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NATURAL DRUGS

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ESTIMATION OF HUMORAL ACTIVITY OF  
ELEUTHEROCOCCUS SENTICOSUS

JANINA DROZD, TERESA SAWICKA and JOANNA PROSIŃSKA

Department of Biological Preparations, Drug Institute,  
30/34 Chełmska Str., 00–725 Warszawa

**Abstract:** The aim of the present work was an estimation of the influence of two plant pharmaceutical preparations containing an extract from the root of *Eleutherococcus senticosus*: Argoeleuter tablets and Immunplant tablets, on the humoral response of immunological system. Experiments were performed with female Balb/c mice six weeks old. In order to reveal the influence of taking preparations, containing an extract from *Eleutherococcus senticosus* on some elements of the immunological system, three ways of their administration have been compared: before illness, during illness and a combination of both.

The obtained results allow formulating the following conclusions:

- the pharmaceutical preparations, containing the extract from *Eleutherococcus senticosus* administered orally, influence on the increase of the level of immunoglobulins comprised in the mice's blood serum,
- the pharmaceutical preparations act with different power, not fully dependent on the content of marker of the active substance – eleutheroside E,
- dosage of the preparations containing the extract from *Eleutherococcus senticosus* should not be established basing only on the extract content,
- best curative results, measured as the stimulation of humoral response of the organism were obtained when a given preparation was administered therapeutically, even though the combined administration – prophylactically with prolonged administration during illness also is correct.

**Keywords:** *Eleutherococcus senticosus*, eleutherosides, immunoglobulins, humoral response, direct hemagglutination.

At present, according to the dominating classification, three different plants are commonly called ginseng. They are *Panax ginseng* (PG), *Panax quinquefolium* (PQ) and *Eleutherococcus senticosus* (ES). Some authors include to them also the fourth – *Tienchi ginseng* (1).

*Eleutherococcus senticosus*, also called Siberian *Panax*, belongs to the plant family – Araliaceae and is growing naturally in East Asia and Russia. It is a raw material for the preparation of extracts applied in the traditional medicine, particularly in Chinese medicine, and beside *Panax Ginseng* is considered to be a panacea for all diseases of body and soul. The advanced studies, lead since the fifties of the last century in the Soviet Union and later in Japan have allowed for more close chemical characteristics of main components of the extracts from *Eleutherococcus* – eleutherosides.

The main active substances of PG and PQ are compounds of triterpenes saponin character called panaxosides (in the former Soviet Union) and ginsenosides (in Japan). Ginseng stimulates biosynthesis of protein and RNA, demonstrates antitumour properties, inhibits aggregation of thrombocytes, exhibits hypoglycaemic action (2).

The therapeutic raw material of ES is rhizomes and stolons dig out in late autumn. It stimulates the human organism, increases intellectual and physical efficiency, improves appetite and memory. It can be used externally to wounds after surgery (3). The active substances are not triterpenes but glycosides of phenyl compounds, among them syringins called eleutherosides (4). Of particular importance in the modulation of immunological response seems to have eleutherosides B, E and F, although there are opinions that the best action is obtained by applying the extract from the whole, non-fractionated, rhizome. For example, the ethanol extract induces, under the in vitro conditions (rats), an increase in the generation of IL-1 and IL-6, but not influences on the generation of IL-2, while the eleutherosides B and E isolated from the extract did not show the above effect (5). Gaffney et al., while trying to explain the „adaptive” mechanism of ES activity, have found that eleutherosides binding to receptors for stress hormones block the activity of catechol-o-methyl transferase which is involved in the degradation of the mentioned hormones. In this way they can modulate the intensity of stress (7).

The preparations, which demonstrate the

so-called adaptive abilities, exert their influence on the organism first of all through the immunological system. Their action should be revealed by the influence on main immunological reactions of the organism, i.e. the cell-mediated immunity and/or humoral response. Of particular importance would be the confirmation of the tonic influence of ES on the formation of immunoglobulins because to generate this response cooperation of both the main immunological cells – lymphocytes T and B is necessary. The humoral response allows destroying exogenous molecules present in the organism in the free state (extraventricular) (8). After interaction with a specific antigen lymphocyte B undergoes activation. At its surface receptors occur for growth factors which stimulate lymphocyte B to proliferation and expression of receptors for differentiation. In the course of primary immunological response, lymphocytes B differentiate in two directions:

- in the plasmocytes which intensively generate and release immunoglobulins,
- in the memory cells, which at their surface possess immunoglobulins receptors and are ready to eventual secondary response by a renewed contact with this antigen (8).

Immunoglobulins produced by lymphocytes B are capable to specific reactions with antigens. During proliferation and independent of antigen differentiation, lymphocytes B produce immunoglobulins of M class (pentamers). However, at the stage of antigen – dependent differentiation, a synthesis of IgG is initiated. In response to the so called thymus dependent antigen (e.g. xenogeneic erythrocytes or serum proteins, hapten-carrier complexes) lymphocytes T play a role of helper cells which activate B cells to differentiation. It would be of interest to confirm the influence of ES on the formation of immunoglobulins.

Immunoglobulins are glycoprotein composed of polypeptide chains (82–96%) and carbohydrates (4–18%). The molecules of antibodies perform a double function, basic i.e. bind of antigen and release of a number of reactions independent of antigenic specificity (e.g. reaction with a complement, with cell receptors).

Immunoglobulins of IgG class appears as the first one in phylogenesis and ontogenesis. Regarding its structure, it is a pentamer of m.w. 970 kDa and a sedimentation constant – 19S. This is about 10% of general pool of immunoglobulins. Antibody of IgG class is a dimer of m.w. 147 kDa and sedimentation constant 6–7S, amounting to 70–75% of general quantity of immunoglobulins and demonstrating great differentiation in structure and function (four subclasses) (8).

Biological meaning of the connection of an antibody with an antigen is not limited only to neutralize and elimination of the antigen from the organism. These complexes take part in the regulation of different links of immunological response: cooperate with non-specific cells of immunological reaction, influence on the chemotaxis of micro- and macrophages and accelerate activation of macrophages (9).

Adaptive action of ES should manifest itself most clearly in organisms characterized by immune-deficiency, leading to increase susceptibility to infection, particularly of the respiratory track. One of the possible ways of treatment of light form of deficiency could be preventive administration of substances whose task would be a non-specific stimulation of the immune system.

*Eleutherococcus senticosus* as a pharmaceutical preparation is available in the form of ethanol or ethanol-water extracts, dry powders, capsules or tablets and also teas from the dried and cut rhizome (10).

The aim of the present work was to study the influence of available on the market pharmaceutical preparations containing the extract from EC on the humoral response of the mice's organism.

## EXPERIMENTAL

### Materials

Pharmaceutical preparations *Argoeleuter* tablets and *Immuplant* tablets containing in their composition *Eleutherococcus senticosus* radix sicum as an active substance were the subject of these studies.

Each of the preparations was prepared in two doses: the first, corresponding exactly (after taking into account the differences in the body weight and metabolism of the rodents) to the maximal dose recommended according to the leaflet for the patients, and the second one, considerably different from the first dose:

- *Argoeleuter* tablets – a dose R and a five-fold lower R1,
- *Immuplant* tablets – a dose D and a three-fold higher D1.

Solutions of both preparations were prepared by dissolving one tablet in a proper quantity of PBS (Biomed, Lublin). The daily dose given to the mice contains about 0.5–2.5 mcg of eleutheroside E. The preparation were administered orally once a day in the quantity of 10–20 mcl. Female Balb/c mice, six weeks old, weighting about 18–20 g have been used for experiments.

Experiments were also carried out for placebo tablets of both preparations. Placebo contain-

ning all the components except the extract from *Eleutherococcus* was prepared and administered to mice in an identical way as the studied preparations.

All the solutions were prepared daily *ex tempore*.

Argoeleuter tablets were applied in three schemes: preventive treatment (P), treatment (T) and preventive treatment + treatment (P+T). Immuplant tablets were investigated in the scheme preventive treatment + treatment (P+T) only.

Mice from groups P or T received Argoeleuter in one of the two doses during 6 successive days. The animals from group P+T received Argoeleuter or Immuplant during 12 (2×6) days with one-day break. Placebo was examined only in the dose corresponding to solutions R and D. Mice from the control group were treated in an identical scheme and received 10 mcl PBS instead of the solution of the studied preparation.

Mice were immunized by peritoneal application of 0.2 ml of 10% SRBC ( $4 \times 10^8$  sheep erythrocytes in 1 ml of PBS) suspension stabilized during 3 days in an Alsever liquid.

## Method

Agglutination's reaction, consisting of specific association of antibodies (agglutinins) with antigen, for instance, erythrocytes, was used in a standard direct hemagglutination test according to Adler (11) and applied in own modification (12).

The cells forming complexes with specific antibodies combine in large aggregations called agglutinates and precipitate from the solution in the form of floccules.

As the titre of the agglutination of serum (estimated quantity of antibodies in serum) its maximum dilution was considered, in which agglutina-

tion appears still (microscopic evaluation). In serum subjected to the action of 0.1 M 2-mercapto-ethanol (DMSO, Sigma) the level of antibodies, class IgG (7S) was determined, while in serum without DMSO the level of IgM and IgG sum.

The Student t-test, calculating standard deviation and the significance of differences between the unpaired groups verified the determination results (number of a well). The value of stimulation index, i.e. the quotient of number of the examined well and number of control well was also calculated.

## RESULTS

### Argoeleuter tablets

#### Preventive treatment

In Table 1 results of the determination of titre of the antibodies in the serum of mice from the prophylactic group are presented: the total sum of IgM+IgG immunoglobulins and IgG immunoglobulins.

Both the studied batches of Argoeleuter tablets equally influence on the statistically significant, about a double increase of level of both: the sum of antibodies and the IgG alone. No difference between the examined doses has been found.

#### Treatment

In Table 2, in a similar manner results of the determination of antibodies in the sera of mice from the treatment group are presented: the total sum of IgM+IgG immunoglobulins and IgG immunoglobulins.

Administration of Argoeleuter tablets prior to the action of stimulus (antigen) similarly influenced on increase of the sum of antibodies in the

Table 1. Influence of Argoeleuter tablets on the production of anti-SRBC antibodies in Balb/c mice serum – preventive treatment

Class of antibody	Mean $\log_2$ titre $\pm$ SD number of mice / stimulation index <sup>#</sup>				
	Control group	Argoeleuter			
		batch a		batch b	
		R	R1	R	R1
IgM + IgG	8.62 $\pm$ 0.738 19 / 1	9.65 $\pm$ 0.777 10 / 1.12**	9.89 $\pm$ 0.481 13 / 1.15*	9.89 $\pm$ 0.525 13 / 1.15*	9.83 $\pm$ 0.514 12 / 1.14*
IgG	8.69 $\pm$ 0.821 21 / 1	9.64 $\pm$ 0.526 11 / 1.11**	9.73 $\pm$ 0.373 13 / 1.12**	10.00 $\pm$ 0.392 13 / 1.15**	9.85 $\pm$ 0.533 13 / 1.13**

\*  $p < 0.01$ ; \*\*  $p < 0.005$ ; <sup>#</sup> the quotient of number of tested and control well

Table 2. Influence of Argoeleuter tablets on the level of anti-SRBC antibodies in Balb/c mice serum – treatment

Class of antibody	Mean log <sub>2</sub> titre ± SD number of mice / stimulation index <sup>#</sup>				
	Control group	Argoeleuter			
		batch a		batch b	
		R	R1	R	R1
IgM + IgG	5.29 ± 0.700 7 / 1	6.63 ± 0.820 8 / 1.25*	6.43 ± 1.116 7 / 1.22	6.00 ± 0.901 8 / 1.13	6.31 ± 0.659 8 / 1.19*
IgG	2.57 ± 1.050 7 / 1	5.56 ± 0.634 8 / 2.16**	5.29 ± 0.921 7 / 2.06**	4.63 ± 1.453 8 / 1.80*	4.13 ± 0.781 8 / 1.61*

\* p < 0.02; \*\* p < 0.0005; <sup>#</sup> the quotient of number of tested and control well

Table 3. Influence of Argoeleuter tablets on the production of anti-SRBC antibodies in Balb/c mice serum – preventive treatment and treatment

Class of antibody	Mean log <sub>2</sub> titre ± SD number of mice / stimulation index <sup>#</sup>				
	Control group	Argoeleuter			
		batch a		batch b	
		R	R1	R	R1
IgM + IgG	6.44 ± 0.497 9 / 1	7.80 ± 0.332 10 / 1.21**	7.67 ± 0.408 9 / 1.19**	8.30 ± 0.600 10 / 1.29**	8.33 ± 0.745 9 / 1.29**
IgG	6.22 ± 0.786 9 / 1	7.65 ± 0.450 10 / 1.23**	7.28 ± 0.343 9 / 1.17*	7.44 ± 0.832 9 / 1.20*	7.67 ± 1.080 9 / 1.23*

\* p < 0.01; \*\* p < 0.0002; <sup>#</sup> the quotient of number of tested and control well

Table 4. Influence of Argoeleuter placebo on the production of anti-SRBC antibodies in Balb/c mice serum

Class of antibody	Mean log <sub>2</sub> titre ± SD number of mice		
	Tested group		
	Control	Preventive treatment	Treatment
IgM + IgG	7.56 ± 1.044 / 8	7.94 ± 0.437 / 9	7.78 ± 0.671 / 9
IgG	6.63 ± 0.740 / 8	6.67 ± 0.624 / 9	6.17 ± 0.471 / 9

serum of mice, as it was observed in the group of animals receiving the preparation in the preventive treatment scheme. At the same time, significant differences have been observed in the level of IgG immunoglobulin compared with the control animals.

#### Preventive treatment + treatment

Table 3 contains results obtained for the animals, which were given Argoeleuter tablets pro-

phylactically, and then after immunization as the medicament.

Administration of Argoeleuter tablets in a mixed system causes a negligible enhancement of the effects observed separately for the preventive treatment and treatment groups. The stimulation index for the sum of antibodies is here higher (about 1.2–1.3 times) than that for the prophylactic (about 1.12–1.15 times) or treatment (about 1.13–1.22) scheme. „B” batch is somewhat more active than „a” batch.

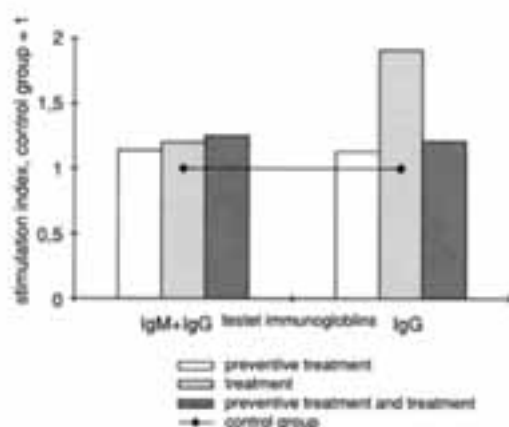


Figure 1. Influence of the Argoeleuter tablets on the production of anti-SRBC antibodies in Balb/c mice serum: IgM+IgG and IgG.

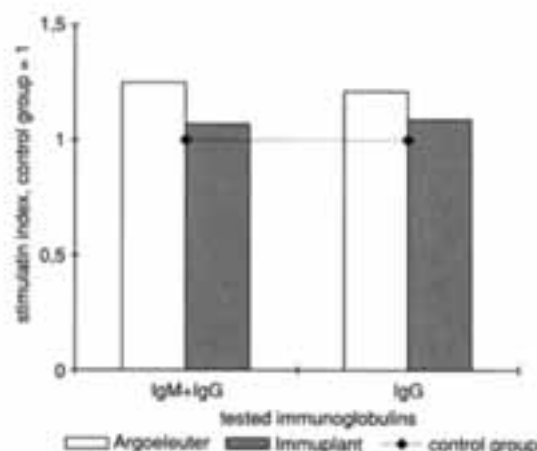


Figure 2. The comparison of influence of two pharmaceutical preparations Argoeleuter tabl. and Immuplant tabl. on the production of IgM+IgG and IgG antibodies in Balb/c mice serum – preventive treatment and treatment.

Table 5. Influence of Immunal preparation on the production of anti-SRBC antibodies in Balb/c mice serum

Class of antibody	Mean log <sub>2</sub> titre ± SD number of mice / stimulation index <sup>#</sup>			
	Tested group			
	Control	Immunal placebo	Immunal tablets	
			D I	D II
IgM + IgG	7.67 ± 0.112 12 / 1	7.25 ± 0.305 12 / 0.94	8.35 ± 0.182 13 / 1.09*	8.00 ± 0.127 13 / 1.04
IgG	7.38 ± 0.152 12 / 1	6.96 ± 0.238 11 / 0.94	8.00 ± 0.123 12 / 1.08*	8.04 ± 0.155 13 / 1.09*

\* p < 0.005 in compare to control group, <sup>#</sup> the quotient of number of tested and control well

There is no significant difference between the studied doses.

#### Placebo tablets

No essential differences have been found in the level of antibodies of both the IgM+IgG sum and the IgG alone in the serum of mice which prophylactically or therapeutically were given placebo. A set of the obtained results is presented in Figure 1 (average values within both batches and doses).

#### Immuplant tablets

##### Preventive treatment + treatment

The determination results of titre of the antibodies in the serum of mice are presented in Table 5.

The administered Immuplant tablets in the proposed, combined system caused only a weak increase in the level of immunoglobulins in the serum of mice. The statistically significant stimulation index for the sum of antibodies was obtained only for the dose D of Immuplant tablets, while for IgG alone this index considerably increased when the animals were given Immuplant in both doses.

#### DISCUSSION

Numerous plant raw materials applied in official and popular medicine are not included in the pharmacopoeia's requirements. In this situation, there is an urgent necessity to perform biological investigations in order to determine their biological activity and, through this, also therapeutic quality.

This particularly concerns cases in which the chemical determination of active components not always is in line with the biological activity of raw material (3).

Gershwin et al. have performed comparative studies on commercially available in the USA 25 preparations containing PG and PQ or ES and determined the content of 7 ginsenosides and 2 eleutherosides by the HPLC method and mass spectrometry. They have found that the real content of the above markers considerably differs from the declaration of the producers. Between seemingly similar preparations the concentration of ginsenosides varied by 15 and 36-fold in capsules and liquids, respectively, while the concentration of eleutherosides varied by 43 and 200-fold for capsules and liquids, respectively (14). These data are very alarming, taking into account that, basing on the analysis of physician's recipes accomplished in pharmacies in the USA, France and Poland, we know that the participation of natural medicaments is amounting to 30–40%.

The problem of *Panax* influence on the level of immunoglobulins in blood serum is not considered in the available literature. One of the papers dealing with the problem is an old report of Czubajev et al., who comparatively estimated the influence of extracts from PG and ES on the level of immunoglobulins in the mice's serum (15). They confirmed its adaptive activity – lack of influence of both extracts on humoral response in healthy animals, and a strong stimulation of humoral activity in a mouse with deficiency of immunological system caused by administration of cyclophosphamide.

The animal model applied in this study is imitating the situation of using a pharmaceutical preparation in humans in the following triple manner: a) preventive, prior to the appearance of infection, b) therapeutically, when infection already appeared and c) a combination of both situations. The difference between two first mentioned groups consists in a different term of carrying out of immunization, i.e. introduction of an antigen (erythrocytes of a sheep) into mice organisms. For a) group after 6 days of administration of the preparation immunization follows, while for b) group proceeds administration of the studied solution. In c) group immunization takes place during administration of the preparation.

To perform experiments, solutions of the studied preparations were prepared from the point of view of similar content of the extract from ES, recounted for eleutheroside E. The investigated batches of Immuplant tablets contained about two times more of eleutheroside than the batches of

Argoeleuter tablets. This was compensated by a proper dilution, so that both pharmaceuticals were prepared in such a way that the mice received approximate quantities of eleutheroside E (Immuplant 0.8 and 2.4 mcg/mouse/day, Argoeleuter – 0.5 and 2 mcg/mouse/day). However, the obtained results of the estimation of biological activity are showing that preparations of a similar content of the marker of active substance – eleutheroside E act with different power. The biological activity of the extract applied to the manufacture of Argoeleuter tablets is higher than that of Immuplant tablets. This can easily be seen while comparing the activity of both preparations in the preventive treatment + treatment scheme (Figure 2, averaged results from two batches and both doses).

An important observation resulting from this work is also the fact that for both pharmaceuticals there is no significant difference between the applied doses, which means that similar effects can be obtained taking a smaller number of tablets. Thus, the dosage of Immuplant tablets seems to be better arranged than that of Argoeleuter preparation. Argoeleuter tablets exerts a positive influence on the generation of immunoglobulins independently of the administration scheme. However, best results are obtained by applying it in parallel to the administration of antigen, which imitates the appearance of disease. Thus, prophylactic administration before a disease is not necessary. If, however, the preparation is taken prior to disease, then in the course of it appearance the administration of the medicine should be continued.

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