated splenocytes.

The statistical significance of the difference in the results was calculated using the “Student” parametric criterion [14].

3. Results and discussion
Research results. Injection of azathioprine at the above dosage causes inhibition in laboratory mice of the development of delayed-type hypersensitivity with repeated subcutaneous injection of a suspension of sheep erythrocytes. In particular, after 24 hours in laboratory animals of the control group, the delayed-type hypersensitivity reaction index was recorded at 19.5, which amounted to 69.9% of the reaction index of the animals of the intact control group, taken as 100% (see the table 1 down below). Giving the animal an oral herbal product for 14 days against the background of immunosuppression (“experiment”) caused a delayed-type hypersensitivity reaction at the level of 23.9, which amounted to 85.6% of the HRT response index of intact animals. Moreover, the value of the HRT reaction index in animals of the “experiment” group is higher than in animals in the “control” group and has a statistically significant difference.
Table 1. The impact of the Chimaphyla umbellata (L.)W.P.C. Barton extract on the severity of the delayed-type hypersensitivity reaction (HRT) (M±m; n=10).

| Groups of animals            | Dose Azathioprine, mg / kg | HRT reaction index, % |
|------------------------------|----------------------------|-----------------------|
| Control (Azathioprine-Az)    | 50                         | 19.5±1.92*            |
| Experiment (Az+wintergreen)  | 50                         | 23.9±2.12***          |
| Intact control               | -                          | 27.9±2.06**           |

* - the difference is statistically significant (p≤0.05) in comparison with control (Az);  
** - the difference is statistically significant (p≤0.05) in comparison with the experiment (Az + wintergreen);  
*** - the difference is statistically significant (p>0.05) in comparison with intact control.

During studying the impact of an herbal remedy on antibody formation, it was found that under immunosuppression conditions in laboratory animals an umbellata wintergreen extract restores the state of the humoral link of the immune response, bringing it closer to the indices of intact animals. It should be noted that the difference between the number of antibody-forming cells in the body of animal groups of "experiment" and "intact control" is not statistically significant. Further research indicates that the administration of azathioprine in the control group significantly reduces the number of antibody-forming cells in comparison with those in the "intact control" animals group (see the table 2 down below). This indicates an immunodeficiency state in the control group animals, which is confirmed by a statistically meaningful difference in the immunocompetent cells number.

Table 2. The impact of the Chimaphyla umbellata (L.)W.P.C. Barton extract for antibody formation (M ± m; n = 10).

| Groups of animals            | Dose mg / kg | Number of AFC to the spleen by 10^6 splenocytes |
|------------------------------|--------------|-----------------------------------------------|
| Control (Azathioprine-Az)    | 50           | 38378±3025**                                 |
| Experiment (Az+wintergreen)  | 50           | 90891±4576***                                |
| Intact control               | -            | 97559±7256*                                 |

* - the difference is statistically significant (p≤0.05) in comparison with control (Az);  
** - the difference is statistically significant (p≤0.05) in comparison with the experiment (Az + wintergreen);  
*** - the difference is statistically significant (p>0.05) in comparison with intact control.

In the “experiment” group, where laboratory animals, after immunosuppression with azathioprine for 14 days were given the umbellata wintergreen extract, the number of antibody-forming cells was more significant both in absolute terms (2.36 times) and in relative terms in terms of 10^6 nucleated splenocytes (1.84 times). A statistically vital difference was established between the antibody-forming cells number in the “control” and “experience” animals group.

4. Conclusions
The results obtained indicate that the course application of the umbellata wintergreen extract for 14 days against the background of experimentally modeled immunosuppression helps to accelerate the
restoration of reduced immunobiological reactivity. In particular, this is confirmed by the identified positive dynamics in terms of the cellular status and humoral parts of the immune response.

In general, the revealed immunocorrective effect allows us to recommend an aqueous-alcoholic umbellata wintergreen extract for the prevention of conditions and the treatment of diseases associated with inhibition of the immune system.

The research results obtained in these studies are consistent with our previously obtained data on the study of the specific pharmacological activity of the umbellata wintergreen extract. In particular, the extract studied by us, separated from the aerial parts of the umbellata wintergreen, is a low-toxic agent and has a pronounced anti-inflammatory effect, exhibiting pronounced analgesic activity [15], significantly reduced “formalin” edema, involution of granulofibrous formations [16], hypothermic action in pyrogenic fever [17].

We consider that the biologically active substances complex contained in the aerial part of umbellata wintergreen extract determines the pronounced immunostimulating effect of the tested agents, specifically phenolic compounds: flavonoids, phenol carboxylic acids, phenolic glycosides, and oak substances.

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