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The Effect of Cannabinoids and Terpenes on the Activation of HMC3 Microglial Cells

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THE EFFECT OF CANNABINOIDS AND TERPENES ON THE ACTIVATION OF HMC3 MICROGLIAL CELLS

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ABSTRACT

MOHAMED MARZOUK: THE EFFECT OF CANNABINOIDS AND TERPENES ON THE ACTVATION OF HMC3 MICROGLIAL CELLS

(Under the direction of Dr. Nicole Ashpole)

Life expectancies of people living with HIV have significantly lengthened due to the availability of antiretroviral therapies. Despite their ability to increase survival, these treatments do not "cure" HIV, nor do the stop the onset of neurological symptoms associated with infection, termed neuroHIV. NeuroHIV describes a myriad of neurological impairments including mood disorders (depression and anxiety), cognitive impairment, neuropathic pain, and motor disinhibition that reduce quality of life for people living with HIV. Mechanistically, the neurological impairments may involve actions of neurotoxic proteins directly produced by the virus. One of these proteins that has been well-characterized is the HIV trans activator of transcription (Tat). Tat exerts neurotoxic effects via activation of microglia, the first line of immune defense of the central nervous system, to induce a pro-inflammatory state in the brain.

Cannabis is more often smoked by HIV-positive individuals than the general population to reduce neuroinflammation. Cannabinoids or cannabis terpenes can attenuate HIV-induced neuroinflammation, this study was carried out to elucidate the potential anti-inflammatory constituents of cannabis for their efficacy on Tat-mediated activation of human microglia cell lines.

Herein, we exposed human microglia to Tat in the presence of a variety of compounds found in cannabis. Toxicity of the cannabis compounds themselves were also assessed. Several active compounds were then tested in a concentration-response curve to determine efficacy and potency. Two compounds, namely, β- caryophyllene and CBN reduced microglial activation in a concentration- dependent manner such that 1000 nM of β-caryophyllene and CBN significantly reduced activation compared to all other concentrations. In regards to CBN acid, 100nM significantly reduced activation compared to 1000nM. As such, the anti-inflammatory effects of β- caryophyllene, CBN, and CBN acid were observed at 1000 nM and 100 nM.

Further studies are needed to be carried out on cannabis and its individual cannabinoids for better understanding of their potential anti-inflammatory effects that the cannabis constituents can exert over HIV or HIV proteins and their mechanisms of actions.

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LIST OF ABBREVIATIONS

BBB	Blood Brain Barrier
CB_1/CB_2	Cannabinoid Receptor 1/2
CBC	Cannabichromene
CBD	Cannabidiol
CNS	Central Nervous System
TRPV1	Transient receptor potential vanilloid 1
CUD	Cannabis Use Disorder
ROS	Reactive Oxygen Species
HIV	Human Immunodeficiency Virus
HMC3	Human Microglial Clone 3
Tat	Trans-Activator of Transcription
THC	Δ-9-Tetrahydrocannabinol

1. Introduction

Many therapeutic studies have been conducted in an effort to treat human immunodeficiency virus (HIV), however, no cure has been developed to this day. According to the CDC (2018), and UNAIDS (2020), approximately 39.2 million people around the world suffer from HIV. Of those 39.2 million, 1.2 million are cases in the U.S. This disease can be transmitted through different routes such as unprotected anal or vaginal sex, birth, breastfeeding, from a mother to child during pregnancy, and through shared drug injection equipment like needles and syringes.

Various neurological symptoms come along after acquiring HIV. NeuroHIV is specific term that is used to describe the host of these neurological symptoms. Symptoms may include anxiety, depression, neuropathic pain, cognitive impairment, and behavioral disinhibition (reviewed in Eggers et al., 2017; Navia et al., 1986; Robinson-Papp et al., 2008). An important question about HIV promotion of neurological dysfunction is what specifically causes it. Neurological dysfunction is caused mainly by virotoxic proteins produced by infected glial cells (Robinson-Papp et al., 2008). The trans-activator of transcription (Tat) is one of the proteins produced by HIV known to drive neurotoxicity. Tat can damage neurons directly by increasing the intracellular Ca^{2+} by activating L-type calcium channels which leads to the over-excitation of neurons leading to excitotoxicity and cell damage (Napier et al., 2014; Krogh et al., 2014; Liu et al., 2000). Tat can also lead to neuron damage and death by stimulating the production of reactive oxygen species (ROS) by promoting the dysfunction of the mitochondrial electron transport chain (Godai et al., 2019; Teodorof-Deidrich and Spector, 2018). Additionally, Tat can also cause cell damage and death by producing cytokines through the activation of the nervous system's proinflammatory glial cells (Kaul et al., 2005; Nath et al., 1999).

People living with HIV use cannabis at a high frequency as a safe and effective treatments for NeuroHIV symptoms (Sohler et al., 2018). Approximately 79% of people living with HIV used cannabis in their lifetime, and 23% of them had used it in the last month to decrease their anxiety and depression and improved appetite (Prentiss et al., 2004). This goes to show that statistically cannabis is used at a greater rate by HIV patients than by the general population (Center for Behavioral Health Statistics and Quality, 2016). Cannabis has been shown to be effective in relieving some NeuroHIV symptoms, a double-blind placebo- controlled study that examined the effects of cannabis on pain relief in HIV patients found that the percentage of subjects with at least 30% pain relief was much higher in the cannabis group compared to the placebo group (Ellis et al., 2009). Another study suggests that smoking cannabis has beneficial psychological effects in HIV positive men, including better medical management, and better future orientation (Bruce et al., 2020). More studies are needed to be done for further investigation the effectiveness of cannabinoids and terpenes on people living with HIV.

Cannabinoids, a class of chemicals found in cannabis, are analogous to an endogenous class of chemicals produced by the human body called endocannabinoids. Humans have an entire complement of endocannabinoids that influences neuronal synaptic communication, and affects biological functions including 452eating, anxiety, learning and memory, reproduction, metabolism, growth and development. One study has even found that endocannabinoids downregulate inflammation *ex vivo* (Jäger et al., 2020). Thus, cannabis smokers may experience euphoric feelings associated with 'being high', and may find additional benefits that cannabinoids provide through the intrinsic endocannabinoid system.

Cannabis is an aromatic medicinal plant belonging to the family Cannabaceae. It is considered a highly promising plant for the development and discovery of new medicinal agents after being consumed by humans for its pain-modulating and psychoactive effects. It is a chemically complex

species with over 500 reported compounds (ElSohly et al. 2017). Cannabis constituents are broadly classified into cannabinoids, which is a unique class of compounds, and other non-cannabinoids including the terpenes, which play a notable role in the cannabis culture (ElSohly et al. 2005). So far, 120 cannabinoids have been isolated from *Cannabis* which are present in the fresh plant as cannabinoid acids (ElSohly et al. 2017). The main component of cannabinoids that is responsible for the psychoactive effects is Δ^9- tetrahydrocannabinol (Δ^9-THC). THC acts as a partial agonist at the CB1 and CB2 receptors (Cascio et al. 2017). CB1 receptors are mostly concentrated in the central nervous system (CNS) (Reviewed in Howlett et al., 2002; Reviewed in Pertwee et al., 2008). Most CB1 agonists can also suppress L- type and T-type Ca^{2+} channel flux, while CB2 agonists suppress T-type channel flux. Because Tat is an L- type Ca2+ channel activator, a CB1 agonist such as THC could theoretically offset Tat's effect on these channels (Qian et al., 2017).

Cannabichromene (CBC), Cannabigerol (CBG), Cannabivarine (THCV), CBD acid, CBG acid, Δ^9-THC acid, Cannabidivarin (CBDV) and Cannabidivarinic acid (CBDVA) are examples of cannabinoids for which some pharmacological effects have been reported. Cannabidiol (CBD) is a major non-psychotropic cannabinoid and has attracted much attention for development as a medicine for several conditions because of its reported anxiolytic, anti- psychotic, antiemetic, anti-convulsant, and anti-inflammatory properties (Leo et al. 2016). CBD is devoid of the undesirable psychotropic properties (Pertwee 2008; McPartland et al. 2015) known for Δ^9-THC. CBD, in combination with THC in Sativex®, an oral spray licensed in UK, was effective in the treatment of multiple sclerosis that is associated with tremor, spasticity and neuropathic pain (Cascio et al. 2017). Moreover, repeated oral treatment of CBD reversed both mechanical and thermal hyperalgesia in chronic inflammatory and neuropathic pain models in rats (Costa et al. 2007).

The anti-inflammatory activity of cannabichromene (CBC) was evaluated by both carrageenan-induced rat paw edema and erythrocyte membrane stabilizing methods. It was

administered by both orally and intraperitoneally (IP) routes. CBC at 240 mg/kg dose was more active than the non-steroidal anti-inflammatory drug, phenylbutazone (Turner and ElSohly 1981). CBC also showed weak analgesic effect in the mice tail-flick test compared to THC (Davis and Hatoum 1983). Δ^9-tetrahydrocannabivarine (THCV), the propyl analogue of Δ^9-THC activated CB_2 receptor *in vitro* and displayed anti-oedema activity in the carrageenan model of inflammation and anti-hyperalgesic activity in the formalin model of inflammatory pain (Bolognini et al. 2010).

Terpenes are another prominent set of non-cannabinoid constituent of cannabis, classified as phytotherapeutic constituents of its essential oils. The primary types of terpenes found in cannabis are typically mono- and sesquiterpenes including, β-caryophyllene, myrcene, limonene, α-pinene, β-pinene, humulene, and linalool (ElSohly and Slade 2005). They are not only associated with the taste and smell of the plant but also are therapeutically active. Studies in animal models and humans have identified analgesic, anti-microbial, anti-inflammatory, antioxidant, anti-nociceptive, anti-histaminic, anti-spasmodic, anti-convulsant, anti-depressant, anti-cancer, and anesthetic effects (Russo et.al., 2011, de Cassia da Silveira et.al., 2017). Literature reports suggest that besides the individual activities of terpenes, they are responsible for improving the cannabinoids' activities in a phenomenon known as the entourage effect (Baron 2018).

Terpenes can easily cross the blood-brain barrier (BBB) due to their lipophilic nature. This possibly means they account for some of the therapeutic effects because pain signals are regulated in the spinal cord and in the periphery. For example, administration of limonene (one of the most common terpenes found in nature) (Noma and Asakawa 2010) resulted in anti-allodynic effects in an injury-induced pain model as well as analgesic effects in an immune activated pain model (Piccinelli et al. 2015). Mechanistically, limonene was thought to carry out these effects by stimulating opioid receptors (Piccinelli et al. 2017). Monoterpenes like myrcene and linalool are associated with pain relief in multiple rodent models. Myrcene has potent anti-inflammatory, analgesic, and anxiolytic

properties (Van Cleemput et al. 2009). A study with neuropathic mouse models showed that myrcene exerts anti-nociceptive effects in mechanical, thermal, and neuropathic pain tests. These effects may by mediated by alpha-2-adrenoreceptor stimulated release of endogenous opioid (Rao et al. 1990).

β-caryophyllene is the most common sesquiterpenoid in Cannabis and is a selective CB2 agonist with the lack of attributed psychoactivity of CB2 agonists (Gertsch et al. 2008). Oral administration of β-caryophyllene resulted in analgesic effects in mouse models of inflammatory and neuropathic pain (Klauke et al. 2014). These effects may be explained by its higher affinity to CB2 receptors, resulting in improving the efficacy of signaling (Shoemaker et al. 2005). Interestingly, a low dose of β-caryophyllene (1 mg/kg) was more effective than large doses in thermal hyperalgesia, reflecting a bell-shaped dose-response curve (Calabrese 2008). Whether β- aryophyllene could reduce HIV mediated pain and inflammatory is not yet known.

The anti-inflammatory properties of cannabis make it a promising candidate for study to potentially offset the neuroinflammatory effects of HIV (Lima et al., 2021). In order to investigate the effects of cannabis extracts, cannabinoids and cannabis terpenes on HIV Tat-mediated neuroinflammation, an experiment was conducted exposing HMC3 cells (a type of neuroglia cell located throughout the brain and spinal cord) to the tested compounds and/or Tat.

2. Materials and Methods

2.1 – Chemicals Involved in Experiment

Chemicals

Cannabinoids and terpenes were obtained from the Marijuana Research Laboratory at the University of Mississippi (University, MS, USA). EMEM media was obtained from ATTC (Lot # 80520201). PBS was obtained from Gibco (Lot # 2085516). 0.25% Trypsin-EDTA was also obtained from Gibco (Lot # 2193366). Phalloidin stain was obtained from Thermo Fisher Scientific (Lot # 2189151). Lastly, DAPI stain was also obtained from Thermo Fisher Scientific (Lot # VK3126282).

2.2- Description of Cells and Cell Culture

HMC3 human brain microglial cells

HMC3 cells were procured and grown following a previous study (Martin, *et.al* 2022). 75 cm^2 flasks in 13 mL of media (89.9% EMEM, 10% heat-inactivated fetal bovine serum, 0.5% antibiotic/antimycotic mixture), and maintained in a 37^oC incubator (5% CO_2). For this experiment, the HMC3 cells were split into 96 well plates where they were split into control and cells treated with different types of cannabinoids and terpenes. After the treatment of the cells, they were fixed and then stained with dapi or phalloidin.

First, cells were passaged into 3 plates and washed with phosphate buffered saline (PBS at a pH of 7.4). PBS is a non-toxic solution used to prevent cells from rupturing or shriveling up due to osmosis. After washing the cells, 6mL of PBS (7.4 pH) and 4mL of 0.25% Trypsin-EDTA were added, and the cells were left to incubate (37^oC, 5% CO_2) for 5 minutes. Trypsin-EDTA is a digestive protease used for its strong proteolytic action. It is used to detach cells from tissue

culture dishes and to dissociate cells from one another. Cells were then transferred into 3 different tubes and centrifuged for 5 minutes at a speed of 2000 rpm. Finally, cells were combined into one tube and using an electronic pipette, cells were pipetted into 4 and a half 96 well plates and left them in the incubator overnight (37^{o}C, 5% CO_2).

Treatment

First, 8 15mL centrifuge tubes were counted and labeled 50µM, 25µM, 5µM, 2.5µM, 0.5µM, 0.25µM, 0.05µM, and 0.025µM for Hexane Ext. Hemp and EtoAc Ext. Hemp (16 tubes total). Then 1000µL of EMEM media was added each tube based on the amount labeled (50µM to 2.5µM). EMEM media is a cell culture media that provides balanced energy sources to support protein production and nucleic acid metabolism. Several dilutions of the tested extracts were performed in EMEM, and 100µL of each dilution were added to two incubated 96 well plates. Lastly, HIV Tat (50 ng/mL) was added to the 96 well plates. The plates were put in the incubator overnight (37^{o}C, 5% CO_2).

Cell Fixation

To fix the cells, the media from two previously prepared 96 well plates was removed from the wells in columns 2-10 (10 acting as control) and 50µL of 4% sodium phosphate (7.4 pH) were added. The plates were then returned incubate overnight (37^{o}C, 5% CO_2).

Staining and Imaging

After calculating the amount needed of each stain, media was removed from columns 2-10 in both plates and added 40μL of the mixed stain (includes phalloidin and dapi stains). They were then returned and allowed to incubate for 48 hours (37^{o}C, 5% CO_2).

Screening

To get the total number of cells, a Nikon inverted fluorescent microscope was used. Cells were divided into three types: resting, active/reactive, and ameboid/phagocytic intermediate. Resting cells showed extended and thin processes. Active/reactive cells showed a retracted process and phagocytic/ameboid intermediate cells showed a rounded cell body without any processes. Each cell type was counted and expressed a percentage from the total number of cells. Along with the types of cells, they were also divided into a microglial activation scale from 1-3. This method was used in previous studies (Paris et al., 2015; Davis et al., 1994; Yoichi, 1999). A score of 1 indicated that the microglial cells were considered resting and possess long processes. A score of 2 indicated that the microglial cells were neither resting nor phagocytic and expressed a shorter process. Finally, a score of 3 indicated that the microglial cells were considered phagocytic and expressed no processes.

Statistical Analysis

Cell activation was assessed using a two-way ANOVA with β-caryophyllene, CBN, and CBN acid concentrations (1, 100, or 1000 nM) and HIV Tat exposure (control or Tat 50 ng/mL) as factors. The same process was administered for figure 3, except HMC3 cells were treated with only once concentration of 50 ng/mL. For figure 2, nuclei/field were counted through a concentration curve for hexane extract and etoAc extract. The concentrations varied from 0 to 50 μg/mL. Group differences were measured via Dunnett's method. All analysis were considered significant when $p < 0.05$.

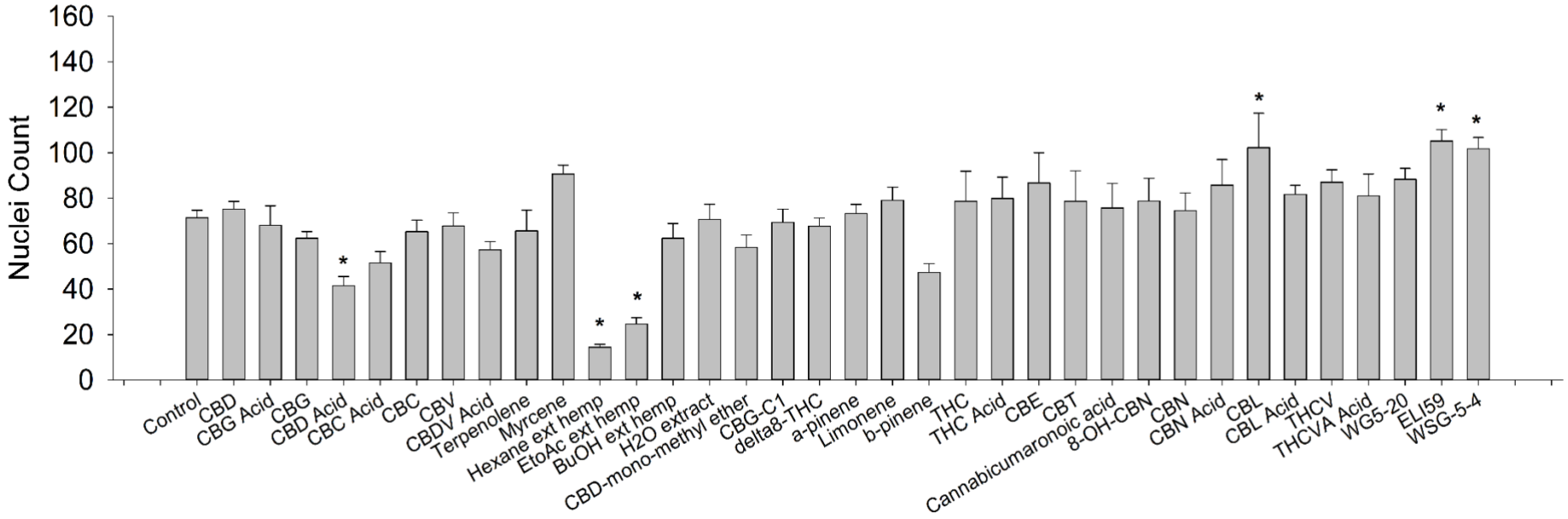
3. Results

Prior to assessing efficacy against Tat-mediated inflammation, toxicity of our test compounds was assessed in HMC3 cells. In total, 4 extracts, 19 phyto-cannabinoids, 6 synthetic cannabinoids, and 5 terpenes were included. Exposure to CBD acid, Hexane ext. hemp, EtoAc ext. hemp showed a decrease in the nuclei count compared to the control suggesting cytotoxicity (Fig 1; Two-way ANOVA $F(2, 13) = 7.052$, $p < 0.001$) (Fig 1; Two-way ANOVA $F(2, 14)= 5.458$, $p < 0.001$), while CBL, ELI-59, and WSG 5-increased nuclei count showing non-toxic effects (Fig 1; Two-way ANOVA $F(2,31)= 3.815$, $p = 0.006$) (Fig 1; Two-way ANOVA $F(2,36)= 4.171$, $p = 0.002$) (Fig 1; Two-way ANOVA $F(2,37) = 3.753$, $p = 0.008$, respectively). In an effort to identify a concentration of hemp hexane ext. and hemp EtoAc ext. that would not cause cell toxicity, a concentration-response curve was generated using a range of concentrations between 50 μg /mL to 0.025 μg /mL. The results showed that as the concentration increased for hemp hexane ext. and hemp EtoAc ext., a downward slope relating to the nuclei/field was observed (Fig 2A; Two-way ANOVA $F(15,23) = 8.978$, $p < 0.001$) (Fig 2B Two-way ANOVA $F(15,23) = 14.298$, $p <0.001$). This shows that the toxicity of the tested hemp extracts on the nuclei/field is less when lower concentrations are used (<0.025μg/mL).

To identify whether the non-cytotoxic cannabinoids and terpenes will have an effect against HMC3cells treated with Tat, a screening test was generated to see the effect of the tested compounds against a control group. The results show that CBN and CBN acid decreased the concentration of activated cells showing their anti-inflammatory effects (Fig 3; n=6-8, Two-way anova, post-hoc Tukey, $p < 0.05$). Lastly, we tested the effects of different concentrations of β- caryophyllene, CBN, and CBN acid on the proportion of each microglial phenotype. For β-caryophyllene, as the concentration increased from 1nM to 1000nM, the concentration for both activated and resting microglia decreased while the concentration for intermediate/phagocytic microglia increased (Figure 4A). For CBN, the concentration for all three phenotypes did not significantly change (Figure 4B).

Lastly, for CBN acid, there was an increase in the concentration of activated microglia and a decrease in the resting and intermediate microglia (Figure 4C). Regarding the absence of the data representing the 1nM and 10nM of CBN acid, this was possibly due to contamination which in turn killed the cells. Therefore, data was not obtained for these concentrations.

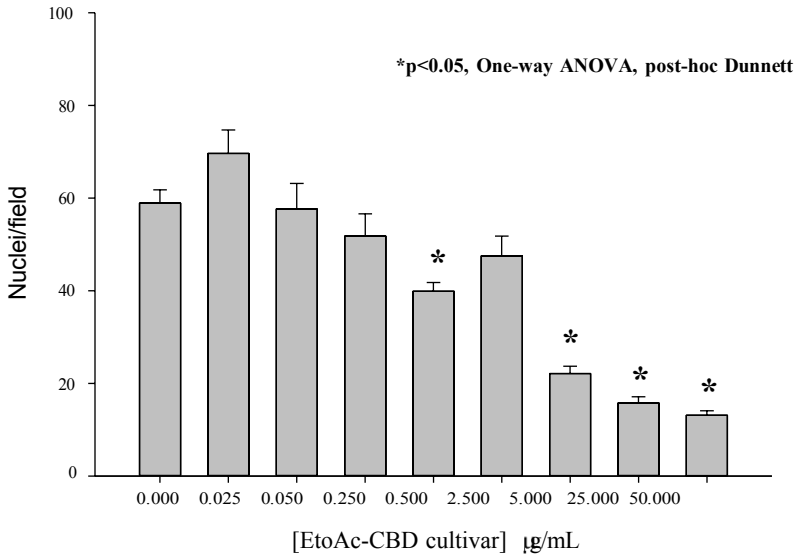
Figure 1



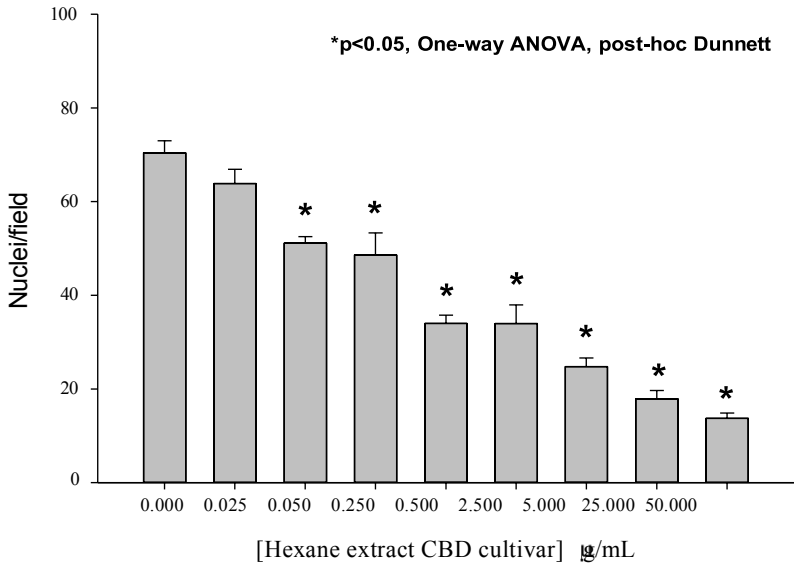
Measurement of toxicity levels of phytocannabinoids, synthetic cannabinoids, terpenes, and extracts on HMC3 microglial cells. Hexane extract hemp, EtoAc extract hemp, CBD acid, and beta-pinene showed the most toxicity. CBL, ELI59, and WSG-5-4 showed the least toxicity.

Figure 2

(A)

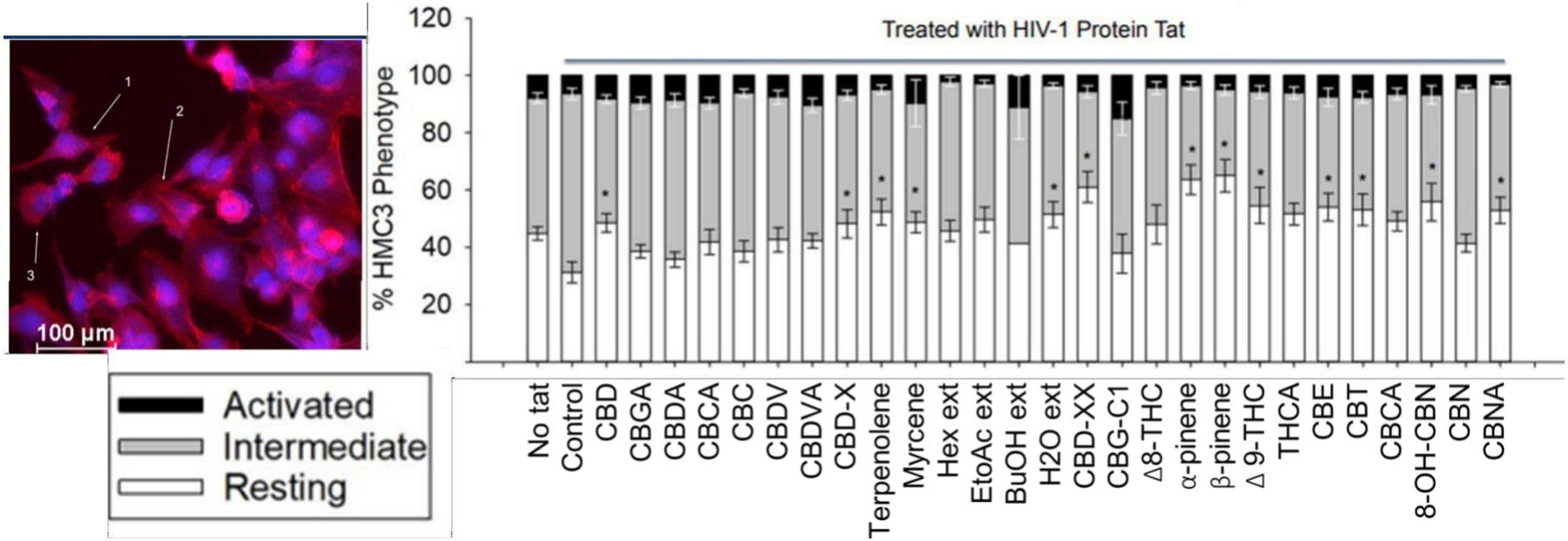


(B)



Testing the effect of EtoAc-CBD cultivar (A) and Hexane extract CBD cultivar (B) on the nuclei/field of HMC3 microglial cells from 50µg/mL to 0.025µg/mL.. **A)** EtoAc-CBD cultivar at a concentration of 50 µg/mL was the most toxic. **B)** Hexane extract CBD cultivar at a concentration of 50 µg/mL.

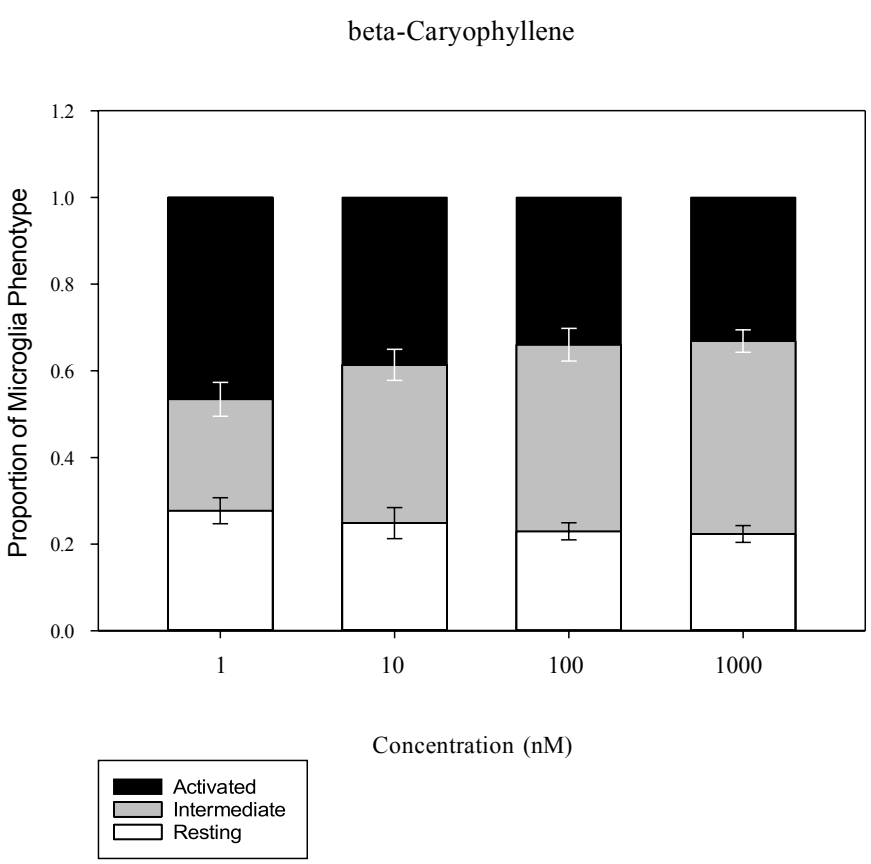
Figure 3



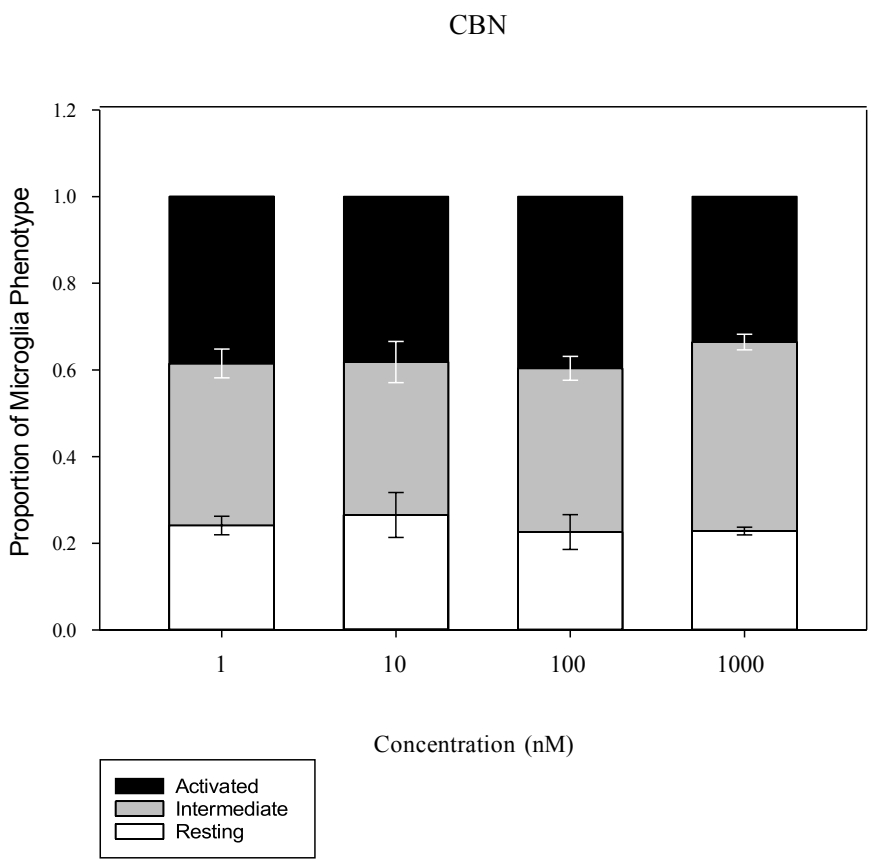
Human microglial cell line-3 (HMC3) cells were treated with recombinant HIV-1 Tat (50ng/mL) and/or 1 µM cannabinoids, terpenes, or extracts. The activation state of the cells was observed 24 hours later to determine the ratio of resting vs reactive/phagocytic cells.
*Significant difference from Tat-treated control, n=6-8 independent observations, $p < 0.05$.

Figure 4

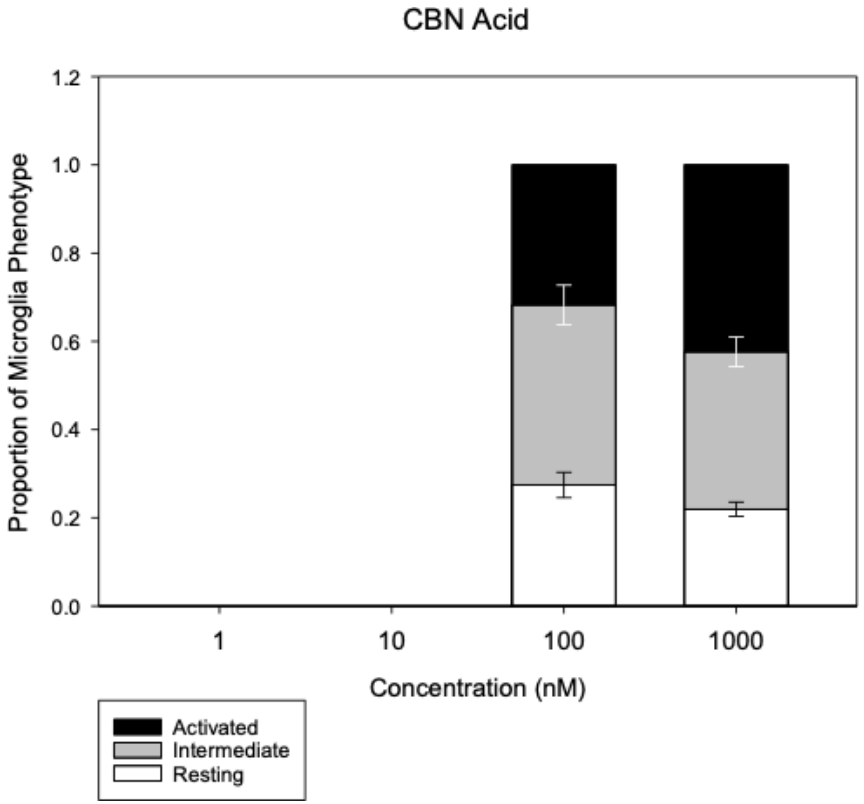
(A)


beta-Caryophyllene
Proportion of Microglia Phenotype
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1
10
100
1000
Concentration (nM)
Activated
Intermediate
Resting

(B)


CBN
Proportion of Microglia Phenotype
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1
10
100
1000
Concentration (nM)
Activated
Intermediate
Resting

(C)



The effect of β-caryophyllene, CBN, and CBN acid compounds on microglial activation in a concentration-dependent manner at different concentrations of (0, 1, 10, 100 and 1000nM). **A)** The β-caryophyllene reduced microglial activation at 1000nM. B) CBN reduced microglial activation at 1000nM. **C)** CNB Acid reduced microglial activation at 100nM.

4. Discussion

The experiments described here examined whether the chemical constituents of cannabis were capable of reducing HIV Tat mediated inflammation within human microglial cells. Tat is known for promoting neuroinflammatory effects by activating microglia and astrocytes (Kaul et al., 2005; Nath et al., 1999). The inflammation caused by this protein does not just occur to the infected cells, it also affects the surrounding cells and causes their activation through a process called 'bystander effect' (reviewed in Ajasin and Eugenin, 2020). This process occurs when the neighboring cells endocytose Tat through one of two ways; a clathrin-dependent endocytic route, and a caveolae mediated route (reviewed in Ajasin and Eugenin, 2020). Once Tat enters the cells it activates the cell by hijacking its normal transcription function leading to the production of inflammatory cytokines. The transcription factors that are hijacked by tat are part of the NF-κB signal transduction pathway. NF-κB functions by regulating different genes such as chemokines, cytokines, and cell adhesion molecules. Tat increases the activity of NF-κB, which in turn leads to the increased production of pro-inflammatory cytokines (McElhinny et al., 1995; DeLuca et al., 1996). Our results suggest that Tat did in fact cause microglia to become activated which is consistent with existing literature (Thangaraj et al., 2018; Periyasamy et al., 2019) and our lab's prior findings.

According to previous studies, Δ-9-tetrahydrocannabinol (THC), cannabidiol (CBD), and other cannabinoid extracts have decreased inflammation and pain in chronically ill patients. CBD for example has shown effects on inflammation in many ways. Some of these include decreasing the monocyte attraction via CCL2, reducing IL-1B and IL-6 release, decreasing GFAP release, and more (Kozela et al., 2010; Stella et al., 2010). Specifically, in microglial cells, studies have shown that CBD reduces the expression of IL-1b, iNOS, and other cytokines induced by LPS (Burstein et al. 2015). CBD also acts on decreasing the production of NF-κB, which in turn

decreases the pro-inflammatory cytokine production. These effects help attenuate the inflammatory effect produced by Tat and could lead to a positive impact on symptoms of neuroHIV, like mood disorders. Based on our results, CBD is not cytotoxic to microglia, and is capable of promoting the resting, anti-inflammatory phenotype.

Common anxiety disorders that HIV patients suffer most from are mood disturbances and major depression (Atkinson et al., 1988). Depression has been linked to an increased number of inflammatory cytokines acting on the brain (Arseniou et al., 2014). A study on effects of Tat on mice, showed that Tat expression in mice causes anxiety and depression like behaviors (Paris et al., 2013; McLaughlin et al., 2017). Mice whose brain was injected with Tat displayed depression like behaviors. (Lawson et al., 2011). However, to counteract this reaction caused by Tat, there is evidence that marijuana acts as an antidepressant (Feingold and Weinstein, 2021). A current treatment option for anxiety is THC. Our results suggest THC is not cytotoxic to microgalia and can enhance their resting phenotype. If microgalial inflammation plays a casual role in anxiety disorders, our findings would support a potential benefit of THC use. Increase THC also increases anxiety in some so this may be an issue.

There are a few ways that this experiment could been improved. An aspect that could have been improved in terms of its generalizability and applicability is through considering the evaluation of differences in vitro in a clonal cell line. In vitro studies limit cell cell interaction and clonal lines often have mutation that differentiate their response compared to primary cells. Additionally, we cannot assess sex specificity within clonal cell line despite knowing Tat as well as cannabinoids have sex specific effects.A study shows that cannabis use disorder (CUD) is more common in men than women (Hasin et al., 2016). Men and women differ in their rate of marijuana use, with men being more frequent users (Cuttler et al., 2016). Men and women are also affected by marijuana in different ways. This may include different levels of withdrawal, cardiovascular

effects, and visuospatial memory impairment (Fattore and Fratta, 2010). To try and understand why marijuana use is different in men and women, several mechanisms have been put forward to explain the differences in marijuana use and effect as well as the difference in cannabinoid receptor system and cannabis metabolism (Calakos et al., 2017). These results bring to light that there may be a difference between the effects of marijuana on HIV-induced neuroinflammation between males and females. In conclusion this experiment demonstrated that β-caryophyllene at a concentration of 1000nM, CBN at a concentration of 1000nM, and CBN acid at a concentration of 100nM expressed anti-inflammatory effects. Future studies may better assess the potential anti- inflammatory effects of THC or other Cannabis constituents at different concentrations using primary microglia on HIV or HIV proteins.

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