

# Stimulation of humoral immunity in mice by complex herbal remedy PERVIVO

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## Abstract

The *in vivo* effect of multicomponent digestive herbal remedy PERVIVO on humoral immunity in mice was studied. Stimulatory effect on anti-SRBC antibody production was presented by feeding mice PERVIVO diluted with water  $\times 2$ ,  $\times 4$ ,  $\times 8$ ,  $\times 16$  and  $\times 32$ .

These doses corresponded to 10 ml, 5 ml, 2.5 ml, 1.25 ml and 0.625 ml of PERVIVO given to 70 kg person.

**Key words:** herbal remedy, humoral immunity, mice.

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## Introduction

In our previous papers we presented the effects of some natural essential oils and synthetic volatile substances (undecanones, fragrances) on specific and non-specific immunity in rodents [1-7]. The majority of these substances, introduced to mice by inhalation has increased their antibody response to antigen sheep red blood cells (SRBC). In the present paper we describe the effect of feeding mice multicomponent bitter digestive herbal remedy PERVIVO on above parameter of humoral immunity.

## Material and methods

PERVIVO (Bittner) liquid remedy is composed from 27 components dissolved in 32% ethyl alcohol. The main substances are extracts from *Angelicae radix*, *Gentianae radix*, *Menyanthis folium*, *Absinthii herba*, *Zingiberis radix* and *Camphora racemica*.

## Mice

The study was performed on 10-12 weeks old female Balb/c mice, weighing 25-28 g, delivered from the Polish Academy of Sciences.

## Study of antibody production

Mice were fed PERVIVO diluted in water, or diluted ethyl alcohol (controls) for 9 days. Animals belonging to

8 experimental groups received daily 40  $\mu$ l (feeding with use of Eppendorf pipette) of following preparations: PERVIVO diluted with water  $\times 2$ ,  $\times 4$ ,  $\times 8$ ,  $\times 16$ ,  $\times 32$ ,  $\times 64$ ,  $\times 128$ , and  $\times 256$ .

These doses corresponded to 10 ml, 5 ml, 2.5 ml, 1.25 ml, 0.625 ml, 0.312 ml, 0.156 ml and 0.078 ml of Pervivo given to 70 kg person (applying the counter 7 for differences between mouse and human in relation of the surface to body mass).

Mice were immunized with 10% SRBC (0.2 ml intraperitoneally) 3 days after start of feeding, and bled in anaesthesia from retroorbital plexus 7 days after immunization.

The antibody level was evaluated with haemagglutination assay in inactivated (56°C, 30 min) sera. After performing a series of sera dilutions, 0.5 % SRBC were added and the mixture was incubated for 60 min at room temperature, then centrifuged (10', 150 g) and shaken. The hemagglutination titer was evaluated in a light microscope – as the last dilution in which at least 3 cell conglomerates were present in at least 3 consecutive fields at objective magnification 20 $\times$ . Stimulation indices were calculated as a ratio of log titer of each experimental serum to the mean log titer of simultaneously performed control.

Experiments were approved by the Local Ethical Committee.

## Statistical analysis

Statistical evaluation of the results was done by 1-way analysis of variance ANOVA (GraphPadInStat3) and the significance of differences between the groups was verified by Tukey – Kramer Multiple Comparisons Test.

## Results

The effect of PERVIVO on antibody production is presented on the Table 1 (mean log titers  $\pm$  SE) and on the Figure 1 (mean stimulation indices).

According to one way analysis of variance (ANOVA) the  $p$ -value is  $< 0.0001$ , considered extremely significant. Variation among column means is significantly greater than expected by chance. PERVIVO in dilutions from 2 to 32  $\times$  highly significantly stimulated this parameter of humoral immunity. In higher dilutions stimulatory effect disappeared.

## Discussion

In this paper we present for the first time the evidence of stimulatory activity of complex herbal remedy PERVIVO on humoral immunity in mice. This effect appeared even in very low doses, up to 32  $\times$  dilution.

PERVIVO is composed from many substances of natural origin. Main function on digestive tract exert group of bitter substances, among them *Radix Gentianae*, *Menyanthes trifoliata* and *Herba Absinthii*.

*Radix Gentianae* is the dried root of *Gentiana lutea*. The root contains bitter secoiridoid glycosides such amarogentin and amaroswerin. The toxicity of *Radix Gentianae* is low. It acts on the liver, gallbladder and bladder channels. Some authors investigating anti-inflammatory properties of medicinal herbs used in Chinese medicine, in order to develop a new herbal formulation to treat inflammation, reported that *Radix Gentianae* demonstrated anti-inflammatory effects in various experimental models. The primary ingredient in *Radix Gentianae* is gentiopicoside which was shown to have anti-inflammatory effects in a murine model of hepatic injury [8-10].

Gentian is a favourite bitter tonic. Fresh gentian root is largely used in Germany and Switzerland for the production of an alcoholic beverage.

The second bitter compound of PERVIVO, *Menyanthes folium* (bogbean leaf) is used in loss of appetite and peptic discomforts. It stimulates the secretion of saliva and gastric juices. The chief constituents are a small quantity of volatile oil and a bitter principle, a glucoside called Menyanthin.

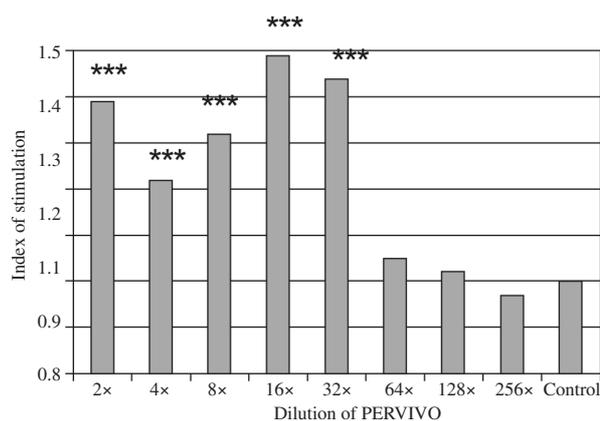
Medicinal Action and Uses Tonic, cathartic, deobstruent and febrifuge. An extract is made from the leaves of *Menyanthes trifoliata* L., which possesses strong tonic properties, and which is beneficial in rheumatism, scurvy,

and skin diseases [11]. One paper described the immunomodulatory effects of polysaccharide fractions of this plant on human blood lymphocytes and granulocytes [12].

No more information of its immunotropic or anti-inflammatory activity is available.

*Angelica radix* is the next important component of PERVIVO.

The root, seed, and fruit of *Angelica* are used to make remedies. *Angelica radix* stimulates the secretion of saliva and gastric juices and is used for intestinal gas (flatulence),



**Fig. 1.** The effect of PERVIVO on anti-SRBC antibody production in mice

**Table 1.** The effect of PERVIVO on humoral immunity in mice

Group of mice	Number of mice	Mean log titer $\pm$ SE	Statistical significance of difference from the control ( $p$ -value)
Control	36	4.1 $\pm$ 0.14	–
PERVIVO Dil. 2 $\times$	15	5.7 $\pm$ 0.19	$< 0.001$
PERVIVO Dil. 4 $\times$	15	5.0 $\pm$ 0.17	$< 0.001$
PERVIVO Dil. 8 $\times$	15	5.4 $\pm$ 0.19	$< 0.001$
PERVIVO Dil. 16 $\times$	10	6.1 $\pm$ 0.23	$< 0.001$
PERVIVO Dil. 32 $\times$	10	5.9 $\pm$ 0.18	$< 0.001$
PERVIVO Dil. 64 $\times$	6	4.3 $\pm$ 0.21	NS
PERVIVO Dil. 128 $\times$	6	4.2 $\pm$ 0.31	NS
PERVIVO Dil. 256 $\times$	6	4.0 $\pm$ 0.10	NS

loss of appetite (anorexia), arthritis, circulation problems, “runny nose” (respiratory catarrh), nervousness. The main active compounds of *Angelica* radix are essential oil, coumarin and coumarin derivatives, ferulic acid and adenosine. The furanocoumarins present in *Angelica* root sensitize the skin to light and during treatment with the drug prolonged exposure to UV radiation should be avoided. Some women use *Angelica* to start their menstrual periods. Sometimes this is done to cause an abortion. *Angelica* is also used to increase urine production, improve sex drive, stimulate the production and secretion of phlegm, and kill germs. In combination with other herbs, *Angelica* is also used for treating premature ejaculation.

*Angelicae* radix has inhibitory effect on platelet aggregation, promotes hemato- and thrombopoiesis and possess anti-oxidant and anti-inflammatory activities. Its anti-oxidative effect is probably connected with the presence of phenolic compounds, especially caffeic and ferulic acids. *Angelicae* radix inhibits expression of cyclooxygenase-2 (COX2) and inducible nitric oxide synthase in a dose-dependent manner as well as phosphorylation of ERK1/2 [13-20].

Another bitter component of PERVIVO, *Artemisia absinthium* L. (Wormwood) has long been used as traditional herbal medicine in Asia and Europe for the treatment of gastric pain, cardiac stimulation, to improve memory and mental function. It is one of the five plants commonly used to treat intestinal worms. The results of controlled clinical trials in patients with Crohn’s disease strongly suggest that wormwood has a steroid sparing effect, suppresses tumor necrosis factor alpha and accelerates healing. Experimental studies in rats suggested that *Artemisia absinthium* is neuroprotective and might be useful adjunct in the treatment of stroke [21-24].

Rhizoma *Zingiberis* is the dried rhizome of *Zingiber officinale* Roscoe (Ginger). The plant, native to south-east Asia, is commercially grown in Africa, China, India (the world’s largest producer) and Jamaica. It possess antiemetic, anti-inflammatory and cholagogic activity.

The drug is a potent inhibitor of cyclooxygenase and thromboxane synthase [25]. It was reported that Ginger essential oil recovered the humoral immune response in immunosuppressed mice. Contrary to the ginger essential oil response, sage essential oil did not show any immunomodulatory activity [26].

So, stimulation of anti-SRBC antibody response in our study by PERVIVO might be connected with its ginger content, or may be the result of synergistic action of some other compounds present in this remedy.

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