Immuno-stimulatory activities of a blend of natural extracts in human immune cells H. Wang and B. Daggy



593.7

ABSTRACT

Polysaccharides are naturally present as cell wall components in plant and fungi. Certain polysaccharides with specific structures such as β -D- (1, 3/1, 6)-glucan are the active ingredients in many well-known immuno-modulatory foods including yeast¹ and mushrooms². β -D- (1,3/1,6)-glucan is recognized and taken up by the mammalian immune cells such as macrophages/dendritic cells via β glucan receptors (dectin-1/TLR-2) on the cell membrane³. Due to the structural similarity between certain β -glucans and polysaccharides found in microorganisms, our immune cells regard them as "pathogen-associated molecules" and will elicit an activated immune response. This activity has been demonstrated in both laboratory^{4, 5} and clinical studies⁶⁻⁸. We investigated the *in* vitro immuno-stimulatory effects on cytokine production and lymphocyte proliferation of a yeast extract polysaccharide with and without additional polysaccharides from other fungi and herbal extracts, total tested sample weight held constant. The polysaccharide preparations were incubated with human immune cells isolated from blood of a healthy donor. The results showed that a specific yeast extract blend had potent immuno-stimulatory effects and was superior to yeast β-glucan alone in several cytokine parameters and lymphocyte proliferation. This suggests that extract blend may potentiate the immunostimulatory effects of β -glucan and enhance the efficacy.

METHODS

Lymphocyte proliferation

Blood was obtained from a single healthy donor and peripheral blood mononuclear cells (PBMCs) were prepared by centrifugation over a ficoll hypaque density gradient, followed by washing. Viable PBMCs were adjusted to a concentration of 2 x 10⁶/mL in complete medium consisting 100 µL/well were placed in triplicate in 96-well microtiter plates. Following this, 100 µL of complete medium, complete medium containing 1% DMSO (background controls), various concentrations of the compounds were added. The final DMSO concentration was kept constant at 0.5% for the DMSO-soluble compounds and the accompanying background control wells.

Cultures was incubated at 37°C in 5% CO2 for 3 days, pulsed with 1 µCi of tritiated thymidine (H3-TdR) for the final 6-16 hours, harvested, and counted to determine H3-TdR incorporation (a readout of proliferation).

Cytokine Production

PBMCs were prepared as described above and 100 μ L/well was placed in triplicate in 96- well microtiter plates. Following this, 100 µL of complete medium, complete medium containing 1% DMSO (background controls), various concentrations of the compounds were added. After approximately 24 hours of incubation at 37°C in 5% CO2, culture supernatants (SNs) were removed and frozen. The SNs were analyzed by multiplexing in the Luminex 200 System for various cytokines.

References:

1. Samuelsen, A.B., J. Schrezenmeir, and S.H. Knutsen, *Effects of orally administered yeast-derived beta-glucans: a review.* Mol Nutr Food Res, 2014. **58**(1): p. 183-93.

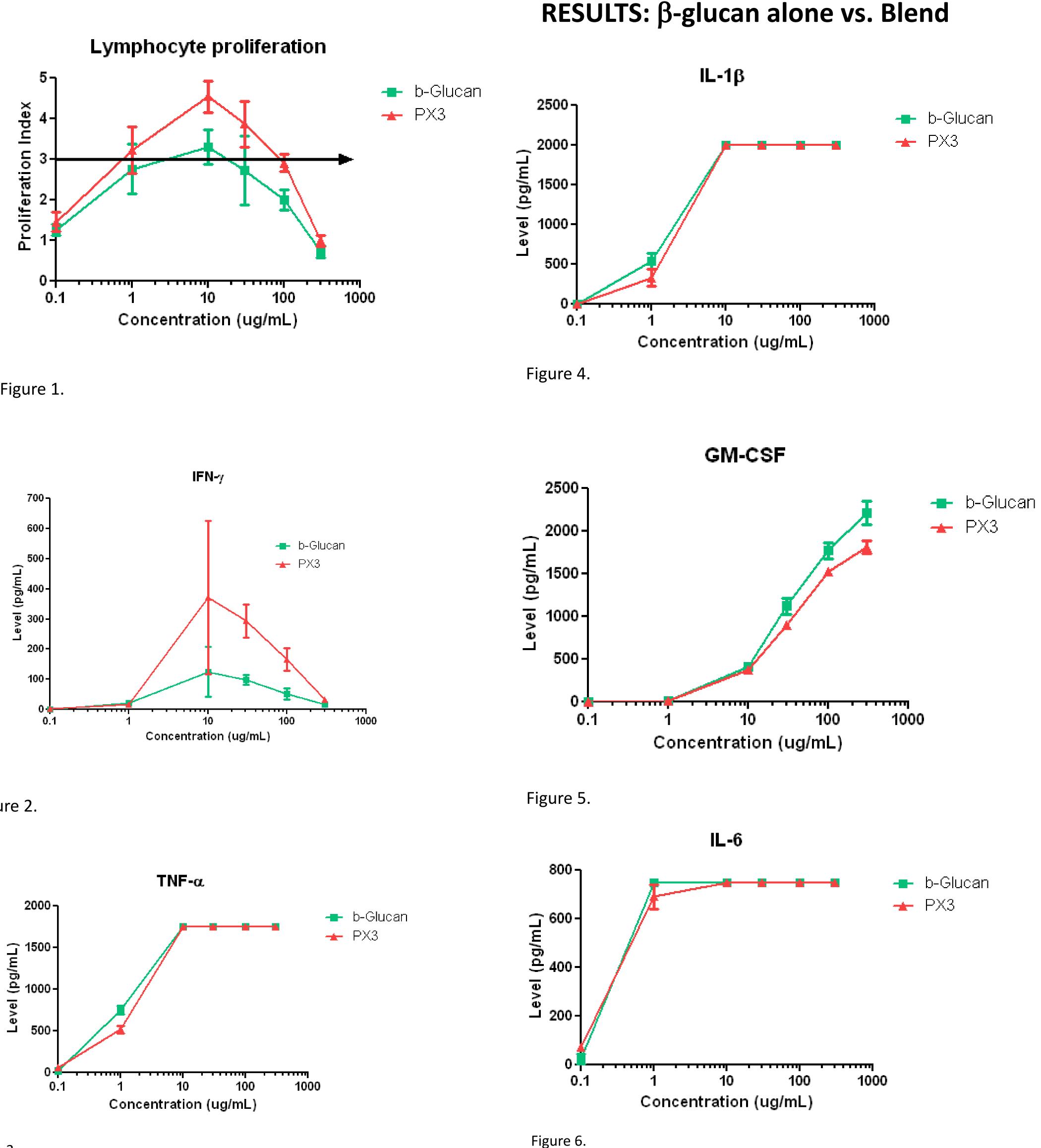
2. Vannucci, L., et al., Immunostimulatory properties and antitumor activities of glucans (Review). Int J Oncol, 2013. 43(2): p. 357-64.

3. Batbayar, S., D.H. Lee, and H.W. Kim, *Immunomodulation of Fungal beta-Glucan in Host Defense Signaling by Dectin-1.* Biomol Ther (Seoul), 2012. **20**(5): p. 433-45.

4. Hong, F., et al., Mechanism by which orally administered beta-1,3-glucans enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models. J Immunol, 2004. 173(2): p. 797-806. 5. Yatawara, L., et al., Aureobasidium-derived soluble branched (1,3-1,6) beta-glucan (Sophy beta-glucan) enhances natural killer activity in Leishmania amazonensis-infected mice. Korean J Parasitol, 2009. 47(4): p. 345-

6. Fuller, R., et al., Influence of yeast-derived 1,3/1,6 glucopolysaccharide on circulating cytokines and chemokines with respect to upper respiratory tract infections. Nutrition, 2012. 28(6): p. 665-9. 7. McFarlin, B.K., et al., Baker's yeast beta glucan supplementation increases salivary IgA and decreases cold/flu symptomatic days after intense exercise. J Diet Suppl, 2013. 10(3): p. 171-83.

8. Bergendiova, K., E. Tibenska, and J. Majtan, *Pleuran (beta-glucan from Pleurotus ostreatus) supplementation,* cellular immune response and respiratory tract infections in athletes. Eur J Appl Physiol, 2011. **111**(9): p. 2033R & D, Shaklee Corporation, Pleasanton, CA 94588





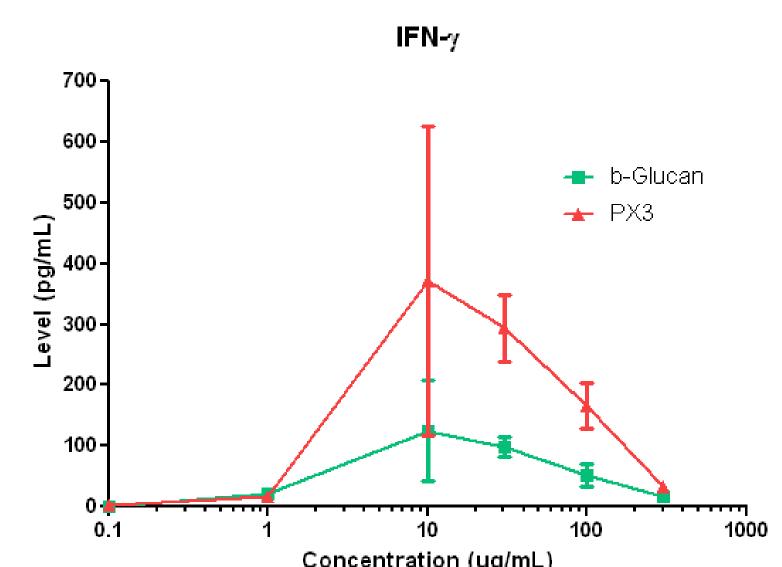


Figure 2.

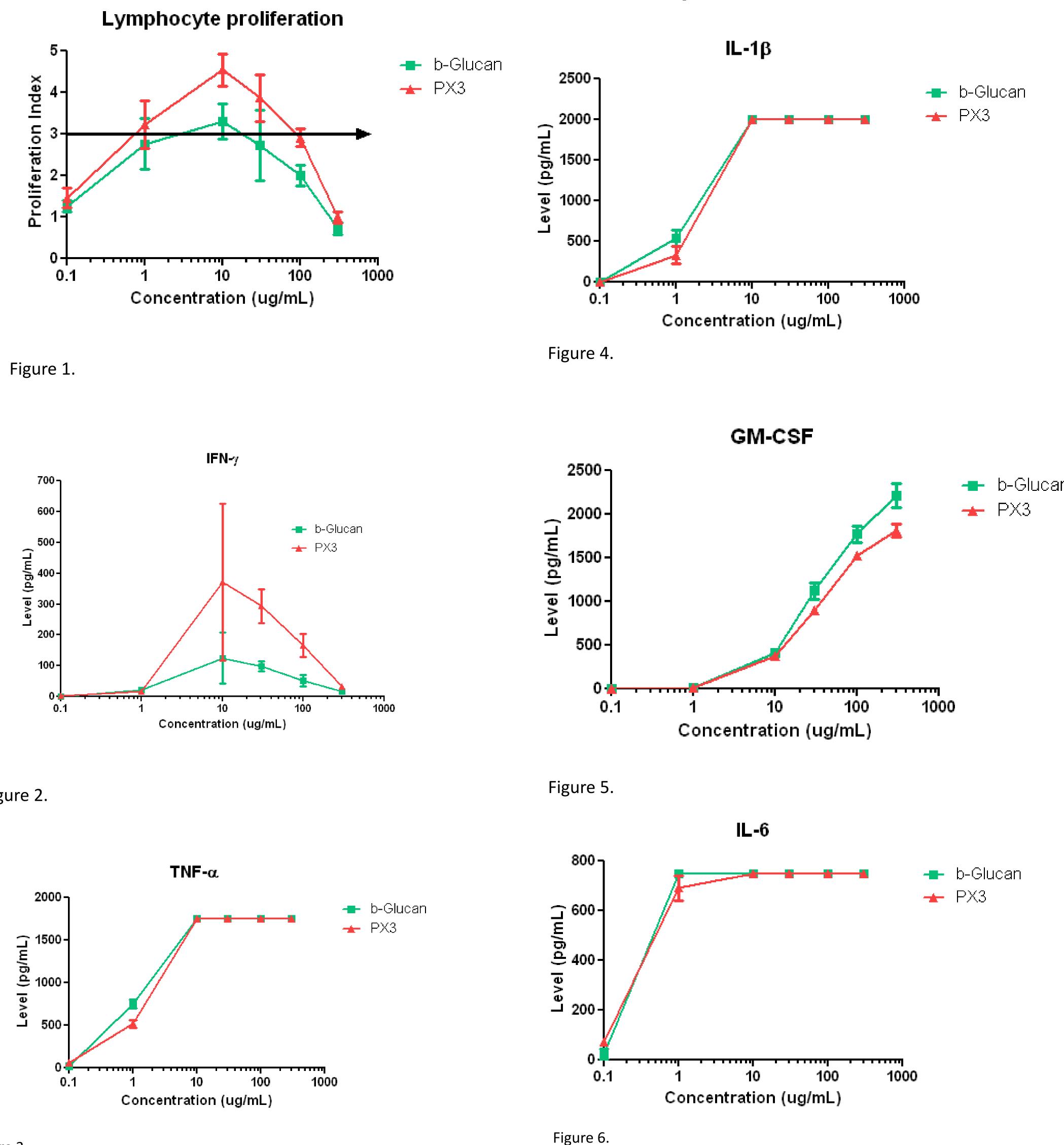
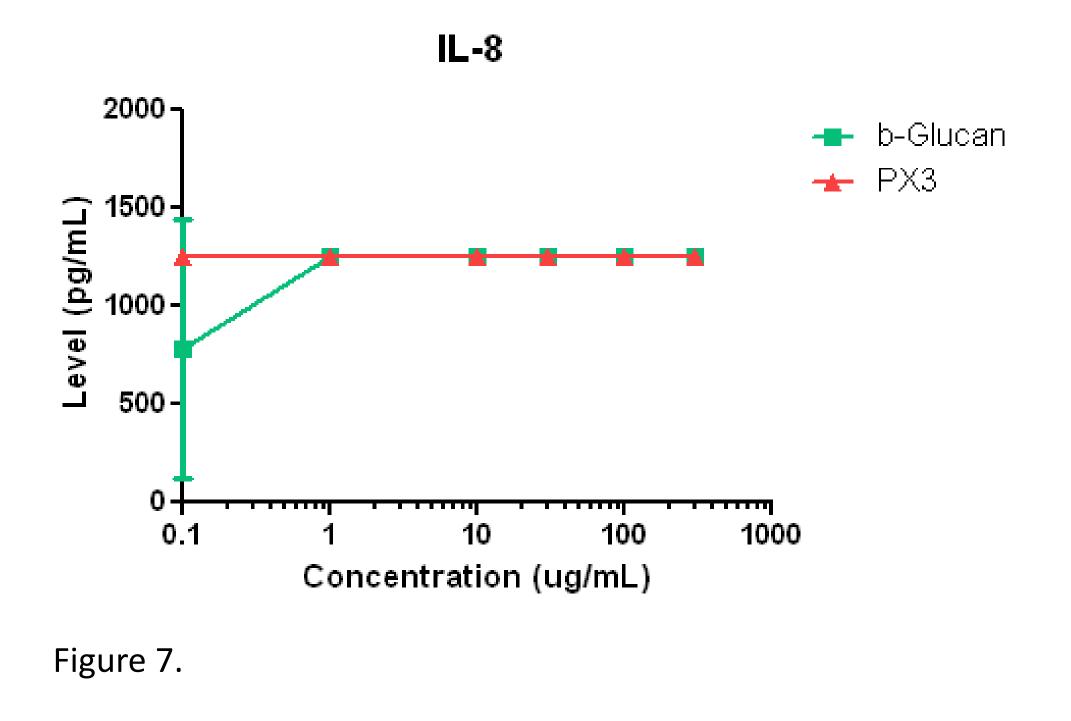
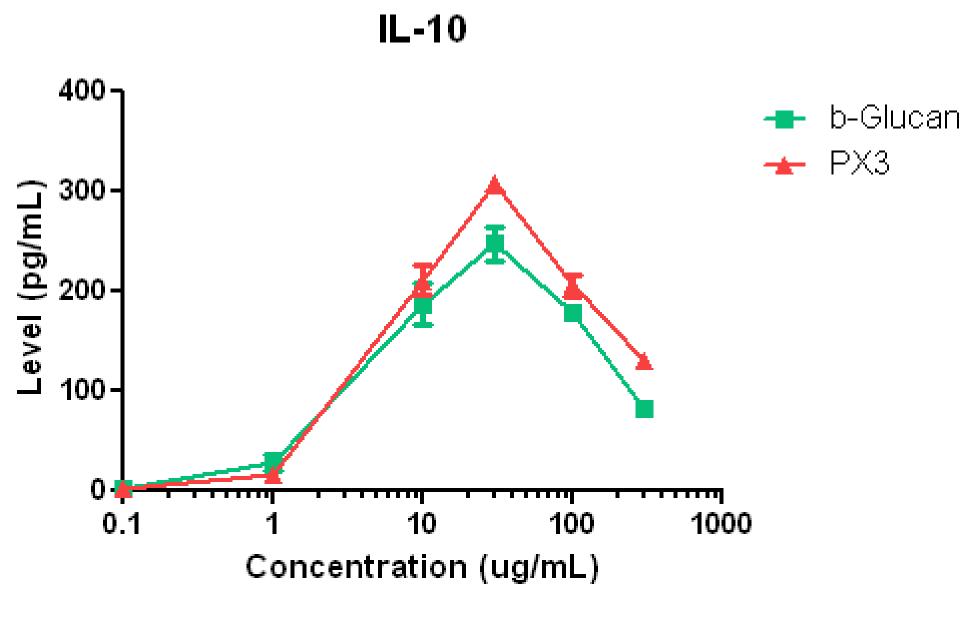


Figure 3.







```
Figure 8.
```

CONCLUSIONS

Our results showed that a specific yeast extract blend (PX3*) had potent immuno-stimulatory effects and was superior to yeast β -glucan alone in several cytokine parameters and lymphocyte proliferation. This suggests that extract blend may potentiate the immuno-stimulatory effects of β -glucan and enhance the efficacy. * may contain combinations of yeast, ginseng,

Princess Matsutake or Reishi mushroom extract.