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Cannabis Oil Extract (COE) POTential:

The Effect of COEs on Gene Expression in Kidney Cells Exposed to Varying Amounts of Glucose In Vitro

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Problem

- 38.0% of U.S. population, age 18+, is prediabetic and 11.6% of U.S. population has diabetes[1].
- Between 1999 and 2023, the rate of diabetes in the U.S.increased from 9.7% to 14.3%[2].
- 45% of people with diabetes also suffer from diabetic nephropathy[3].
- Approximately 10% of the global population has chronic kidney disease[4].

Purpose

- This study investigates the gene expression of podocalyxin (*PODXL*), maternally expressed gene 3 (*MEG3*), and fibronectin (*FN1)* in Human Glomerular Epithelial Cells (HGEC) exposed to varying glucose levels, as well as different cannabis oil extracts (COEs).

Review of Literature

- Cannabis plants have anti-diabetic properties[5].
- Cannabidiol (CBD), cannabichromene (CBC), and cannabigerol (CBG) are 3 of many COEs found in *Cannabis sativa*[6] (Figs. 1-3).
- *PODXL* RNA codes for a protein that supports podocyte foot adhesion in the podocytic slit diaphragm, which is essential to the proper functioning of the glomerular filtration apparatus[7].
- *MEG3,* a nuclear long noncoding RNA (lncRNA), exhibits increased levels in hyperglycemic cells, which is associated with excessive mitochondrial fission and podocytopathy (kidney disease affecting the podocytes)[8].
- Fibronectin (*FN1*) codes for a glycoprotein that gets upregulated in patients with diabetic nephropathy (kidney disease)[9].

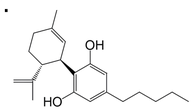

Fig. 1: CBD

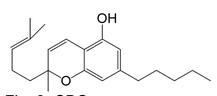

Fig. 2: CBC

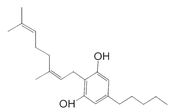

Fig. 3: CBG

Methods

- Retrotranscription (RT) of HGEC RNA to cDNA
- qPCR of *PODXL, MEG3*, and *FN1* using Bio-Rad CFX96 with C1000 Touch Thermal Cycler (Fig. 4)
- C_q analysis using Bio-Rad CFX Maestro 2.0
  - Removal of outliers using 1.5IQR method
  - ΔC_q normalization: *GAPDH* housekeeping gene
  - Fold change (FC) calculated using $2^{-\Delta\Delta Cq}$ method
- qPCR localization of *MEG3*
  - Nuclear:cytoplasmic fold index (FI) calculated using (2^-ΔCt nucl)/(2^-ΔCt cyt) method

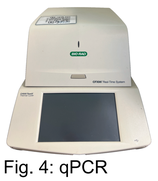

Fig. 4: qPCR

Results

- Increased levels of *PODXL* with COEs in normal glucose (5 mM), but decreased levels of *PODXL* with CBC and CBG in high glucose (25 mM) (Figs. 5-6, Tab. 1)

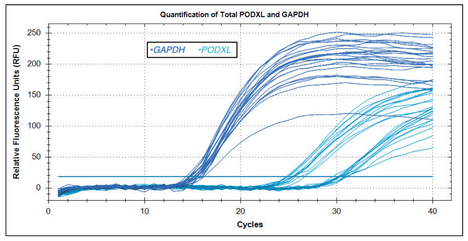

Fig. 5: *PODXL* qPCR amplification

Glucose Level	Treatment (5 µM, 24 hr)	PODXL mRNA Levels
normal (5 mM)	None	1
	CBD	1.5
	CBC	1.7
	CBG	2.5
high (25 mM)	None	0.05
	CBD	0.07
	CBC	0.0000014
	CBG	0.0000014

Table 1: *PODXL* mRNA Levels

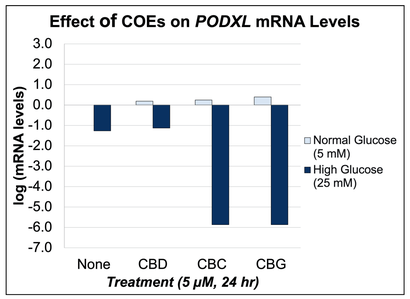

Fig. 6: Effect of COEs on *PODXL* levels

- Increased *MEG3* levels at 3 weeks in high glucose, but not as much with COEs, especially CBC and CBG (Fig. 7)
- Increased *MEG3* levels in high glucose with COEs (Fig. 7)
- Increased *FN1* levels with COEs compared to no treatment in normal glucose (Fig. 8)
- No significant differences in *FN1* levels between COE treatments at 3 weeks or long term in high glucose (Fig. 8)

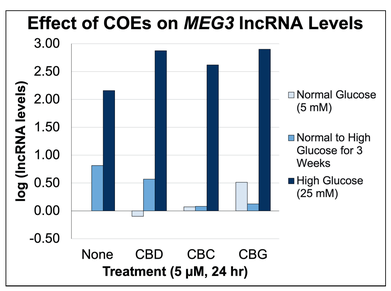

Fig. 7: Effect of COEs on *MEG3* Levels

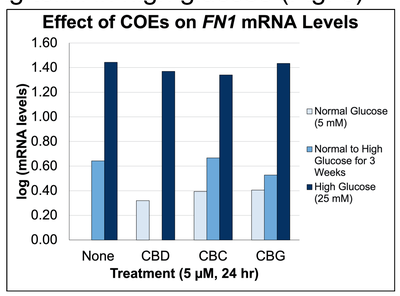

Fig. 8: Effect of COEs on FN1 Levels

- Decrease in nuclear to cytoplasmic *MEG3* ratio in high glucose at 4 weeks and beyond

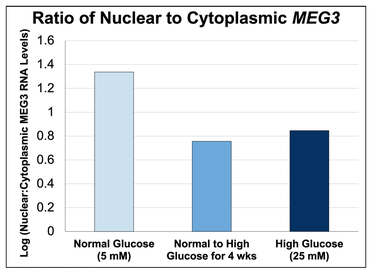

Fig.9: *MEG3* localization

Discussion

- Significant downregulation of *PODXL* in high glucose compared to normal glucose samples (p=0.007) using a t-test (α=0.05)
- Insignificant difference (p=0.48) in *MEG3* at week 3 in high glucose using ANOVA post hoc Tukey HSD (α=0.05)
- Significant upregulation (p=0.001) in *MEG3* after week 3 in high glucose.

Conclusion

- CBD neither increases nor decreases *PODXL* levels in HGEC when compared to the control.
- CBC and CBG do not appear to be good candidates for protecting HGEC from *PODXL* downregulation.
- COEs appear to increase *FN1* levels compared to normal glucose with no treatment. This undesirable effect could lead to an immune infiltration response in vivo[9].
- The increase in *MEG3* appears to be in the cytoplasm due to the lower nuclear to cytoplasmic ratio.

Future Research

- Two more biological replicates for each condition so that further statistical analysis can be completed
- Repeat with other COEs such as CBDA, CBDV, CBDA, CBE, CBGA, CBN, or CBCT

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