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Does CBD Induce Apoptosis in Diffuse Large B Cell Lymphoma?

Sophia Xu

This study assessed the ability of cannabidiol (CBD), a compound derived from cannabis, to induce apoptosis in diffuse large B-cell lymphoma (DLBCL). This experiment consisted of cell viability assays and apoptosis assays, both of which measure important qualities needed in cancer treatment. CBD demonstrated a strong capability in inhibiting cell viability, at concentrations near 50-100 uM, thereby emphasizing its potential in minimizing relapse. CBD actively induced apoptosis at concentrations above 25 uM, with apoptosis frequencies reaching nearly 100%. These results reinforce the idea that CBD induces apoptosis throughout many different types of cancers, including DLBCL. Thus, this study demonstrates that CBD has a great potential in cancer treatment by eradicating cancer cells in DLBCL.

Keywords: CBD, DLBCL, Apoptosis, Cell Viability, Cancer Treatment

Introduction

The need to find a solution to the medical world's leading problem of cancer rises each year, as the projected increase of deaths as a result of cancer are expected to grow from 8.2 million in 2012 to 23 million in 2030 ("Cancer Statistics," n.d.). Within that statistic, Non-Hodgkin lymphoma is recognized as one of the most common cancers in the United States, responsible for a predicted 74,680 diagnoses in 2018 ("Key Statistics for Non-Hodgkin Lymphoma," n.d.). This increase in diagnoses emphasizes the importance of developing effective treatments for this cancer. Non-Hodgkin lymphoma primarily affects T lymphocytes and B lymphocytes, which results in a decreased ability to resist pathogens ("American Cancer Society," n.d.). In a very aggressive form of Non-Hodgkin lymphoma, known as diffuse large B-cell lymphoma (DLBCL), B-lymphocytes enlarge and multiply out of control to form tumours. DLBCL accounts for 30-40% of all Non-Hodgkin lymphoma cases and is often be fatal, if untreated, suggesting the importance of addressing this disease (Li et al., 2018). Currently, there are a number of treatments for Non-Hodgkin lymphoma, ranging from chemotherapy and radiation to immunotherapy ("American Cancer Society," n.d.). However, each method of treatment has its own negative aspects that indicates the need for more effective treatment. For example, these treatments are extremely expensive, averaging at 5,871 USD a month, making this option unaffordable to many families and often cutting treatment short, consequently affecting the potential of relapse (Kutikova et al., 2006). Additionally, chemotherapy and radiation are associated with negative side effects such as extreme fatigue, nausea, vomiting, and most notably, hair loss, which can be uncomfortable for patients and should be addressed in the development of new treatments ("American Society of Clinical Oncology," 2017). Most importantly, chemotherapy and radiation are nonspecific techniques, meaning that these treatments will not only target malignant cells, but will also attack the body's own healthy cells ("Chemotherapy to Treat Cancer," 2015). This consequently weakens the immune system and the patient's overall health, resulting in a longer and extremely debilitating recovery process. Thus, there arises a clear need to find better treatment for

DLBCL that is able to address the problems associated with current treatments.

Recently, studies have pointed towards cannabinoids, compounds derived from cannabis, as a plausible treatment for cancers (Lee, 2012). These cannabinoids have long been known for their therapeutic agents by alleviating pain and nausea, but their relationship in the study of cancer is relatively new (Grotenhermen & Muller-Vahl, 2012). Even so, positive effects of cannabinoids have been noted for certain cancers. In 2008, a team of Harvard scientists demonstrated that the cannabinoid Δ9-tetrahydrocannabinol (Δ9-THC) significantly reduced the spread of lung cancer (Preet et al., 2007). Similarly, cannabidiol (CBD) activates cell death in aggressive breast cancer cells through the intrinsic apoptotic pathway (Ligresti et al., 2006). These benefits have contributed to the increased interest in cannabinoids and their potential in the realm of cancer treatment. While these cannabinoids have been increasingly researched among different cancers, there is a notable gap in research concerning cannabinoids and their functions in lymphoma, presumably because lymphoma already has effective treatment described above, whereas in other cancers, such as breast cancer, these treatments are not as effective, especially in the latter stages. Yet, as previously indicated, these treatments come with severe consequences and need to be addressed with the development of new cancer treatments. As such, this paper will focus on the therapeutic relations that cannabinoids have with Non-Hodgkin Lymphoma.

There are many different types of cannabinoids, each of which has notable positive effects on the human body. Out of those cannabinoids, Δ9-THC and CBD have been most commonly researched in terms of cancer treatment. However, there are certain concerns that arise with the use of cannabinoids as drugs. These cannabinoids are extracted from cannabis, more commonly known as marijuana. While these cannabinoids would be used strictly for its medical applications, cannabis is known for its psychoactive or "high" effect, and this drug can be abused for other reasons. However, it is important to note that the key difference between these two cannabinoids is that Δ9-THC demonstrates this psychoactive effect, whereas CBD lacks it (Kohn, 2016). In fact, CBD has been shown to reduce these psychoactive effects, due to the absence of a large binding affinity for CB1 receptors (Wilcox, 2017). These receptors are responsible for releasing chemical signals, producing the "high" effect of marijuana in the brain (Mackie, 2008). Thus, in order to minimize any ethical concerns, this paper will be limited to CBD rather than the psychoactive cannabis compound Δ9-THC.

CBD represents a strong potential candidate for cancer treatment as it addresses many of these problems associated with current treatments. Not only is cannabidiol associated with therapeutic effects such as the alleviation of pain and nausea, thereby countering the negative side effects from current treatment, but CBD has proven to solely target cancer cells, ensuring that the body's own healthy cells will not be attacked (Massi et al., 2006). Thus, CBD addresses the most important concern with current cancer treatments, in that CBD would result in a stronger recovery process. Furthermore, a CBD drug might be more financially affordable for families. Altogether, CBD could provide a much better option for cancer patients in terms of cost, health, and efficiency. This study will focus on the effect of CBD on DLBCL.

Ultimately, the purpose of cancer treatment is to eradicate cancerous cells that form tumours. In terms of current cancer treatments, radiation uses electromagnetic waves to eradicate these cells. Chemotherapy focuses on the special ability of these cancerous cells to multiply rapidly in order to target and effectively eradicate these cells. In this case, apoptosis would be the mechanism by which CBD would possibly eradicate DLBCL cells. As a result, this paper will evaluate the degree at which CBD induces apoptosis in DLBCL cells as an indicator of its potential as cancer treatment.

Apoptosis refers to programmed cell death in that this type of cell death follows a very systematic approach. There are generally two methods to induce cell death: apoptosis and necrosis. Necrosis involves an external factor, such as a puncture, that induces death. As a result, necrosis causes harm in the human body due to inflammation in the area surrounding the wound. In contrast, apoptosis involves the collapse of the cell and is therefore what this paper will measure since apoptosis would harm the patient less in comparison to necrosis. The process of apoptosis can be conducted in numerous ways. Ultimately, when the cell prepares for its death, proteins known as caspases are activated to break down the cellular components

that are required for the cell to survive (Elmore, 2007). For example, the activation of caspases initiates the enzymes, DNases, to destroy the nucleus, including the DNA, of the cell, which contributes to its demise (Enari et al., 1998). Once the cell collapses, macrophages are sent to remove these cells, making the process overall very clean, and thus producing no harmful effect parallel to that of necrosis (Fink & Cookson, 2005). The two major pathways involved in apoptosis are the extrinsic pathway or death receptor activated pathway and the intrinsic or mitochondrial pathway (Elmore, 2007). Each of these mechanisms of apoptosis is critical to understanding the processes that CBD may undertake in conducting apoptosis in DLBCL.

This study consists of two experiments: one which assesses the effect of CBD on cell viability and one which assesses the effect of CBD on apoptosis. Both of these measures are important qualities of cancer treatment that needs to be evaluated. Cell viability evaluates the potential for a cell to recover from treatment, thereby determining the extent at which CBD can reduce the potential for relapse in patients. On the other hand, the apoptosis assay measures the ability of CBD to induce cell death in DLBCL and determine the best concentration for a drug.

Literature Review

The research of cannabinoids in the field of cancer has largely pertained to other cancers and is specifically lacking in the realm of lymphoma. Thus, the gap in research is determining how CBD interacts with DLBCL. However, it is also important to understand the mechanisms by which CBD interacts with various cancers to provide information that could be used for this experiment. As such, this paper recognizes the relationship between CBD and breast cancer, glioma, and leukemia, as these studies have proven to yield promising results that are comparable to those of this research.

In breast cancer, CBD was found to effectively inhibit breast cancer cell proliferation through the down regulation of Id-1 expression, which is responsible for tumour proliferation, migration, and invasion when found in high quantities (Ligresti et al., 2006). Thus, CBD has been shown to effectively inhibit the activities of tumours, correlating to a strong response towards breast cancer cells. The study also indicated that among the possible underlying mechanisms involved in this inhibition, CBD directly activated the capsaicin receptor (TrpV1). This receptor functions primarily to detect and regulate body temperature, which could affect these tumour-related activities. Additionally, the activation of TrpV1 may have indirectly activated CB2 receptors via oxidative stress. These CB2 receptors are directly involved in immune suppression, the induction of apoptosis, and the induction of cell migration (Basu et al., 2011). In 2011, another study concluded that CBD induces apoptosis through mitochondrial-mediation in breast cancer (Shrivastava et al., 2011). Thus, CBD alters tumour-related activities in breast cancer and potentially induces apoptosis through various mechanisms including the activation of CB2 receptors and mitochondrial-mediation, as demonstrated by both studies (Ligresti et al., 2006; Shrivastava et al., 2011).

In glioma, numerous mechanisms for apoptosis have been identified, including the release of cytochrome C and the activation of caspase-9 and caspase-8 pathways (Massi et al., 2006). Cytochrome c plays an active role in ATP synthesis in the mitochondria, and its release from the mitochondria to the cytosol is associated with the activation of a caspase cascade, thereby triggering apoptosis (Ow et al., 2008). Additionally, while CBD suppressed proliferation of human glioma cell lines, there was no reported effect of CBD on healthy monocytes (Massi et al., 2006). This suggests that CBD is specific to cancerous cells and solely induces apoptosis in affected, cancerous cells in glioma. This finding is significant since healthy human body cells were unaffected, while cancer cells were destroyed. Furthermore, this result directly addresses issues with chemotherapy and radiation and their inability to differentiate between healthy and cancer cells.

In Leukemia, CBD led to CB2 mediated reduction and activation of caspase-8, caspase-9, and caspase-3 (McKallip et al., 2006). Furthermore, CBD exposure initiated the breakdown of the mitochondrial membrane and release of cytochrome C (McKallip et al., 2006). All of these mechanisms are correlated with the induction of apoptosis. Correspondingly with glioma, CBD had no effect on healthy human monocytes, whereas it induced apoptosis in cancer cells (Gallily et al., 2003). This suggests that this characteristic could be replicated across different types of cancers.

The similarities and relationships in how CBD induces apoptosis in various cancers suggests a link between intrinsic and extrinsic apoptotic pathways. For example, an increasingly dominant reaction of CBD on cancer cells is the release of cytochrome c, which is associated with the intrinsic pathway in apoptosis, suggesting that this may be a common characteristic of CBD-induced apoptosis. Based upon these studies, CBD seems to conduct apoptosis in a consistent method through the release of cytochrome c and the activation of caspases. On the other hand, it is presently unclear as to how CBD induces apoptosis and the method through which this occurs, specifically in DLBCL. However, based upon the successful conduction of apoptosis in breast cancer, glioma, and leukemia, it is likely that CBD will also induce apoptosis in DLBCL.

Materials and Methods

DLBCL cell lines were established in the laboratory. These cell lines were extracted from a total of 24 patient samples. As a basic growth medium, 1% penicillin/streptomycin (Hyclone, Logan, UT, USA) was used with cell culture of RPMI-1640 medium (Gibco, Rockville, MD, USA) and 15% fetal calf serum (Gibco). All 24 cell lines (CJ, LP, RC, TMD-8, WP, LY-19, MZ, 8LR, HT, MS, Toledo, BJAB, u2392, DS, Pheiffer, McA, SUDHL-4, HF, SUDHL-6, HBL-1, DB, EJ, Val) were routinely tested for any small parasitic bacteria, also known as mycoplasma, using a MycoSEQ™ Mycoplasma Detection kit (Invitrogen, Carlsbad, CA, USA). This step is particularly important as mycoplasma can induce morphological changes, alter growth rates, and effectively alter the cell viability assays to be conducted ("Mycoplasma Detection and Elimination," n.d.). These stocks of authenticated cell lines were then stored in liquid nitrogen to guarantee that the cells and growth medium would not evaporate. Two experiments were conducted: the cell viability assay and the apoptosis assay. The cell viability assay measures the ability of the DLBCL cells to recover after exposure to CBD, thereby measuring its ability to relapse. The apoptosis assay was used to measure the degree at which CBD would be able to induce cell death in cancerous cells and is the precursor in determining the efficacy of a drug for treating cancer. For the cell viability assay, concentrations of 0 uM, 1.5 uM, 3.1 uM, 6.25 uM, 12.5 uM, 25 uM, 50 uM, and 100 uM of CBD were used. These concentrations were established by a serial dilution to evaluate cell viability on a greater spectrum. The apoptosis assays used the same concentrations, with the exception of 100 uM. The primary reason for the omission of 100 uM is that an apoptotic trend can be established in lower concentrations. However, in order to consider CBD's implications as a drug, it will be necessary to evaluate the most effective concentration at which CBD inhibits both cell viability and induce apoptosis. Thus, the same concentrations from the cell viability assays were used for the apoptosis assays (with the exception of 100 uM) in order to effectively compare each concentration point and determine possible drug concentrations required for treatment in the future. Cannabidiol was obtained from Tocris Cookson Inc. (Ellisville, MO). For therapeutic compound screenings, CBD was prepared as 1000-fold stock solutions in tissue culture grade dimethyl sulfoxide (DMSO) in order to freeze the cells to prevent the formation of crystals that may damage the cells and produce inaccurate data. The final concentration of DMSO in media was 0.1%, a concentration that has shown no toxicity or effect on the DLBCL cells. For the cell viability assay, cells from representative DLBCL cell lines were plated at 5,000 cells per well, with 20 uL medium and 10% fetal bovine serum to acquire ample data to accurately establish a conclusion. These cells were incubated for 96 hours, as per apoptosis protocol, and DMSO was added. Fetal bovine serum is used primarily for its low level of antibodies and many growth factors. The cell viability assays were performed using the Celltiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI, USA) by adding CellTiter-Glo Reagent and recording luminescence. For the apoptosis assays, the cells were incubated for 48 hours with the appropriate CBD dilution and DMSO was added. Only the cell line RC was used in the assay in order to minimize time constraints. Unlike the cell viability assays, the induction of apoptosis will not differ per cell line. Thus, a general trend can be concluded and extended from the apoptosis assay, suggesting that one cell line will suffice. The Annexin V/PI staining method, with flow cytometry, was used to assess apoptosis. The assays were performed in duplicate. Statistical analysis consisted of standard deviation in the cell viability assays to determine the precision of these trials.

Results

Cell Viability Assays

Control- The control for the cell viability assays was established through untreated cells in each cell line, exhibited by the 0 uM column in Figure 1, Figure 2, and Figure 3 below. This information is important because cell viability differs per trial per cell line, as demonstrated by the various starting points of each trend for the cell lines. Using this control, a comparison can be made to determine the effectiveness of various CBD concentrations by contrasting the number of viable cells left to the control.

Variables- The primary variable that was manipulated in the cell viability assay was the CBD concentration in order to establish both the effectivity of each concentration and address the implications of CBD as a drug. This information will be particularly

Cell Lines 1-8

Table 1. Average Cell Viability count of DLBCL cell lines 1-8 (CJ, LP, RC, TMD-8, WP, LY-3, LY-19, MZ). Averages are taken from three trials of each cell line.

CBD-99 (uM)	(1) CJ Avg	(2) LP Avg	(3) RC Avg	(4) TMD-8 Avg	(5) WP Avg	(6) LY-3 Avg	(7) LY-19 Avg	(8) MZ Avg
0	14.74	20.84	16.34	22.32	19.06	18.71	16.61	19.66
1.5	15.95	21.52	16.47	23.76	21.17	18.37	17.13	18.62
3.1	18.07	19.57	16.40	22.67	20.70	18.26	18.34	19.43
6.25	16.71	19.94	15.42	23.17	21.32	19.72	18.83	17.92
12.5	14.53	17.15	14.30	20.36	20.33	17.72	16.55	13.78
25	9.46	10.28	7.07	14.62	14.17	10.71	10.99	8.19
50	1.51	3.14	0.12	4.49	2.83	1.16	0.22	2.22
100	0.30	0.14	0.26	0.32	1.51	0.90	0.07	4.71

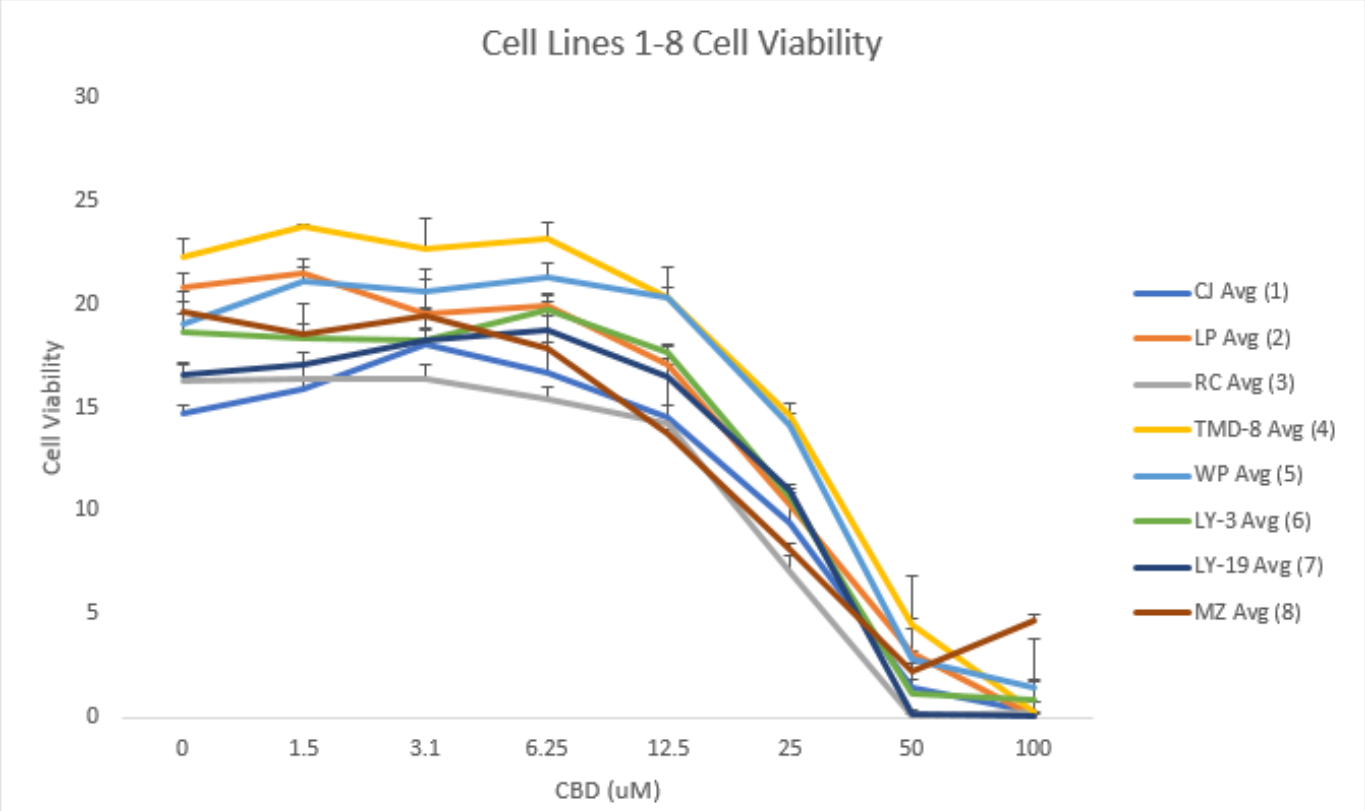

Figure 1. *This graph corresponds to the set of values in Table 1. In each cell line, a general, predicted downward trend is established in each cell line. From 50 - 100 uM, each cell line exhibits the lowest cell viability count. Error bars represent standard deviation.*

Cell Lines 9-16

Table 2. Average Cell Viability count of DLBCL cell lines 9-16 (8LR, HT, MS, Toledo, BJAB, u2392, DS, Pheiffer). Averages are taken from three trials of each cell line.

CBD-99 (uM)	(9) LR Avg	(10) HT Avg	(11) MS Avg	(12) Toledo Avg	(13) BJAB Avg	(14) U2932 Avg	(15) DS Avg	(16) Pheiffer Avg
0	14.96	31.93	15.99	23.35	22.17	12.75	20.22	17.99
1.5	12.57	31.58	17.17	26.69	32.91	10.80	25.10	21.20
3.1	16.00	29.41	19.99	28.11	29.47	10.42	23.66	19.84
6.25	13.89	30.45	19.70	29.07	30.02	10.74	25.25	21.46
12.5	14.00	30.27	20.68	25.74	24.53	10.25	24.58	18.06
25	13.68	18.16	15.83	26.93	22.32	11.19	22.33	19.06
50	12.58	5.30	12.40	16.80	18.94	11.89	15.58	17.23
100	3.40	0.16	1.32	0.29	3.61	0.53	0.70	5.57

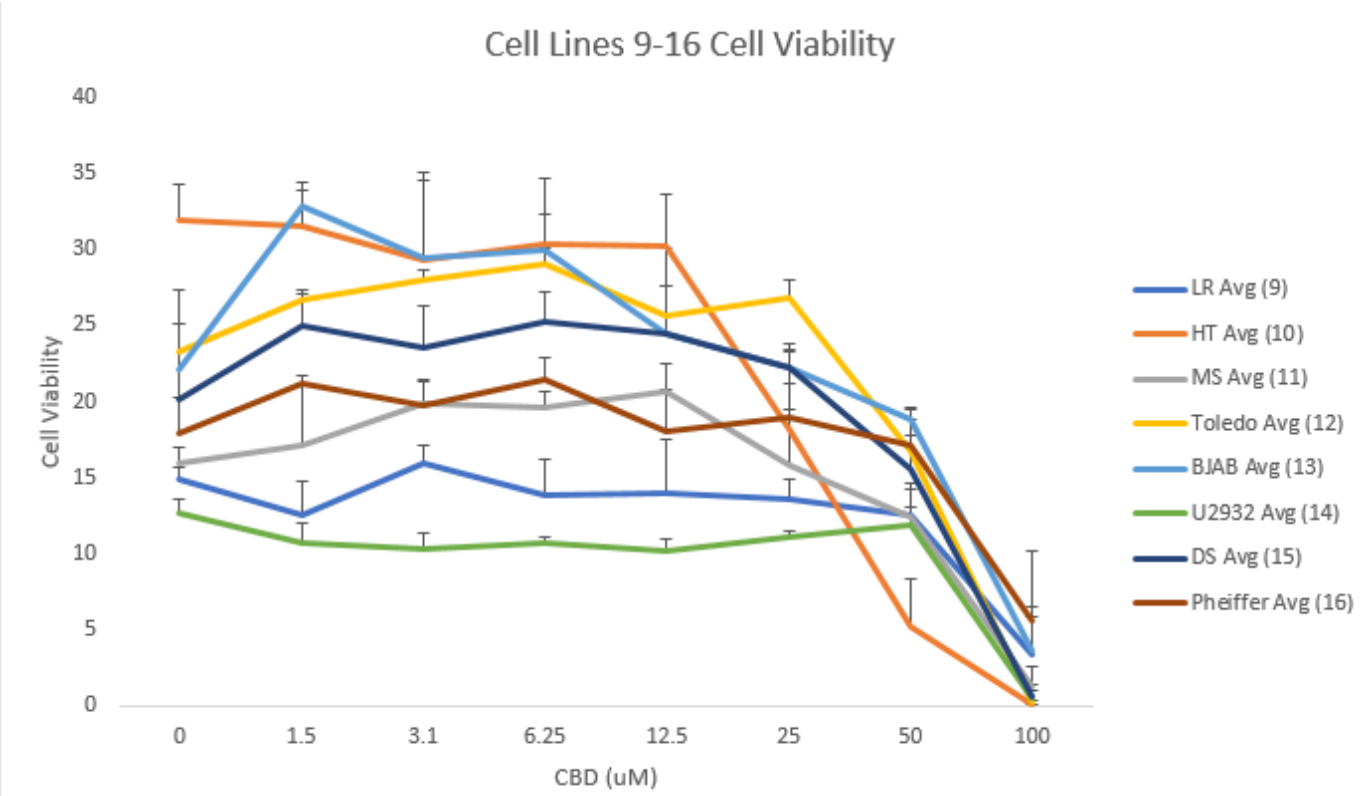

Figure 2. *This graph corresponds to the set of values in Table 2. In each cell line, a general, predicted downward trend is established in each cell line. Once again, each cell line exhibits the greatest reduction in cell viability count from 50 -100 uM.*

important in understanding the necessary concentrations of CBD for a viable drug treatment. Thus, this experiment is dose-dependent and should produce a dose-dependent trend downwards. A serial dilution was established in which each dosage was half of the previous concentration. Thus, with these concentrations ranging from 0 uM to 100 uM, a wide spectrum is created to examine the ability of CBD to inhibit cell viability at all concentrations, in comparison to a limited scope that may exclude this potential. Ideally, these dose response graphs would depict a relatively stable decrease in cell viability, from the control to 0 viable cells at 100 uM. Another variable includes the 24 cell lines that were tested. In Figure 1, Figure 2, and Figure 3, the results of the cell lines are divided into three groups of eight cell lines, labelled accordingly. The groupings of these cell lines demonstrate the difference in days in which the assays were performed. The structuring of these experiments into three groups helps to ensure that any possible mistakes that may have occurred would not affect all 24 cell lines.

Cell Lines 17-24

Table 3. *Average Cell Viability count of DLBCL cell lines 17-24 (McA, SUDHL-4, HF, SUDHL-6, HBL-1, DB, EJ, Val). Averages are taken from three trials of each cell line.*

CBD-99 (uM)	(17) McA Avg	(18) SUDHL-4 Avg	(19) HF Avg	(20) SUDHL-6 Avg	(21) HBL-1 Avg	(22)DB Avg	(23) EJ Avg	(24) Val Avg
0	17.73	15.31	20.35	14.50	17.54	10.83	17.50	14.87
1.5	18.25	16.45	21.09	12.35	19.41	10.85	19.64	16.81
3.1	17.37	16.64	20.80	11.19	19.25	11.69	20.19	16.85
6.25	16.07	15.20	19.89	13.52	18.61	11.43	18.69	14.89
12.5	13.83	15.97	17.43	11.16	18.68	10.40	17.46	16.13
25	9.42	14.19	16.23	10.18	17.18	8.49	17.31	13.82
50	4.59	5.55	3.32	2.57	8.41	1.28	12.31	12.40
100	1.15	0.05	0.09	0.03	1.00	0.15	6.11	3.58

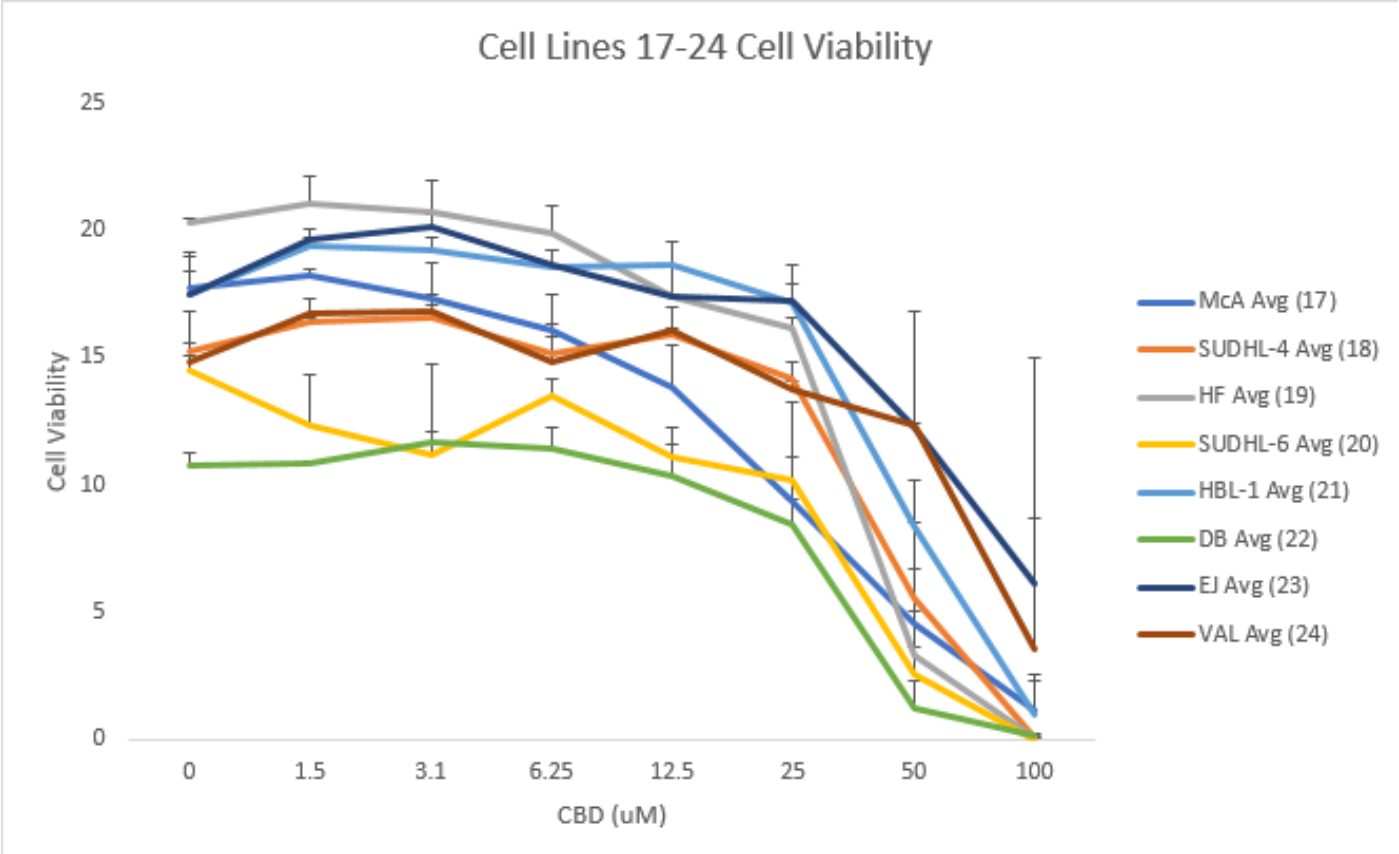

Figure 3. *This graph corresponds to the set of values in Table 3. In each cell line, a general, predicted downward trend is established in each cell line. There are minimal increases depending on the cell line. In these trends, the lowest cell viability count and greatest decrease begins at 25 uM and continues until 100 uM.*

Results (Figures 1-3). As seen in Figures 1-3, a general downward trend is established in each cell line. This trend fluctuated near the initial cell viability values and remained stagnant until a certain concentration. In Figure 1 and Figure 2, an abrupt decrease is depicted at 50 uM. On the other hand, this same decrease occurs at Figure 3 at 25 uM. Additionally, in Figures 1-3, all graphs demonstrate a significant reduction in cell viability by 100 uM, suggesting that CBD is effective in inhibiting cell viability. Generally, at 100 uM, cell viability is most effectively inhibited, with the exception of cell line MZ. The tables correspond with the data presented in the graph, in order to concisely show the points used. The cell viability data listed in the table were taken as the average of three trials in order to ensure accuracy.

Cell Line RC

Table 4. This table indicates the frequency of apoptosis in cell line RC (cell line 3). These concentrations were established from the cell viability assays.

Concentration CBD-99 (uM)	Frequency of Apoptosis
0	27.90%
3.1	22.50%
6.2	34.70%
12.5	66.40%
25	99.00%
50	99.80%

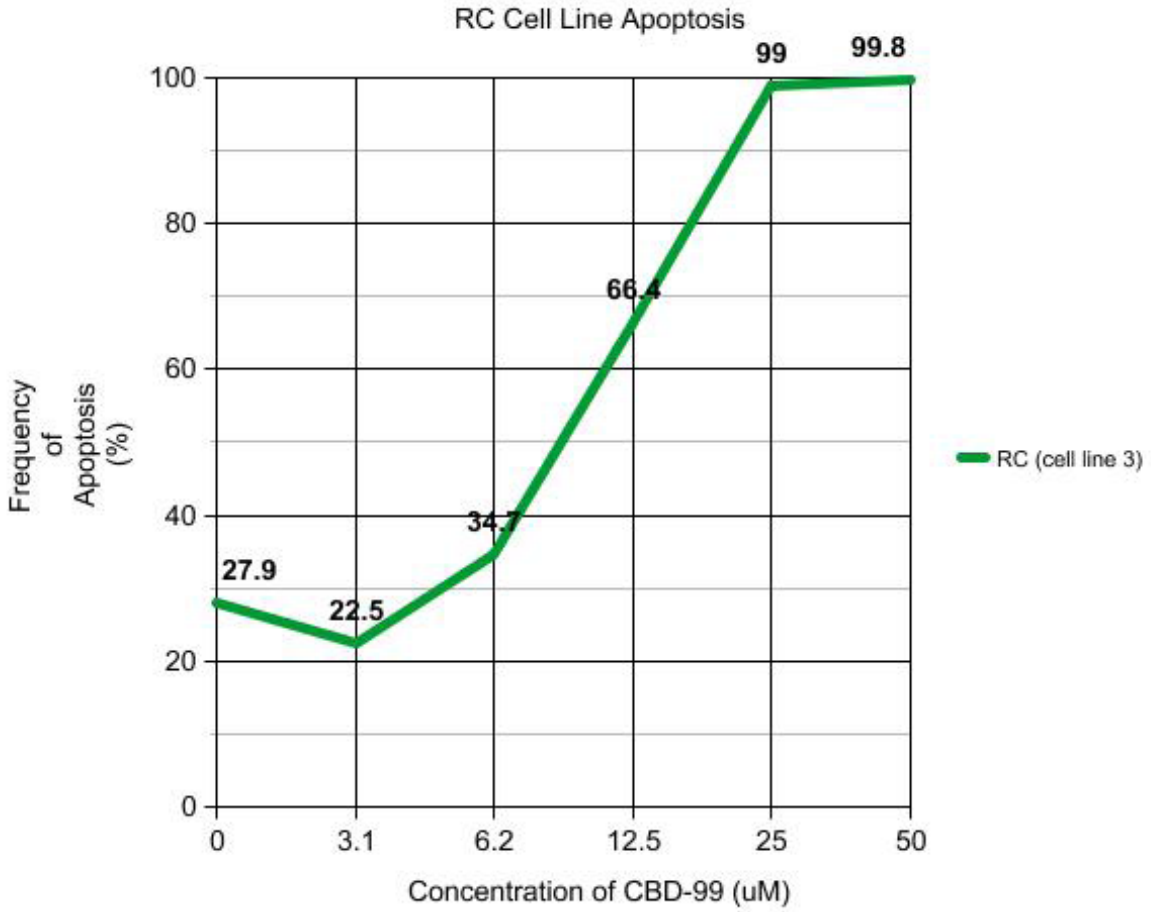

Figure 4. This graph corresponds to the set of values in Table 4.

Apoptosis Assays

Control- In the apoptosis assays, the control was established when CBD had a concentration of 0 uM, as seen in Figure 4. This control installs the basis for the apoptosis assays, allowing for comparison to see if CBD induces apoptosis or inhibits apoptosis. Since the cells can conduct apoptosis themselves, the establishment of a control at 0 uM allows for an effective comparison to determine the apoptotic frequencies that were induced as a result of CBD, rather than by the cell's own function.

Variable- The primary variable that was manipulated was CBD concentration. While a serial dilution was also utilized, the concentrations ranged from 0 uM to 50 uM as apoptotic frequency as 50 uM suf-

ficed to indicate a successful conduction of apoptosis in the cell line. The dependent variable measured the frequency of apoptosis.

Results (Figure 4/ Table 4). As seen in Figure 4, an increasing, positive trend is established in the apoptosis assays in cell line RC. Interestingly, the frequency of apoptosis at 3.1 uM is lower than the control. Generally, a dosage curve for apoptosis should increase exponentially when corresponded with a serial dilution. Between 6.2 uM and 25 uM, a steady increase in apoptotic frequency is established. The highest frequency of apoptosis is established at 25 uM and 50 uM, at nearly 100%.

Discussion and Analysis

Analysis of Cell Viability Assays (Figure 1-3). The decreasing values for cell viability demonstrate that DLBCL was actively inhibited through the exposure of CBD. On the other hand, if cell viability increased from the control, CBD would have adverse effects by promoting DLBCL. Since cell viability was inhibited, CBD demonstrates a greater potential in cancer treatment. In Figure 1, each line indicates the general trend of decreasing cell viability, affirming the hypothesis that cell viability will significantly decrease as CBD concentration increases. The exception is cell line MZ that increases in cell viability from 50 uM to 100 uM. This directly proposes that CBD reduces apoptosis frequencies in high concentrations, refuting the hypothesis that cell viability is inhibited. However, this event can be explained by sources of error. It is likely that the well-received varying amounts of CBD due to air bubbles in the pipet or cells may have been picked up from earlier trials, which will all contribute to inconsistent data readings. Since the other cell lines follow the trend without variations, this is likely a consequence of human error.

In all of the figures, the general trend seems to be a stabilizing effect with minimal changes to cell viability, followed by a large decrease in cell viability. The points at which this extensive reduction occurs will be important in determining adequate concentrations for a potential CBD drug. In both Figure 2 and Figure 3, this reduction occurs at 50 uM and continues until 100 uM, whereas in Figure 1, this trend begins at 25 uM.

Standard deviation varies per cell line per trial suggesting a wide variance in the data. This would suggest that these results are not consistent and can vary thoroughly. However, the calculated coefficient of variation, used to measure relative variability, is less than 100% for the cell lines. Thus, while the standard deviation is large at some concentrations for certain cell lines, this variability is accounted for by the coefficient of variation, thus affirming that these results are plausible.

Analysis of Apoptosis Assay (Figure 4/Table 4). A higher frequency of apoptosis indicates a greater capability of CBD to inhibit cancer cells. Therefore, the expected general trend is a continuous increase in apoptosis frequency as concentration increases. Thus, if this trend is exhibited, it can be deduced that CBD would be effective in inducing apoptosis and can then be considered for cancer treatment. Contrarily, while CBD demonstrated effective inhibition of cell viability, if a negative trend is established in the apoptosis assays, CBD can be ruled out for its implications as a drug. In Figure 4, the positive trend is established where apoptosis frequency increases with concentration. However, the exception is the concentration at 3.1 uM. Since the control has been established at 27.9%, the expectation is that the subsequent concentrations would yield an apoptosis frequency greater than this value. Nevertheless, at 3.1 uM, apoptosis frequency decreased from the control, suggesting that CBD may actually reduce apoptosis in DLBCL at that concentration. This deviation can be attributed to human error, similar to the cell viability assays, but should further be looked into for future studies.

From the data presented in Figure 4, the greatest reduction, found by the slope, occurs at 12.5 uM, with 25-50 uM having the greatest apoptosis frequency at nearly 100%. This suggests that studies looking into the plausibility of CBD as a drug for DLBCL should focus on the concentration range of 25-50 uM.

Discussion (Figures 1-4/ Tables 1-4). The purpose of this research is to ultimately determine whether CBD can induce apoptosis in DLBCL, as this represents the essential marker for cancer treatment. The induction of apoptosis in cancerous cells represents the first step in developing cancer treatment, since the primary goal is to eradicate cancerous cells. While apoptosis was the primary focus of this experiment, both cell viability and apoptosis were measured. These cell viability assays represent a measure of the ability

of the DLBCL cells to recover from CBD treatment, which is a significant factor to consider for treatment. As such, this assay provides insight on the implications of a CBD drug, as an anticancer drug would not only have to induce apoptosis in cancerous cells but also minimize the potential for relapse. Figure 1 and Figure 2 suggest that CBD is capable of inhibiting cell viability, specifically at concentrations around 50 uM to 100 uM, as indicated by the abrupt reduction in cell viability at 50 uM. Similarly, Figure 3 suggests that CBD is capable of inhibiting cell viability at concentrations from 25 uM to 100 uM. Thus, a higher concentration, between 50 uM to 100 uM, should be used to account for all cell lines. However, the key factor that determines whether or not CBD could function as potential cancer treatment lies in its capabilities to eradicate DLBCL through the induction of apoptosis. While cell viability is an important measure to evaluate, if CBD does not induce apoptosis, CBD could be ruled out for treatment altogether. Overall, the apoptosis assays hold greater priority than the cell viability assays as the induction of apoptosis in DLBCL represents the ultimate marker for cancer treatment. From Figure 4, the positive trend established asserts that CBD does induce apoptosis in DLBCL, especially at concentrations greater than 25 uM. This suggests that a concentration point between 50 uM to 100 uM would be successful in inhibiting cell viability and inducing apoptosis. Altogether, these results suggest that CBD induces apoptosis and can be considered as a candidate for cancer treatment, given its benefits in reducing cell viability. Statistical analysis illustrates that these results, while varied, are therefore reliable.

These results overall represent a consensus among the scientific community that CBD does induce apoptosis in cancer. As previously stated, CBD has proven to induce apoptosis through varying, but similar, mechanisms in breast cancer (Ligresti et al., 2006), glioma (Massi et al., 2006), and leukemia (McKallip et al., 2006). This paper has effectively shown that CBD does induce apoptosis in DLBCL, thus indicating that in a larger context, CBD induces apoptosis in multiple cancers.

Limitations

Although the apoptosis assays provided reliable results, the methods of apoptotic inductions by CBD were not examined. Therefore, the mechanisms of apoptosis could not be compared to previous studies presented in the Literature Review. This remaining lack of knowledge does limit the potential of CBD as a drug for DLBCL and suggests a need for further research in this area. Furthermore, time limitations restrained the number of trials performed for the apoptosis assay, thereby obstructing statistical analysis due to lack of ample data. However, the apoptosis assay demonstrates a significant difference between the control concentration and subsequent concentrations. With nearly a 70% difference in apoptotic frequency, the assertion that CBD induces apoptosis in DLBCL is indisputable. Additionally, since this experiment was conducted in vitro, it cannot fully replicate results in the environment of the human body. Thus, the results produced from this experiment cannot be guaranteed in the human body. Nonetheless, since human cell lines were utilized and the induction of apoptosis was ascertained by the assays, this experiment does indicate the ability of CBD to induce apoptosis in human DLBCL cell lines. Given these circumstances, this paper represents a degree to which CBD would induce apoptosis in the human body but is not fully representative. Despite these limitations, the successful conduction of apoptosis in cell line RC and the successful inhibition of all twenty four cell lines in terms of cell viability affirms the possibility of CBD as an anticancer drug.

Conclusion and Future Directions

The results of this study directly suggest that CBD has a strong potential for treating DLBCL. The results concluded from the cell viability assays indicate that CBD is effective in reducing the potential for relapse in cancerous large B lymphocytes. This assay is particularly important in addressing relapse in cancer, in that certain cancerous cells may escape treatment and metastasize to other parts of the body. The apoptosis assays were the main focus of this paper and the results from Figure 4 indicate that CBD is effective in inducing apoptosis in DLBCL. This confirms the hypothesis that CBD induces apoptosis in DLBCL.

The results from this paper indicate that CBD does induce apoptosis in DLBCL, which is the first step in addressing its implications as a drug. However, in

order to fully address this possibility, other factors should be looked into. First, this paper indicates the concentrations of CBD at which it is most effective in reducing cell viability and promoting apoptosis. These concentrations should be regarded in terms of toxicology levels that are acceptable and healthy for the human body. Furthermore, testing should be done to determine any possible side effects that have not previously been accounted for. Other possible considerations include a combination of current treatment techniques with CBD. This combination therapy would be able to minimize the negative limitations of current treatment such as cost, side effects, and weakened immune system while using CBD in moderation (Fisher et al., 2016). Moreover, the administration of CBD as a drug should be considered. Already, certain studies have noted that CBD would be most effective when administered orally due to a high lipid content (Zgair et al., 2017). However, this form of administration could differ depending on the cancer and the site of tumours. Lastly, this paper solely focused on CBD's ability to induce apoptosis but did not look into the different pathways that may have been used, unlike other aforementioned studies. Given that a link between the intrinsic and extrinsic apoptotic pathway in inducing apoptosis was not examined in DLBCL, no conclusions regarding whether this method of apoptosis induction is consistent or similar to other cancers can be made. A further, in-depth analysis of these pathways would be useful in understanding CBD's ability to conduct apoptosis and should be the next focus in the steps leading up to drug development.

Overall, CBD has demonstrated a great capability in targeting DLBCL and substantiates the need for further research in this area to develop treatments. This paper represents the first step in developing a CBD cancer treatment, based on the successful conduction of apoptosis.

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