Immunotropic Activity of Plant Extract Echinosol

N. V. Isaykina, T. V. Perevozchicova, and G. I. Kalinkina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 8, pp. 188-191, August, 2008
Original article submitted August 2, 2007

The formulation of medicinal herbal tea Echinosol was developed. Immunotropic properties of aqueous-alcohol extract Echinosol were studied. This extract (300 mg/kg) had a stimulatory effect on the following parameters: formation of T cell precursors and production of T cell effectors; migration of lymphocytes, monocytes and neutrophils into the blood; digestive activity of neutrophils; migration of monocytes and lymphocytes into the peritoneal exudate; expression of C_{3b} receptors on macrophages; phagocytic capacity of cells due to the existence of C_{3b} receptors; number of antibody-producing cells in the spleen; and synthesis of immunoglobulins.

Key Words: *medicinal herbal tea; extract; immunotropic properties*

Adverse ecological conditions and stress factors contribute to immune dysfunction in humans. Synthetic, biotechnological, and natural medicinal preparations are used for the correction of immunological disorders. These drugs have a modulatory effect on the immune system and modulate the degree, type, and direction of immune reactions. Much recent attention is paid to plant immunocorrectors. These drugs are characterized by mild effect, low toxicity, allergenicity, and complex action on the organism. The preparations of bioactive substances from roots and herb of purple coneflower (*Echinacea purpurea*) are of particular interest in this respect. They are used in Russia and foreign countries. However, Russian pharmaceutical market is characterized by narrow assortment of these preparations. The majority of them are expensive imported medicinal agents [1]. It is necessary to develop inexpensive Russian drugs from purple coneflower and other plants, which should have a complex effect on the immune system. Medicinal herbal teas can satisfy these requirements.

Medicinal herbal tea Echinosol was developed on the basis of published data and results of clinical treatment with officinal medicinal plants. This preparation consists of purple coneflower herb, beggar-ticks herb, hill-growing saltwort herb, nettle leaves, licorice roots, and *Laminaria* thallus [3-5]. Liquid aqueous-alcohol extract Echinosol was obtained from this formulation by the rational method. The extract was standardized by the content of ethanol, extractive substances, and total hydroxycinnamic acids (relative to chicory acid and polysaccharides). These compounds determine immunotropic activity of the extract [6,7].

This work was designed to study immunotropic properties of aqueous-alcohol extract Echinosol from medicinal herbal tea.

MATERIALS AND METHODS

Experiments were performed on 162 adult male outbred albino mice weighing 18-20 g. The animals were maintained under standard vivarium conditions. The mice were divided into 5 groups (10-12 animals per group).

The animals were intraperitoneally immunized with 10% suspension of sheep erythrocytes (SE) in a dose of 0.2 ml. The extract in a dose of 300 mg/kg was administered intragastrically. The dose of this extract was comparable with the therapeutic dose of the reference drug Immunal. Control animals intragastrically received pure water (0.2 ml). The mice were decapitated under light ether anesthesia.

Siberian State Medical University, Russian Ministry of Health, Tomsk, Russia. ***Address for correspondence:*** nadezhda.isaykina@gmail.com. N. V. Isaikina

0007-4888/08/1462-0223

Activation of the humoral immune response was estimated from the number of leukocytes, B lymphocytes, and antibody-producing cells (APC) in the spleen and antibody formation in the blood. Activation of T cell immunity was evaluated from the delayed-type hypersensitivity response [10]. Changes in the nonspecific resistance were determined by the following parameters of phagocytosis: percent of active neutrophils and macrophages, phagocytic capacity of these cells, index of phagocytosis completeness, and expression of C_{3b} and $F_{c\gamma}$ receptors on macrophages (MP). Blood cells and peritoneal exudate cells were examined.

Nucleated cells of the spleen and peritoneal exudate were counted by the standard blood test. The following parameters were estimated: peripheral blood B lymphocyte count (modified micromethod) [8,10]; number of APC in the spleen (Cunningham method); hemagglutinin titer in blood plasma (Jg, reaction of hemagglutination) [10]; phagocytic activity of blood neutrophils (method of V. M. Berman and E. M. Slavskaya); $F_{c\gamma}$ receptors on MP [2]; and C_{3b} receptors on MP [9].

The results were analyzed by Student's *t* test and nonparametric Mann—Whitney test for two independent samples.

RESULTS

The course of intragastric treatment with Echinosol was followed by a significant increase in the number of monocytes (by 26%) and splenic APC (by 97%) and total Jg titer in blood plasma (by 75%). These data indicate that the extract has a strong effect on specific organism's resistance (Table 1). Examination of the peritoneal exudate revealed a significant increase in the number of monocytes (by 32%), lymphocytes (by 83%), C_{3b} receptor-expressing mononuclear cells (by 1.6 times), and phagocytizing macrophages (by 1.5 times) and completeness of neutrophil phagocytosis (by 1.7 times; Tables 2 and 3). Hence, course administration of the extract significantly increased the number of peripheral blood monocytes, peritoneal exudate lymphocytes, and splenic APC, plasma Jg titer, and parameters of macrophage and neutrophil phagocytosis. Our findings confirm the immunotropic effect of Echinosol.

Echinosol did not modulate the induction phase of the humoral immune response.

This extract significantly increased the number of blood monocytes and splenic APC during the production phase of the humoral immune response (by 34 and 44%, respectively; Table 1). Hence, Echinosol stimulates migration and accumulation of APC (antibody precursors) in the studied lymphoid organ.

The effect of Echinosol on humoral immune memory was evaluated after repeated immunization of animals. We revealed a significant increase in the number of stab neutrophils, segmented neutrophils, and monocytes in the peripheral blood of laboratory animals (by 90, 39, and 42%, respectively). The number of blood B lymphocytes decreased by 32% (Table 1), which was probably associated with cell migration in the spleen and differentiation to APC. Our findings indicate that repeated administration of the antigen after extract

TABLE 1. Immunotropic Effects of a Liquid Extract Echinosol (Cell Indexes of the Peripheral Blood and Humoral Immune Response)

Parameter	Stab neutrophils, 10^6/liter	Segmented neutrophils, 10^6/liter	Monocytes, 10^6/liter	Lymphocytes, 10^6/liter	B lymphocytes, 10^6/liter	APC, 10^6/organ	Jg, log T
Course of extract administration							
control	118.00±0.03	2669.0±0.4	345.10±0.05	2297.04±0.30	205.52±0.08	4.14±0.30	1.0±0.5
treatment	121.00±0.03	2464.0±0.5	436.12±0.06*	2361.03±0.40	200.33±0.06	8.15±0.30*	1.75±0.50*
Production phase							
control	96.40±0.06	1899.7±0.5	278.17±0.05	2539.08±0.50	319.76±0.06	4.61±0.50	5.05±0.50
treatment	119.40±0.04	2388.0±0.4	371.75±0.06*	3119.92±0.50	333.47±0.06	6.65±0.50*	4.97±0.50
Formation of humoral immune memory							
control	77.50±0.01	1965.2±0.5	275.00±0.06	3867.5±0.4	421.56±0.05	8.99±0.30	6.63±0.50
treatment	147.20±0.05*	2730.9±0.5*	390.88±0.06*	2725.2±0.5	287.44±0.06*	7.98±0.20	6.97±0.50

Note. Here and in Tables 2 and 3: *$p<0.05$ compared to the control.

TABLE 2. Peritoneal Exudate Cells and Parameters of Phagocytosis after the Course of Treatment with a Plant Extract Echinosol (Cytogram and Phagocytosis of Peritoneal Exudate Mononuclear Cells)

Group	Monocytes, 10^6/liter	Lymphocytes, 10^6/liter	MP, 10^6/liter	C_{3b} receptors on MP, %	$F_{c\gamma}$ receptors on MP, %	MP activity, %	Phagocytic activity of MP
Control	463.36±0.04	468.82±0.04	1963.82±0.10	6.66±0.50	8.05±0.10	34.0±0.9	7.13±0.20
Treatment	611.33±0.04*	856.44±0.04*	1698.67±0.10	10.94±0.40*	6.32±0.20	52.0±1.0*	6.5±0.2

TABLE 3. Phagocytic Activity of Blood Neutrophils

Group	Neutrophil activity, %	Phagocytosis completeness, %	Phagocytic activity of neutrophils
Control	22.00±1.00	38.00±2.00	4.88±1.00
Treatment	22.80±1.00	64.71±2.00*	4.89±1.00

treatment is followed by an increase in the number of blood monocytes, stimulation of migration in lymphoid organs, and cell differentiation.

The effect of Echinosol on T cell immunity was evaluated from the delayed-type hypersensitivity response. Immunal served as a reference drug [1]. After intragastric administration of Immunal and treatment with SE in the sensitizing dose, the delayed-type hypersensitivity response increased by 1.5 times compared to SE-sensitized animals (20.8± 1.0 and 13.92%, respectively). The delayed-type hypersensitivity response in mice receiving Immunal and sensitizing dose of SE was 1.5-fold lower (9.14±1.00%) than in SE-sensitized animals. Therefore, Immunal has a stimulatory effect on the formation of T cell precursors. After intragastric administration of Echinosol and treatment with SE in a sensitizing dose, the delayed-type hypersensitivity response decreased by 2 times (7.38±1.00%) compared to SE-sensitized animals. Administration of SE in a sensitizing dose to Echinosol-receiving mice was followed by 1.6-fold increase in the delayed-type hypersensitivity response (22.07± 1.00%). As differentiated from Immunal, Echinosol stimulated the formation of T cell precursors and T cell effectors.

We conclude that liquid extract Echinosol modulates various stages of the immune response. This extract increases the number of lymphocytes, monocytes, and neutrophils in the blood, stimulates migration of monocytes into the spleen and peritoneal exudate, increases the count of splenic APC and immunoglobulin synthesis during the production phase of the immune response, stimulates the expression of C_{3b} and $F_{c\gamma}$ receptors on peritoneal exudate macrophages and phagocytic activity of cells (through these receptors), and improves digestive properties of neutrophils. A liquid extract Echinosol stimulates the formation of antigen-specific T lymphocytes and mature T cells.

REFERENCES

1. *State Register of Medicinal Products* [in Russian], Moscow (2003).
2. V. M. Zemskov, S. V. Rodionov, V. I. Pantin, *et al*., *Immunologiya*, No. 6, 53-56 (1985).
3. N. V. Isaikina, G. I. Kalinkina, and V. V. Klimov, *Novaya Apteka*, No. 3, 57-61 (2002).
4. N. V. Isaikina, V. V. Klimov, and G. I. Kalinkina, *Byull. Sib. Otd. Ros. Akad. Med. Nauk*, No. 3, 15-18 (2001).
5. T. L. Kiseleva, A. V. Chauzova, and A. A. Karpeev, *Farmatsiya*, No. 2, 15-18 (2000).
6. V. A. Kurkin, *Phenylpropanoids as Promising Natural Biologically Active Compounds* [in Russian], Samara (1996).
7. V. A. Kurkin, O. I. Avdeeva, E. V. Avdeeva, and P. G. Mizina, *Rast. Resursy*, **34**, No. 2, 81-85 (1998).
8. D. K. Novikov and V. I. Novikova, *Study of the Immune Status* [in Russian], Moscow, Vitebsk (1996).
9. I. S. Freidlin, *Mononuclear Phagocyte System* [in Russian], Moscow (1984).
10. R. M. Khaitov, I. S. Gushchin, B. V. Pinegin, *et al*., *Vedom. Farmakologich. Kom.*, No. 1, 31-36 (1999).