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



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## ABSTRACT

Polysaccharides are naturally present as cell wall components in plant and fungi. Certain polysaccharides with specific structures such as  $\beta$ -D- (1, 3/1, 6)-glucan are the active ingredients in many well-known immuno-modulatory foods including yeast<sup>1</sup> and mushrooms<sup>2</sup>.  $\beta$ -D- (1,3/1,6)-glucan is recognized and taken up by the mammalian immune cells such as macrophages/dendritic cells via  $\beta$ glucan receptors (dectin-1/TLR-2) on the cell membrane<sup>3</sup>. Due to the structural similarity between certain  $\beta$ -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<sup>4, 5</sup> and clinical studies<sup>6-8</sup>. 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  $\beta$ -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 10<sup>6</sup>/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  $\mu$ 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:**

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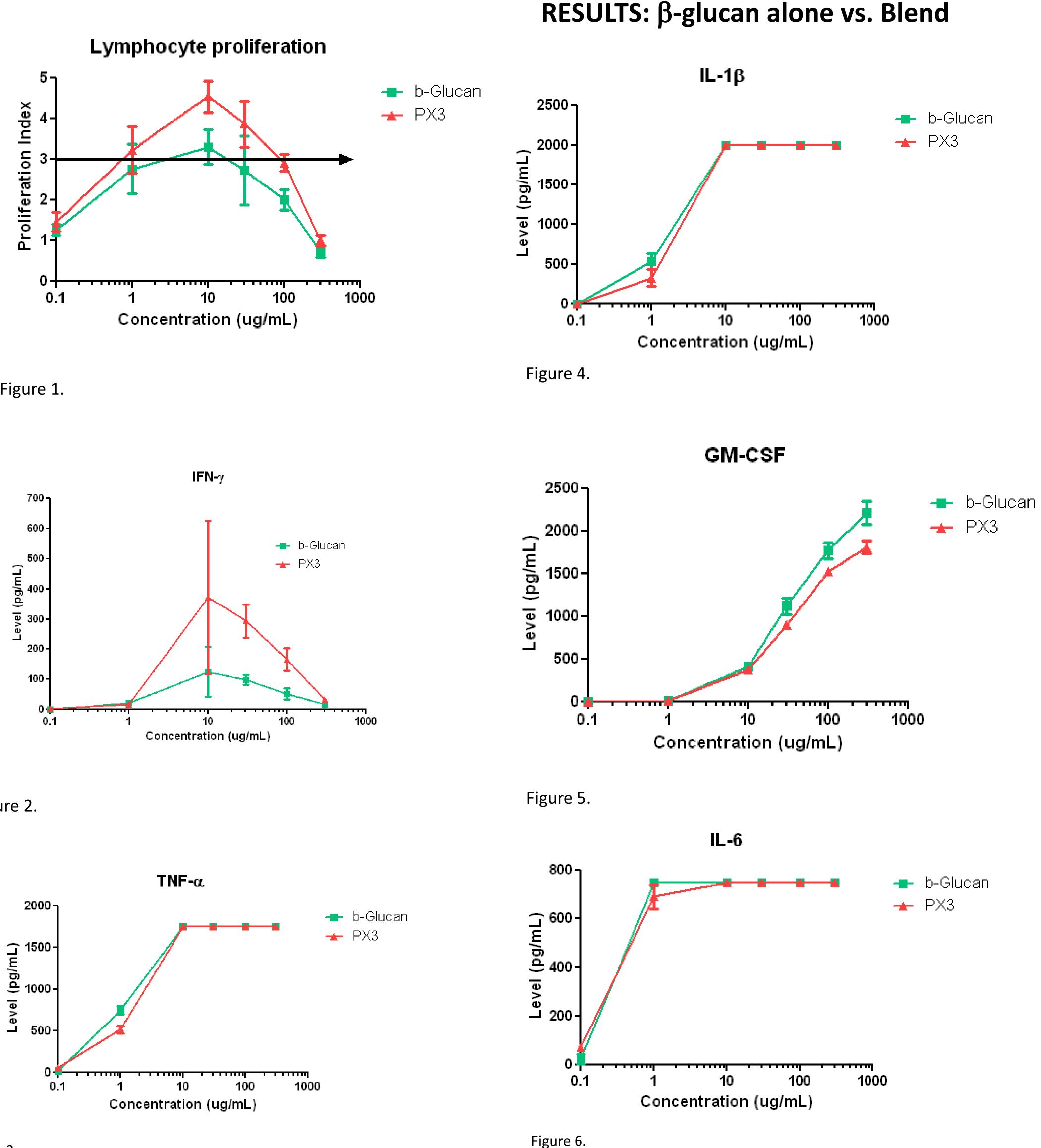
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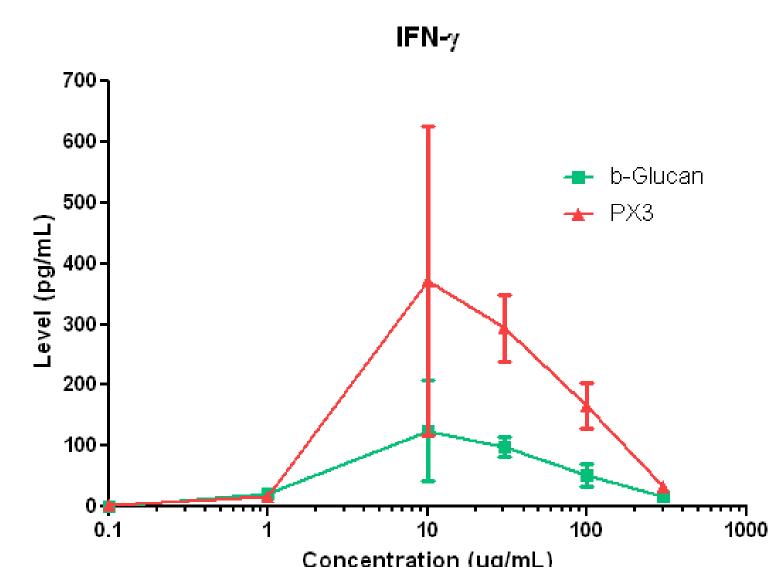


Figure 2.

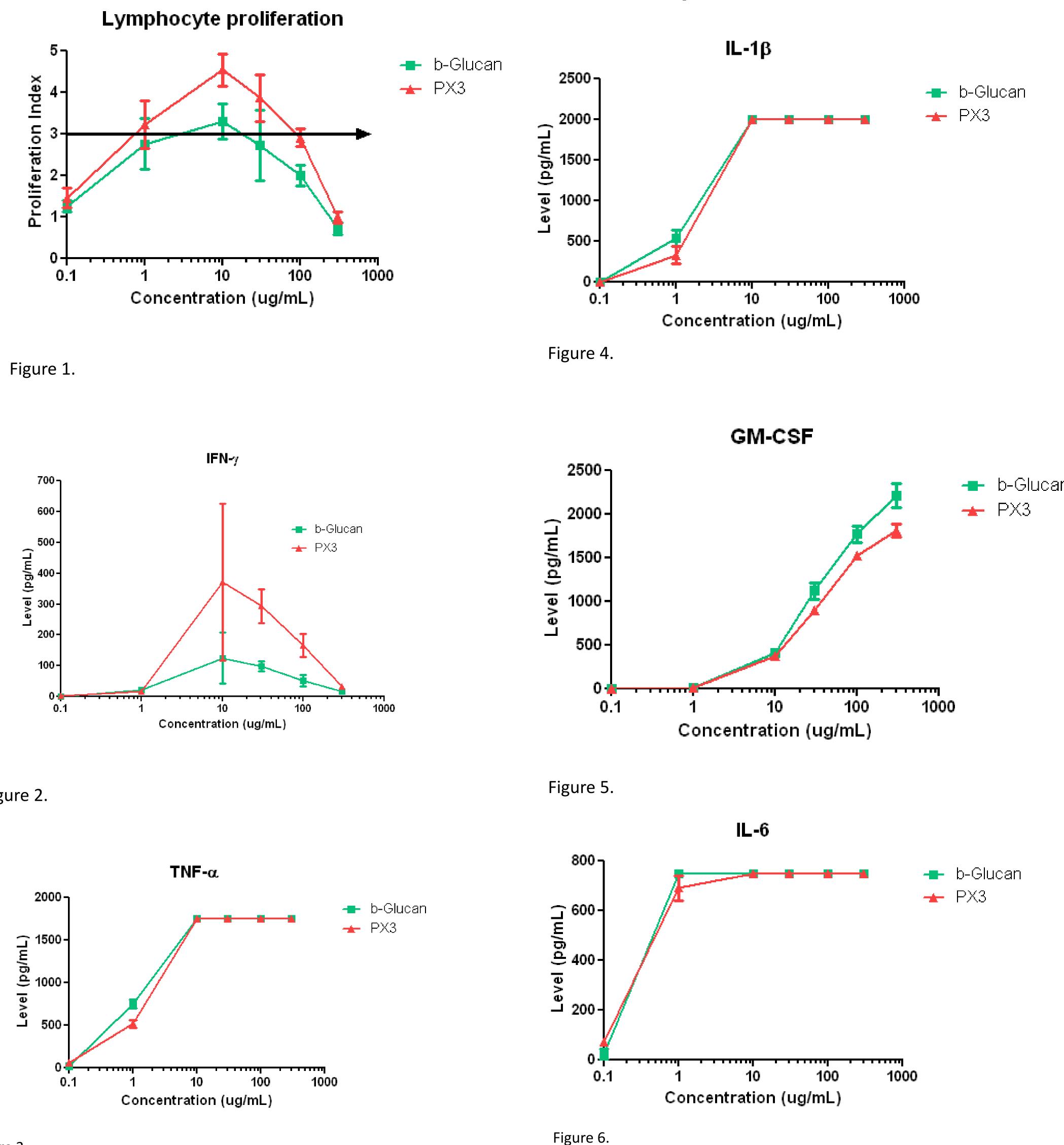
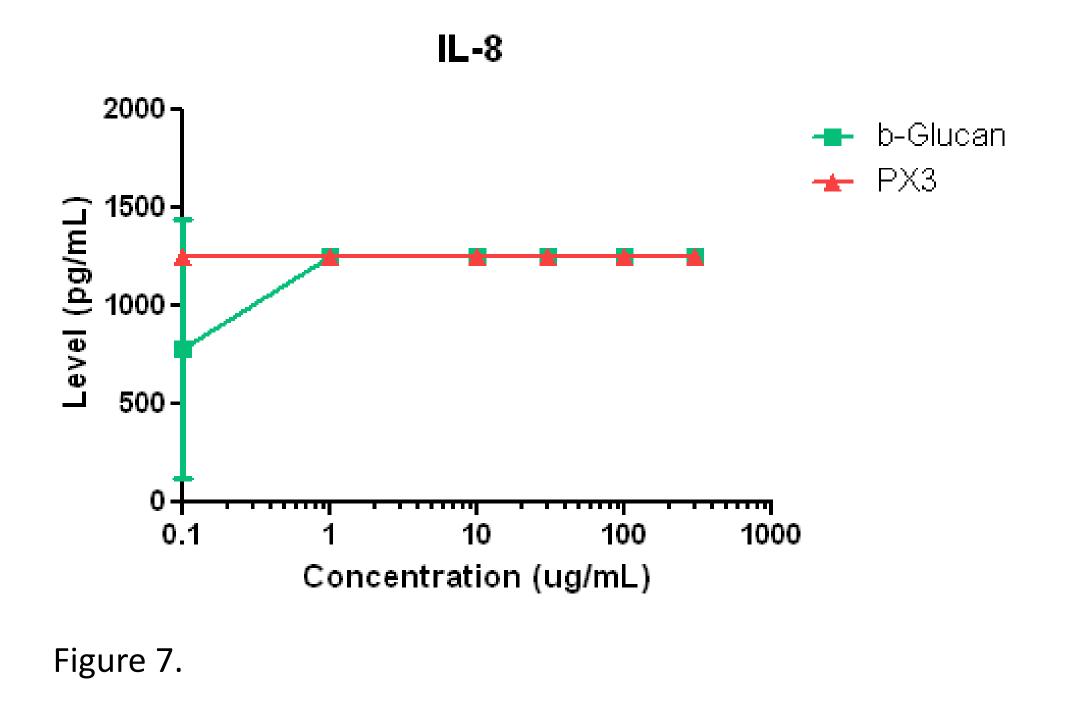
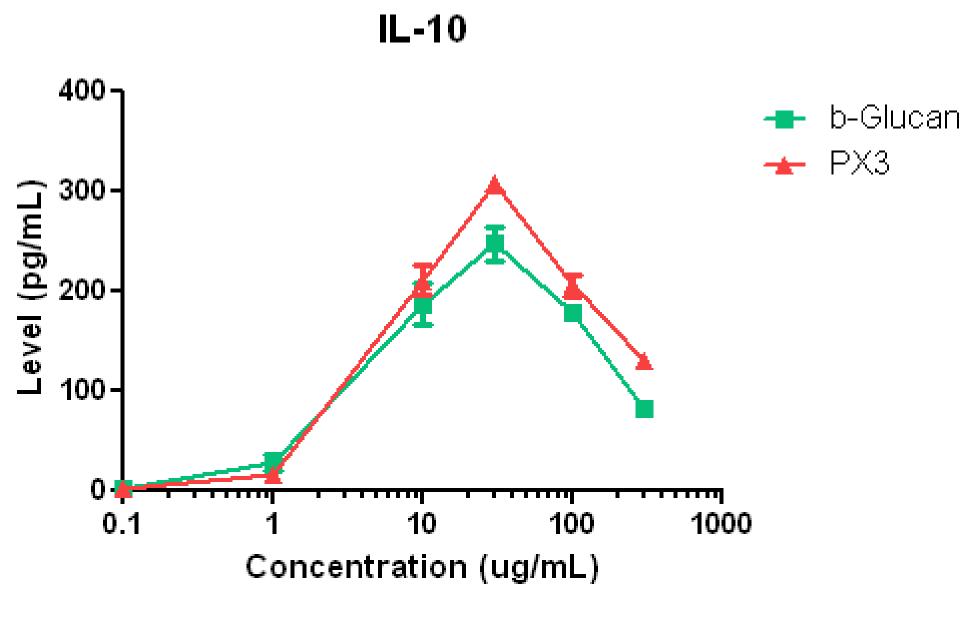


Figure 3.







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Figure 8.
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# CONCLUSIONS

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

Princess Matsutake or Reishi mushroom extract.