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Research Article

Phenolic Compounds and Immunomodulating Activity of Chicory (*Cichorium intybus* L.) Extract

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ABSTRACT

Introduction: This research aims to determine the immunomodulating activity of chicory (*Cichorium intybus* L.) herb extract and to evaluate the prospects of development of the medicine based on it. **Methods:** Object of the research was dry chicory extract obtained from herb (aerial part) of a wild plant. The chemical composition of the extract was determined by HPLC-MS method. Its immunomodulating action has been explored *in vivo* experiments involving intact animals, as well as immunosuppressed animals, treated with azathioprine cytostatic agent. Chicory extract was administered at a dose of 30 mg/kg per os 1 time per day for 14 days. As a reference drug, Immunal (Lec Pharma, Slovenia) was used. Chicory extract action on the state of cell immune component was evaluated in delayed hypersensitivity reaction. The humoral immunity condition was evaluated by the count of antibody-forming cells determined by the local hemolysis method. The state of the macrophage component of the immune response was evaluated in the phagocytosis reaction of peritoneal macrophages in relation to colloid liquid ink particles. **Results:** Dry chicory extract is capable to reduce the suppressive azathioprine effect on the cell-mediated immune response, antibody response, and phagocytosis with macrophages; it does not change the immunity indicators in intact animals. **Conclusion:** Dry chicory extract contains phenolic complex of biologically active substances, namely oxycoumarins, hydroxycinnamic acids, and flavonoids. Dry chicory extract is an effective immunocorrecting agent; it should be recommended for further study and application aiming for the prevention and treatment of immunodeficiency states.

Key words: Chicory herb, Phenolic compounds, Dry extract, Immunomodulating activity.

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INTRODUCTION

In the context of the increasing rate of scientific and technological development of society, the vital activity of a modern man is also changing significantly. The intense dynamics in the life rhythm accompanied by stress situations and psychoemotional tension against the background of the environment deterioration causes often a decrease in the body's resistance and, as a result, weakening in the immune mechanisms of its protection.

Immunomodulating agents are used to correct immunodeficiency conditions. Herbal medicines are especially promising in this context. Herbal preparations in comparison with synthetic products are of less toxicity, nave better tolerance and minimal adverse reactions even with prolonged use.[1]

One of the promising sources of biologically active substances (BAS) is common chicory (*Cichorium intybus* L.), an herbaceous plant belonging to the *Asteraceae* family. This species is distributed as a wild plant from the White Sea coast in the north to the Black Sea coast in the south and from the Baltic in the west to the Pacific coast in eastern part of Russia.[2]

At the same time, chicory is domesticated, it has been cultivated in Russia since the end of the 17th century, mainly in the territory of Yaroslavl region. Roots of cultivated chicory are used after roasting as ersatz coffee, named "chicory coffee".

Both underground and aerial parts of chicory are widely used in traditional medicines including Chinese and Mongolian ones as an immunomodulatory, choleretic, hepatoprotective, hypoglycemic agent. This plant is described in the Chinese Pharmacopoeia, is used to obtain homeopathic medicine agents in Germany. Extract of chicory herb is included into complex preparation Liv.52 (India).

It was found according to published data concerning results obtained *in vitro* and *in vivo* experiments that different parts of a chicory plant (roots, leaves, seeds, fruits) have pharmacological activity.[3-7] Moreover, this action is due to the complex of BAS, represented by the main groups of secondary metabolites, namely polyfructosans, hydroxycinnamic acids, coumarins and flavonoids.

The most promising medicinal plant material can be just the aboveground part of this plant. Unlike underground organs, herb of wild chicory is convenient to collection during the long flowering season (from June till August).

The aim of this research was to examine the immunomodulatory activity of chicory herb extract to assess the prospects of a medicine creating on its basis.

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MATERIALS AND METHODS

The object of the research was dry chicory extract obtained from herb (aerial part) of wild plants collected in flowering phase on the territory of Lipetsk region of Russian Federation in 2018. The extract preparation technique is the following: dried up to moisture content of 6.83 ± 0.68 %, and grinded chicory herb was under extraction with ethanol 70 % in the ratio of 1:10 for three times. The combined extracts were fused, concentrated on a rotary evaporator to an aqueous residue, and treated with dichloroethane. The purified extract was dried up to moisture content of 3.52 ± 0.35 %.

Determination of phenolic compounds sum in terms of chicoric acid was carried out by direct spectrophotometry using the spectrophotometer UV-1800 (Shimadzu). The qualitative BAS composition in the extract was determined on an LCMC-8040 liquid chromatograph-mass spectrometer (Shimadsu, Japan) consisting of Nexera liquid chromatograph in combination with the triple quadrupole mass-spectrometer (ionization method was electrospray (ESI), mass scanning was in the mode of positive and negative ions recording in the range m/z 240-2000, the voltage on a capillary of the ionization source was 5 kV, the temperature of the heating unit was 400 °C, the flow of nitrogen (desiccant gas) was 20 l/min) and a diode-matrix detector. Chromatography was performed on a Luna 5µm C18 100 Å (250 x 4.6 mm) column, at a column temperature of 30 °C, a mobile phase flow rate was 1 ml/min, the injection volume of the test solution was 10 µl. As the mobile phase, solvent systems were used: 0.1% solution of trifluoroacetic acid (A) and acetonitrile (B) in the gradient elution mode: (0-20 min - 10%, B, 20-30 min - 25% B, 30-40 min - 40% B, 40-44 min - 60% B, 44-48 min - 80% B, 48-60 min - 10% B).

The research of immunomodulating activity was carried out involving male mice of the F1 line (CBAxC57Bl/6) having body weight of 18-20 g. The effect of the extract on the indicators of cellular, humoral and macrophage immunity components was studied in intact animals, as well as animals in the state of immunosuppression caused by cytostatic azathioprine, which was administered to the control group of animals at a dose of 50 mg/kg orally 1 time per day for 5 days.[8]

Dry chicory extract was administered to the 1st experimental group of mice against the azathioprine background and to the 3rd experimental group of intact mice at a dose of 30 mg/kg per os 1 time per day for 14 days. The experimental therapeutic dose of dry chicory extract equal to 30 mg/kg was determined empirically in preliminary tests conducted involving 30 mice. As a reference drug, Immunal (Lec Pharma, Slovenia) was used. Immunal was administered to the 2nd experimental group of mice against the azathioprine background, and to the 4th experimental group of intact mice in an isoeffective dose of 5 ml/kg per os 1 time per day for 14 days. The intact and control groups of animals received purified water according to a similar treatment plan. The effect' evaluation were performed on day 20 of the experiment.

Chicory extract action on the state of the cellular component of the immune response was evaluated in a delayed hypersensitivity reaction (DHR) according to a standard method.[9] The state of humoral immunity was evaluated by the number of antibody-forming cells (AFCs) determined by local hemolysis according to A.J. Cunningham (1965).[10] The state of the macrophage component of the immune response was evaluated in the phagocytosis reaction of peritoneal macrophages in relation to colloid liquid ink particles. The optical density of the peritoneal exudate cell lysate reflecting the amount of ink absorbed by peritoneal macrophages was determined using CECIL-2011 spectrophotometer at a wavelength of 620 nm.[9] The results obtained were processed by a statistical method using the Student's t-criterion.

RESULTS AND DISCUSSION

Dry extract of chicory herb is hygroscopic amorphous yellow-brown powder. The content of phenolics sum in terms of chicoric acid is 8.3 ± 0.4%. According to HPLC-MS, the following oxycoumarins are identified in the phenolic complex of the extract: esculetin (4) and chicoryin (1), hydroxycinnamic acids: chicory (10), chlorogenic (3), kaftaric (2), isochlorogenic A (9), coffee (5); flavonoids: isocvercetin (7), astragaline (8) and rutin (6). The dominant substances are esculletin, chicoryin, and chicoryic acid (Figure 1).

In a result a study how the antibody formation processes is influenced by dry chicory extract applied at a dose of 30 mg/kg, it was found out that this agent restores the humoral immune response under azathioprine immunosuppression. With the introduction of the test medicine against the background of immunosuppression, a significant increase in AFC amount was observed both in absolute values and when calculating for 10^6 splenocytes; the first indicator exceeded the level of azathioprine suppression by 1.4 times, and the second by 1.6 times. It was also found out that this medicine does not affect the indicators of humoral immunity in intact animals (Table 1).

At examination of dry chicory extract effect on cell-mediated DHR it was found out that tested medicine restore the index of this reaction (DHRI) in the condition of azathioprine immunosuppression.

At the test medicine administration against the background of immunosuppression, DHRI increase by 1.5 times compared with control was observed, whereas no significant change in this indicator was noted in intact animals under application of the test medicine (Table 2).

At the study of dry chicory extract effect on phagocytic activity of peritoneal macrophagocytes in intact mice in relation to colloid liquid ink particles it was found out, that this medicine do not cause any significant change in phagocyte index comparing with the data obtained in the intact group. If the medicine examined was administered to the immunodeficient animals, phagocyte index increase by 1.9 timed comparing with the data obtained in the control group was observed (Table 3).

Thus, the examination of immunomodulatory properties of dry chicory extract revealed its efficiency in relation to the reactions of cell, humoral and macrophagal components of immune response at the experimental immunodeficiency provoked with azathioprine cytostatic agent. Dry chicory extract applied in a dose of 30 mg/kg is capable to reduce the suppressive azathioprine effect on the cell-mediated immune response, antibody response and phagocytosis with macrophages. The medicine under study does not change the immune indices in intact animals. It should be noted that this property is inherent only to true immunomodulatory agents that are active in conditions of the immune system damage only. Efficiency of dry

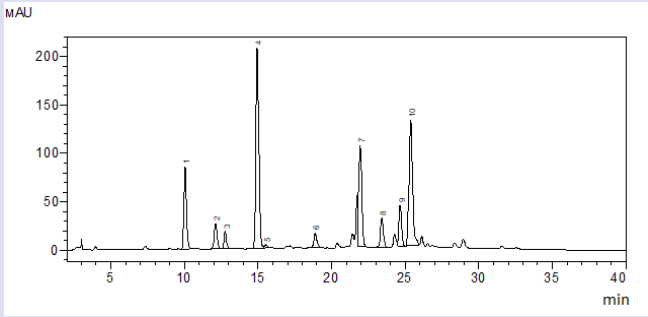

Figure 1: HPLC-UF-chromatogram obtained with chicory herb extract (360 nm).

Table 1: Dry chicory extract effect on antibody formation.

Groups of animals	Absolute AFC count in a spleen	AFC count in 10^6 splenocytes
Intact, n=10	46199±2203	272±22
Control (azathioprine), n=10	29328±2368*	163±10*
Experimental 1 (azathioprine + chicory extract), n=10	42022±3629**	252±12**
Experimental 2 (azathioprine + Immunal), n=10	41059±2763**	212±13**
Experimental 3 (chicory extract), n=10	40655±3821	244±18
Experimental 4 (Immunal), n=10	39842±2546	237±21

Note: P-values ≤0.05 represented significant differences between data: * – in the intact group, ** – in the control group; n – animal amount in a group.

Table 2: Dry chicory extract effect on the severity of the delayed hypersensitivity reaction (DHR).

Groups of animals	DHRI, %
Intact, n=10	37.4±2.56
Control (azathioprine), n=10	23.3±1.84*
Experimental 1 (azathioprine + chicory extract), n=10	35.5±2.27**
Experimental 2 (azathioprine + Immunal), n=10	30.7 ± 2.14**
Experimental 3 (chicory extract), n=10	33.92.46
Experimental 4 (Immunal), n=10	32.52.46

Note: P-values ≤0.05 represented significant differences between data: * – in the intact group, ** – in the control group; n – animal amount in a group.

Table 3: Dry chicory extract's effect on phagocytic activity of peritoneal macrophagocytes.

Groups of animals	Phagocyte index, opt. dens. units
Intact, n=10	0.680±0.043
Control (azathioprine), n=10	0.332±0.026*
Experimental 1 (azathioprine + chicory extract), n=10	0.623±0.038**
Experimental 2 (azathioprine + Immunal), n=10	0.498±0.025**
Experimental 3 (chicory extract), n=10	0.5970.024
Experimental 4 (Immunal), n=10	0.5840.037

Note: P-values ≤0.05 represented significant differences between data: * – in the intact group, ** – in the control group; n – animal amount in a group.

chicory extract efficiency is comparable to the effect of Immunal used as a reference drug.

CONCLUSION

Dry chicory extract contains a phenolic complex of biologically active substances that are presented by oxycoumarins, hydroxycinnamic acids, and flavonoids. The results of the immunomodulating activity examination confirm that dry chicory extract is an effective immunocorrecting agent; it can be recommended to further study and application for the prevention and treatment of diseases caused by human immune system disorders. Novelty and priority of the research is confirmed by the Russian Federation patent.[11]

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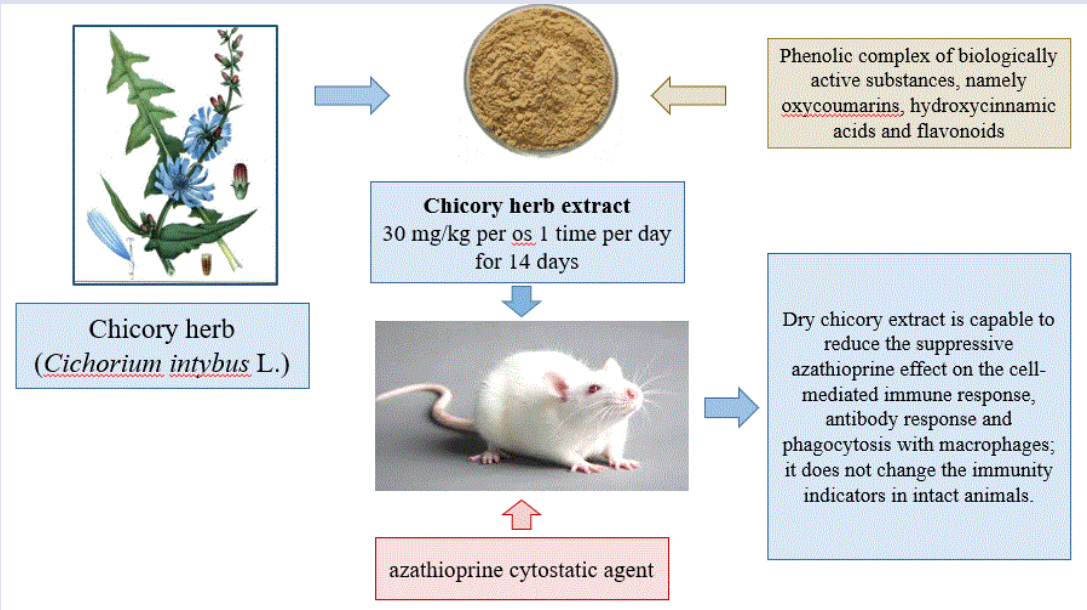
GRAPHICAL ABSTRACT



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