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# Immunomodulatory Activity of *Sambucus mexicana* and *Trichostema lanatum* on LPS

## Stimulated RAW 264.7 Macrophage Cells

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### Abstract

Chumash medicinal plants *Sambucus mexicana* (Mexican elderberry) and *Trichostema lanatum* (woolly blue curls) were tested for immunomodulatory activity. Anti-inflammatory effects were determined by treating LPS induced RAW 264.7 macrophage cells with plant extracts and measuring the levels of cytokines: tumor necrosis factor alpha (TNF-alpha) and interleukin 10 (IL-10). We hypothesized that both plants would exert immunomodulatory activity by reducing the pro-inflammatory production of TNF-alpha or by promoting M2 polarization with a concurrent increase in IL-10 production. At concentration 0.01 mg/mL woolly blue curls and Mexican elderberry demonstrated anti-inflammatory activity by reducing the concentration of TNF-alpha *in vitro*, while levels of IL-10 were indistinguishable.

### Introduction

The Chumash people, natives of Southern California, used a variety of medicinal flora to treat ailments. Plants, Mexican elderberry and woolly blue curls were recorded to be used to treat infections, cuts, and injuries<sup>1</sup>. We were interested in the immunomodulatory capabilities of these plants given that medicinal plants have a prominent position in drug discovery

The term "immunomodulatory" refers to the ability of a substance to activate, suppress, or regulate one or more functions of the immune system.

Anti-inflammatory immunomodulators can be identified by measuring TNF-alpha, a dynamic pro-inflammatory cytokine produced by stimulated classically activated (M1) macrophages<sup>2</sup>. Healing immunomodulators can be identified by measuring the concentration of IL-10, an anti-inflammatory cytokine released by alternatively activated (M2) macrophages<sup>2</sup>. IL-10 down regulates the pro-inflammatory response induced by M1 macrophages, which is essential for angiogenesis and wound repair<sup>2,3</sup>.



### Methods

All assays were preceded by a 16 hour incubation and used controls: DMSO at 0.01% and LPS at 0.001 µg/µL. All components were added during the same time period; no pre-treatments.

**Cell Line:** RAW 264.7 Macrophage cells (ATCC).

Cultured in DMEM complete with 10% FBS and 1% Antibiotic-Antimycotic. Incubated at 37°C with 5% CO<sub>2</sub>.

**Plant Extraction:** Plants collected from the Santa Monica Mountains were extracted and dried thrice with 100% methanol, rotary evaporation, and centrifugal evaporation. Variables were Mexican elderberry and woolly blue curl extract

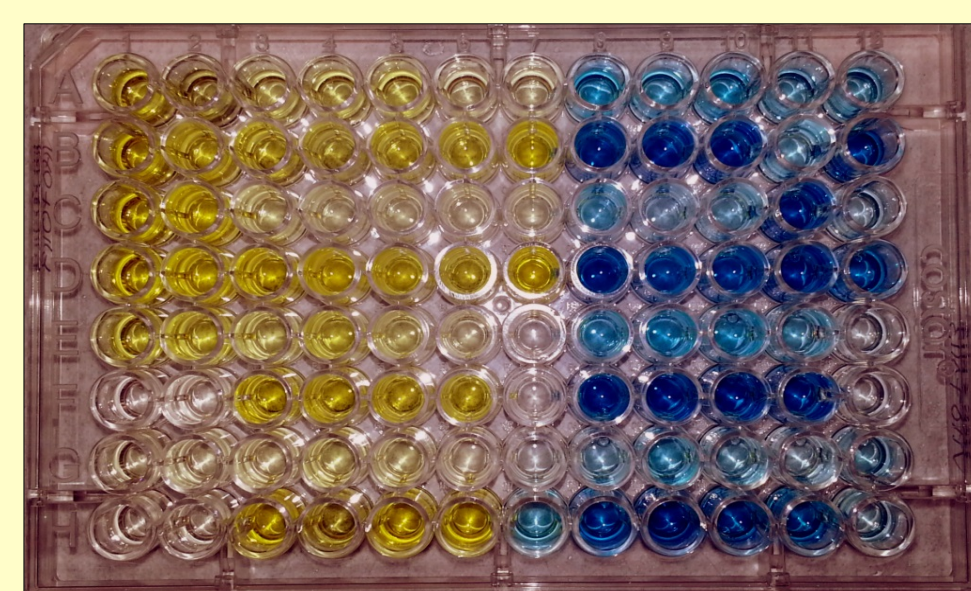
dissolved in DMSO and added to cells at 0.001 mg/µL and 0.01 mg/µL concentrations.

**Macrophage Proliferation:** Cell proliferation was measured using CCK-8 (Dojindo Laboratories).

**Cytokine Production:** TNF-alpha and IL-10 levels were measured by collecting cell supernatants at t=12 and t=24 and performing an ELISA according to the manufacturer (eBioscience).



RAW 264.7 Cells in culture medium



completed (left) and ongoing (right) ELISA

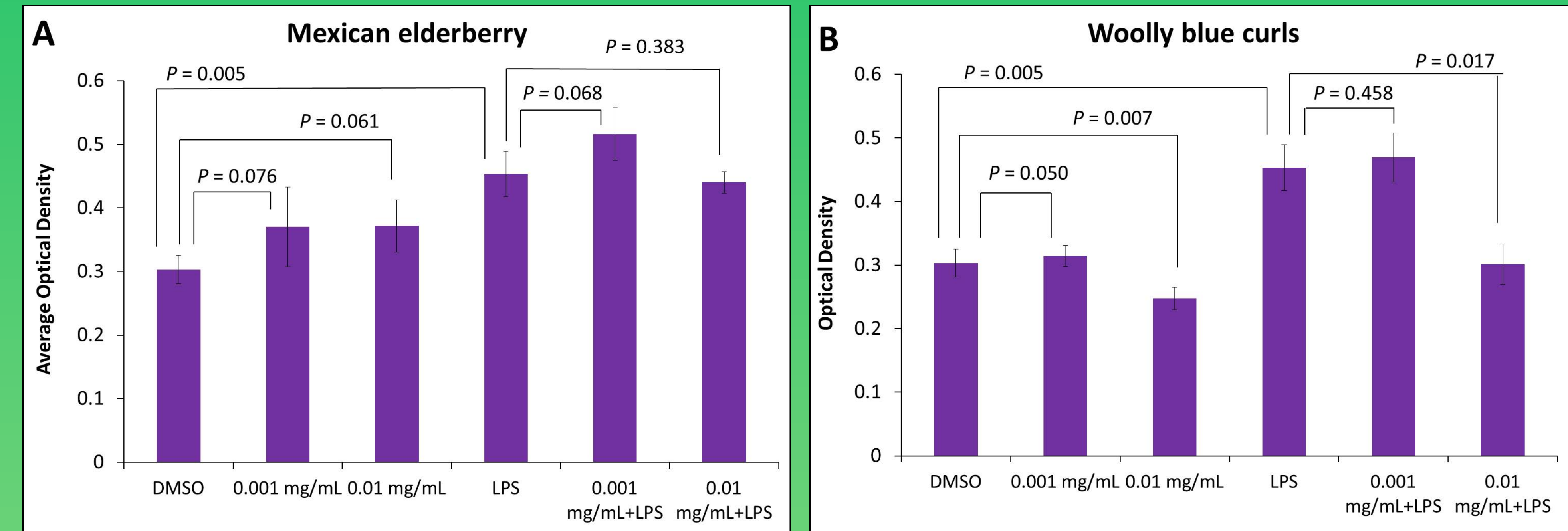
### References

1. Timbrook, J. *Chumash Ethnobotany: Plant Knowledge Among the Chumash People of Southern California*. (Santa Barbara Museum of Natural History, 2007).
2. Laskin, D. L. *Chem. Res. Toxicol.* 22, 1376–1385 (2009).
3. Mosser, D. M. & Edwards, J. P. *Nat. Rev. Immunol.* 8, 958–969 (2008).
4. Soromou, L. W. *et al. Molecules* 17, 3574–3585 (2012).
5. Xaus, J. *et al. Blood* 95, 3823–3831 (2000).
6. Dale, N. *Flowering plants: the Santa Monica Mountains, coastal & chaparral regions of Southern California*. (Capra Press, 1986).

### Results

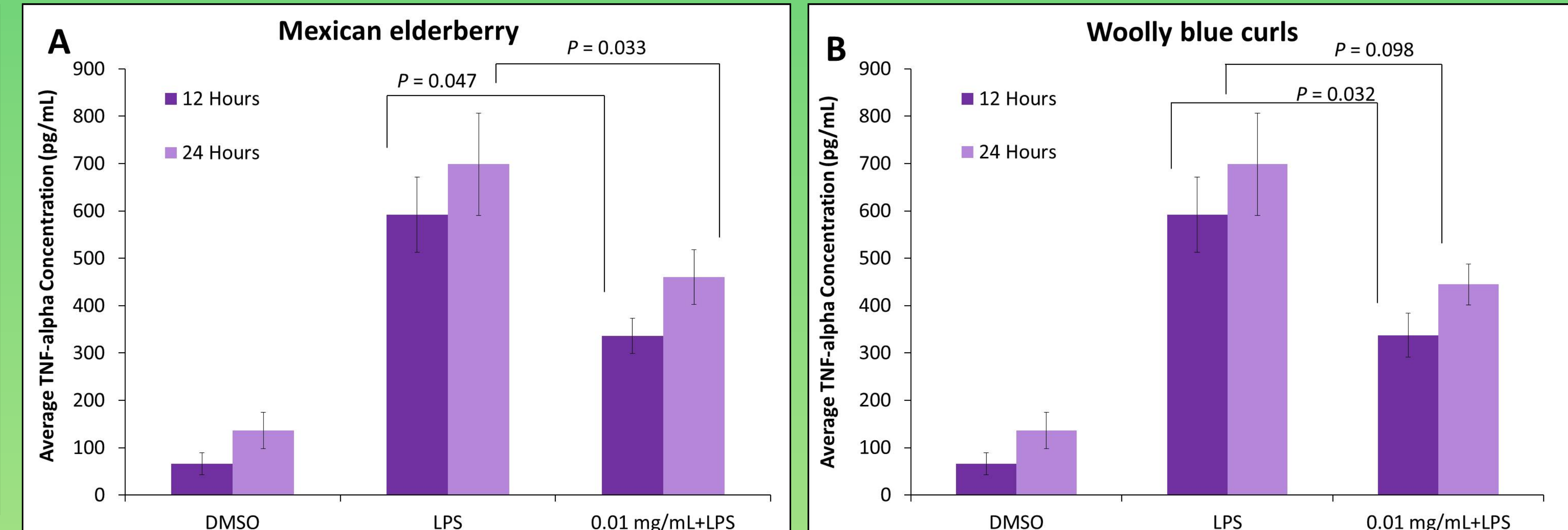
**Key Result #1:** Cell proliferation was unaffected by Mexican elderberry and reduced by woolly blue curls.

**Figure 1: Affect of plant extracts on cell proliferation**  
(A) Mexican elderberry demonstrates no effect on cell proliferation at concentrations 0.001 mg/mL and 0.01 mg/mL (B) Woolly blue curls decreased cell proliferation (P=0.017) at 0.01 mg/mL, but had no effect at 0.001 mg/mL.



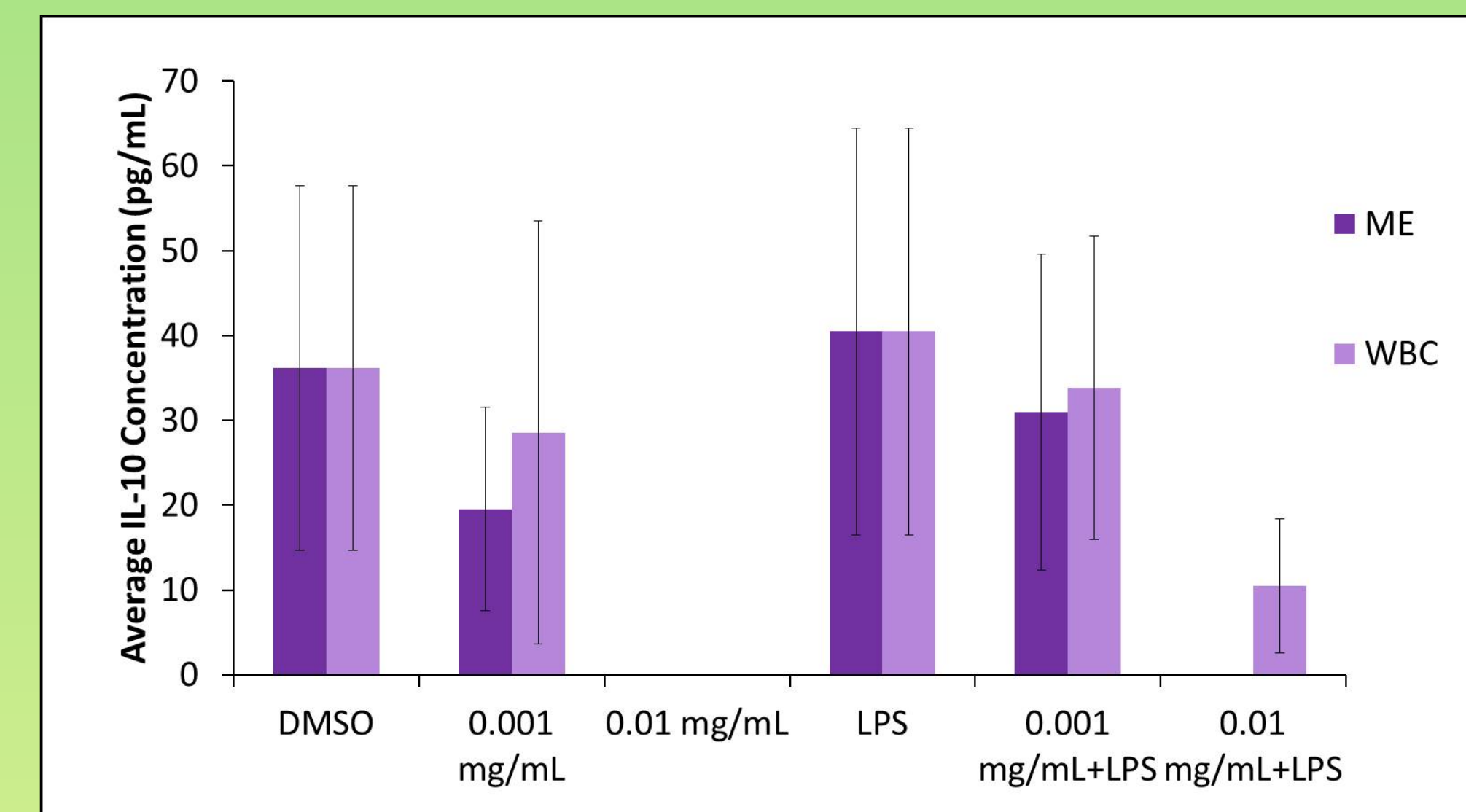
**Key Result #2:** Mexican elderberry and woolly blue curls reduce inflammatory effect of LPS in macrophages.

**Figure 2: 0.01 mg/mL concentrations of Mexican elderberry and woolly blue curls reduce TNF-alpha levels**  
(A) Cells treated with Mexican elderberry at 0.01 mg/mL exhibited a 255 pg/mL decrease (P=0.047) in TNF-alpha levels at t=12 and a 237 pg/mL decrease (P=0.033) at t=24, both in comparison to the LPS control. (B) Cells treated with woolly blue curls at 0.01 mg/mL exhibited a 254 pg/mL decrease (P=0.032) in TNF-alpha levels at t=12 and a 253 pg/mL decrease (P=0.098) at t=24 in comparison to the LPS control.



**Key Result #3:** Mexican elderberry and woolly blue curls have no detectable effect on IL-10 levels.

**Figure 3: Results of IL-10 ELISA after treatment with plant extracts**  
For both concentrations of Mexican elderberry IL-10 was unobserved and/or undetected by the ELISA. The same result occurred with both concentrations of woolly blue curls.



### Discussion

Medicinal plants that modulate cytokine production by macrophages are an important focus of inflammation research<sup>4</sup>. In particular, TNF-alpha is responsible for the regulation of pro-inflammatory responses including cytokine recruitment, apoptosis of cells, and phagocytosis of pathogens<sup>2,4,5</sup>. Deregulation of TNF-alpha production can induce sepsis, tissue injury, and long term inflammation<sup>2,4</sup>. The data presented in this experiment poses possible pharmacological control of TNF-alpha production. Because IL-10 secretion was not observed, the extracts are not immunomodulators of M2 macrophages.



### Conclusions and Further Directions

- Mexican elderberry and woolly blue curl extracts are capable of reducing TNF-alpha secretion by macrophages.
- No significant change in IL-10 secretion was observed.
- Future experiments should include isolation of a bioactive chemical from plants or identification of a TNF-alpha inhibition pathway.

### Acknowledgements

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