



Immuno-stimulatory activities of blends of natural extracts in human immune cells

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Conflict of Interest:

Authors are Shaklee Corporation employees.

ABSTRACT

Polysaccharides are naturally present as part of the 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 yeast and mushrooms. β -D- (1,3/1,6)-glucan is recognized and are taken up by the mammalian immune cells such as macrophages/dendritic cells via beta-glucan receptors (dectin-1/TLR-2) on their cell membrane. Due to these polysaccharides are similarly found in microorganisms, our immune cells regard them as "pathogen-associated molecules" and will elicit an activated immune response to prepare our body for defense. Previously we investigated the in vitro immuno-stimulatory effects on cytokine production and lymphocyte proliferation of a blend of extracts containing polysaccharides including yeast beta-glucan in human immune cells isolated from blood of a healthy donor. The results showed that the blend had potent immuno-stimulatory effects and was superior to β -glucan alone (10 – 100 μ g/mL) in several cytokine parameters (IFN- γ , IL-10, IL-12) and lymphocyte proliferation index. We further explored other potential blends of natural extracts with that were known to have immune enhancing properties. Interestingly, while some combination showed synergistic enhancement in activities, others showed reduced efficacy compared to β -glucan (10 μ g/mL) alone. Based on our results, we were able to develop a dietary supplement that has the optimal combination of natural extracts to potentiate the immuno-stimulatory 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 2×10^6 /mL in complete medium consisting 100 μ L/well were placed in triplicate in 96-well microtiter plates. Following this, 100 μ L 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.

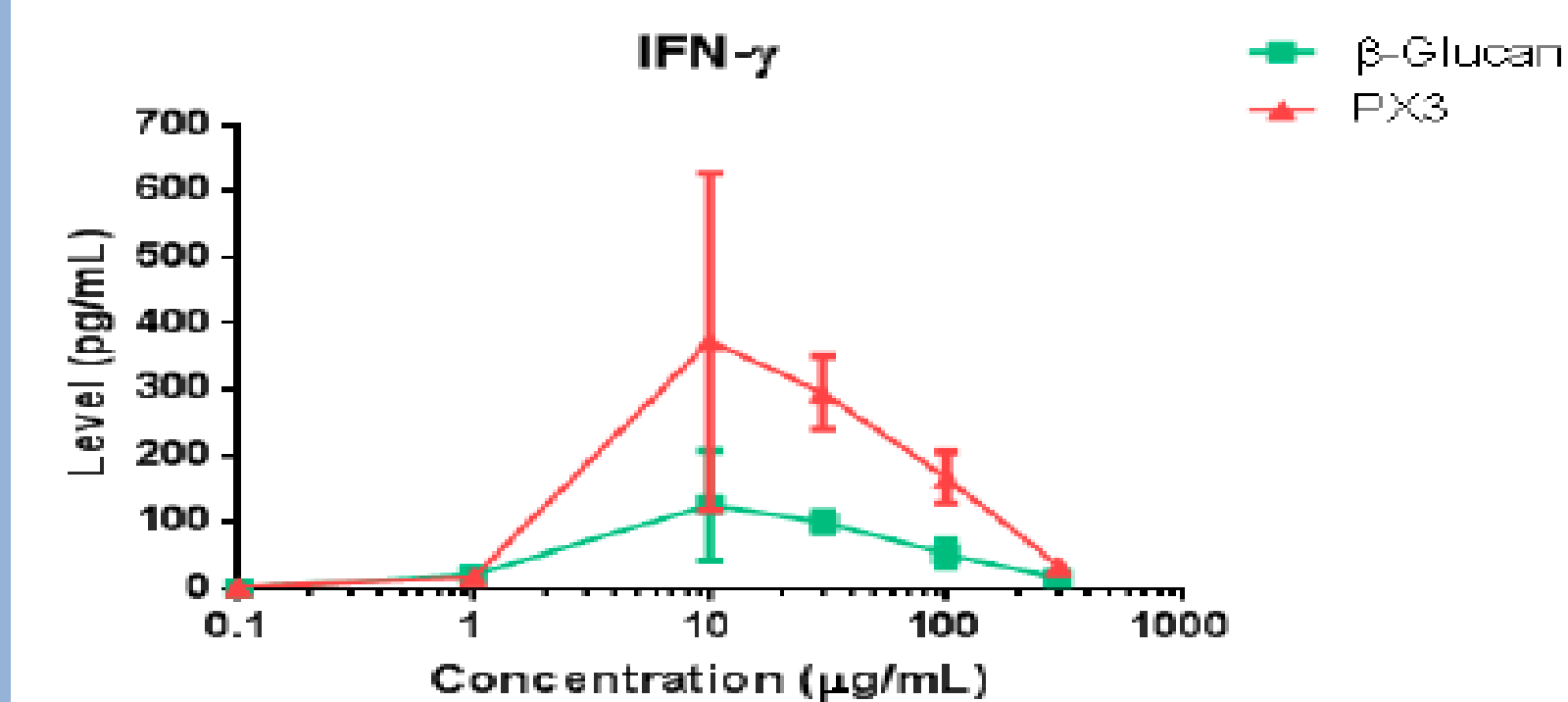
Cultures was incubated at 37°C in 5% CO₂ for 3 days, pulsed with 1 μ Ci of tritiated thymidine (H₃-TdR) for the final 6-16 hours, harvested, and counted to determine H₃-TdR incorporation (a readout of proliferation).

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 μ L 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.

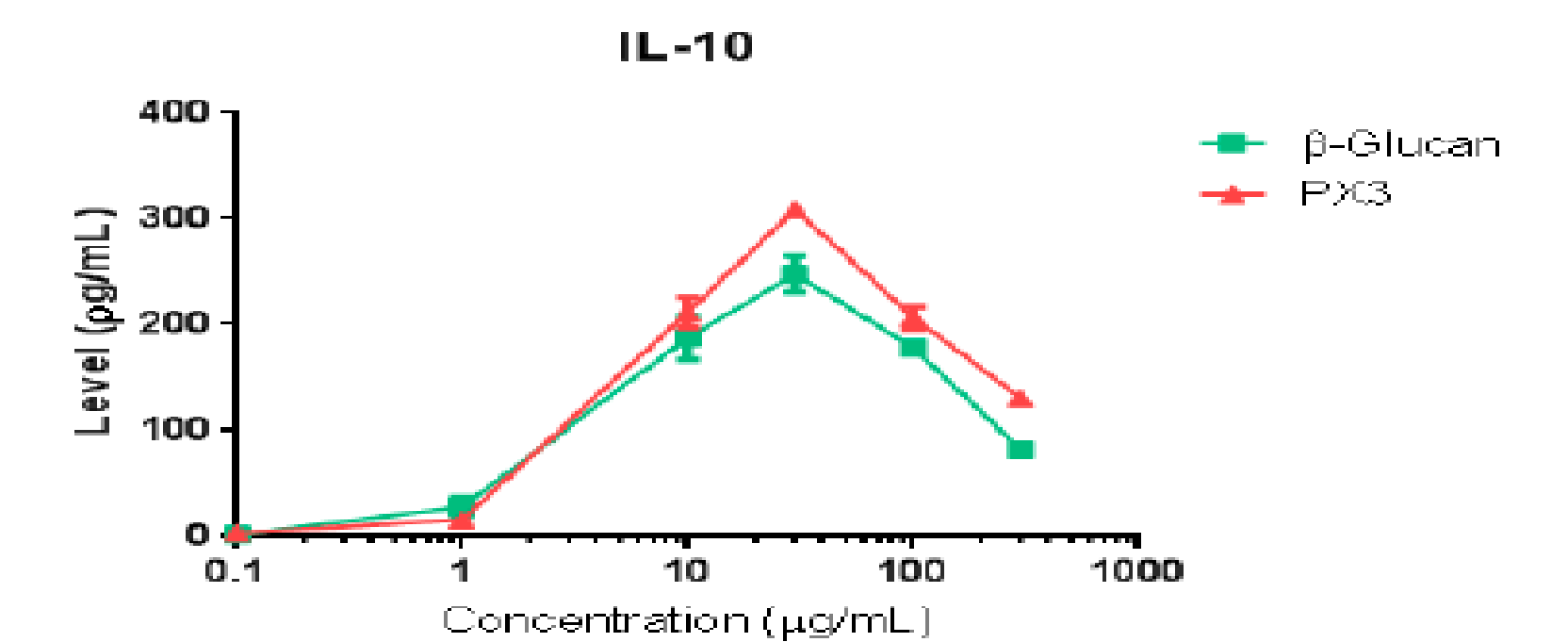
RESULTS

Figure 1.



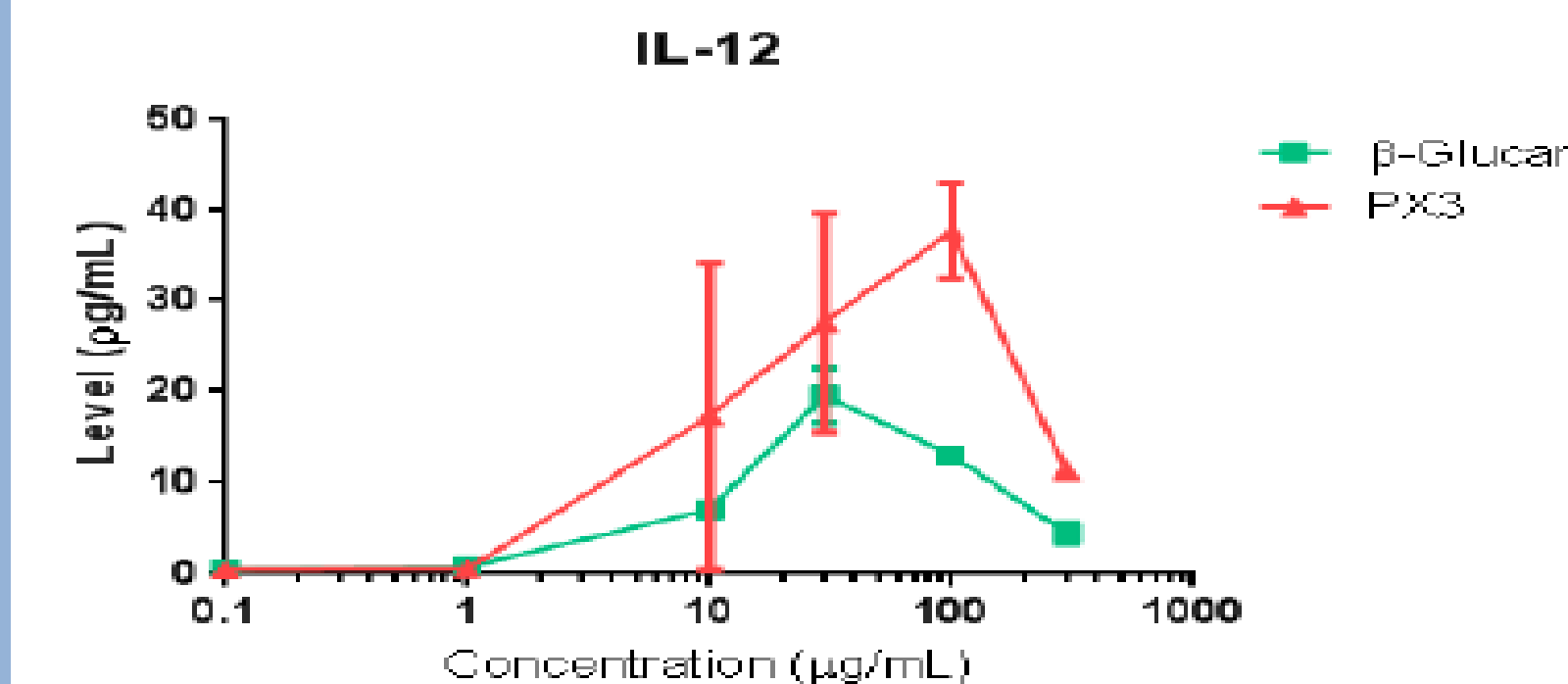
Two way ANOVA:
** Group $p = 0.0047$
**** Concentration $p < 0.0001$
* Interaction $p < 0.0488$

Figure 2.



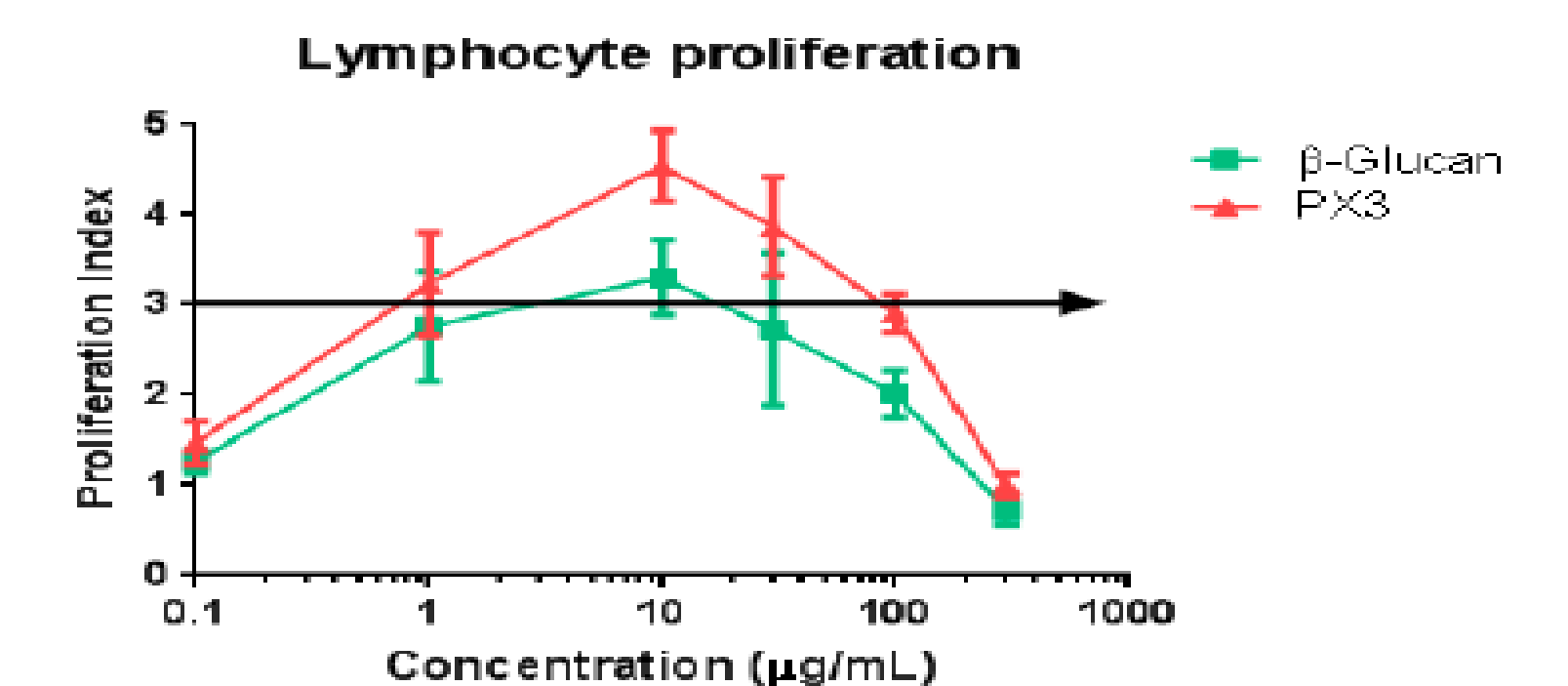
Two way ANOVA:
**** Group $p < 0.0001$
**** Concentration $p < 0.0001$
**** Interaction $p < 0.0001$

Figure 3.



Two way ANOVA:
** Group $p = 0.002$
**** Concentration $p < 0.0001$
* Interaction $p = 0.0264$

Figure 4.



Two way ANOVA:
**** Group $p < 0.0001$
**** Concentration $p < 0.0001$
Interaction $p = 0.077$



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RESULTS CONTINUED

Figure 5.

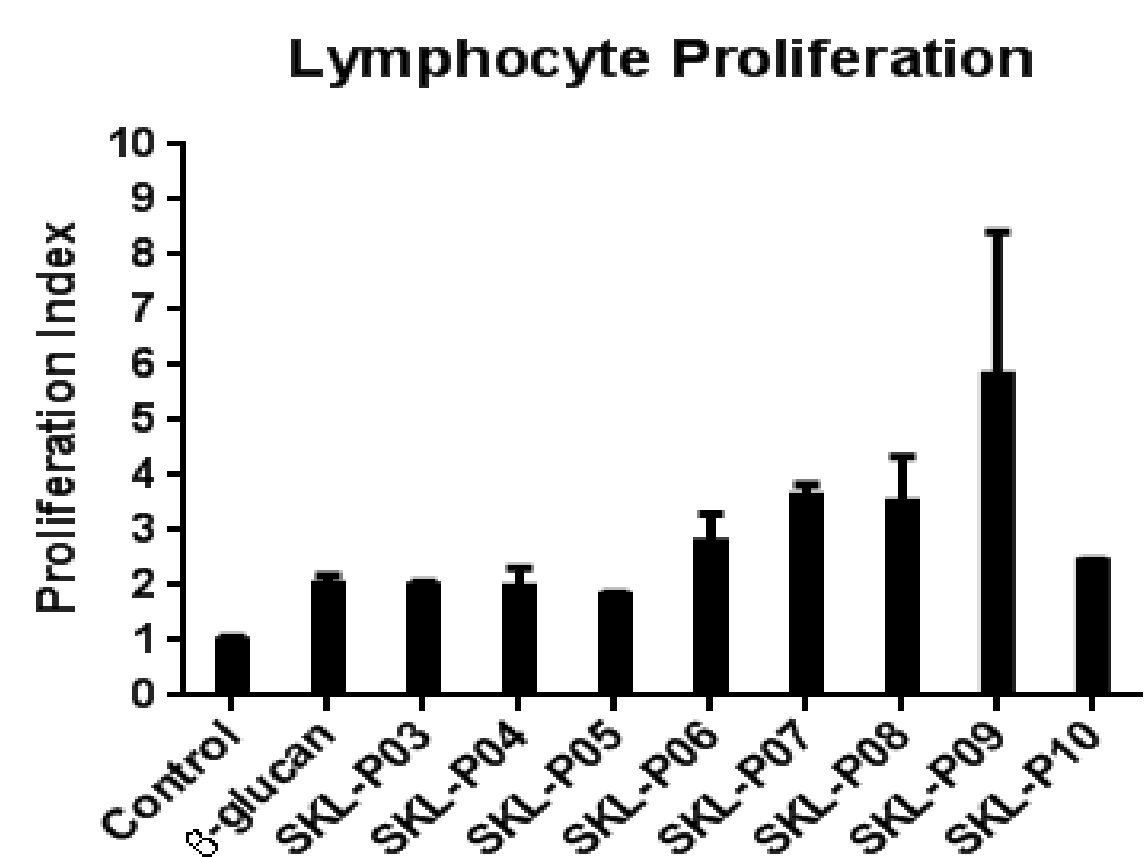


Figure 6.

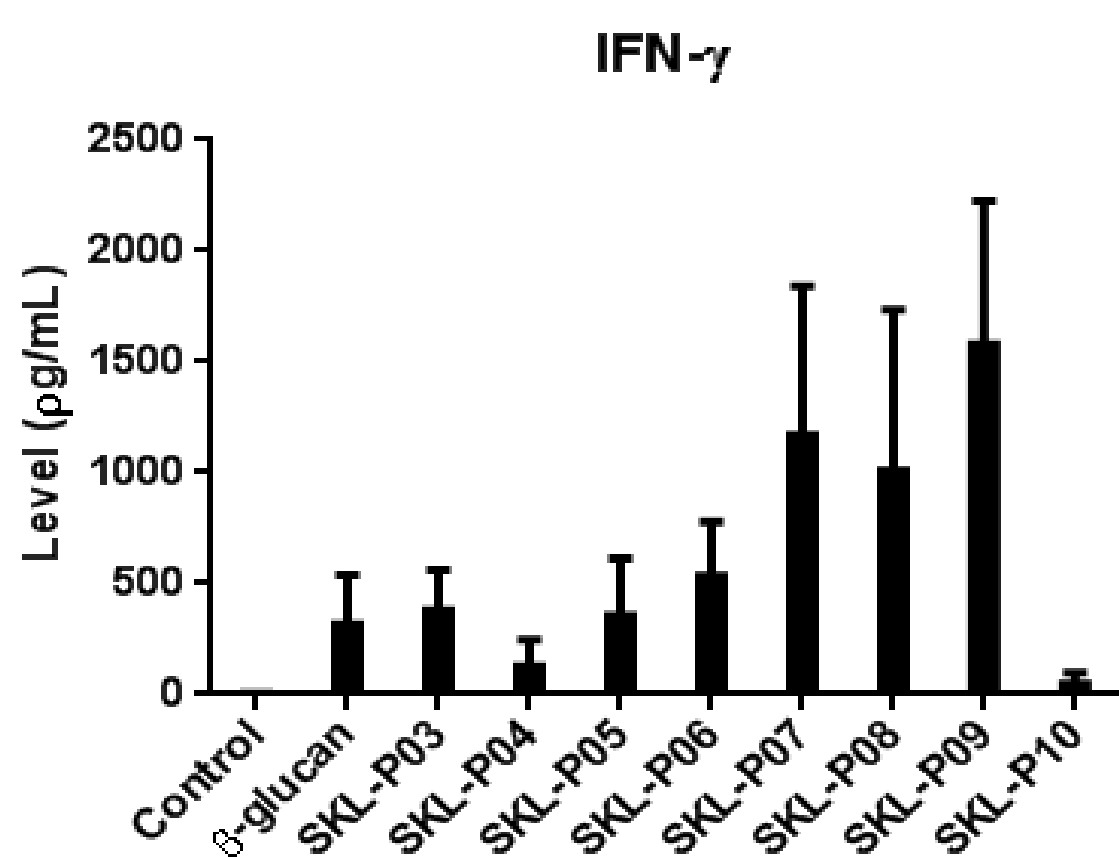


Figure 7.

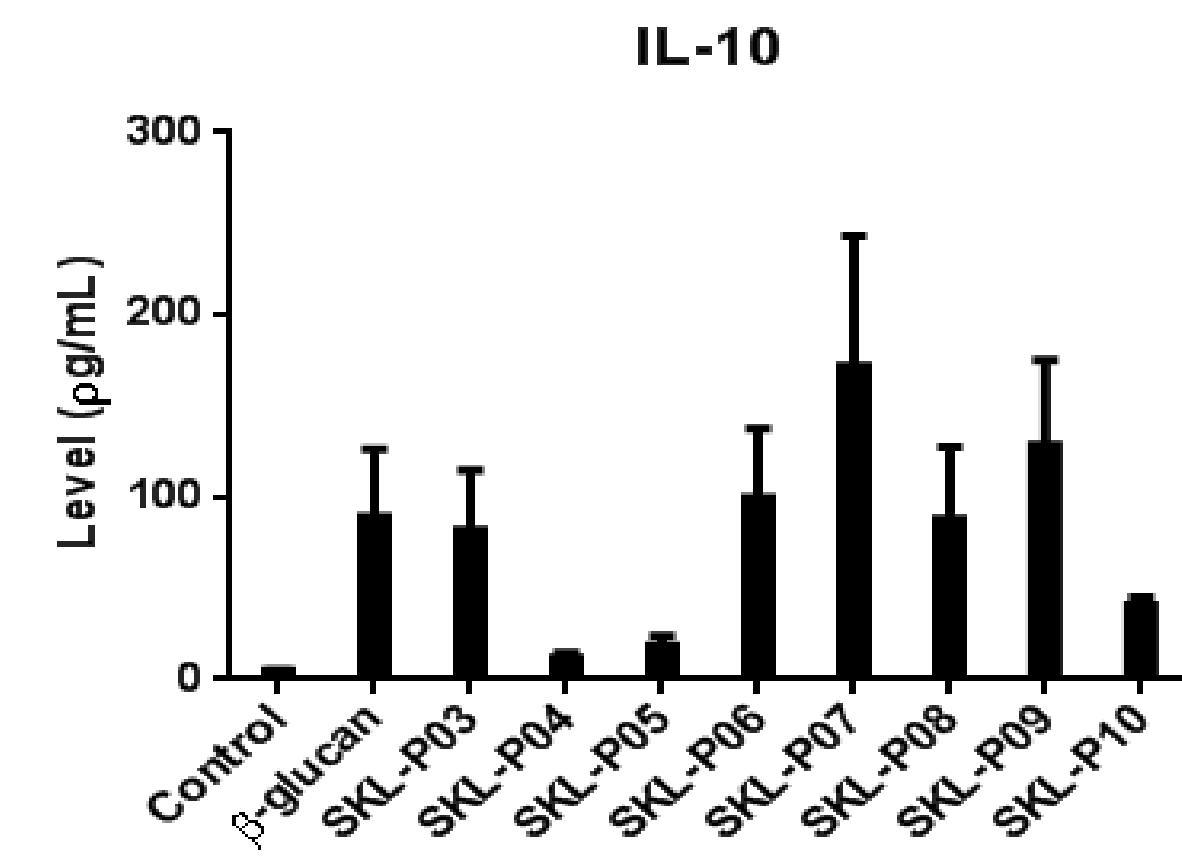


Figure 8.

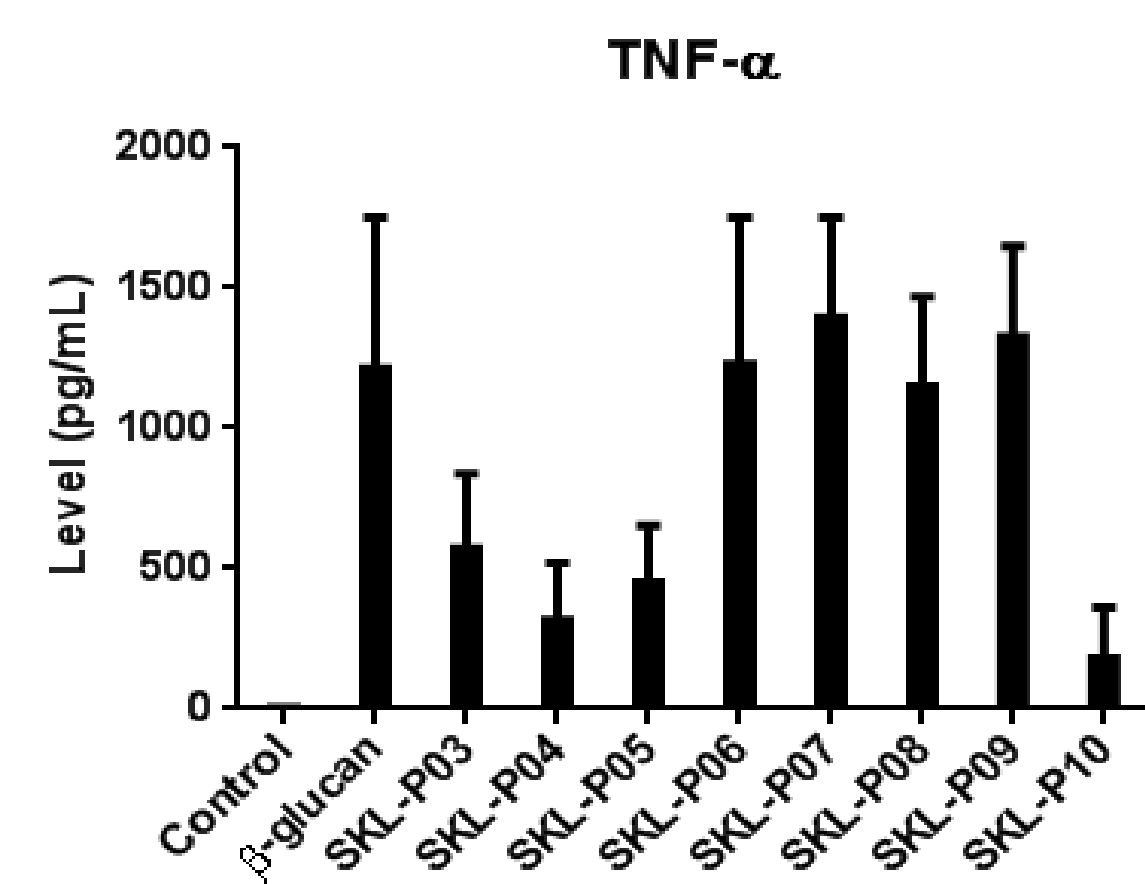


Figure 9.

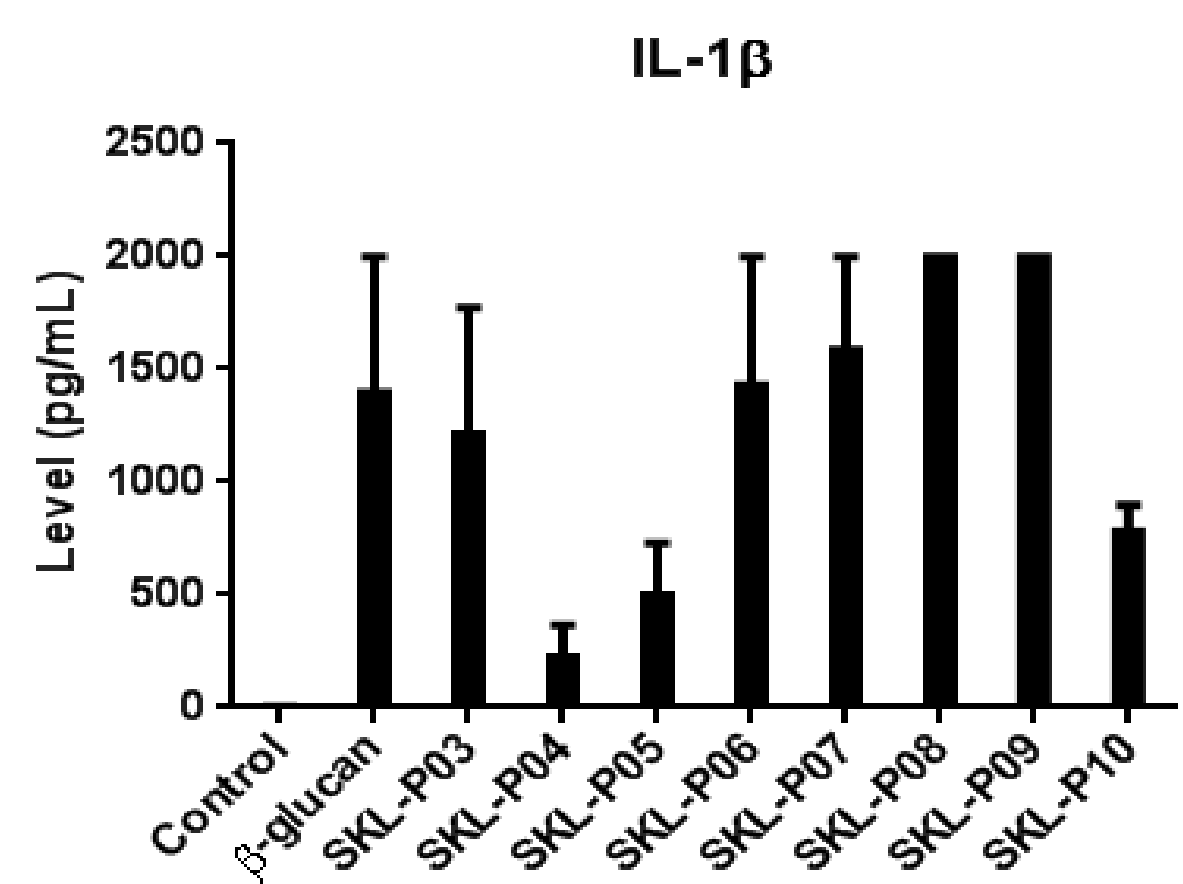
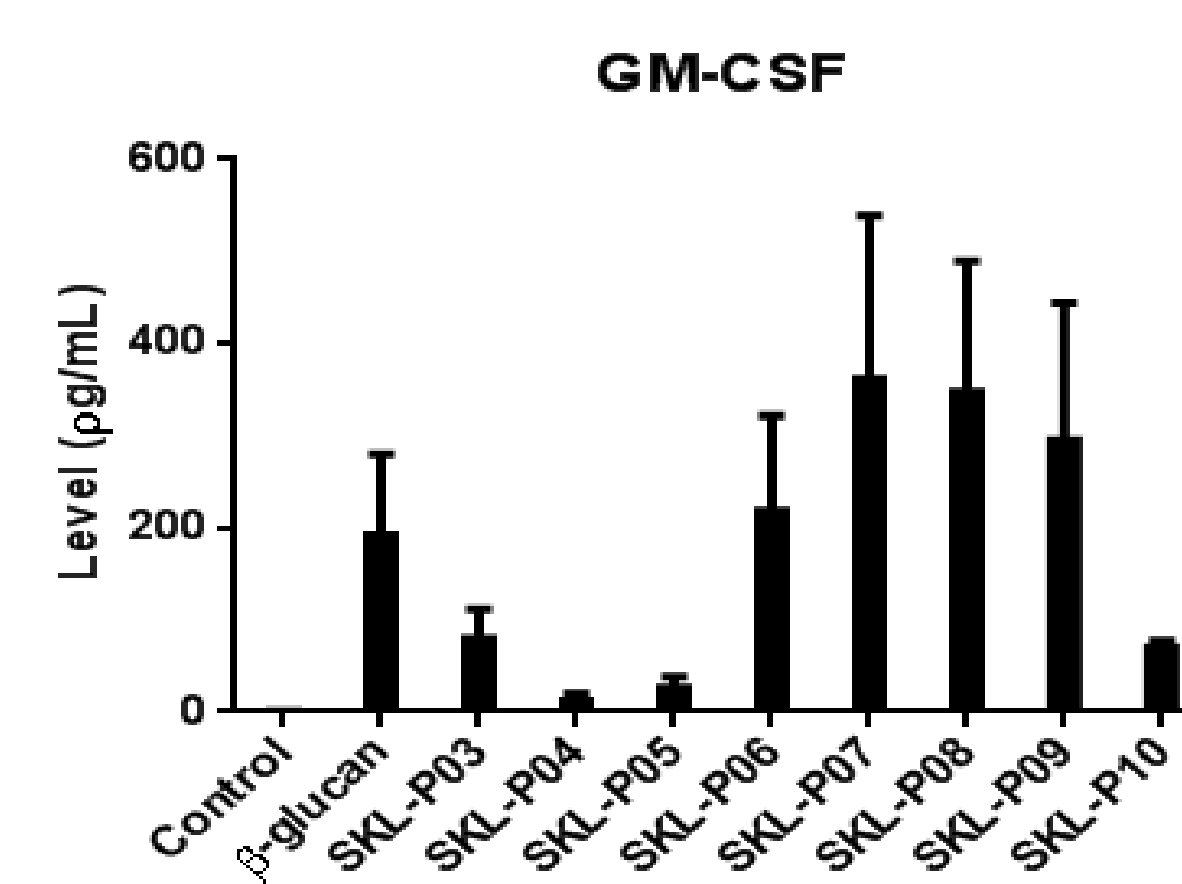


Figure 10.



CONCLUSIONS

Our previous 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 (IFN-γ, IL-10, IL-12) and lymphocyte proliferation. Additional experiments with different combinations of natural extracts (Panax ginseng/American ginseng, Princess Matsutake mushroom /Reishi mushroom, Astragalus root) showed interesting results. While some combination showed synergistic enhancement in activities, others showed reduced efficacy compared to β-glucan (10 μg/mL) alone. Based on our results, we were able to develop a dietary supplement that has the optimal combination of natural extracts (SKL-P09) to potentiate the immuno-stimulatory effects of β-glucan and enhance the efficacy.

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(Abbreviation: IFN-γ: Interferon-gamma; IL-10: Interleukin-10; TNF-α: Tumor necrosis factor-alpha; IL-1β: Interleukin-1beta; GM-CSF: Granulocyte-macrophage colony-stimulating factor)