

## NATURAL DRUGS

### THE EFFECT OF THE *MELISSA OFFICINALIS* EXTRACT ON IMMUNE RESPONSE IN MICE

JANINA DROZD and ELŻBIETA ANUSZEWSKA

Department of Biochemistry and Biopharmaceuticals, National Institute of Public Health,  
30/34 Chełmska Str., 00-725 Warszawa

**Abstract:** The effect of an extract from *Melissa officinalis* on immune response in mice was analysed using the cytotoxicity test in three dilutions (undiluted extract and extract diluted 10 and 100 times) and hemagglutination and rosette tests with various routes of administration (oral and subcutaneous). The immunostimulating activity of the extract was compared with that of a synthetic compound – levamisole, which influence on the immune system is well known. The present results confirm the effect of water extracts from leaves of *Melissa* on the immune system, in both humoral and cellular response.

**Keywords:** *Folium Melissa*, humoral and cellular immune response

Previous research conducted in our laboratory showed that herbal raw materials and their active compounds have considerable immunostimulating activity *in vitro* and *in vivo* (1–6).

The immunostimulating activity of herbal raw materials was compared with that of synthetic compounds, which have a known effect on the immune system, such as levamisole and isoprinosine (7), and the combined effect of antibiotics and ellagic acid on steroid resistance of mouse thymocytes was tested (8, 9).

Herbal raw materials containing biologically and chemically active compounds such as polyphenolic acids may be a very interesting group of substances considering their immunological activity. One of such materials is *Melissa officinalis* containing substantial amounts of caffeic acid and chlorogenic acid. The sedative effect of *Melissa officinalis* is widely known but it was also proven, that its water extracts, comprising among others easily soluble tannins and polyphenolic acids, have clear antibacterial and even antiviral properties. Until now the possibility of immunostimulating activity of this material has not been considered.

The experimental object was *Melissa officinalis* of the *Lamiaceae* (*Labiatae*) family (10), which leaves (*Folium Melissa*) are used as a material. Apart from the leaves the shoot apexes are sometimes collected (*Herba Melissa*). The material contains ethereal oils (geraniol, nerol, citronellal, linalool) phenolic acids (rosemary, caffeic, ferulic, chlorogenic), triterpene acids (ursolic and oleanolic), flavonoids and minerals.

Ethereal oil acts spasmolytically and sedatively on the central nervous system and it also has bacteriostatic and virostatic properties. Polypheno-

lic substances and their ester compounds have antiviral properties as well, being especially effective towards *Herpes* virus (a component of herpes ointment); moreover their biligenic properties and stimulation of gastric juice secretion were described. *Folium Melissa* also used as a sleep-inducing drug, tranquilizer in vegetative neurosis and it reduces the concentration of thyrotropic hormone.

The purpose of the present study included experiments which allowed us to demonstrate the immunomodulating effect of pharmacopoeial extract from *Melissa officinalis* leaves. To attain this, three tests were used: cytotoxicity, active hemagglutination and E rosette formation test.

The cytotoxicity test enables the estimation of influence of a particular agent on viability of mouse thymocytes in 18–20 h cultures with hydrocortisone. Immature thymocytes have steroid receptors on their surface, what makes them sensitive to lytic properties of hydrocortisone, inducing the active process of autodestruction leading to apoptosis (11, 12). During maturation and the acquisition of immunocompetence, the cells lose those receptors and become steroid resistant. Thymocyte cultures are a good model system for testing various factors, which may accelerate the maturation of T lymphocytes.

Active hemagglutination test is based on the response of specific binding of the antibodies with antigen such as erythrocytes (13). After coupling with their specific antibodies, the erythrocytes form complexes called agglutinins.

E rosette formation test is grounded on spontaneous formation of rosettes of ram erythrocytes around the lymphocytes.

In order to rule out the influence of the route of administration, the extract from *Melissa of-*

*ficinalis* leaves in the hemagglutination and rosette tests was administered orally and subcutaneously.

## EXPERIMENTAL

### Chemicals

Hydrocortisonum hemisuccinatum (HC) 500 mg, from Jelfa Poland; Levamisole – levamisole hydrochloride, substance, from Sigma USA; culture medium containing Hepes 20 mM/l, RPMI-1640, IITD Wrocław Poland; phosphate buffered saline, PBS, IITD Wrocław; Hank's buffer (with 0.5% lactoalbumin hydrolyzate), IITD Wrocław Poland; Ram erythrocytes (SRBC), Biomed, stabilized in Alsewer buffer Poland; Alsewer buffer: glucose (POCH), sodium citrate (POCH), sodium chloride (POCH), citric acid (POCH) Poland; fetal Calf serum, Bioprodukt Hungarn; trypan blue, substance, Merck Germany.

### Animals

Mice from inbred line Balb-c, females (Ethical Commission permission No. ZT/1/2001, ZT/4/2001, ZT/6/2001).

### Plant raw material

Folium Melissae, Biofix, Tuszyn  
Water extracts were prepared from the plant raw material according to the procedure described in P.Ph. IV. Undiluted extracts and extracts diluted with PBS buffer 10 and 100 times were used in the experiments.

## METHODS

### Cytotoxicity test

Thymocytes obtained from homogenized thymus glands were washed three times with RPMI buffer and centrifuged (1800 rpm, 7–8 min). After the last centrifugation, the thymocytes at concentration  $4 \times 10^6$  were suspended in the RPMI buffer enriched with 10% bovine foetal albumin. The suspension was poured into sterile test tubes and 10  $\mu$ l of the extracts was added to each test tube. After 1 h, HC was added at the concentration of 50  $\mu$ g per 1 ml of thymocytes suspension. Samples prepared this way were incubated for 18–20 h in 37°C, in the atmosphere containing 5% CO<sub>2</sub>. After the incubation period, the cells were stained with 0.04% trypan blue and the number of living and dead cells was determined with use of microscope.

Statistical analysis of the obtained results was performed with the use of the computer program Medistat (14) and the arithmetic deviation and

statistical significance of differences for two related samples were calculated (the percent of relative survival rate in the control was considered as 100%).

### Active hemagglutination test

To immunize the mice, ram erythrocytes (SRBC) at concentration  $4 \times 10^8$  were used. On the first day, the animals were given orally and subcutaneously the water extracts from leaves of *Melissa* two hours before immunization. The extracts have been administered for the next three days. On the fifth day from immunization blood samples were taken and the serums were prepared for analysis. Subsequently the suspension of 1% SRBC was added to the prepared serum dilutions.

The serum agglutination titer (estimated amount of antibodies in serum) was taken as the maximum sera dilution, where agglutination was still observed under microscope.

### E rosette formation test

The mice used in hemagglutination test were also the source of splenocytes. The splenocytes obtained from the spleen were properly prepared, exposed to 1% SRBC suspension in Hanks buffer and afterwards the percentage of cells around which rosettes were formed was estimated under microscope. The rosette was considered as a splenocyte surrounded by at least three ram erythrocytes.

Experimental groups (rosette and hemagglutination tests):

1. control – (PBS) subcutaneous administration;
2. investigated sample – subcutaneous administration;
3. control – (PBS) oral administration;
4. investigated sample – oral administration

The experimental design used in the rosette and hemagglutination tests was expected to demonstrate the possible differences in immunological activity connected with the route of administration of the investigated extract.

## RESULTS AND DISCUSSION

The immune mechanisms of resistance to infection can be divided into (15):

- non-specific (innate) immunity, which is composed of: mechanical barrier i.e. intact skin and mucous membranes, humoral (lysozyme, low pH) and mechanical (cilia movement, washing the surface by tears) agents acting on body surface, humoral agents acting in cells and cellular fluids (interferons, complement system, acute phase proteins), macrophages, NK cells, granulocytes;

– specific (acquired) immunity composed of two immunological responses: humoral response – dependent on formation of specific antibodies, which neutralize the pathogenic microbe; and cellular response, in which clones of helper and cytotoxic T-lymphocytes recognize the antigens of microorganisms.

The mechanisms of immune response may be altered not only by synthetic compounds but also by natural compounds, called biomodulators (16).

Herbal raw materials are drugs of complex chemical composition (10) and usually all the substances contained in the material have a biological effect. But sometimes only the final product of degradation (created during plant material storage) shows the biological activity, which makes herbal raw materials useful. The herbal raw materials, which have been used for a long time in the treatment of various conditions (of digestive and respiratory systems, skin diseases, metabolic and others), have been used in recent years as immunomodulating drugs.

There are two types of immunomodulating activity – intensifying (stimulating) and inhibiting (suppressive). It is a simplified division, because some biological compounds affect the immunological response in both stimulating and suppressive way, and it depends on the dose, application regimen, experimental model (*in vitro*, *in vivo*) and the state of immune reactivity of the body.

The results presented hereafter, investigating the effects of extract from *Melissa officinalis* leaves on immune activity, confirmed the influence of water extracts from leaves on the immunological system, in both the humoral and cellular response.

Table 1 shows the viability of mouse thymocytes from Balb-c line, growing in the presence of hydrocortisone, after addition of undiluted and 10 and 100 times diluted extract from *Melissa officinalis* leaves. The control cultures were growing in the presence of hydrocortisone only and the viability of thymocytes from these cultures was considered as 100%. The addition of *Melissa officinalis* extract increased statistically thymocytes viability for dilutions 10x and 100x, but at the same time thymocyte viability did not differ much from further dilutions.

The immunostimulating activity of *Melissa officinalis* leaves extract was compared with the activity of a synthetic immunostimulator – levamisole (Figure 1).

Table 2 presents the results of the effect of *Folium Melissa* extract on the number of splenocytes forming spontaneous E rosettes, depending on the way of administration. The extract diluted 10x was used, because this dose generated the highest stimulation in the cytotoxicity test. The

Table 1. The percentage of thymocyte viability after addition of *Folium Melissa* extracts in 18–20 h cultures previously treated with hydrocortisone

Extract dilution	<i>Folium Melissa</i>
Undiluted	109.9±9.19 n=19**
Diluted 10 x	118.6±12.32 n=21*
Diluted 100 x	112.8±11.35 n=13***

\*p<0.0001 \*\*p<0.001 \*\*\*p<0.005

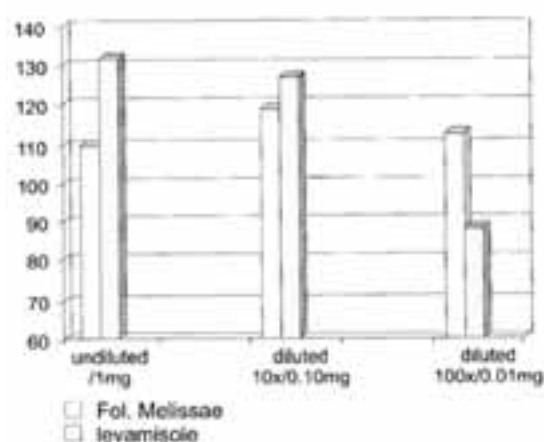


Figure 1. The effect of *Folium Melissa* extract on thymocyte cultures *in vitro* in comparison with levamisole

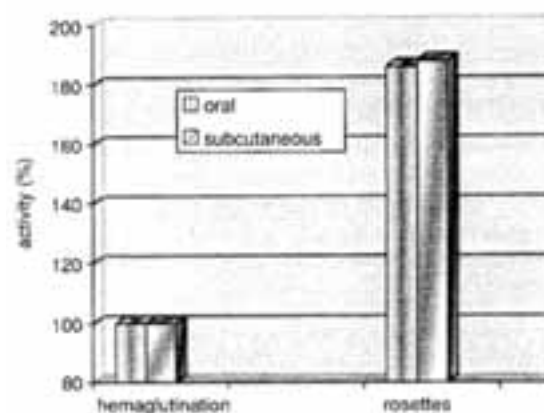


Figure 2. The comparison of immunological activity of *Melissa officinalis* extract depending on the route of administration (oral or subcutaneous) in active hemagglutination and E rosette formation tests

control group were mice receiving only PBS.

Table 3 displays the results of the influence of *Folium Melissa* extract on the titer of anti-SBRC antibodies in the active hemagglutination test. The results indicate that there is no immunomodulating activity irrespective of the route of administration.

Table 2. Immunological activity of *Melissa officinalis* extract diluted 10 x determined in E rosette formation test

Route of administration	<i>Folium Melissa</i>
Oral	185.9±34.90 n=14*
Subcutaneous	188.3±37.53 n=16*

\*p&lt;0.0001

Table 3. Immunological activity of *Melissa officinalis* extract diluted 10 x determined in the hemagglutination test

Route of administration	<i>Folium Melissa</i>
Oral	100.0±0.00 n=14
Subcutaneous	99.9±8.66 n=15

Data from Figure 2 and Tables 2 and 3 suggest that the extracts enhance the splenocyte ability to associate with ram erythrocytes, regardless of the route of administration (orally – 186%, subcutaneously – 188%). Neither has the route of administration any effect on the increased titer of anti-SBRC antibodies in the active hemagglutination test. Thus, in practice, the oral route of administration may be of greater importance, as simpler and not requiring any additional equipment (syringe).

There are two basic types of immune response: humoral and cellular.

Free antibodies present in blood, lymph and mucous and serous excretions are the key elements of the humoral response. In the first phase, B lymphocytes synthesize antibodies of the IgM class (in the shape of pentamers). Then, B lymphocytes begin the production of another classes of antibodies, usually IgG (as monomers), more specific to the offending antigen. To determine the titer (level) of antibodies produced in response to the applied antigen (ram erythrocytes), the active hemagglutination test was used. The amount of antibodies detected 5 days after immunization was the sum of antibodies from IgM and IgG classes. At first, a production of IgM immunoglobulins is observed and then the immunoglobulins from the IgG class appear.

In the cellular response, the antigen is directly bound by the cells, T-lymphocytes. The cytotoxicity test performed on mouse thymocytes allowed us to estimate the effect of exogenous factor on the central lymphatic organ – the thymus. T-lymphocytes mature in the thymus and under the influence of certain substances they acquire the competence necessary in their mature

form. This phenomenon is known as immunocompetence.

One of the characteristic features of human T-lymphocytes is their ability to form rosettes with ram erythrocytes. On the basis of this property, E rosette formation test were used, which allowed us to assess the immunological activity in the cellular type response.

Usually a particular antigen induces cellular and humoral response simultaneously. Which of the two responses prevails, depends on the form of antigen and the manner and way of stimulation.

The stimulation of immunological activity observed in cytotoxicity and rosette tests and its lack in active hemagglutination test may suggest a different mechanism of immunological action of *Melissa officinalis* extracts in each of the applied tests.

## REFERENCES

1. Sawicka T., Drozd J., Prosińska J., Borkowski B.: *Herba Pol.* 40, 31 (1994).
2. Drozd J.: *Biul. Inst. Leków* 4, 60 (1997).
3. Sawicka i wsp.: *Terapia* 3 (2), 43 (2001).
4. Prosińska J., Sawicka T., Drozd J.: *Acta Polon. Pharm.–Drug Res.* 59, 267 (2002).
5. Drozd J., Sawicka T., Prosińska J.: *Acta Polon. Pharm.–Drug Res.* 59 (2002).
6. Drozd J., Anuszevska E.: *Acta Polon. Pharm.–Drug Res.* 60, 3, 199 (2003).
7. Drozd J., Tautt J.: *Biul. Inst. Leków* 46 (1/2), 43 (2002).
8. Prosińska J., Sawicka T., Drozd J.: *Biul. IL* 4, 60 (1997).
9. Prosińska J., Sawicka T., Drozd J.: *Biul. IL* 2, 44 (2002).
10. Kohlmünzer S.: in *Farmakognozja*, PZWL, Warsaw 2000.
11. Fearnhead H.O., Chwaliński M., Snowden R.T., Omerod M.G., Cohen G.M.: *Biochem. Pharmacol.*, 48, 1073 (1994).
12. Ahmed S.A., Sriranga N.: *Immunopharmacol.* 28, 55 (1994).
13. Sokolnicka et al.: *Immunother.* X, 83 (1994).
14. Sęk St., Skierski J.: in *Wprowadzenie do metod statystycznych, teoria i praktyka – mikrokomputerowy system Medistat*, CMKP, Warsaw 1990.
15. Jakóbsiak M.: *Immunologia*, PWN, Warsaw 1995.
16. Kostowski W.: *Farmakologia*, PZWL, Warsaw 1998.

Received: 18.09.2003