

Ouachita Baptist University

## Scholarly Commons @ Ouachita

---

Honors Theses

Carl Goodson Honors Program

---

2015

### The Effect of Cannabinoids on Triple Negative Breast Cancer Cells

Haley Dahl

*Ouachita Baptist University*

Follow this and additional works at: [https://scholarlycommons.obu.edu/honors\\_theses](https://scholarlycommons.obu.edu/honors_theses)



Part of the [Alternative and Complementary Medicine Commons](#), [Diseases Commons](#), and the [Other Chemicals and Drugs Commons](#)

---

#### Recommended Citation

Dahl, Haley, "The Effect of Cannabinoids on Triple Negative Breast Cancer Cells" (2015). *Honors Theses*. 183.

[https://scholarlycommons.obu.edu/honors\\_theses/183](https://scholarlycommons.obu.edu/honors_theses/183)

This Thesis is brought to you for free and open access by the Carl Goodson Honors Program at Scholarly Commons @ Ouachita. It has been accepted for inclusion in Honors Theses by an authorized administrator of Scholarly Commons @ Ouachita. For more information, please contact [mortensona@obu.edu](mailto:mortensona@obu.edu).

# **SENIOR THESIS APPROVAL**

This Honors thesis entitled

**“The Effect of Cannabinoids on Triple Negative Breast Cancer  
Cells”**

written by

**Haley Dahl**

and submitted in partial fulfillment of  
the requirements for completion of  
the Carl Goodson Honors Program  
meets the criteria for acceptance  
and has been approved by the undersigned readers.

Dr. Lori Hensley, thesis director

Dr. Barbara Pemberton, second reader

Dr. Chris Mortenson, third reader

Dr. Barbara Pemberton, Honors Program director

April 22, 2015

## **Abstract**

Triple Negative Breast Cancer (TNBC) is a difficult type of cancer to treat because it is negative for progesterone, estrogen, and HER-2 receptors. Because TNBC is negative for these three receptors, it does not respond to normal hormonal therapies. The purpose of my experiment is to see if different cannabinoids, compounds from the Cannabis plants, could be used as alternative treatment options. These experiments employed three different cannabinoids: ajulemic acid, cannabidiol, and hemp oil. Cell viability was measured after 72 hours of treatment using a MTT assay. The results showed that the three cannabinoids could be used to effectively destroy the TNBC cells. We used this data to calculate the median lethal dose (LD-50), the concentration of the cannabinoid that can be given to destroy half of the cells. Our data suggest, cannabinoids could potentially be used as an alternative treatment for TNBC. However, much more testing would need to be done before it could be confirmed as a viable treatment option.

## **Introduction**

Many people today use Cannabis for medicinal purposes. Many cancer patients use cannabis to help with the pain and the nausea produced as a side effect of chemotherapy. There has been research that shows Cannabis' potential to not only help with symptoms of chemotherapy but to also help prevent angiogenesis and metastasis of cancerous tumor cells (McCarthy). A major issue that surrounds the use of cannabis is the psychoactive effect that follows its usage. To get around this issue, the cannabinoids that were used for the experiment do not produce these psychoactive effects. We want to know whether these cannabinoids are effective in preventing the growth of breast cancer cells.

## *Triple Negative Breast Cancer*

Triple Negative Breast Cancer (TNBC) is so called because it is negative for the three receptors that normally fuel breast cancer growth. TNBC is negative for the estrogen receptor (ER), the progesterone receptor (PR), and the hormone epidermal growth factor receptor 2/neu (HER-2) (Fig. 1) (Crown). A lack of the three receptors means the growth of the cancer is not supported by the hormones estrogen or progesterone or by the presence of an abundance of HER-2 (Crown). Many breast cancer treatments will target any of these three receptors via hormonal therapy. However, because TNBC does not contain these receptors, this particular type of breast cancer is difficult to treat. Earlier stages of TNBC may respond well to chemotherapy, but it is not always effective. TNBC is typically treated with a combination of surgery, radiation therapy, and chemotherapy (McCarthy). However, these treatments cause the person to be very sick and weak and there is still a high chance that the cancer will return after treatment.

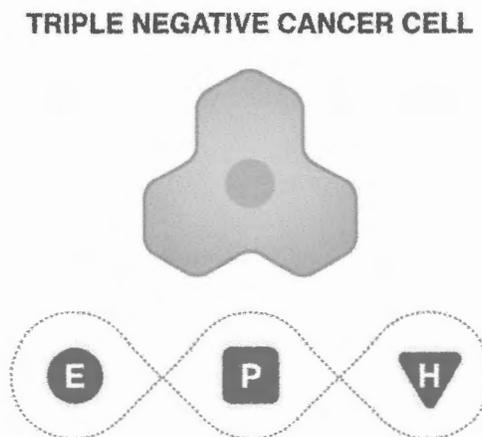


Figure 1: There are no receptor sites on the enzyme that the specific shapes of ER, PR, and HER-2 will be able to bind

About 10-20% of breast cancers in the United States are Triple Negative. This particular type is more likely to affect younger women (between the age of 40 or 50), African American women, Hispanic women, and individuals with a BRCA1 gene mutation (Amirikia). TNBC tends to be more aggressive than other types of breast cancers. TNBC is more likely to spread beyond the breast and more likely to return after treatment. These risks are greatest within the first few years of treatment. Typically, if the cancer has not recurred after about five years, the likelihood of the cancer returning is much smaller (Liedtke). Furthermore, the five-year survival rates for TNBC are typically lower than other breast cancers. A study conducted in 2007 with more than 50,000 women in all stages of breast cancer showed that 77% of women with TNBC survived at least five years compared to the 93% of women that survived with other types of breast cancer. Also, TNBC cells typically had a higher grade than other types of breast cancer. On a scale of one to three, TNBC is typically a grade three. A higher grade means there are less cancer cells that resemble normal, healthy breast cells in their appearance and growth patterns (Boyle).

A typical mutation that can cause Triple Negative Breast Cancer is the mutation in the tumor suppressor gene BRCA1. A normal BRCA1 gene would prevent the development of cancers. The BRCA1 gene plays a role in repairing damaged double-stranded DNA and controlling cell proliferation and cell differentiation. A woman with a mutation at the BRCA1 gene has an increased risk of developing breast cancer. However, a mutation in the BRCA1 gene does not mean TNBC will develop; it will just increase the risk. Furthermore, not all women who develop TNBC will have this specific

mutation; there are other unknown factors that are contributors to developing TNBC as well (Lindeman).

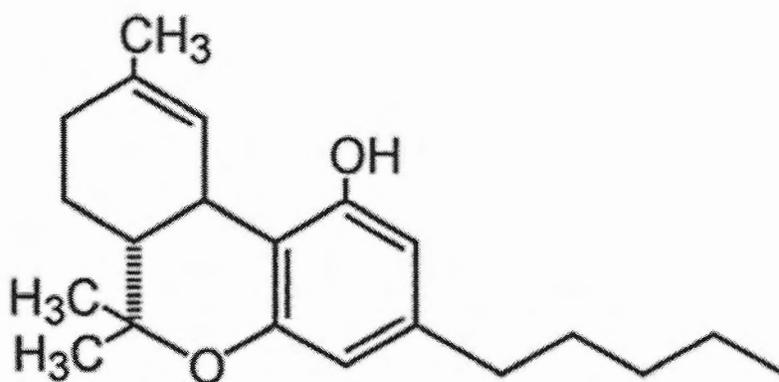
In order to raise the five-year survival rate, researchers have been trying to find targeted therapies for TNBC. Three potential treatments are currently being developed. A Poly-ADP-ribose polymerase (PARP) inhibitor is being developed to prevent the enzyme PARP from repairing the DNA of breast cancer cells. PARP1 acts on single stranded DNA to repair it when breaks occur. If PARP1 is inhibited, there should be an increase in breaks in the DNA. BRCA1 is required to repair the DNA. However, the cells with dysfunctional BRCA1 will not be able to repair the DNA. Therefore, by inhibiting PARP1, the cells with BRCA1 dysfunction should become unstable and undergo apoptosis, leaving behind the normal cells (McCarthy). Potentially, this could make chemotherapy more effective at destroying the cancerous cells. Anti-angiogenic agents are being developed to inhibit angiogenesis. Angiogenesis is the formation of new blood vessels that must occur in tumors for them to receive oxygen and nutrients needed to grow and spread. Drugs such as bevacizumab (Avastin®) and sunitinib (Sutent®) are being developed to inhibit angiogenesis and cell growth. Furthermore, epidermal growth factor receptor (EGFR) targeted therapies are being developed to prevent the overexpression of the protein EGFR. An abundance of EGFR will encourage the growth of the cancer. If a treatment can target EGFR, potentially the cell will be inhibited from growing (McCarthy).

The cells used for the following experiment come from a Caucasian woman in her early 50s. The TNBC cells, referred to as MDA-MB-231 for the experiment, are taken from the patient's mammary gland/breast. The cells used were taken from an aneuploidy

female, which means she contains an abnormal number of chromosomes. The normal chromosomes N8 and N15 are absent. Furthermore, the epidermal growth factor (EGF) and the transforming growth factor alpha (TGF alpha) receptors are expressed in the cells (*MDA-MB-231 ATCC*).

### *Cannabinoids*

Cannabinoids are naturally occurring compounds found in the plant *Cannabis sativa*. Cannabinoids exert their effects by interacting with the cannabinoid receptors, CB1 and CB2, located on the surface of the cells. CB1 receptors are located in the central nervous system (Howard). CB2 receptors are typically found in the immune system. Cannabinoids in the brain typically affect the limbic system, which alters memory, cognition, and psychomotor performance. Cannabinoids will also affect the mesolimbic pathway, which affects the reward and pleasure responses and will also alter pain perception (Howard). Since CB1 receptors are mainly located in the central nervous system, stimulation of these receptors produces a marijuana-like effect on the psyche and circulation, while no such effect is seen by activated CB2 receptors (Howard). There are many different subclasses of cannabinoids, the most well known being delta-9-tetrahydrocannabinol ( $\Delta^9$ -THC or THC) (Fig. 2). THC is the main psychoactive ingredient in cannabis or marijuana. A higher concentration of THC is derived from the leaves and the flowering parts of the cannabis plant (Adams).



### **Δ-9-tetrahydrocannabinol (THC)**

Figure 2: Molecular structure of THC.

Cannabinoids are believed to have medicinal benefits in the treatment of cancer. Cannabinoids have potential to cause antitumor effects by various mechanisms including: induction of cell death, inhibition of cell growth, and inhibition of tumor angiogenesis invasion and metastasis (Vaccani). The downside to using cannabis to help with the cancer is that it causes the patient to be in a daze for an extended period of time. Three alternative drugs that were used in this experiment were ajulemic acid, hemp oil, and cannabidiol. These three cannabinoids are structurally similar to THC. All three of these drugs have little to no THC present, thus there is no psychoactive effect.

#### *Ajulemic Acid*

Ajulemic acid (AJA) is a synthetic cannabinoid that is structurally similar to THC (Fig. 3), but will not have the same psychoactive effects. They both act as analgesics and anti-inflammatory agents (Recht, Salmonsens, et al.). Ajulemic acid also has potential to be used as an antitumor agent. While CB1 and CB2 receptor antagonists blocked the effects of THC, only CB2 receptor antagonists blocked the effects of AJA. This means

that AJA produces antitumor activity by acting at least partially through the CB2 receptors (Recht, Salmonsén, et al.).

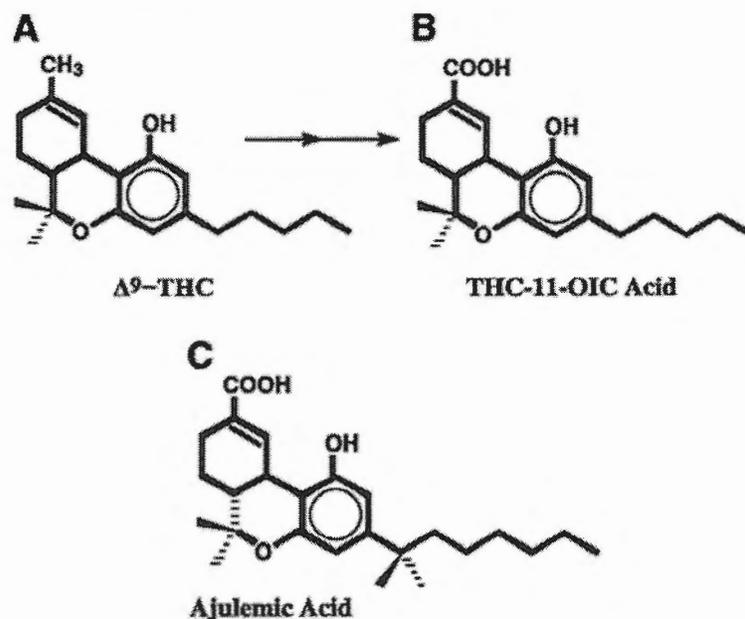


Figure 3: A) THC is responsible for the psychoactive effects of cannabis. B) THC-11-oic acid is the oxidized form of THC. C) Ajulemic Acid is a nonpsychoactive synthetic derivative of THC-11-oic acid.

### *Hemp Oil*

Hemp oil has been extracted from the stalk and stems of the cannabis plant and contains many different cannabinoid compounds. At these two areas of the plant, the THC concentration is less than 0.6%. Hemp oil also contains natural oils, as in flavonoids and terpenoids. Due to the extremely low concentration of THC, hemp oil does not have any psychoactive effects but should produce the same medicinal benefits as THC and AJA.

### *Cannabidiol*

Cannabidiol (CBD) is present in the cannabis plant but has been purified (Fig. 4). CBD is considered to be the most abundant cannabinoid. CBD is similar to AJA and hemp oil in that it maintains the medicinal benefits while producing no psychoactive effects. People exhibiting a wide variety of conditions have used CBD; these conditions

include arthritis, epilepsy, MS, chronic pain, schizophrenia, and diabetes. CBD has shown sedative, anti-epileptic, anti-dystonic, anti-emetic, and anti-inflammatory effects on people with the previous conditions (Grotenhermen).

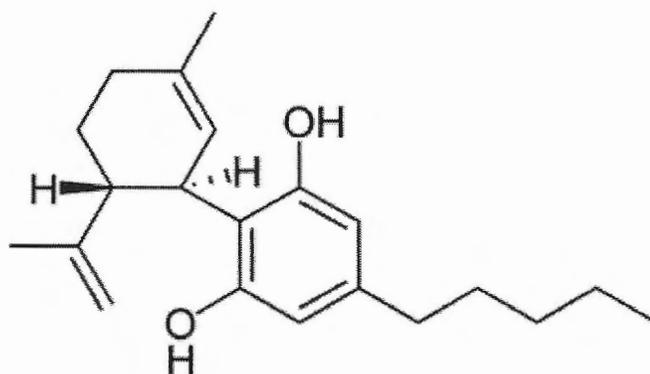
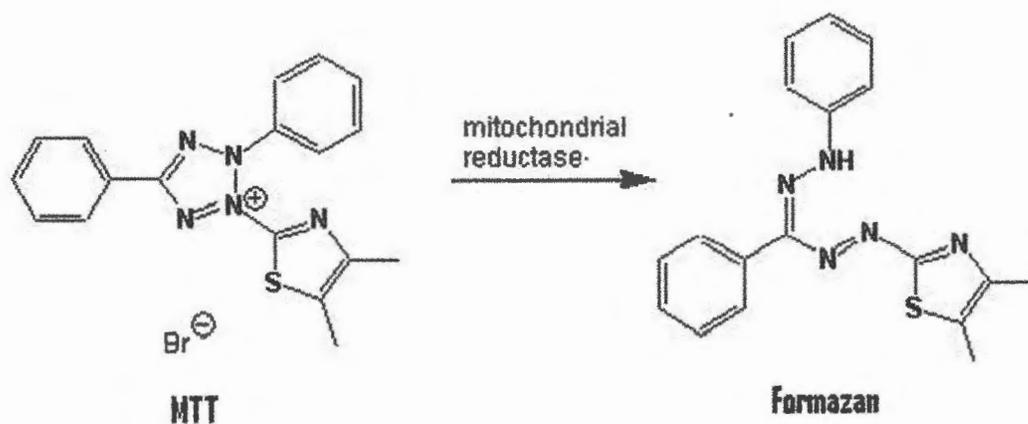


Figure 4: Structure of Cannabidiol.

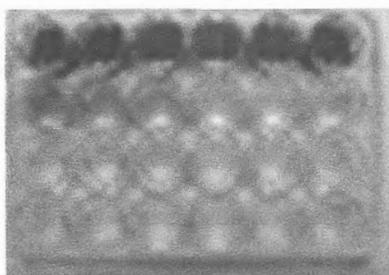
#### *MTT Cell Proliferation Assay*

The MTT Assay was used to measure cell viability in cannabinoid treated and untreated cells. MTT, more specifically yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide), is a chemical that can be cleaved by an enzymatic reaction that occurs in actively respiring mitochondria. A cell that is alive will have respiring mitochondria that have active dehydrogenase enzymes. These enzymes will cleave MTT and generate NADH or NADPH (American Type Culture Collection). The products of this reaction will produce a purple precipitate (Fig. 5).



**Figure 5: MTT reduction in living cells forms formazan, which creates a purple precipitate.**

The purple precipitate can be quantified by measuring the absorbance. If there are more cells alive, there is a darker purple color throughout the liquid in the well. A darker purple color will give a higher absorbance (about 550 nm), which indicates more viable or live cells. An absorbance value that is lower than the control cells will typically be a clear color indicating a reduction in the number of live cells (Fig. 6).



**Figure 6: An example of an MTT Assay. The top row, which is more purple shows, shows that more cells are still respiring. The bottom row, which is a clear color, shows that most of the cells are dead.**

### *LD-50*

A LD-50 is the median lethal dose that is required to kill half the cells of the tested population. The LD-50 is useful to measure the short-term toxicity potential of a material. LD-50 is typically used in the pharmaceutical field to describe the dosage of

drug that may be given before becoming toxic. Generally, the smaller the LD-50 value, the more toxic the chemical is. The higher the LD-50 value, the lower the toxicity (Zbinden).

## **Materials**

For the three experiments performed, all of them used similar methods with a few differing variables. All three involved plating cells and performing the MTT assay on each plate. The main differences amongst the experiments were the concentrations of AJA, CBD, and hemp oil used and the numbers of cells used.

### *Plating Cells*

This method requires a 30 mL flask of the MDA-MB-231 cells that have been grown at least 24-48 hours beforehand. A sufficient amount of grown, living cells is necessary for this experiment to perform properly. Also needed for this method is roughly 30 mL of the cell's medium, 10 mL of PBS (1X), and 3 mL of trypsin. Also required is a vacuum pressure pump to vacuum off the medium, an incubator at 37°C, a 48-well plate, and an automatic pipet aid device that dispenses the liquid into the wells. In addition to these large machines, a large amount of glass Pasteur pipettes and disposable 5, 10, and 25 mL glass pipettes are required. Finally, a hemacytometer is required to count the average number of cells present in the flask.

### *MTT Assay*

This method will use the cells that were previously plated and subsequently treated with the different concentrations of AJA, CBD, and hemp oil. It requires about 25 mL of the MTT reagent that has been added to the medium. The MTT assay also requires about 25 mL of dimethyl sulfoxide (DMSO). This method will also need a vacuum

pressure pump, a automatic pipet aid device, the Belly Dancer shaker, and the Tecan infinite M200 plate reader. In addition, a large amount of glass Pasteur pipettes and disposable 5 and 10 mL glass pipettes are required.

#### *Treating with Higher Concentrations*

This experiment requires a 48-well plate and cells at a concentration of  $3 \times 10^4$  cells/well. ajulemic acid, cannabidiol, and hemp oil will each have concentrations at 20, 40, and 60  $\mu\text{M}$ . Also required for the experiment is DMSO and ethanol (EtOH) that is concentrated at 60 equivalents (eq). This experiment will also use the materials required for plating cells and the MTT assay.

#### *Treating with Lower Concentrations*

The experimental setup for this experiment is almost exactly the same as the above experiment, except the concentrations are decreased. The concentrations for AJA are set at 5, 10, 15, and 20  $\mu\text{M}$ . The concentrations for both CBD and hemp oil are set at 5, 10, and 15  $\mu\text{M}$ . Furthermore, DMSO and ethanol are present at a concentration of 15 eq. This experiment will also use the materials required for plating cells and the MTT assay.

### **Methods**

#### *Plating Cells*

Before beginning the experiment, the trypsin and medium must be pre-warmed for at least fifteen minutes. Using the 30 mL flask of MDA-MB-231 cells that were grown in the 37°C incubator for a period of time, vacuum off the media in the flask. Make sure not to disturb the cells that are stuck to the back of the flask by positioning the glass micropipette towards the front corner of the flask. After vacuuming off the media,

add 10 mL of PBS (1X) to the front of the flask. Rinse the flask gently with the PBS by lightly tilting the flask on its back and swishing the liquid around. Then vacuum off the PBS while making sure not to disturb the cells on the back of the flask. Following this step, add 3 mL of the warmed trypsin to the flask. Place the flask with the trypsin into the incubator at 37°C for a few minutes. The purpose of the trypsin is to loosen the cells from the back of the flask, thus it is no longer necessary to worry about knocking the cells loose. After incubating the cells, observe the cells in the flask under the microscope to ensure the cells are no longer attached to the back of the flask. If there are cells not moving around in the flask, then hit the flask slightly to knock the cells loose. Once all the cells have been knocked loose, add 27 mL of the warmed medium to bring the total volume in the flask back to 30 mL. Using a 25 mL pipette, mix the solution in the flask multiple times. This is to ensure the cells are broken apart from each other (so that the cells are not in large clumps). Place a small amount of this mixture into a vial (~ 20  $\mu$ L). Place 10  $\mu$ L of the mixture on a hemacytometer. The hemacytometer will count the average number of cells/mL in the flask. Place the hemacytometer underneath the microscope and count the number of cells present in the square grids (there are eight square grids). The eight numbers are then averaged and then multiplied by 10,000 ( $X \times 10^4$  cells/mL). In order to figure out how many milliliters of cells are needed, divide the total number of cells needed by the amount of cells/mL previously calculated. The total number of cells needed is  $150 \times 10^4$ . To help determine the total number of cells needed, there are 48 wells that are filled with 1 mL/well of the concentrated cells. To ensure there is enough of the solution, multiply  $3 \times 10^4$  by 50 (instead of 48) to obtain the number  $150 \times 10^4$  cells total. To figure out the amount of media that must be added, subtract the

amount of milliliters of cells needed from 50. Put the milliliters of cells needed and the milliliters of media needed into a separate flask. Mix the mixture thoroughly to ensure that the cells are spread throughout. Place 1 mL of this mixture into each well on the plate. Then move the plate back and forth a couple of times to prevent the cells from being more confluent on the edges of the well. Finally, incubate the plate at 37°C for 24 hours to allow the cells to stick to the bottom of the wells. Make sure to replace the amount of cells pulled out of the original flask with new media, so that new cells can be grown. In order to determine the amount of cells and media needed use the following:

1.  $50 \times 3 \times 10^4 = 150 \times 10^4$  cells total
2.  $\frac{150 \times 10^4}{X \times 10^4} = X$  mL of cells
3.  $50 - X$  mL of cells =  $Y$  mL of media

#### *Determining the concentration of the cells*

To determine the concentration of cells needed for the experiment, determine the amount of cells and medium that should be added together to make the correct concentration by using the plating cells protocol. Determining the concentration is a trial and error type of test. Start with the concentrations of the experiment set at  $3 \times 10^4$  cells/well and  $4 \times 10^4$  cells/well. Make a 13 mL solution of the cells + media, so that 1 mL of cells can be put into 12 wells. In order to determine the amount of cells and media needed:

1.  $13 \times 3 \times 10^4 = 39 \times 10^4$  total cells needed
2.  $\frac{39 \times 10^4}{X \times 10^4} = X$  mL of cells
3.  $13 - X$  mL of cells =  $Y$  mL of media
4.  $13 \times 4 \times 10^4 = 52 \times 10^4$  total cells needed

5.  $\frac{52 \times 10^4}{X \times 10^4} = X$  mL of cells

6. 13-  $X$  mL of cells =  $Y$  mL of media

After adding 1 mL of  $3 \times 10^4$  into all 12 wells and 1 mL of  $4 \times 10^4$  into all 12 wells, incubate the plate for 24 hours. After incubating the plate, observe each well underneath the microscope to ensure there is no contamination and equally grown cells. Then vacuum off the media in each well (one row at a time) and add 1 mL of the cannabinoid treatments to the wells. The cells at the concentration of  $4 \times 10^4$  will be treated with 40  $\mu$ M of CBD, 20  $\mu$ M of hemp oil, and 40  $\mu$ M of hemp oil. The cells at the concentration of  $3 \times 10^4$  will be treated with 20, 40, and 60  $\mu$ M of AJA. Each treatment will be placed in three wells. Also, each concentration of cells will have three wells of untreated, plain media. The 48-well plate will be incubated for 48 hours followed by a MTT assay to measure the effects of the different concentrations.

#### *Treating with Higher Concentrations*

The MDA-MB-231 cells with a concentration of  $3 \times 10^4$  are treated with the cannabinoids after the cells have been plated onto a 48-well plate and incubated for 24 hours. Each well will receive 1 mL of the treatment after the solution in the wells has been vacuumed off. It is necessary to observe each well under the microscope to ensure the cells in each well look similar and normal. One treatment will be placed into four different wells, thus at least 4 mL of the treatment will need to be made. The treatments that will be used for this experiment are as follows: untreated, 60 eq of DMSO, 60 eq of ethanol, 20, 40, and 60  $\mu$ M of AJA, CBD, and hemp oil. In order to make AJA, CBD, and hemp oil stock solutions, use the following:

Using a small vial, add 10  $\mu\text{L}$  of 100 mM AJA stock solution to 90  $\mu\text{L}$  of DMSO to make a 10 mM stock solution. Make the hemp oil and CBD stock solution the same way as the AJA stock solution. The only difference is the CBD stock solution will be dissolved in 90  $\mu\text{L}$  of ethanol (instead of DMSO).

In order to make the treatments (using 10 mM stock solutions of AJA, CBD, and hemp oil and making 5 mL of the treatments), use the standard equation,  $M_1V_1 = M_2V_2$ :

$$(20 \mu\text{M of cannabinoid})(5000 \mu\text{L}) = (10,000 \mu\text{M of stock solution})(X \mu\text{L}) = 10 \mu\text{L}$$

$$(40 \mu\text{M of cannabinoid})(5000 \mu\text{L}) = (10,000 \mu\text{M of stock solution})(X \mu\text{L}) = 20 \mu\text{L}$$

$$(60 \mu\text{M of cannabinoid})(5000 \mu\text{L}) = (10,000 \mu\text{M of stock solution})(X \mu\text{L}) = 30 \mu\text{L}$$

Add 5 mL of media to 12 different vials that have been labeled for each treatment.

Then, using a micropipette, take 10, 20, or 30  $\mu\text{L}$  of media out of the 5 mL vials. Then replace the same amount of microliters taken out with each specific cannabinoid. Once each treatment has been properly added to each well, incubate the plate for 48 hours.

After this period of time, an MTT assay is performed and the data is recorded.

#### *Treating with Lower Concentrations*

The method for treating the cells with lower concentration is the same as treating the cell with higher concentration. The only difference is the concentration of cannabinoids used on the cells. The purpose of treating the cells with a lower concentration of cannabinoids is to determine the LD-50. The treatments that will be used for this experiment are as follows: untreated, 15 eq of DMSO, 15 eq of ethanol, 5, 10, and 15  $\mu\text{M}$  of CBD and hemp oil, 5, 10, 15, and 20  $\mu\text{M}$  of AJA. The amount of stock solution added to the 5 mL vials is as follows:

$$(5 \mu\text{M of cannabinoid})(5000 \mu\text{L}) = (10,000 \mu\text{M of stock solution})(X \mu\text{L}) = 2.5 \mu\text{L}$$

$(10 \mu\text{M of cannabinoid})(5000 \mu\text{L}) = (10,000 \mu\text{M of stock solution})(X \mu\text{L}) = 5 \mu\text{L}$

$(15 \mu\text{M of cannabinoid})(5000 \mu\text{L}) = (10,000 \mu\text{M of stock solution})(X \mu\text{L}) = 7.5 \mu\text{L}$

$(20 \mu\text{M of cannabinoid})(5000 \mu\text{L}) = (10,000 \mu\text{M of stock solution})(X \mu\text{L}) = 10 \mu\text{L}$

### *MTT Assay*

Before beginning the procedure, warm the MTT reagent + medium mixture (49mL plain media + 1 mL MTT reagent). This procedure will be performed on the 48-well plate that was treated with the different concentrations of cannabinoids 48 hours earlier. Every well should be observed underneath a microscope. This is to observe the cells to make sure there is no contamination and they look normal. Using the vacuum pressure pump and a glass micropipette, vacuum off the media in each well. Make sure not to touch the micropipette to the bottom of the well, otherwise it could mess up the results. After vacuuming off one row on the plate, add 0.5 mL of the MTT reagent + medium to each well. Vacuum the wells one row at a time to prevent overexposure of the cells to the environment. After all the wells are filled with 0.5 mL of the MTT reagent + medium, incubate the plate at 37°C or one to two hours. After the plate has been properly incubated, vacuum off the media in the wells (one row at a time) and add 0.5 mL of DMSO to each well. Once all the wells are filled, place the 48-well plate on The Belly Dancer shaker for 10-20 minutes. Then place the plate into a Tecan Infinite M200 plate reader. The wavelength in each well will be measured and these results will be graphed.

### **Results**

#### *Higher Concentration*

After performing three separate trials on the MDA-MB-231 cells, it was concluded that the higher concentrations of cannabinoids could successfully decrease the

amount of cancerous cells that are alive. As can be deduced, all three of the cannabinoids at concentrations of 40 and 60  $\mu\text{M}$  have essentially killed all of the cells present in the wells (Fig. 7). Ajulemic acid at a concentration of 20  $\mu\text{M}$  appears to have the highest percentage of cell viability amongst the treatments. The percentage, being at about 40%, is still very low and is too high a concentration for us to be able to calculate the LD-50. The CBD and hemp oil treatments at a concentration of 20  $\mu\text{M}$  also have very low yields of percent cell viability (less than 20%). As expected, the untreated cells and the cells treated with 60 equivalents of either DMSO or ethanol have little to no effect on the cell viability. The results of the experimentation with higher concentration of cannabinoids showed that AJA, CBD, and hemp oil could effectively kill the MDA-MB-231 cells. However, due to the low yield of percent cell viability, this is not an effective model to determine a LD-50 value. To determine this, we would need to lower the concentrations.

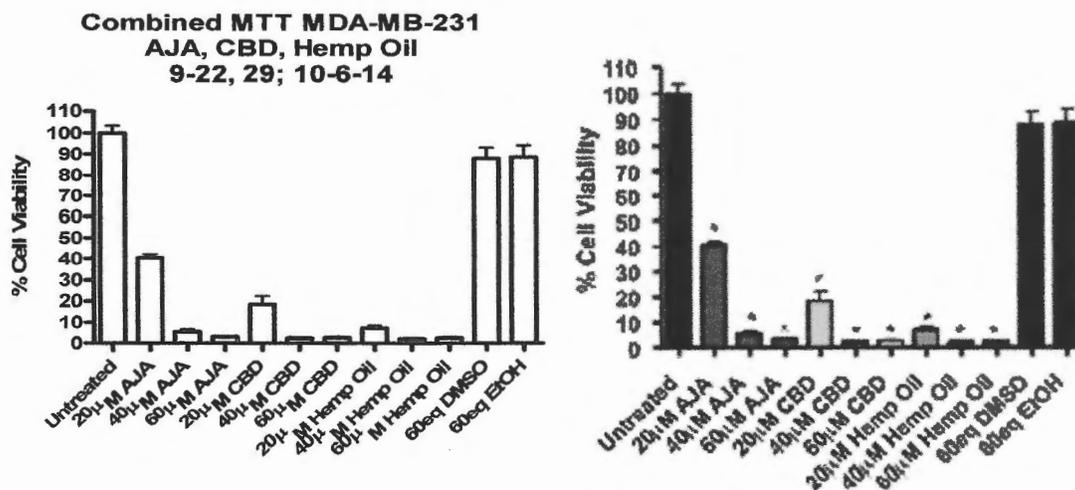


Figure 7: Results of the MTT assay using the higher concentrations of cannabinoids.

### Lower Concentrations

The concentrations were lowered in order to determine the LD-50 for AJA, CBD, and hemp oil. A linear trend is observed when the concentration of each treatment is

increased (Fig. 8). As the concentration of each treatment was increased, the percentage of cell viability decreased. Furthermore, the untreated MDA-MB-231 cells and the cells treated with 15 equivalents of DMSO have a very high percent cell viability, which is expected. An unexpected result that is observed is the consistent low percentage of cell viability in the cells treated with 15 equivalents of ethanol. The most plausible reason for this occurrence is because the ethanol seemed to be degraded. We concluded that the reason the ethanol appeared to be killing the cells was because it had degraded. Another unexpected result is that the cells treated with 5  $\mu$ M of AJA experienced a percent cell viability that is higher than the percent cell viability for the untreated cells. The cause of this is unknown but we do not believe that a very low concentration of AJA could actually increase cell viability.

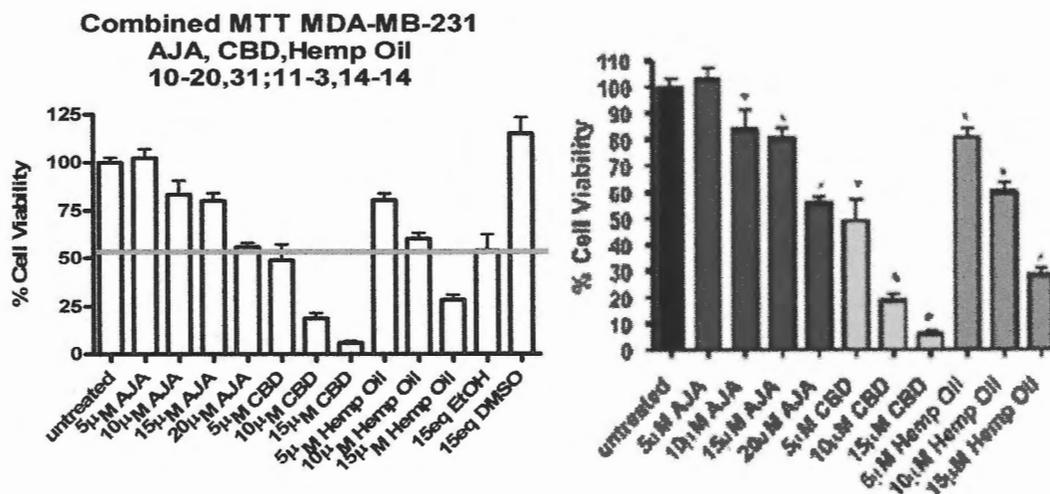


Figure 8: A) Results of the MTT assay using the lower concentrations of cannabinoids. The line is representative of where the LD-50 concentrations will be taken from. B) Color coated version of A.

#### LD-50

Using the data from figure 8, the LD-50 was determined. The data used to make the graph in figure 8 was inserted into an excel spreadsheet, the different treatments were averaged together, and made into a XY scatter plot. By using the trend line function, a

line will be drawn through the data points producing an equation. By setting  $x=50$ , the LD-50 is determined by solving for  $y$ . A rough estimate of the LD-50 can also be determined by drawing a line through the graph at 50% cell viability (Fig. 8A). This will not give an exact number, but is useful in the absence of excel. Based on the results, the LD-50 in MDA-MB-231 cells for ajulemic acid is 27.9  $\mu\text{M}$ , cannabidiol is 4.3  $\mu\text{M}$ , and hemp oil is 9.9  $\mu\text{M}$ .

## **Discussion and Conclusion**

### *Purpose of DMSO and Ethanol*

As discussed earlier, AJA and hemp oil are dissolved in DMSO to make the stock solutions. CBD is dissolved in ethanol to make stock solution. Due to the presence of DMSO and ethanol in each of the treatments, a negative control group is necessary. The negative control group is to ensure that the DMSO or ethanol present in the original stock solutions did not affect the viability of MDA-MB-231 cells. We can determine the DMSO and ethanol had no effect on the cells by observing the graphs in Figure 6. The percent cell viability for 60 eq of DMSO and ethanol is at or very near 100%. This shows that DMSO and ethanol were not the cause in the decrease of cell viability. It was indeed the work of the AJA, CBD, or hemp oil that caused the decrease in cell viability.

### *Determination from Concentrations*

Based on the results from the different concentrations of cannabinoids, ajulemic acid, cannabidiol, and hemp oil could be used to effectively kill the MDA-MB-231 cells characteristic of Triple Negative Breast cancer. The higher concentrations (20, 40, and 60  $\mu\text{M}$ ) showed that AJA, CBD, and hemp oil could definitely kill the cells. The lower concentrations (5, 10, 15, and 20  $\mu\text{M}$ ) provided the information needed to determine the

concentration of AJA, CBD, and hemp oil that could be given to the MDA-MB-231 cells in order to destroy half of the cells present. The two experiments in conjunction with one another are able to provide a model to determine the effectiveness of the cannabinoids and the required concentration of the cannabinoids.

*Are Cannabinoids an effective treatment for Triple Negative Breast Cancer*

Although this experiment was performed on a small scale, there is potential it could also be effective on a larger scale. On a small scale, the different cannabinoids are effective in treating Triple Negative Breast cancer cells. There are still many different experiments that would need to be performed in order to determine the effectiveness of the cannabinoids as an alternative treatment to chemotherapy and surgery. Before becoming a viable treatment for Triple Negative Breast cancer, there would need to be trials that observe the effects in model organism. There would also need to be a better understanding of the pathway used by the treatments to assert its effects on the cells. Potentially, though, AJA, CBD, and hemp oil could be used as alternative and less toxic treatments for Triple Negative Breast cancer.

## Bibliography

- Adams IB, Martin BR: Cannabis: pharmacology and toxicology in animals and humans. *Addiction* 91 (11): 1585-614, 1996. Web.
- Amirikia KC, Mills P, Bush J, Newman LA. Higher population-based incidence rates of triple-negative breast cancer among young African-American women: Implications for breast cancer screening recommendations. *Cancer*. 117(12):2747-53, 2011.
- Boyle, P. Triple-negative breast cancer: Epidemiological considerations and recommendations. *Annals of Oncology*, 23(6), Vi7-Vi12, 2012.
- Crown, J., O'shaughnessy, J., & Gullo, G. Emerging targeted therapies in triple-negative breast cancer. *Annals of Oncology*, 23(6), Vi56-Vi65, 2012.
- Grotenhermen F, Russo E, eds.: Cannabis and Cannabinoids: Pharmacology, Toxicology, and Therapeutic Potential. Binghamton, NY: The Haworth Press, 2002.
- Howard P, Twycross R, Shuster J, et al.: Cannabinoids. *J Pain Symptom Manage* 46 (1): 142-9, 2013.
- Liedtke C, Mazouni C, Hess KR, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. *J Clinical Oncology*, 26:1275-1281, 2008.
- Lindeman, G., & Visvader, J. Cell fate takes a slug in BRCA1-associated breast cancer. *Breast Cancer Research*, 13(2), 306-306, 2011.
- McCarthy, N., Mitchell, G., Bilous, M., Wilcken, N. and Lindeman, G. J. Triple-negative breast cancer: making the most of a misnomer. *Asia-Pacific Journal of Clinical Oncology*, 8: 145–155. doi: 10.1111/j.1743-7563.2012.01533, 2012.
- "MDA-MB-231 (ATCC® HTB-26™)." *MDA-MB-231 ATCC® HTB-26™ Homo Sapiens Mammary Gland/breast*. ATCC, 1 Jan. 2014. Web. 18 Feb. 2015. <<http://www.atcc.org/products/all/HTB-26.aspx#characteristics>>.
- "MTT Cell Proliferation Assay Instruction Guide." *ATCC*. American Type Culture Collection, 1 Jan. 2011. Web. 19 Feb. 2015. <<http://www.atcc.org/~media/DA5285A1F52C414E864C966FD78C9A79.ashx>>
- Recht L.D., Salmons R., Rosetti R., Jang T., Pipia G., Kubiowski T., Karim P., Ross A.H., Zurier R., Litofsky N.S., Burstein S. Antitumour effects of ajulemic acid (CT3), a synthetic non-psychoactive cannabinoid. *Biochemical Pharmacology*. (2001); 62: 755–763. 18 Dec. 2000. Print.

Vaccani A, Massi P, Colombo A, et al.: Cannabidiol inhibits human glioma cell migration through a cannabinoid receptor-independent mechanism. *Br J Pharmacology* 144 (8): 1032-6, 2005.

Zbinden, G. & Flury-Roversi, M. Significance of the LD50-test for the toxicological evaluation of chemical substances. *Archives of Toxicology*, 47(2), 77-99, 1981.