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Experimental data obtained during previous research projects allowed us to confidently conclude that glucans are highly active immunostimulators. The aim of this study was to evaluate a commercial sample IMMUNO-BOO. 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 will be used, both sexes, age 6-8 weeks
2. Biological activity of glucan will be tested after 14 days of oral feeding (100 or 200 ug/mouse). Declared amount of 25% beta glucan, ie 25 μg at 100 μg and 50 μg at 200 μg
3. Glucans will be compared with a negative control (PBS).
4. Five mice will be used for each experiment.
5. Experiment designed for the total of 11 samples.

Experiments:

1. Mice were orally given different daily doses of samples 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 types of cells. A small but clear dose-dependence was observed (Figure 1). Similar results were found when we measured IL-2 production (Figure 2). 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 Ag 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 30-40% 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. Increase 30 - 40 times compared to standard reaction.

3. An increase in antibody production by 200-300% over control Ag was confirmed.

4. A slight increase of 5-10% in the production of own stem cells was confirmed, but it is difficult to evaluate statistically due to the short testing period.

Due to the presence of probiotics, it might be beneficial to further evaluate the possible effects on some problems of the gastrointestinal tract.

Figure 1

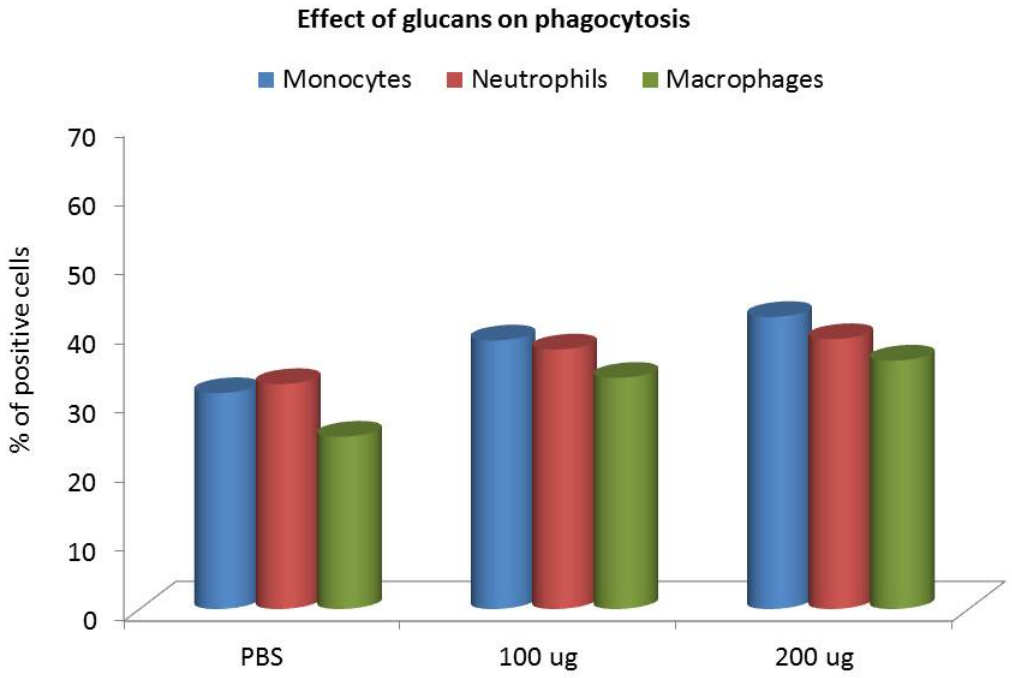

Effect of glucans on phagocytosis
Monocytes
Neutrophils
Macrophages
% of positive cells
70
60
50
40
30
20
10
0
PBS
100 ug
200 ug

Figure 2

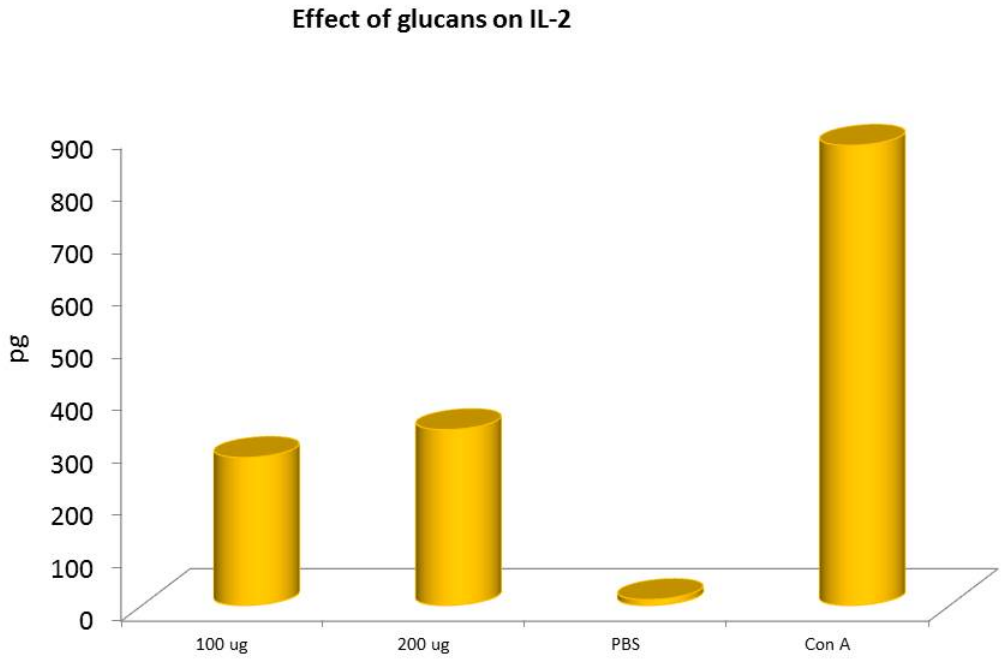

Effect of glucans on IL-2
pg
900
800
700
600
500
400
300
200
100
0
100 ug
200 ug
PBS
Con A

Figure 3

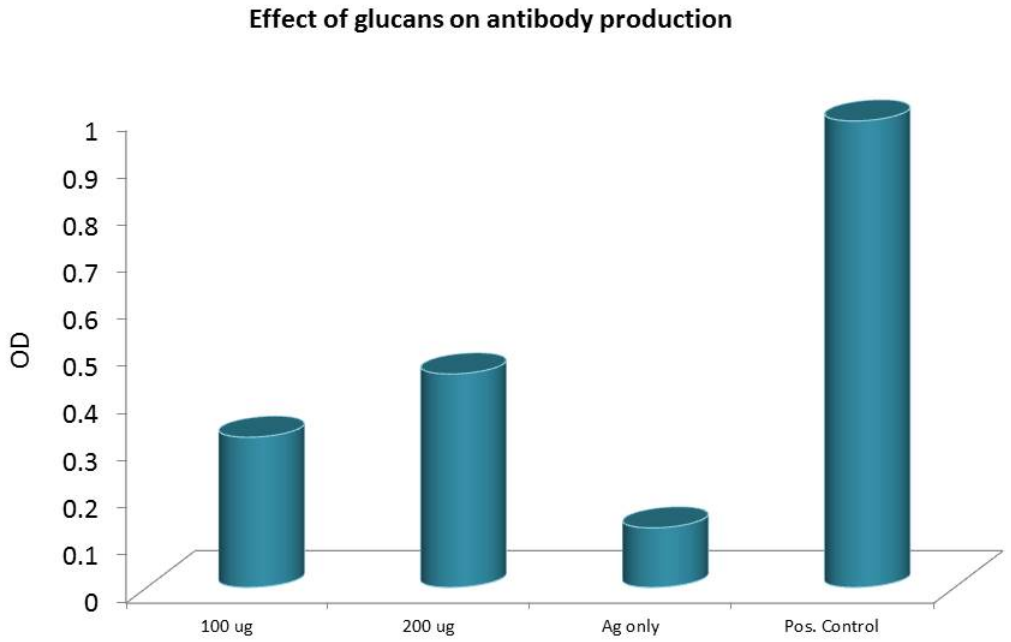
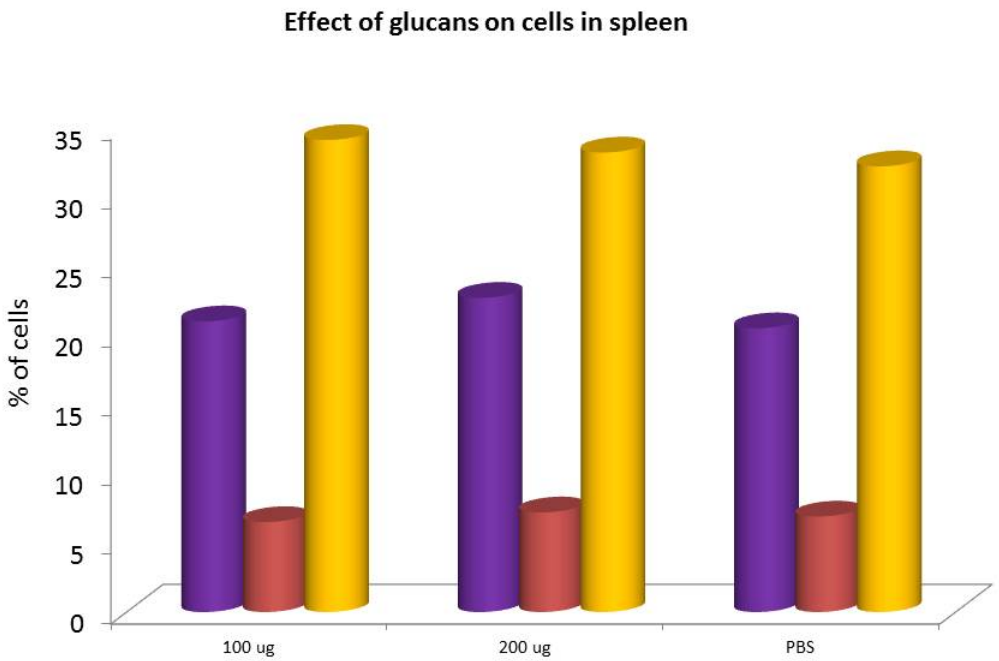

Effect of glucans on antibody production
OD
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
100 ug
200 ug
Ag only
Pos. Control

Figure 4



Left to right: CD4, CD8 and CD19

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