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Effect of Cannabidiol and Δ9-Tetrahydrocannabinol Concentration on Breast Cancer Cell Viability

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Introduction

One in eight women (about 12.5%) in the United States will develop invasive breast cancer over the course of her lifetime (U.S Breast Cancer Statistics, 2018). Not only is breast cancer deadly, but it can spread to other parts of the body such as lymph nodes, lungs, and other organs. Breast cancer cells in the lymph nodes suggest an increased risk of cancer metastasis and decreased rate of survival. Breast cancer is treated in various ways, such as radiation and surgical removal of the breast, which leaves the survivors with physical and emotional trauma. Chemotherapy is another common treatment, but it has side effects such as hair loss, easy bruising, bleeding, and cognitive disorders that can affect concentration and focus (American Cancer Society, 2016). Due to these negative side effects, researchers have been looking for supportive alternate treatments that are equally effective but lack the side effects. One possibility is *Cannabis*-derived cannabinoid drugs. Cannabinoids are being investigated (Qamri et al., 2009) as anticancer treatments with fewer side effects than conventional chemotherapy.

Purpose and Significance

Studies suggest that cannabinoids can kill cancer cells, and researchers have proposed that the concentration of cannabinoids makes a difference in determining cancer cell apoptosis (cell death); however, optimization of the cannabinoids concentration is necessary (Sarfaraz, Adhami, Syned, Afaq, & Murkhtar, 2008). Sarfaraz et al. (2008) proposed that the overexpression of cannabinoid receptors (CB1 and CB2: G-protein couple receptors located throughout the body) may lead to cancer cell death, but little or no expression of these receptors could lead to cell proliferation and metastasis, due to the suppression of the antitumor immune response. It is unknown how the concentrations of two common cannabinoids—cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC)—affect cancer cells. Therefore, studying the effect

of varying concentrations of CBD and THC on breast cancer cells could offer a potential avenue for new treatments of this disease.

Because *Cannabis* is easily grown, the cost of cannabinoid-based cancer treatment could potentially be much lower than other forms of chemotherapy (Gringsppoon, 1999). Furthermore, cannabinoid drugs are known to reduce many side effects associated with other treatments, such as pain, loss of appetite, nausea, and vomiting (Rocha, Stefano, Haiek, Oliveira & Da Silveria, 2008; Tramèr et al., 2001; Ware et al., 2010), which will benefit cancer patients by preventing them from taking additional medications for side effects. The use of cannabinoid drugs can also potentially void the need for traumatic surgeries. Lastly, when an effective concentration is known for treatment, cannabinoids should work more efficiently than most other cancer treatments because they inhibit the cancer growth without interfering with normal cells (Kogan, 2005). Not only are cannabinoid drugs affordable and have anti-proliferative properties, they can also reduce side effects associated with other treatments, making them a potential treatment to research and develop.

Review of Literature

Cancer Pathogenesis

During the cell cycle, there are checkpoints that are responsible for making sure the cell is capable to function and regulate when necessary. At the checkpoint, if a cell has any damage or cannot serve its purpose, the cell is marked for apoptosis. Apoptosis is a normal physiological process that removes unwanted cells by programming the cell's death. Some damaged cells escape the checkpoint without being marked for apoptosis. These abnormal cells can grow out of proportion and become cancer cells (Visconti et al., 2016).

Cancer cells are cells that have lost the ability to function normally or die but can still divide. Uncontrollable growth forms a mass of tissue called a tumor. Because cancer cells are similar to normal cells, it is difficult to selectively kill them without affecting normal cells (Yasukawa, 2014). When the cancer cells spread to different regions of the body, it is called metastasis. Cyclin E is a cell cycle regulator and is important, together with cyclin-dependent kinase (cdk) 2, for the G1/S transition during the cell cycle. In tumors, there is normally an overexpression of the cyclin E protein, causing cell proliferation and bypassing the checkpoint leading to cancer.

There are five types of cancer classified by their tissue/cell types of origin: sarcomas, leukemia, lymphomas, melanoma, and carcinomas (Movva, 2015). Carcinomas, which were the focus of this study, develop in the epithelial cells. They start from the skin or epithelial tissue and spread to the internal organs, and they are caused by the damage of DNA resulting in a mutation (Rosenbreg, 1987). MCF-7, which is a human breast adenocarcinoma cell line, was the selected cell type utilized. Breast cancer is the most common cancer and the leading cause of cancer death in American women. American Cancer Society estimated that 41,400 deaths occurred in 2018 among males and females due to breast cancer.

The method of cancer treatment is determined based on the cancer type, the stage of cancer development, and the person's health. The three common cancer treatments are radiation, surgery, and chemotherapy. Radiation therapy uses high energy waves to make small breaks in the DNA inside cancer cells. During this process, abnormal cells are destroyed and new, normal cells replace the dead ones (Lawrence, Ten Haken & Giaccia, 2008). Surgery is used to remove a tumor prior to metastasis, whereas chemotherapy uses drugs to inhibit cancer cell function (Ho Im et al., 2016).

Cannabinoids

Cannabinoids are compounds originally discovered in the plant *Cannabis sativa.* They are potential chemotherapeutic agents being investigated to treat cancer. The discovery of cannabinoids occurred in the 1940s, and the receptors for these molecules were discovered in the mid-1980s (Pertwee, 2006). To date, approximately 113 cannabinoids have been isolated from *Cannabis* (Namdar et al., 2018).

There are three primary types of cannabinoids: endocannabinoids, synthetic cannabinoids, and phytocannabinoids (Qamri et al., 2009). Endocannabinoids are endogenous cannabinoids that the human body synthesizes and releases; they also interact with the CB1 and CB2 receptors. Anandamide is a type of endocannabinoid that binds to CB1 and activates the receptor (Pertwee, 2008). Synthetic cannabinoids are designed for research purposes, and they also interact with the CB1 and CB2 receptors (Seely, Lapoint, Moran, & Fattore, 2012). Synthetic cannabinoids are mildly psychoactive because of their cannabimimetic properties (Grigoryev et al., 2011).

Phytocannabiniods are cannabinoids that occur naturally in the *Cannabis* plant; the most thoroughly investigated phytocannabinoids are THC and CBD (Figure 1) (Borgelt et al., 2013). CBD has a significantly different action than THC (Gertsch, Pertwee & Di Marzo, 2010). THC directly activates the CB1 receptor, which causes a change in brain function due to its psychoactive properties. Unlike THC, CBD has a low affinity for the CB1/CB2 receptors. However, it causes cellular modulation that leads to an indirect interaction with the CB1 receptor (Pacher, Batkai, & Kunos, 2006). CBD induces endogenous neurotransmitter uptake, which is responsible for signal transmission in the brain (Moreira et al., 2011).

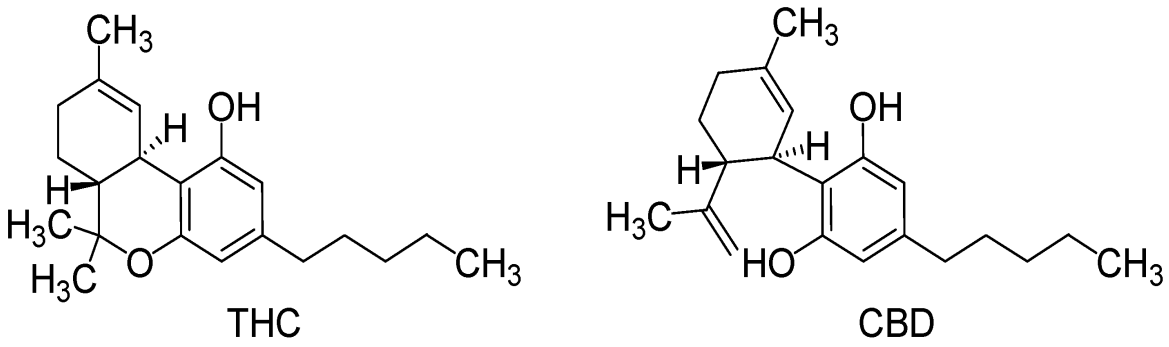

Figure 1. THC and CBD chemical structures.

Cannabinoids serve a purpose in pharmaceutical science, even though *Cannabis* is just becoming accepted by society (Gupta, 2014). Due to powerful therapeutic properties used to treat several medical conditions, cannabinoids have been contributing to pharmaceutical benefits since 1850. Research has shown that cannabinoids can influence factors that help the body respond to injury, defend against viruses and bacteria, and repair damaged tissues (Nagarkatti, Pandey, Rieder, Hegde, & Nagarkatti, 2009). Cannabinoids can also regulate energy in the body by activating the 5-hydroxytryptamine (serotonin) receptor, which contributes to the reduction of addiction, anxiety, depression, nausea, pain, and vomiting as well as improves appetite and sleep (Crippa et al., 2010).

CBD and THC inhibit cancer metastasis by activating the CB1 and CB2 receptors (Patsos, Hicks, Greenhough, Williams, & Paraskeva, 2005). CB1 receptors are in the basal ganglia, brain, cerebellum, limbic system, and reproductive system. CB2 receptors are correlated with anti-inflammatory processes and are found in the spleen in addition to the locations mentioned for CB1. CB2 is expressed on demand; the modulation of CB2 levels is a common feature to cells of macrophage lineage as they participate in the inflammatory response and undergo differential gene expression and acquisition of distinctive functional properties (Cabral, 2009).

Cannabinoids and Cancer

The mechanism by which cannabinoids directly kill breast cancer cells is currently unknown, but researchers have postulated a potential mechanism. Velasco et al. (2016) suggested a pathway that cannabinoids might use to cause cancer cell apoptosis (Figure 2). THC stimulates the stress-regulated protein pathway, which enhances the inhibitory interaction of the pseudokinase homologue 3 with pro-survival kinases, Akt. This leads to inhibition of the mammalian target of rapamycin complex 1 and the subsequent stimulation of autophagy-mediated cell death (Velasco et al., 2016).

Velasco et al. (2016) proposed the mechanism for glioma cancer cells. Even though the mechanism for apoptosis in breast cancer cells is not known, most cancer apoptosis probably follows a similar mechanism. Researchers have frequently determined that THC and CBD can kill cancer cells. However, the specific concentration of CBD and THC is rarely addressed. Sarfaraz et al. (2008) speculated that the concentration makes a difference in whether there will be apoptosis or proliferation in cancer cells.

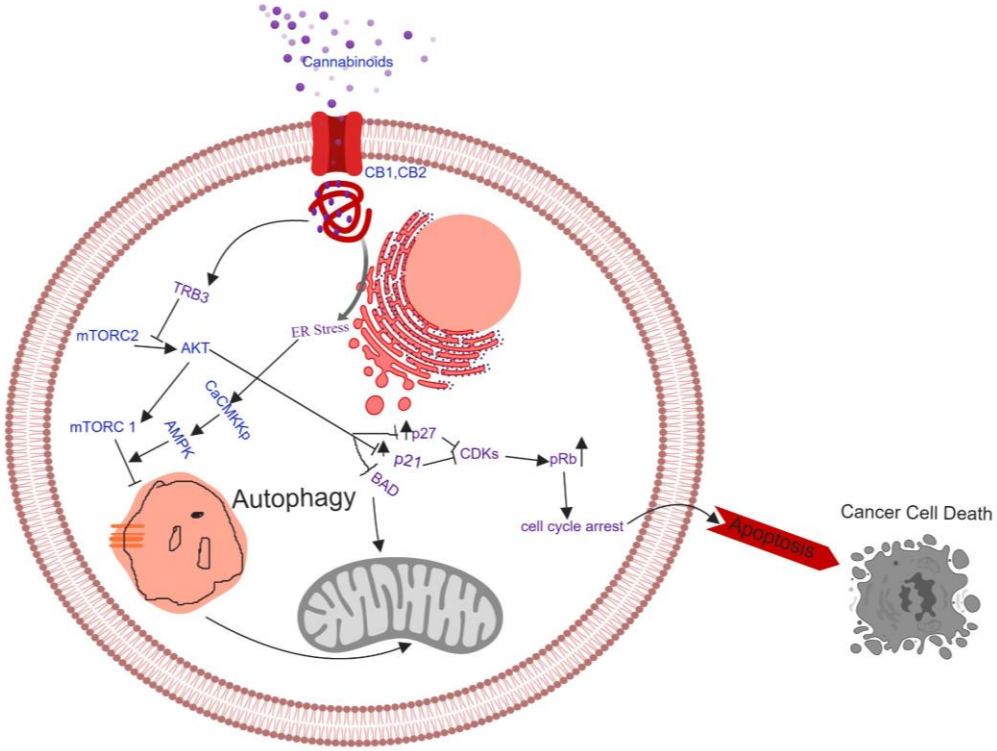

Figure 2. The proposed mechanism of apoptosis caused by cannabinoids on glioma cells (Velasco et al., 2016).

Sarfaraz et al. (2008) proposed that overexpressed CB1 and CB2 receptors may lead to tumor destruction, but low or no expression of these receptors could lead to cell proliferation and metastasis because of the suppression of the antitumor immune response. This suggests that cannabinoids as an anticancer treatment will not exhibit the side effects associated with current cancer treatments, making cannabinoids a promising contribution to current cancer therapies. Because the concentration of cannabinoids affects cancer cells, the precise concentration of THC and CBD needed to cause apoptosis of the breast cancer cells must be examined.

Methods

This study was conducted at the University of Northern Colorado in the Department of Chemistry and Biochemistry and the School of Biological Sciences. Cell culture was performed with a MCF-7 human breast cancer cell line obtained from Bio-Rad (Bio Rad Laboratories, Hercules, CA, USA). Cultures were kept in an incubator at 37 ºC with 95% humidified air and 5% CO_2. Two controls for this experiment were used: cells in Roswell Park Memorial Institute (RPMI) culture medium without cannabinoids and cells in the same medium with ethanol. The ethanol was added to determine the effect ethanol had on cancer cells, due to the fact that the drugs were dissolved in ethanol. Two treatment groups were also used to compare CBD and THC. These treatment groups were treated with CBD or THC at varying concentrations, including 0.1, 1, 10, and 100 micromolar. These concentrations were arbitrarily chosen because there is not an established baseline of concentrations that might affect the cancer cells. To study whether CBD and THC cause apoptosis or proliferation in MCF-7 cells, an MTT assay was performed to determine cell proliferation through a colorimetric measurement of metabolic mitochondrial activity.

Media

Fresh media was made by adding either a mixture of fetal bovine and horse sera (FBE, cat. No. VWR) or using an RPMI base. Additionally, penicillin 100 unit/L/streptomycin (100 ug/L (ThermoFisher, 10378016), sodium pyruvate 1 mM (ThermoFisher, 11360070), bovine insulin 10 mg/mL (ThermoFisher, 12585014), L-glutamine 2 mM (ThermoFisher, 25030081), Hepes (buffer agent) 10 mM (ThermoFisher, 15630080), and 2-mercaptoethanol 0.142 M (ThermoFisher, 35602BID) were added to the RMPI to provide a complete media (cRPMI) with everything the MCF-7 cancer cells need to grow.

Cell Growth and Passing

After incubation for 24 hours (37 ˚C, 95% humidified air, 5% CO_2), old medium was discarded and cells were trypsinized by adding 3 mL of trypsin (1x) concentration (ThermoFisher, 25200-056) to the flask and incubating the flasks at 37 ºC and 5% CO_2 for 1-2 min, until cells lifted from the culture flask surface. Six mL of fresh media were added to each flask to neutralize the trypsin reaction. The cells, now in fresh media, were centrifuged at 1200 rpm in conical tubes for 5 min. Supernatant was discarded, and the cell pellets were resuspended in 1 mL of fresh media each. A 10 µL sample of MCF-7 cells was stained with trypan blue (1:1) and counted using a Countess II Automated Cell Counter. These results were used to determine the cell density to determine the volume containing the proper number of cells needed to be grown in each well. The calculated number of the cells were then resuspended in fresh media (12 mL in the T-75 flask), then transferred constantly to 96 wells plates and incubated until they were 80% confluent (roughly 48-64 hr.).

Adding Cannabinoids to the Cancer Cell Cultures

The first control group was suspended in media without any cannabinoids or ethanol (the vehicle control); the second control group was suspended in media with ethanol equal to the amount of ethanol in which the cannabinoids were delivered. Each concentration treatment had three replicates. For the first treatment of breast cancer cells, 0.1 µM CBD was added to each of the three wells in the first column. The second column had 1 µM of CBD, while the third column had 10 µM CBD, and lastly the fourth column had 100 µM of CBD. A second set of breast cancer cells followed the same protocol with the concentrations of THC using a different plate.

MTT Assay

The plates were incubated (37 ºC, 95% humidified air, 5% CO_2) for 24 hours. Tetrazolium dye-MTT assay (ThermoFisher, V13154) was added to the MCF-7 cancer cells to measure cell metabolic activity and determine the actual percent of the MCF-7 cancer cell apoptosis (Kowalczewska et al., 2016).

Western Blot

The protocol by Pullen et al. (2012) was used in determining cell viability through Western blot. MCF-7 cells were analyzed for apoptosis through Western blotting by detecting PARP cleavage. This allowed comparison of control group apoptosis (baseline) to cells treated with cannabinoids and served as a preliminary measure of induction of apoptosis, which could be the basis for later studies examining the timing of apoptosis. MCF-7 cells were treated with lysis buffer (from Cell Signaling Technology, a detergent containing phosphatase and protease inhibitors to preserve protein structure) (ThermoFisher, 89900) to obtain intracellular proteins; immediately after lysis, samples were microcentrifuged for 5 min at 13,000xg. Proteins were denatured and chemically reduced at 95-100 ºC in 1X Laemmli buffer containing β-mercaptoethanol for 10 min, then cooled on ice. The supernatant was subjected to SDS-PAGE with 4%-20% Tris-glycine polyacrylamide gels for resolution based on size (kDa), and it was then electro-transferred to a nitrocellulose membrane. Twenty-five milliliters of 1X Tris-buffered saline (TBS) were used to wash the nitrocellulose membrane for 5 min at room temperature. Then, to block unspecific binding, the membrane was incubated at room temperature for 1 h in a mixture of TBS with 5% (v/v) milk and 0.05% (v/v), (TBST). The membrane was then rinsed with TBST and incubated with a primary antibody specific for detecting human poly (ADP-robosyl) polymerase 1 at 1:1000) diluted in 1X TBST containing 5% bovine serum albumin

(BSA), with gentle agitation overnight at 4 °C. The membrane was washed three times for 5 min each with TBST. The membrane was then incubated for 45 min with anti-rabbit IgG conjugated to horse radish peroxidase (HRP). TBST was used to wash the membrane, and detection was made by using enhanced chemiluminescence substrate activated by HRP (PerkinElmer, Rodgau, Germany). Densitometric analysis was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

An ANOVA was performed to compared cannabinoid concentrations among themselves and with control to determine if there was a significant difference in percent of the breast cancer cell death in both MTT assay and Western blot. Poc-host test was performed to determine which effects due to concentrations differ from one another. The treatment groups were compared with the control since the control was set as the standard.

Results

The first study determined the exact concentration of CBD and THC needed to kill MCF-7 breast cancer cells. CBD was more effective at stimulating apoptosis compared to THC. However, the most effective concentration of CBD varied between the MTT assay and the Western blot assay. Since the THC was not as effective as we predicted, we could test the THC in higher concentrations in the future to gauge effectiveness. Because these are preliminary results, the assays will need to be replicated. This study will be replicated to determine if the basic findings of the original work are accurate. Due to the therapeutic potential of CBD and THC, the findings of this study could contribute to the ever-growing body of knowledge regarding cancer treatments.

MTT ASSAY

The MTT assay detects the proliferation of MCF-7 breast cancer cells. As the proliferation increases, it indicates that the concentration causes stimulation of proliferation in the MCF-7 breast cancer cells. The lower proliferation indicated that the MCF-7 breast cancer cells were inhibited by the concentration of the CBD or THC. The media was the control, which showed typical/expected proliferation of breast cancer cells. As illustrated in Figure 3, CBD is the more effective inducer of apoptosis of the MCF-7 breast cancer cells compared to the THC and the control. The CBD and THC concentrations at 100 µM were the most effective among the concentrations.

However, THC concentrations had mixed effects on the MCF-7 breast cancer cells. At 10 µM, THC stimulated the cancer cell growth compared to media alone, and that is the opposite effect hypothesized. Nevertheless, the rest of the THC concentrations had no effect on the proliferation of the MCF-7 breast cancer cell.

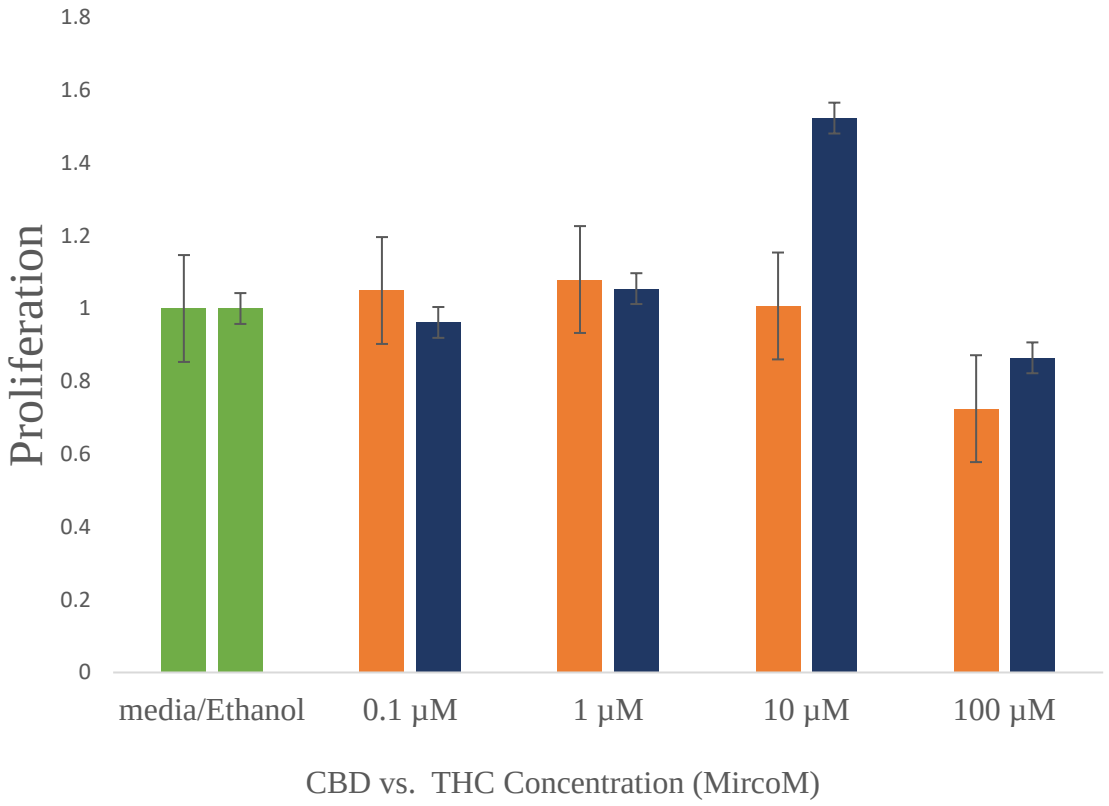

Figure 3. MCF-7 proliferation as a function of CBD and THC. The first bar of each concentration is CBD (orange), and THC is the next bar of each concentration (blue).

Western Blot

The Western blot assessment illustrates the ratio between cleaved and uncleaved parp protein. The ratio is directly proportional to the apoptosis of the MCF-7 breast cancer cells. The higher ratio of the cleaved to uncleaved PARP protein indicates the apoptosis of the breast cancer cells with influence by CBD or THC.

As illustrated in Figure 4, THC concentration caused more apoptosis in MCF-7 breast cancer cells compared to CBD. However, CBD and THC concentration at 0.1 µM showed an increase in the ratio of the cleaved and uncleaved PARP protein, indicating that there is an apoptosis of breast cancer. A decrease in ratio shows no changes in MCF-7 breast cancer cells, or perhaps stimulation occurs, leading to proliferation of the MCF-7 breast cancer cells. As

illustrated in Figure 4, CBD concentrations of 1 µM and 100 µM decrease in the ratio, which indicates a lack of apoptosis or perhaps a proliferation of the MCF-7 breast cancer cells.

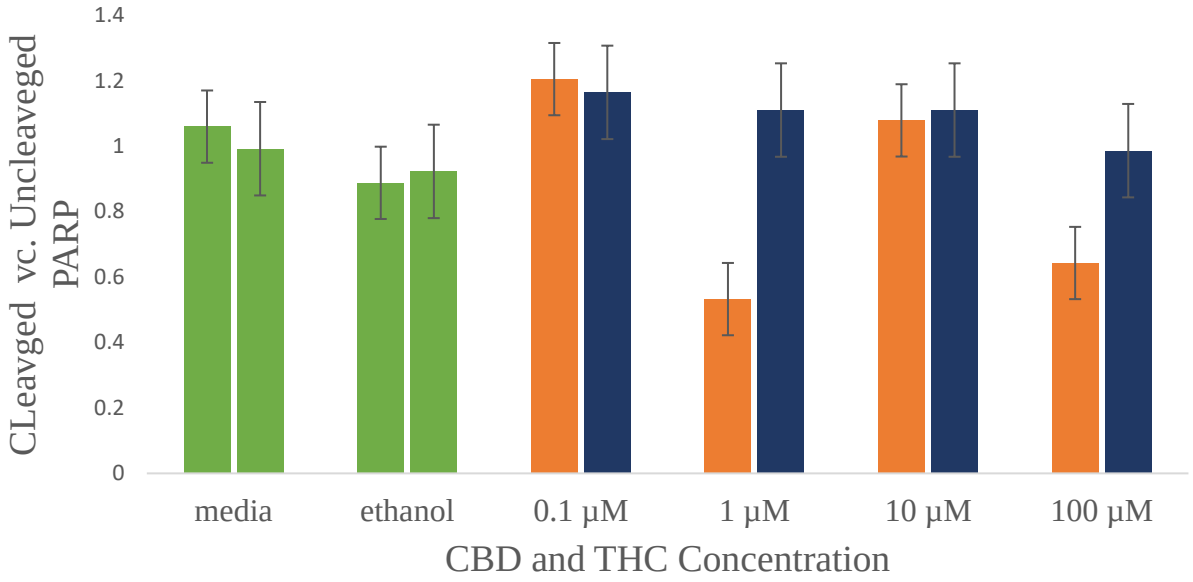

Figure 4. Detection of Parp-protein cleavage. An increase in the bar graph, higher than the media, indicates MCF-7 breast cancer cell apoptosis. CBD (orange), THC (blue), and the Controls (green).

Statistical Analysis

ANOVA - MTT Assay

A t-test of CBD or THC to media shows no significant different; however, comparison of each concentration to the media differs. The two-way analysis of variance is an extension of the ANOVA that examines the influence of two different categorical independent variables on one continuous dependent variable. The use of two-way ANOVA is to understand if there is an interaction between the two independent variables, which in this case are CBD and THC, on the dependent variable, which in this case is the apoptosis of the MCF-7 breast cancer cells shown in Figure 5 and 6. The P-values need to be equal to or less than 0.0500 to account for statistical significant differences between the variables.

As illustrated in Table 1, the comparison of CBD and THC to media is P value = 0.0598, which indicates that there is no statistical significance; however, there is a significant difference between the concentrations. As illustrated in Table 2, each concentration, including the control, was compared with all the concentrations to find a significant difference. All the concentrations show no significant difference except for the relationship between 10 µM THC and 100 µM CBD, which stipulates that at 100 µM CBD concentration, the concentration has an opposite effect on the MCF-7 breast cancer cells compared to all the other concentrations and the media.

Table 1: MTT assay comparison of CBD and THC treatment to the media. Significant difference is P valve of 0.05

ANOVA	
P value	0.0597
Exact or approximate P value?	Approximate
P value summary	Ns
Are means signif. different? (P < 0.05)	No
Number of groups	9
Friedman statistic	14.97
Data summary	
Number of treatments (columns)	9
Number of subjects (rows)	12

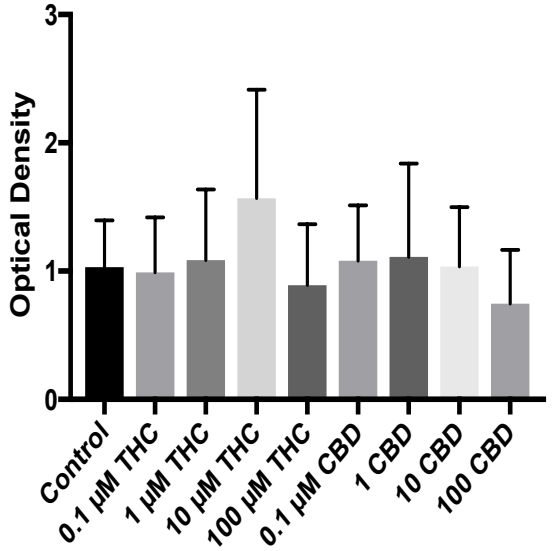

Fig.5. Visual presentation of the comparison of CBD and THC to the media.

Table 2: The mean significant at the 0.5 level. Multiple comparison of t-test of each group to every other group. The difference is significant at the P-valve 0.05 level.

Dunn's multiple comparisons test	Significant?	Adjusted P Value
Control vs. 0.1 µM THC	No	>0.9999
Control vs. 1 µM THC	No	>0.9999
Control vs. 10 µM THC	No	>0.9999
Control vs. 100 µM THC	No	>0.9999
Control vs. 0.1 µM CBD	No	>0.9999
Control vs. 1 CBD	No	>0.9999
Control vs. 10 CBD	No	>0.9999
Control vs. 100 CBD	No	>0.9999
0.1 µM THC vs. 1 µM THC	No	>0.9999
0.1 µM THC vs. 10 µM THC	No	0.1868
0.1 µM THC vs. 100 µM THC	No	>0.9999
0.1 µM THC vs. 0.1 µM CBD	No	>0.9999
0.1 µM THC vs. 1 CBD	No	>0.9999
0.1 µM THC vs. 10 CBD	No	>0.9999
0.1 µM THC vs. 100 CBD	No	>0.9999
1 µM THC vs. 10 µM THC	No	>0.9999
1 µM THC vs. 100 µM THC	No	>0.9999
1 µM THC vs. 0.1 µM CBD	No	>0.9999
1 µM THC vs. 1 CBD	No	>0.9999
1 µM THC vs. 10 CBD	No	>0.9999
1 µM THC vs. 100 CBD	No	>0.9999
10 µM THC vs. 100 µM THC	No	0.1868
10 µM THC vs. 0.1 µM CBD	No	>0.9999
10 µM THC vs. 1 CBD	No	>0.9999
10 µM THC vs. 10 CBD	No	>0.9999
10 µM THC vs. 100 CBD	Yes	0.0108
100 µM THC vs. 0.1 µM CBD	No	>0.9999
100 µM THC vs. 1 CBD	No	>0.9999
100 µM THC vs. 10 CBD	No	>0.9999
100 µM THC vs. 100 CBD	No	>0.9999
0.1 µM CBD vs. 1 CBD	No	>0.9999
0.1 µM CBD vs. 10 CBD	No	>0.9999
0.1 µM CBD vs. 100 CBD	No	>0.9999
1 CBD vs. 10 CBD	No	>0.9999
1 CBD vs. 100 CBD	No	>0.9999
10 CBD vs. 100 CBD	No	>0.9999

ANOVA- Western Blot

As illustrated in Table 3, the comparison of CBD and THC to media (P value = 0.7364) indicates that there is not any statistical significance. In addition, the P value for all the concentrations indicates that there is not any statistically significant difference between them.

Table 3: Western blot comparison of CBD and THC treatment to the media.
Significant difference is when the P valve is 0.05

ANOVA	Column2
P value	0.7364
Exact or approximate P value?	Approximate
P value summary	Ns
Do the medians vary signif. (P < 0.05)?	No
Number of groups	10
Kruskal-Wallis statistic	6.035
Data summary	
Number of treatments (columns)	10
Number of values (total)	24

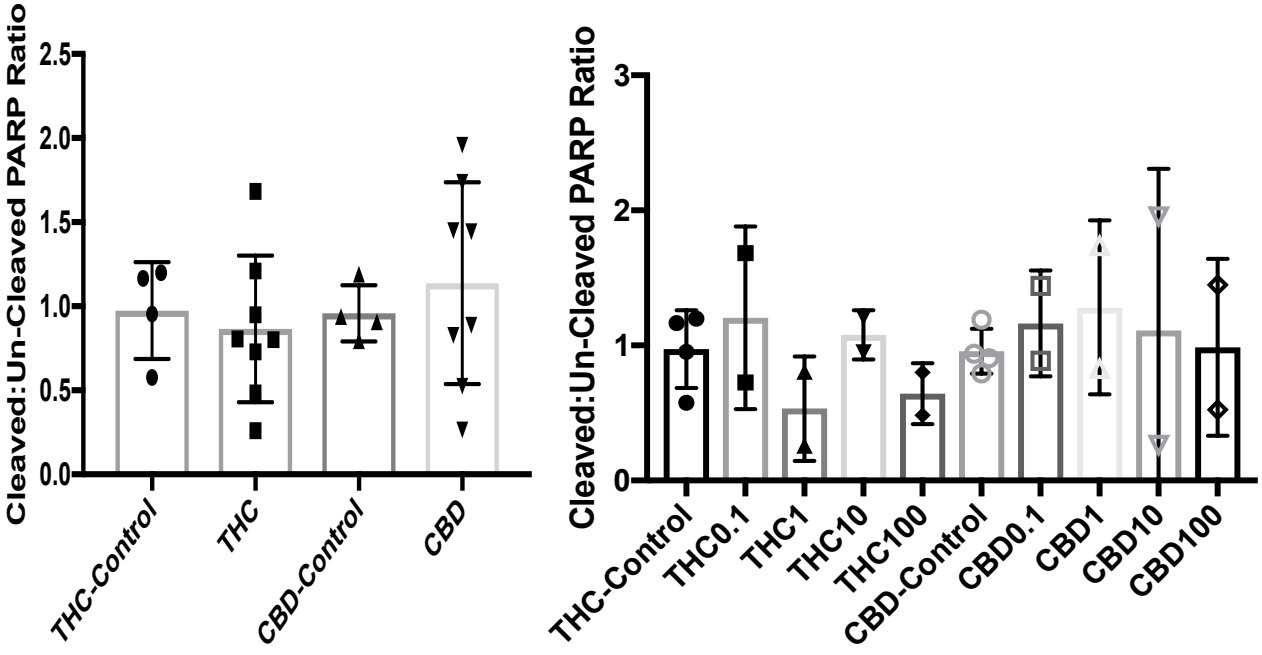

Figure 6: Comparison of the THC and CBD to the media. B. Multiple comparison, t-test of each group to every other group.

Discussion

This study focused on the ascertainment of various concentrations of CBD and THC that influence MCF-7 breast cancer apoptosis. Studies have shown that certain cannabinoids cause the apoptosis of cancer cells, but only with a certain concentration, which has not been well documented. Therefore, this study helped determine the exact concentration of CBD and THC needed to kill MCF-7 breast cancer cells rather than create mass multiplication leading to more growth of the cancer. An MTT assay and Western blot were the methods used to conduct this experiment. The previous research states that CBD was more effective at stimulating apoptosis compared to THC. However, the most effective concentration of CBD varied between the MTT assay and the Western blot assay. The previous research results were preliminary; however, this research is a replication with larger sample sizes. This is a better representative of the population, making the data more accurate and precise.

As illustrated in the MTT Assay, 100 µM is lower in proliferation in both CBD and THC, which indicates inhibition of the MCF-7 breast cancer cells. The most salient finding is that 100 µM CBD was the most effective concentration in the MTT assay assessment. However, THC concentration results vary. At a concentration of 100 µM, THC inhibits the cancer growth, but at 10 µM concentration, THC increases proliferation, meaning the drug stimulates a growth in the MCF-7 breast cancer cell. Visually, the MTT assay illustrated that CBD was more effective overall than THC; however, T-Test results indicate that there is not any statistically significantly difference. Blasco-Benito et al. (2018) suggested that the combination of cannabinoids with estrogen receptor- or HER2-targeted therapies (tamoxifen and lapatinih, respectively) or with cisplatin produced additive anti-proliferative responses in cell cultures. THC and CBD did not show any significant difference compared to the control due to the lack of these combinations.

Takeda et al. (2008) concluded with RT-PCR analysis that there was no detectable expression of CB receptors in MCF-7 cells, and therefore cannabinoids had no effect on the cells. However, the presence of CB receptors cause Δ^9-THC to inhibit the proliferation of MCF-7 cells.

As illustrated in the Western blot assessment, as the CBD concentrations increase, the apoptosis decreases in MCF-7 breast cancer cells. In Figure 4, the 0.1 µM CBD concentration resulted in the most apoptosis compared to all the other CBD concentrations. Western blot results conflict with the MTT assay; this may be due to fact that there was a larger sample size in the MTT assay then there was in the Western blot. THC concentration had mixed effects on the MCF-7 breast cancer cells. Figure 4 also shows that 0.1 µM concentration was most effective for both CBD and THC.

However, the most effective concentrations of CBD and THC on the MCF-7 breast cancer cells as assessed by Western blot were different from the MTT assay's most effective concentration. Even though Western blot and MTT assay have different values for effective concentrations, both methods show that there is indeed an apoptosis of MCF-7 breast cancer cells due to the influence of CBD or THC. However, this effective concentration did not have a statistically significant difference. The research by Petreocells et al. (1998) indicated that anandamide works best on the MCF-7 breast cancer cell with concentrations of 5-10 µM, which works by blocking human breast cancer cell proliferation through CBI-like receptor-mediated inhibition of endogenous prolactin action at the level of prolactin receptor.

Focusing on the Western blot, THC has the opposite effect at concentrations of 1 µM and 100 µM, indicating that there was no apoptosis among MCF-7 breast cancer cells or perhaps that proliferation was stimulated. Comparing the information to the MTT assay, 10 µM THC caused proliferation, but in the Western blot, 1 µM and 100 µM caused proliferation. The proliferation

occurred in both methods, proving that THC could perhaps stimulate proliferation instead of inhibiting the MCF-7 breast cancer cells. Sarfaraz et al. (2008) stated that low doses of cannabinoids cause acceleration of proliferation of the cancer cells instead of inducing apoptosis. Sarfarz et al. results were supported by THC concentration at 10 µM from MTT assay and 1 µM, 100 µM from Western blot assay.

Statistically, there is no significant difference between CBD and THC from the Western blot. Furthermore, there is no significant difference among all the concentrations from the Western blot, but visually there is trend. Even though the statistical data show no significant difference between the media and the concentration, multiple reports, such as Blasco-Benito et al. (2018), Sarfaraz et al. (2008), and Velasco et al. (2016), have shown that cannabinoids do cause anti-proliferation of cancer cells. The lack of statistically significant difference in this research could be because the sample sizes were not quite large enough.

THC and CBD appeared to be effective on the MCF-7 cancer cells; 100 µM or 0.1 µM showed inhibition of MCF-7 cancer cells depending on the use of Western blot or MTT assay. Moving forward, an alternative to the MTT assay could be used, since MTT assay is not sensitive enough for the time points examined. The next focus will be using Western blot to narrow down between these two concentrations at which the drug causes inhibition of the proliferation of breast cancer and testing enough to get a statistically significant difference between the concentration of CBD and THC to media. Favorable findings may provide an accessible and affordable cannabinoid-based treatment for patients.

Conclusions

CBD and THC appeared to be effective at stimulating apoptosis of the MCF-7 breast cancer cells; however, there were no statistically significant differences. Western blot illustrated that at 0.1 µM, CBD and THC are the most effective at stimulating inhibition of the MCF-7 human breast cancer cells, while MTT assay illustrated that at 100 µM, CBD and THC are most effective at stimulating apoptosis of the MCF-7 breast cancer cells. Since Western blot is the more reliable method, the concentration provided from the Western blot should be examined further. However, the sample size for the Western blot was small, and larger sample sizes for the Western blot will therefore be the next focus.

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