Transcriptomic Analysis of Cannabidiol and Tetrahydrocannabivarin Revealed New Molecular Targets for Treatment of Experimental Diabetic Neuropathy

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Abstract

PURPOSE: To identify the transcriptomic signatures of Cannabidiol (CBD) and tetrahydrocannabivarin (THCV) in Streptozotocin induced experimental diabetic neuropathy (DN)

METHODS: Animals were rendered diabetic using STZ (55 mg/kg, i.p). CBD was administered (10 & 20 mg/kg, i.p) and THCV (15 & 30 mg/kg, i.p) during the last 4 weeks of 12 week diabetic period. The animals' pain perception was assessed using the Hargreaves plantar test, hot and cold plate method, vonfrey aesthesiometer, and Randal Sellito apparatus, and nerve functional assessment using the Laser Doppler oxymeter. After the study, the animals' blood was drawn to measure blood glucose levels and their DRGs were isolated for transcriptomic studies

RESULTS: Diabetic animals after eight weeks significantly ($P<0.001$) increased hypersensitivity to thermal and mechanical pain and also significantly ($p<0.001$) reduced nerve blood flow when compared to the age matched control animals. CBD and THCV treatment reversed these effects in a dose-dependent manner while having no effect on the animals' body weights or blood glucose levels. Differently expressed genes (transcriptomic analysis) have been discovered in the isolated DRGs of control, diabetic, and treated animals, with 32 genes in the control group, 33 in the THCV group, and 45 in the CBD group, all of which differ from the genes expressed in diabetic animals' DRGs. These genes regulating nerve function by affecting the RAP1 signaling pathway, MAP kinase signaling pathway, neurotrophin signaling pathway, Parkinson's disease, Alzheimer's disease, focal adhesion, insulin signaling pathway, microRNAs in cancer, and others according to KEGG analysis.

Conclusion: Despite the fact that CBD and THCV are non-psychoactive medical marijuana components, they differ in their ability to regulate different genes that contribute to the health of neurons in diabetic condition. More research is needed to understand how these two compounds work together to reduce diabetic pain.

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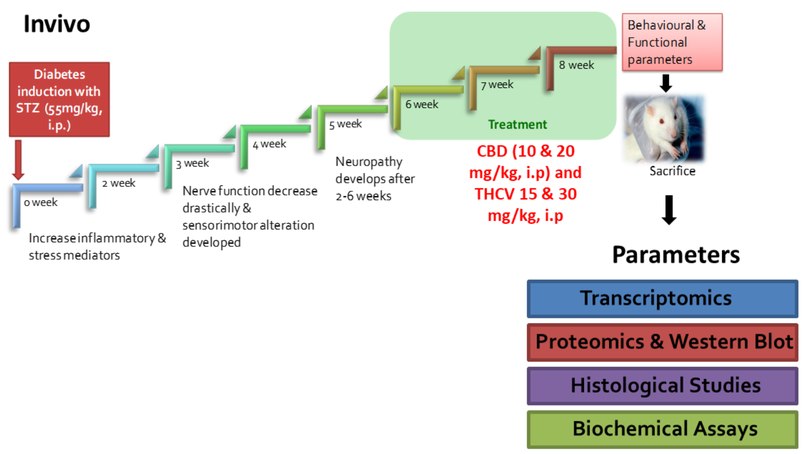
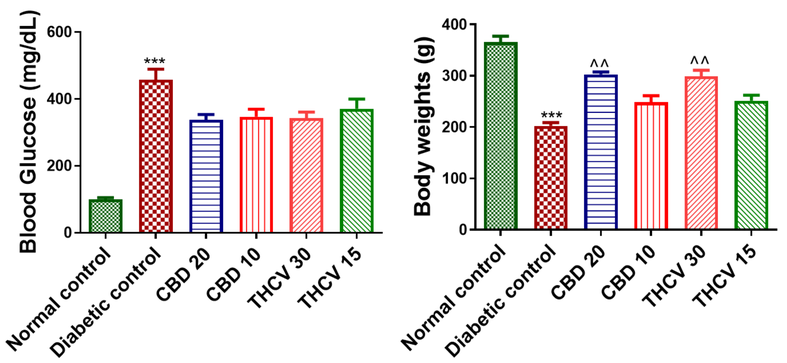
Background

- Diabetic neuropathy is a chronic devastating complication which can approximately affects 50-60% of the diabetic subjects.
- Distal symmetric polyneuropathy is the core manifestation of the diabetes which results in hyperalgesia, burning pain in the limbs which if ignored can develop into foot ulcers.
- Oxidative stress and neuroinflammation are found to be the preponderant mechanisms of hyperglycaemia induced neuronal damage.
- These factors might underlie the peripheral sensitization of nociceptors, demyelination, endothelial damage and can cause hyperalgesia, conduction velocity and blood flow deficits respectively.
- Medical marijuana provides symptomatic relief clinically for pain associated with diabetic peripheral neuropathy (DPN). In diabetic patients, nerve injury is a common complication that leads to chronic pain, numbness and substantial loss of quality of life
- The non-psychoactive minor phytocannabinoids, Cannabidiol (CBD), Cannabigerol (CBG) and Tetrahydrocannabivarin (THCV) have shown their potential in reducing neuropathic and musculoskeletal pain by regulating inflammation and oxidative stress via antagonizing CB1 receptors and agonizing CB2 receptors and transient receptor potential cation channel subfamily V member 1 (TRPV1) ion channels
- THCV reduced obesity and diabetes in rodent studies by acting as a direct antioxidant through functioning as a neutral antagonist for CB1 receptors and partial agonist for CB2 receptors
- CBD was under clinical trials for the indication of diabetes and diabetic complications, however these phytocannbinoids pharmacological mechanisms were not well explored

Experimental Design

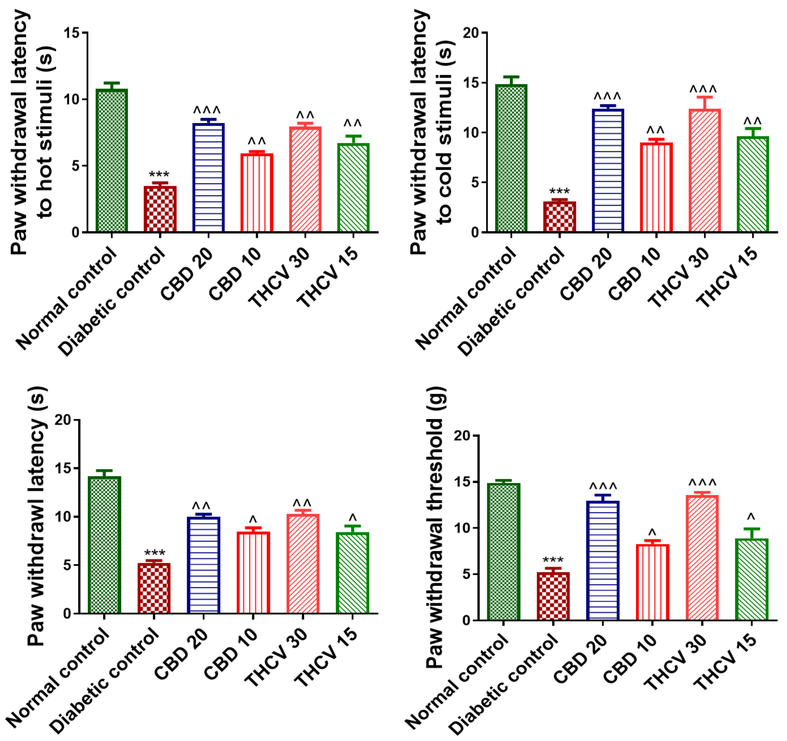


Blood glucose levels & Body weights



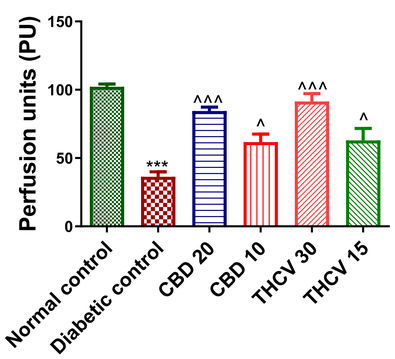
*Effects of minor phytocannabinoids (CBD and THCV) on animal body weights and blood glucose levels. Results are expressed as mean ± SEM (n=6). ***P<0.001 Vs Normal control & ^^p<0.01 Vs Diabetic control*

Neurobehavioral changes



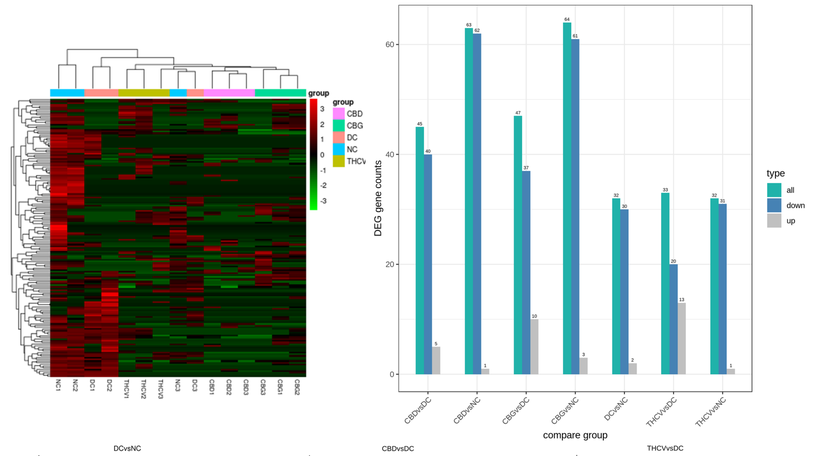
*Effects of minor phytocannabinoids (CBD & THCV) on thermal pain in diabetic animals. Representative histograms of paw withdrawal latencies to hot stimuli, cold stimuli and infrared heat applied by hot plate, cold plate and Hargreaves apparatus respectively and paw withdrawal threshold in grams (Von Frey) after the last day dose to diabetic animals of eight weeks study. Results are expressed as mean ± SEM (n=6). ***P<0.001 Vs Normal control & ^p<0.05, ^^p<0.01 & ^^^p<0.001 Vs Diabetic control.*

Nerve blood flow

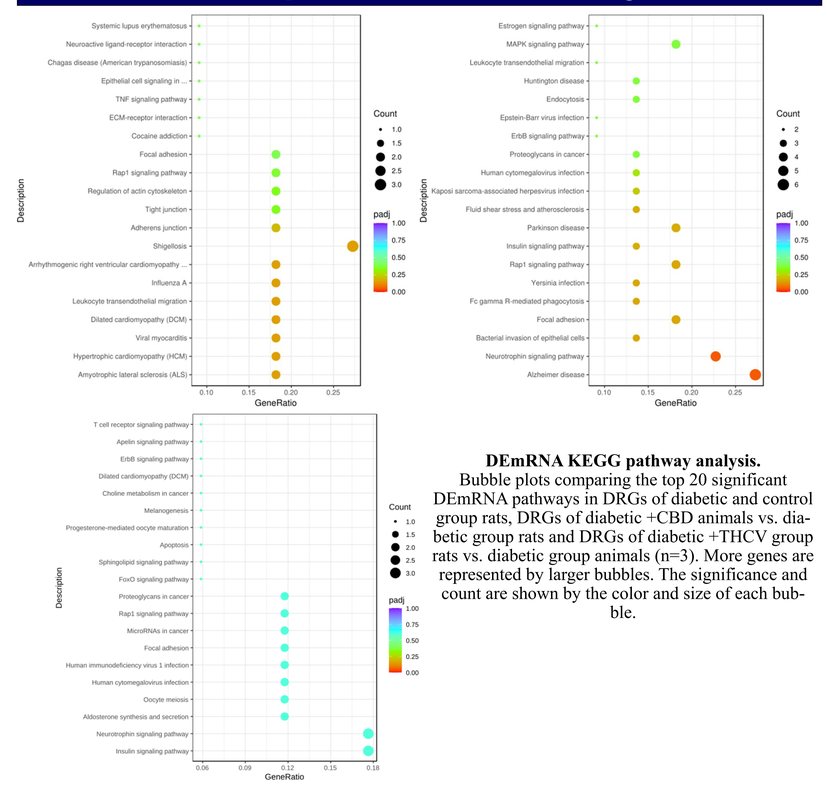


*Effects of minor phytocannabinoids (CBD and THCV) on sciatic nerve blood flow in diabetic animals. Representative histograms showing nerve blood flow measured in perfusion units after the last day dose to diabetic animals of eight weeks study. Results are expressed as mean ± SEM (n=6). ***P<0.001 Vs Normal control & ^p<0.05, ^^p<0.01 & ^^^p<0.001 Vs Diabetic control.*

Transcriptomics-DEG



Transcriptomics-KEGG Analysis



DEmRNA KEGG pathway analysis. Bubble plots comparing the top 20 significant DEmRNA pathways in DRGs of diabetic and control group rats, DRGs of diabetic +CBD animals vs. diabetic group rats and DRGs of diabetic +THCV group rats vs. diabetic group animals (n=3). More genes are represented by larger bubbles. The significance and count are shown by the color and size of each bubble.

Conclusions

- *CBD and THCV showed neuroprotective benefits in diabetes-induced neuropathic rats, as measured by a variety of neurobehavioral and nerve functional characteristics.*
- *MAPkinase, Insulin, Rap1, Neurotropin, TNF, Apelin, FoXO, and ErbB signaling pathways, along with MicroRNAs, were found to be involved in the pathogenesis of diabetic peripheral neuropathy by transcriptome analysis of DRG homogenates from diverse treatment groups of rats.*
- *Future knock-down/knock-in experiments are needed to fully comprehend the pharmacology of CBD and THCV in the treatment of diabetic neuropathic pain.*