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Experimental data obtained during previous research projects published in more than 40 research studies allowed us to confidently conclude that glucans are highly active immunostimulators. The aim of this study was to evaluate a commercial sample EPI-PV. We used two different doses and 14 days oral supplementation. Both cellular and humoral immune response was evaluated, so this project will be able to answer the question whether the sample stimulate one or both branches of the immune system.

Conditions:

1. Balb/c mice (Jackson) will be used, both sexes, age 6-8 weeks
2. Biological activity of sample will be tested after 14 days of oral feeding (100 or 200 ug/mouse).
3. Tested sample will be compared with a negative control (PBS).
4. Five mice will be used for each experiment.

SAMPLE: EPI-PV (Inomed, Czech Republic)

Experiments:

1. Mice were orally given different daily doses for 14 days interval.
2. Evaluation of phagocytosis in peripheral blood. In order to evaluate the effects of glucan on phagocytosis, synthetic microspheres prepared from 2-hydroxyethylmethacrylate copolymer, were used. Using well established techniques, phagocytic activity of macrophages isolated from peritoneal cavity and monocytes and neutrophils in peripheral blood were tested.
3. Evaluation of the effects of glucan on IL-2 production by splenocytes using a commercial IL-2 ELISA kit. A control group with Concanavalin A was used.
4. Evaluation of the effects of glucan on antibody response. The glucan-fed mice (3 weeks in these experiments) were injected twice (day 0 and day 14) with ovalbumin. On day 21, the mice were killed, serum collected and evaluated for anti-ovalbumin antibodies by ELISA. As control, antigen (ovalbumin) injected sc. with Freud adjuvants, was used.
5. Evaluation of CD4, CD8 and CD19 surface markers by flow cytometry. Cells isolated from the spleen were tested.

Results:

Studies of phagocytosis (both in peritoneal macrophages and blood neutrophils and monocytes) showed that the sample has significant stimulating activity in all three cell types. A small but clear dose-dependence was observed (Figure 1). Similar results were found when we measured IL-2 production (Figure 2), the only difference was more clearly defined dose-dependency. The IL-2 production without any stimulation is usually very low (sometimes even 0), so the observed IL-2 production is statistically significant.

Next, we focused our attention of possible stimulation of antibody response. Both doses significantly improved antibody response (compared to antigen only), again with clear dose-dependence (Figure 3). The last part of the project was evaluation of possible effects on composition of spleen cells. CD4-positive and CD-8 positive T lymphocytes and B lymphocytes (CD19 cells) were measured by flow cytometry. Small improvements in numbers of B lymphocytes were observed, but these changes were not statistically significant (Figure 4).

Conclusions:

Several conclusions can be reached.

1. Immunostimulatory activity of the test sample to cellular immunity (phagocytosis) was confirmed. There was an increase of app. 40% compared to the control sample without supplementation.

2. The immunostimulatory activity of the test sample on humoral immunity (antibody production and IL-2 secretion) was confirmed. The stimulation was statistically significant.

3. An increase in antibody production by 260-300% over control Ag was confirmed.

4. The tested material was a mixture of nine different nutraceuticals, so it is not possible to determine which individual part is responsible for the effects described above. From the literature we can assume that most of the effects is probably caused by glucan/s, but to be sure, the individual parts would need to be evaluated separately. These experiments would not only determine the most active substance, but also allowed the preparation of optimal mixture.

5. Current results clearly show that the tested material supports both cellular and humoral branch of immune reactions.

Figure 1

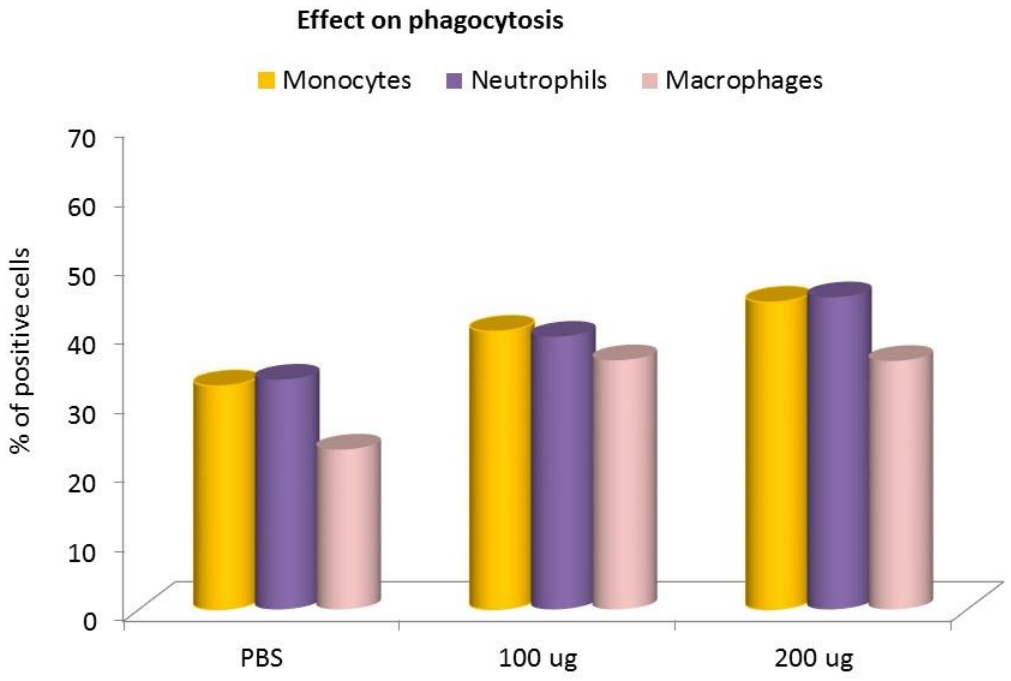

Effect on phagocytosis
Monocytes
Neutrophils
Macrophages
% of positive cells
70
60
50
40
30
20
10
0
PBS
100 ug
200 ug

Figure 2

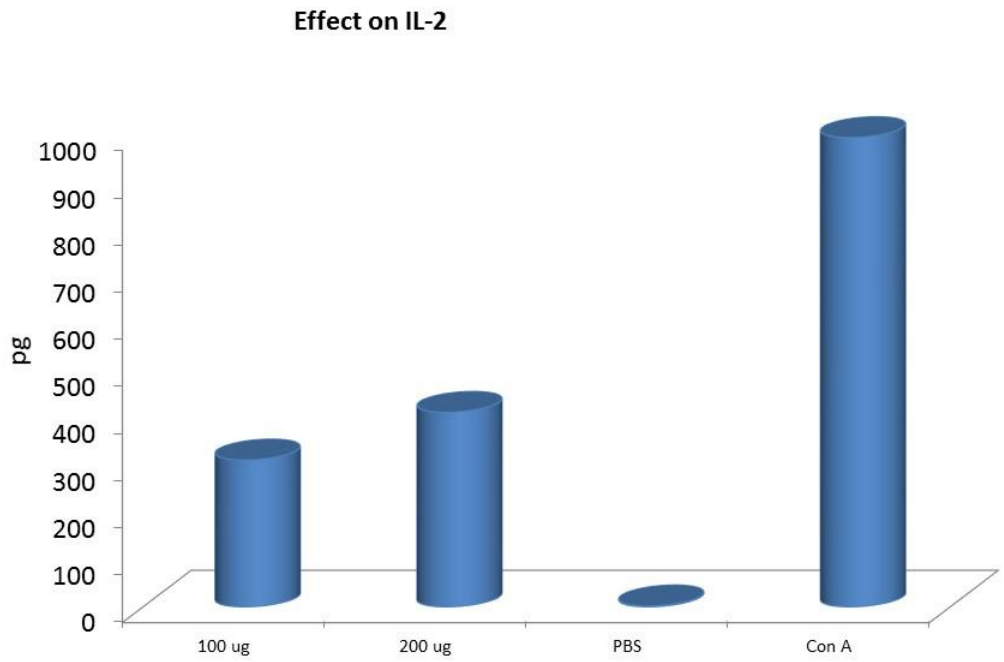

Effect on IL-2
pg
1000
900
800
700
600
500
400
300
200
100
0
100 ug
200 ug
PBS
Con A

Figure 3

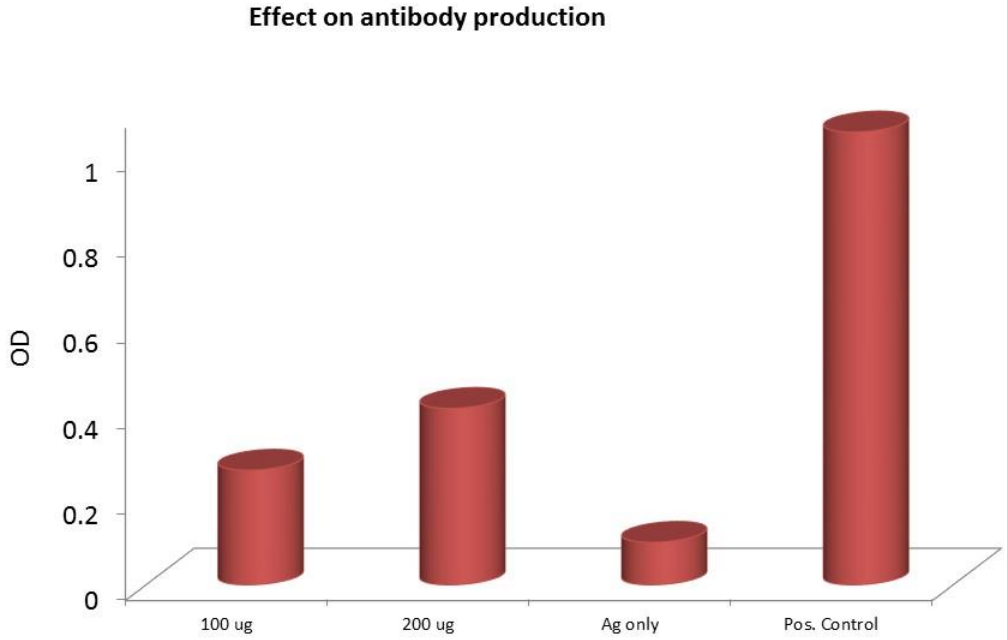
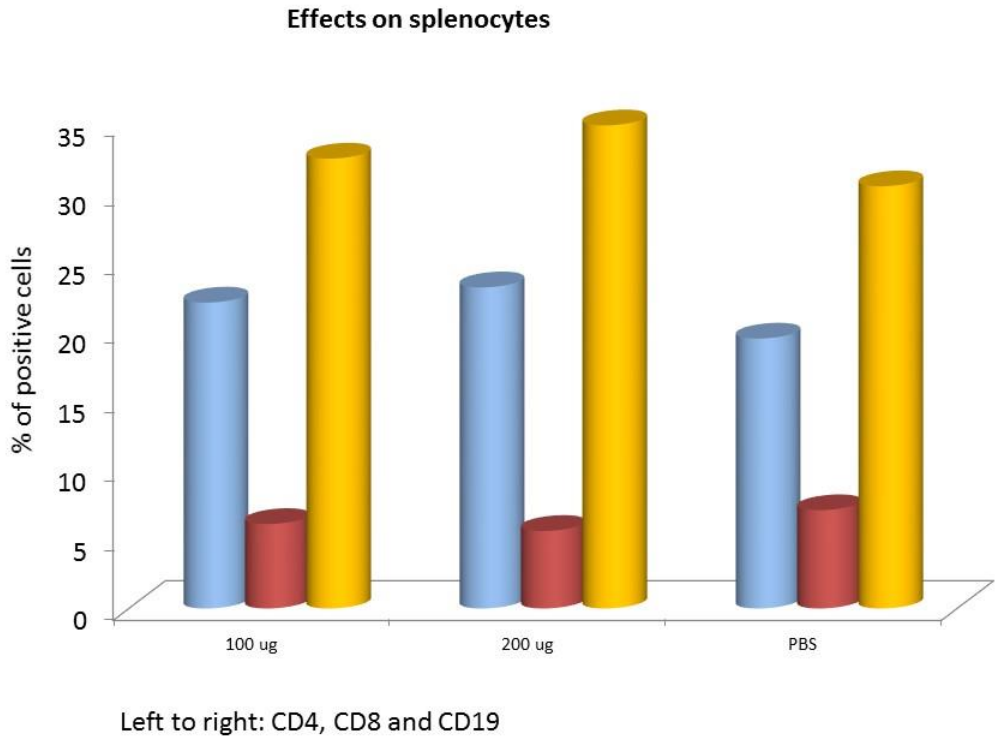

Effect on antibody production
OD
1
0.8
0.6
0.4
0.2
0
100 ug
200 ug
Ag only
Pos. Control

Figure 4



Left to right: CD4, CD8 and CD19

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