# CBD Attenuates Hyperglycemia Induced Pro-Inflammatory Cytokines, Chemokines and M1 Polarization in THP1 monocytes

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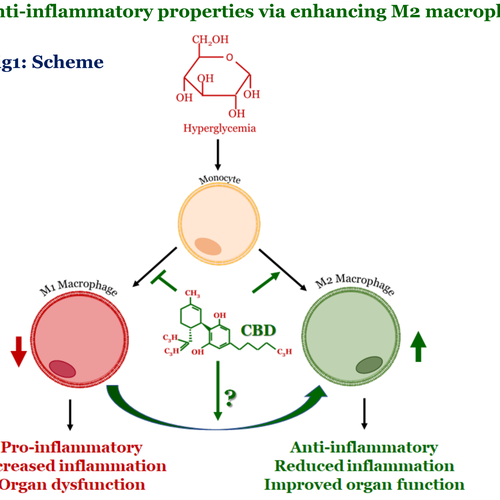
## INTRODUCTION

- Infiltrating monocytes are a major player in the development and pathogenesis of various diseases such as atherosclerosis, diabetes, and Alzheimer's.
- Cannabidiol (CBD) therapeutics is a growing area of research and becoming a major public concern on its use in pain management, diabetes, Alzheimer's, and psychological disorders.
- The underlying mechanism of inflammation management using CBD i. e. whether CBD enhances the differentiation of anti-inflammatory M2 macrophages from monocytes remains unknown, which could be used as a potential therapeutic intervention in diabetes and many other inflammatory disease conditions.
- In this study, we investigated whether CBD polarizes monocytes to M2 macrophages, and ameliorates hyperglycemia-induced pro-inflammatory microenvironment by enhancing anti-inflammatory M2 macrophages. Further, we investigated the mechanisms of anti-inflammatory properties of CBD attenuation under hyperglycemic conditions of monocyte infiltration.

## HYPOTHESIS

**We hypothesize that CBD attenuates the hyperglycemia in monocytes as well as provide anti-inflammatory properties via enhancing M2 macrophages.**

**Fig1: Scheme**




## METHODS

- THP-1, a human monocyte cell line obtained from American Type Culture Collection (ATCC) and was cultured in RPMI-1640 medium following the supplier's instructions.
- Cells were incubated with CBD (0, 2.5, 10, and 20µg/ml) to determine whether THP1 monocyte cells differentiate into pro-inflammatory M1 macrophages or anti-inflammatory M2 macrophages.
- THP1 cells were examined under high glucose conditions to simulate the diabetic model.
- Cells were incubated with glucose (50mM) for 24hrs, followed by CBD for additional 24hrs.
- After 48hrs of incubation, cells and cell culture supernatants were examined by immunocyto-chemistry (ICC), RT-PCR, and ELISA respectively.
- Additionally, Cytokine array (120 cytokines) was performed for all the three groups to evaluate inflammatory cytokine profile.
- Cell fate of differentiated cells was determined using different markers such as CD206, and Arginase-1 for M2 macrophages and iNOS, TNF-α, and IL-6 for M1 macrophages and pro-inflammatory cytokines.

## RESULTS

### Monocyte treatment with a dose-dependent increase of CBD enhances M2 macrophage markers CD206 and Arginase1

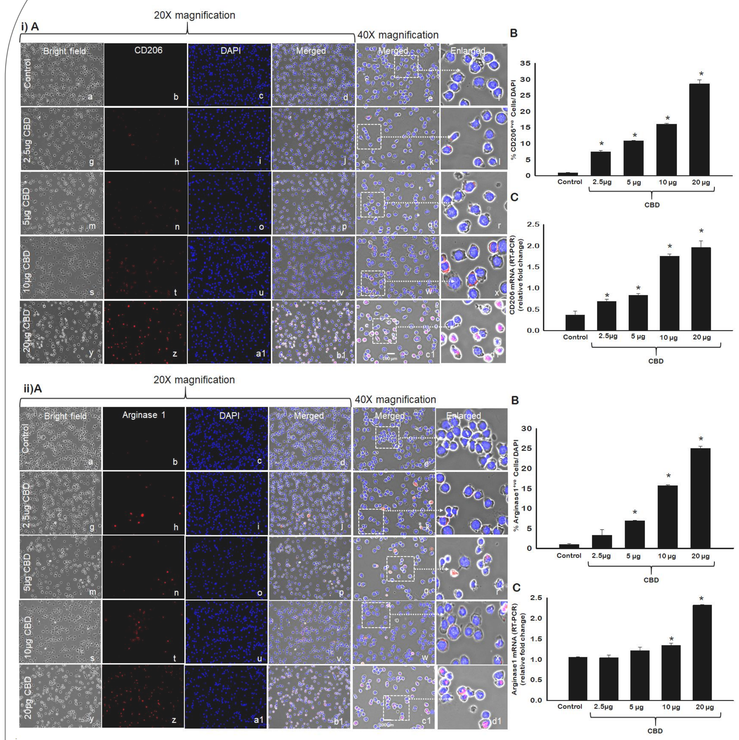



**Fig.2: Dose-dependent increase of CBD polarizes monocytes into M2 macrophages:** CBD treatment polarizes monocytes to M2 macrophages. Representative images (20X) of ICC staining for M2 macrophage markers i) CD206, ii) Arginase1 after 24hrs dose-dependent treatment of CBD. Representative images were taken with a Keyence microscope. As shown in panel (A) all groups that exhibit CD206/Arginase1-positive cells are in red (b, h, n, t, z), DAPI in blue (c, i, o, u, a1), merged images (d, j, p, v, b1), 40X magnification merged images (e, k, q, w, c1), white dotted boxes and arrows indicate the enlarged section of merged images(f, l, r, x, d1). Scale bar = 100µm. Quantitative analysis in bar graphs for ICC (B) and gene expression (C) shows increased expression of CD206 and Arginase1 with increased concentration of CBD. Error bars = mean ± standard error of the mean (SEM). One-way ANOVA and Tukey tests were performed to assess statistical significance* p<0.05 vs. control; n=3-4.

### Dose-dependent increase of CBD increases anti-inflammatory cytokine IL-10

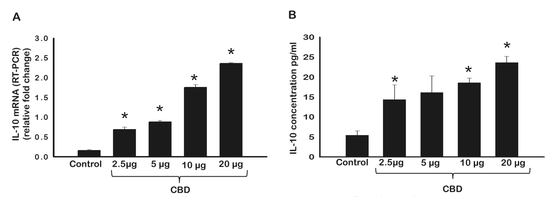



**Fig.3: Dose-dependent increase of CBD increases anti-inflammatory cytokine IL-10.** Bar graphs represent the A) gene expressions of IL-10 and B)ELISA was observed as dose increased. Error bars = mean ± standard error of the mean (SEM). One-way ANOVA and Tukey tests were performed to assess statistical significance * p<0.05 vs. control; n=3-4.

### CBD treatment reduces Monocyte polarization to M1 macrophages under diabetic conditions

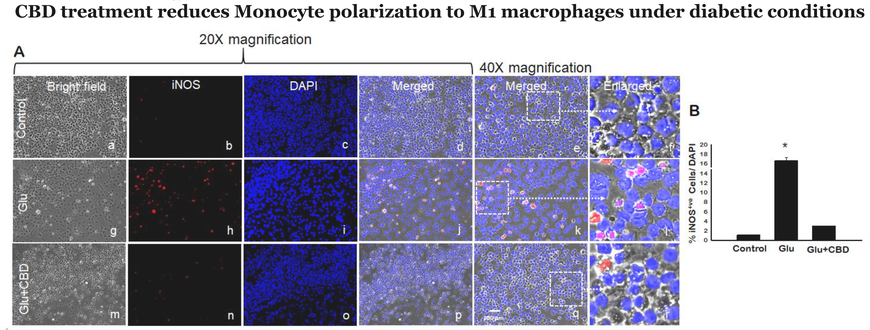



**Fig.4: CBD reduces monocyte polarization to M1 macrophages under diabetic conditions:** CBD reduces hyperglycemia-induced monocyte differentiation to M1 macrophages. Representative images (X20) of ICC staining for M1 macrophage markers iNOS. As shown in panel (A) all groups that exhibit iNOS-positive cells are in red (b, h, n), DAPI in blue (c, i, o), merged images (d, j, p), 40X magnification merged images (e, k, q), white dotted boxes and arrows indicate the enlarged section of merged images (f, l, r). Scale bar = 100µm. Quantitative analysis in the bar graph for ICC (B) shows increased expression of iNOS under hyperglycemic conditions whereas CBD significantly reduced the iNOS expression. Error bars = mean ± standard error of the mean (SEM). One-way ANOVA and Tukey tests were performed to assess statistical significance* p<0.05 vs. control; n=3.

### CBD treatment enhances Monocyte polarization to M2 macrophages under diabetic conditions

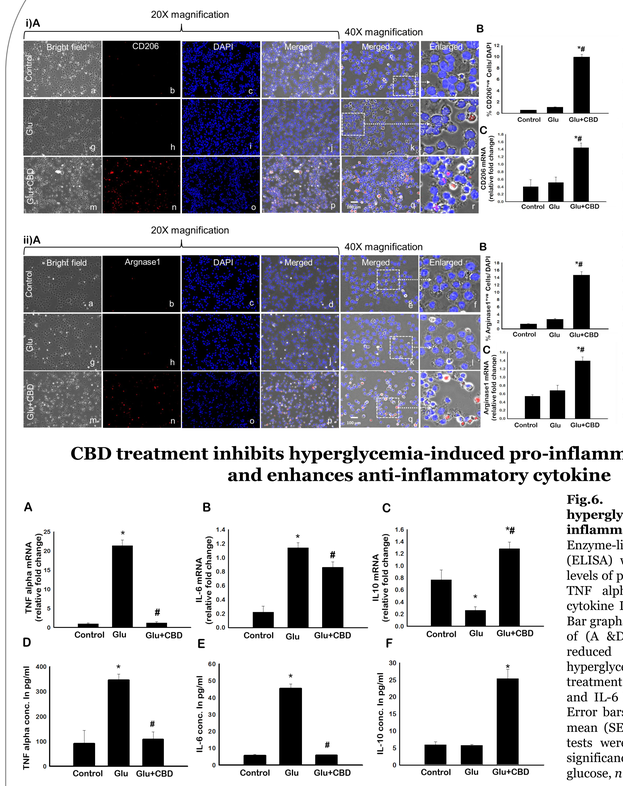



**Fig.5: CBD enhances monocyte polarization to M2 macrophages under diabetic conditions:** Representative images (20X) of ICC staining for M2 macrophage markers i) CD206, ii) Arginase1. As shown in panel (A) all groups that exhibit i) CD206 and ii) Arginase1-positive cells are in red (b, h, n), DAPI in blue (c, i, o), merged images (d, j, p), 40X magnification merged images (e, k, q), white boxes and arrows indicate the enlarged section of merged images(f, l, r). Scale bar = 100µm. Quantitative analysis in a bar graph for ICC (B) shows increased expression of CD206 and Arginase1 under diabetic conditions with CBD treatment. Error bars = mean ± standard error of the mean (SEM). One-way ANOVA and Tukey tests were performed to assess statistical significance* p<0.05 vs. control; #P < 0.05 glucose; n=3.

### CBD treatment inhibits hyperglycemia-induced pro-inflammatory cytokines and enhances anti-inflammatory cytokine




**Fig.6. CBD treatment inhibits hyperglycemia-induced pro-inflammatory cytokines.** RT-PCR and Enzyme-linked immunosorbent assay (ELISA) was performed to determine the levels of pro-inflammatory cytokine levels of TNF alpha and IL-6, anti-inflammatory cytokine IL-10 in cell culture supernatants. Bar graphs represent gene and protein levels of (A &D) TNF alpha, (B&E) IL-6 and reduced (C&F)IL-10 levels under hyperglycemic conditions, whereas CBD treatment potentially reduced the TNF alpha and IL-6 levels by enhancing IL-10 levels. Error bars = mean ± standard error of the mean (SEM). One-way ANOVA and Tukey tests were performed to assess statistical significance. *$P < 0.05$ vs. control, #$P < 0.05$ glucose, $n = 3$

### CBD attenuates hyperglycemia-induced pro-inflammatory microenvironment by enhancing anti-inflammatory M2 macrophage markers

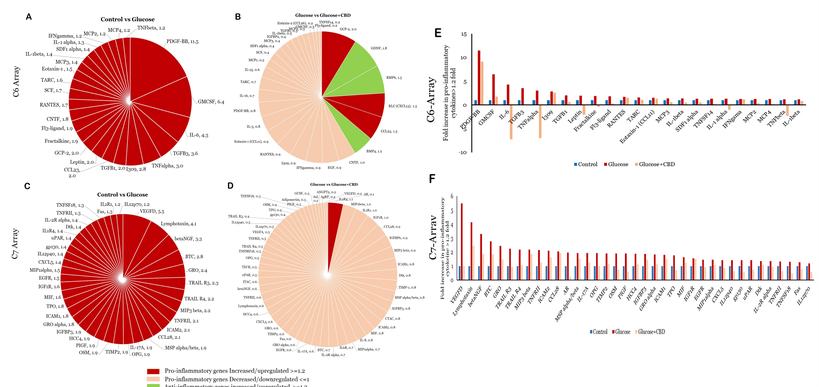



**Fig.7: CBD attenuates hyperglycemia-induced pro-inflammatory microenvironment by enhancing anti-inflammatory M2 macrophages:** Pie charts (A&C) represent the hyperglycemia upregulated pro-inflammatory cytokines that are involved in inflammation, apoptosis, angiogenesis, macrophage inflammatory proteins, growth factors and adhesion molecules as compared to control, whereas CBD treatment reduced differentially the pro-inflammatory cytokine expression (B & D). Bar graphs (E&F) represents the Pro-inflammatory cytokines that are upregulated >1.2 fold when compared to control.

## CONCLUSION

❖ We suggest that CBD has a greater therapeutic potential in diabetic conditions by decreasing pro-inflammatory monocytes, macrophages, and cytokines. We also provide evidence that CBD enhances anti-inflammatory M2 macrophages.