

# THE EFFECTS OF CANNABIDIOL ON PROINFLAMMATORY CYTOKINES

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## ABSTRACT

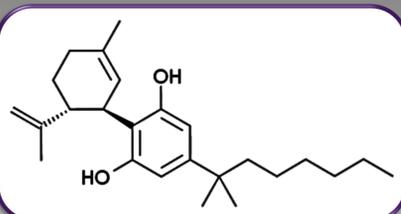
Cannabidiol (CBD) is a non-psychoactive compound of *Cannabis sativa* that has acquired recent interest as a natural supplement with several proposed health benefits. One of the main being its possible therapeutic use as an anti-inflammatory drug. We investigated the effectiveness of CBD at reducing and preventing the inflammatory response on human peripheral blood mononuclear cells (PBMCs). Lipopolysaccharide (LPS) was used to initiate the response, while simultaneously being treated with different concentrations of CBD alone, common drugs alone, and a combination of the two. The inflamed and treated cells were placed into a 96-well plate to test the levels of five pro-inflammatory cytokines: **IL-1 $\alpha$** , **IL-1 $\beta$** , **IL-6**, **IL-12 (p40)**, and **TNF-alpha**. The plate was run on a Luminex MAGPIX<sup>®</sup> analyzer. An Analysis of Variance (ANOVA) test was ran to compare the mean values of each drug and its effect on the five cytokines. A TukeyHSD test was used as well to determine significance across the different concentrations. It is believed that CBD will be effective at reducing the amount of cytokine present.

### Full Spectrum CBD

### CBD Isolate

"Whole plant" or full spectrum CBD contains pure cannabidiol along with all other cannabinoids found in the marijuana plant including CBDV, CBG (Cannabigerol), CBC (Cannabichromene) and CBN (Cannabinol). It is thought (with little research) that the highest level of effectiveness comes from the synergistic interactions of all cannabinoids. We used a brand of full spectrum CBD that can be bought over-the-counter at your local pharmacy.

Isolated pure scientific grade cannabidiol was purchased from Fisher Scientific. Isolated CBD is extracted through supercritical CO<sub>2</sub> extraction.



## RESULTS

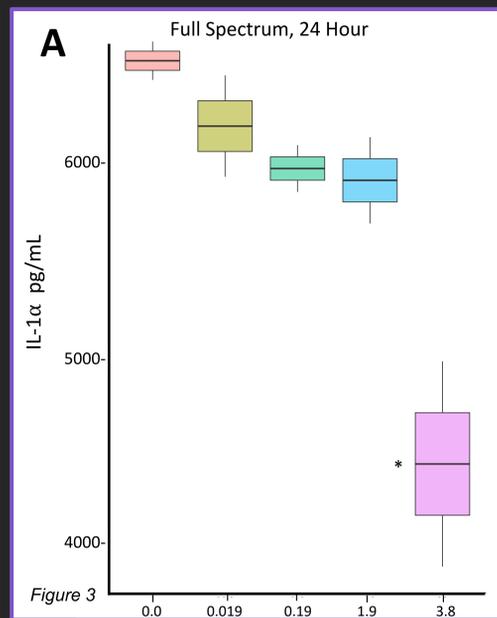


Figure 3

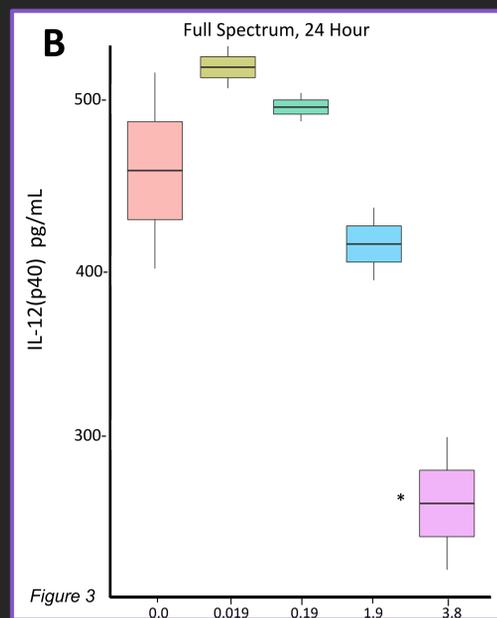


Figure 3

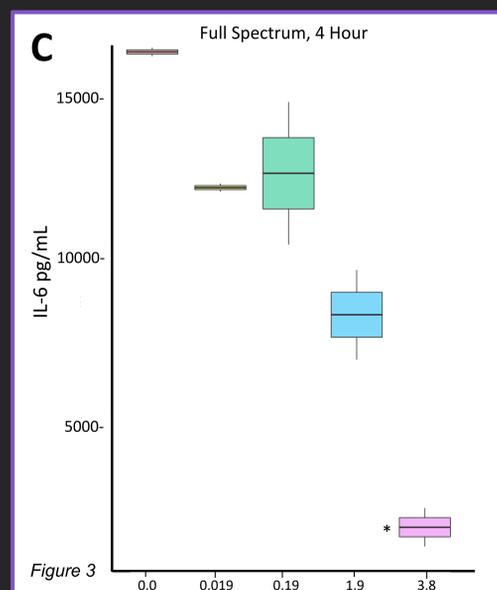


Figure 3

Figure 3.A – A boxplot display of Full Spectrum CBD's result on the IL-1 $\alpha$  cytokine. All concentration values are in a mg/mL unit. \* (p value = 0.02). Positive control is the 0.0 mg/mL and is what was compared against in TukeyHSD test.

Figure 3.B – A boxplot display of Full Spectrum CBD's result on the IL-12(p40) cytokine. All concentration values are in a mg/mL unit. \* (p value = 0.043). Positive control is the 0.0 mg/mL and is what was compared against in TukeyHSD test.

Figure 3.C – A boxplot display of Full Spectrum CBD's result on the IL-6 cytokine. All concentration values are in a mg/mL unit. \* (p value = 0.0018). Positive control is the 0.0 mg/mL and is what was compared against in TukeyHSD test.



## METHODS

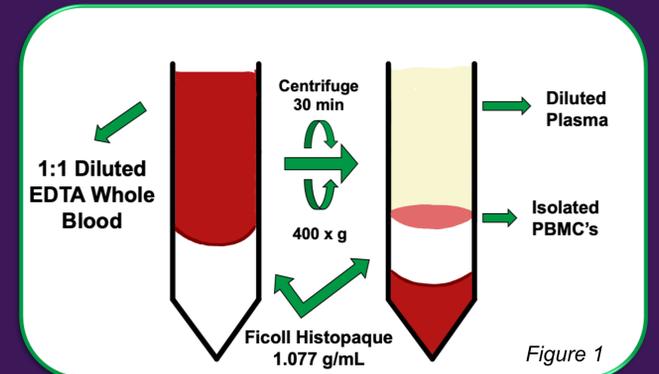


Figure 1

Figure 1. PBMC's were isolated from blood of student researcher through standard ficoll separation. Cells were removed, washed and enumerated with Beckman-Coulter CBC analyzer. The total cells were diluted to a concentration of ~ 5.0x10<sup>5</sup> cells/well.

Table 1 CONCENTRATIONS

Isolated CBD	Full Spectrum CBD	Lipopolysaccharide
200 ng/mL	3.8 mg/mL	100 ng/mL
100 ng/mL	1.9 mg/mL	
50 ng/mL	0.19 mg/mL	
25 ng/mL	0.019 mg/mL	
12.5 ng/mL		

Table 1. CBD concentrations were all incubated with LPS and PBMC's for 1, 4 and 24 hour time points. Positive controls were incubated with LPS and neither CBD. Negative controls were incubated with no LPS or either CBD.

### Magpix<sup>®</sup> Magnetic Bead Technology:

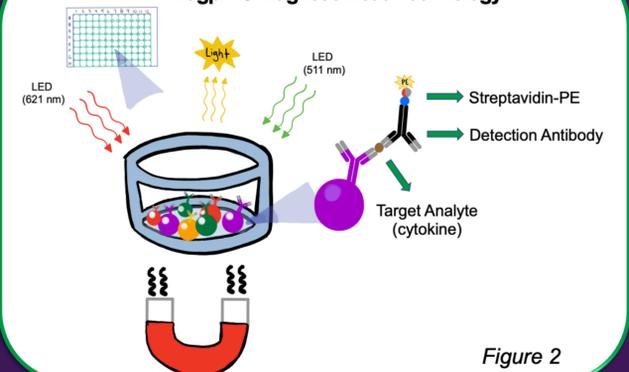


Figure 2

Figure 2. Magpix analyzer was used to determine values of IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-12(p40), and TNF- $\alpha$ . All conditions were ran in duplicate. Standards were provided by Millipore and ran according to their protocol.

## DISCUSSION

- The data that is displayed is the only ones showed significance to reject the null hypothesis that CBD would not reduce cytokine production. None of the pure CBD values across any concentration at any time periods showed significance when Tukey test was performed. There was no cytokine production across all cytokines, drugs and concentrations at the one hour time point. We believe that the LPS had yet to illicit an inflammatory response.
- After calculating significant p-value for 3 different cytokines with the highest Full Spectrum CBD concentration another plate is to be run to try and duplicate the results with more data points. Along with using the 3.8 mg/mL as the new lowest dose and increasing it from there to see if the trend continues.
- To further the research it is planned in our second plate to run Ibuprofen USP and Hydrocortisone USP which are known anti-inflammatory drugs to also use as a comparison the CBD which will be ran with it.



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