Assessing Intervals of Epigallocatechin Gallate (EGCG) and Cannabidiol (CBD) on *Saccharomyces cerevisiae* Cell Reproduction as a Possible Application on Cancer Cell Proliferation

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Cancer is one of the most unknown and prevalent diseases of today. There are methods to cure the disease, such as chemotherapy, however, these treatments cause a lot of pain and discomfort to patients. The purpose of this study was to find an alternative and more natural treatment to decrease the rate of cell reproduction of a cancer cell model using epigallocatechin gallate (EGCG) or cannabidiol (CBD). It was hypothesized that if Saccharomyces cerevisiae were exposed to increased concentrations of epigallocatechin gallate or cannabidiol, then the rate of cell production after they were exposed would decrease. Yeast was placed in a spectrophotometer to measure initial cell density. For CBD, the yeast was transferred to the water-soluble CBD solution along with maltose needed for the fermentation process. The test tube was covered to force anaerobic respiration. For EGCG, the respective amounts of capsules were cut and the contents were poured into the test tubes and mixed in with the yeast and medium. After 24 hours, final cell densities were measured. The average difference between the initial and final absorbances for EGCG and CBD decreased as the concentrations increased. Two one-way ANOVAs were run at an $\alpha = 0.05$, the difference between all three concentration groups for CBD had a p-value <0.0001, rejecting the null hypothesis. The EGCG groups rejected the null hypothesis with a p-value of ~0.003. In conclusion, the hypothesis was supported and as levels of CBD and EGCG increased, the rate of yeast reproduction decreased.

Introduction

Cancer is one of the most unknown and prevalent diseases of today. Many scientists have tried methods of curing the disease, such as chemotherapy, however, these treatments can cause a lot of pain and discomfort to the patient. It also can cause many side effects such as a weakened immune system, fatigue, hair loss, easy bruising and bleeding, and many others[2]. As an alternative to that, many people have been gravitating towards more natural methods of curing diseases. Natural, over the counter supplements have been something that some people have been gravitating towards recently. Over the counter, non-FDA regulated drugs are commonly used by people because of the claims they make about benefiting overall health.

Over the past two years, local stores have been leaning towards selling products containing CBD and marketing them to say they have health benefits. Products include everything from CBD-infused lip balm to CBD lotions and foot scrubs. Cannabidiol is a phytocannabinoid that comes from the plant *Cannabis sativa* that has pain-relieving, anti-inflammatory, and chemopreventive activities. CBD has been shown to be very effective in various EMV-linked pathologies[4]. EMVs, exosomes and microvesicles, are lipid bilayer-enclosed structures that are released by the cell and involved in intercellular communication through the transfer of proteins and genetic material. They also help carry molecules that are characteristic of their parental cells to their recipient cells[4]. This allows for intercellular communication and it also affects many different physiological and pathological processes including cell differentiation, angiogenesis (development of new blood vessels), and cell migration. Very recent studies on pharmacological inhibition of EMV release shows that EMV that sheds off of cancer cells allows it to reject drugs better and it contributes to their resistance against chemotherapeutic agents[4].

In green tea, there is a compound called epigallocatechin-gallate or EGCG that is very abundant. EGCG is said to have anti-cancer properties, however, this is only now beginning to be understood on a more cellular level. Cancer biology is raising more and more attention to EGCG because of its pro-oxidative, pro-apoptotic, and anti-proliferative properties[6]. Three of the main protein receptors that are targeted with the EGCG are 67LR, Pin1, and TGFR-II[6]. Further research will show how the interaction of EGCG with protein targets in cancer cells will allow for the development of many new pharmacological treatments targeting EGCG-activated master regulators of vital pathways in cancer.

Cancer is the uncontrolled growth of abnormal cells in the body. Cancer begins to develop when the body's normal control mechanisms in the cell cycle stops working. These cancerous cells do not die and instead they begin to grow out of control and form new abnormal cells. The mass of extra abnormal cells form a tumor. *Saccharomyces cerevisiae*, or common baker's yeast, is a suitable model organism because it has a similar cell cycle as cancer cells and reproduces very fast. Yeast uses fermentation as the main metabolic pathway and then switches to oxidative metabolism only when the carbon source is limited. It, like any mammalian cell, undergoes a process called apoptosis which is the death of cells at a normal rate. This means that the cell also shares similar properties as a mammalian cell including oxidative stress and a major role played by mitochondria[3]. Essentially, the main characteristics of a cancer cell are an evasion of apoptosis and sustained proliferation (rapid growth). This would cause the genetically mutated cells to proliferate at a rapid pace, which is what cancer is defined as.

Due to the lack of natural treatments for cancer spreading, many patients have to undergo very drastic procedures even for smaller tumors. This study shows a possible alternative or assisting factor to a treatment already given to the patient. The primary purpose of this study was to find an alternative and more natural treatment to slow down cell reproduction of a cancer cell using EGCG or CBD. It was hypothesized that if *Saccharomyces cerevisiae* are exposed to epigallocatechin gallate (EGCG) or cannabidiol (CBD), then as the concentration of the supplement increases, the rate of regeneration after they are exposed would decrease.

To conduct this experiment, a pure strain of *Saccharomyces cerevisiae* was taken and fed a mixture of sugar and either epigallocatechin gallate (EGCG) or cannabidiol (CBD). These were both tested in different intervals of concentrations using a spectrophotometer to find if different concentrations of EGCG or CBD had either a negative or positive effect on the decline of cell growth.

Methods

The first step was to collect all materials from Carolina Biological and Kazmira needed for this study. Water soluble CBD was obtained from Kazmira after speaking to Dr. Sharma about this study. Then, the surface where the study was conducted, as well as test tubes and stirring rods, were sterilized using bleach and a flame. Next, a micropipette was used to suction approximately 50 µL of *Saccharomyces cerevisiae* in their respective assignment test tube. Each test tube rack was labelled either EGCG or cannabidiol. Then thirty six clear cuvettes for the first trials of EGCG or CBD were placed on the lab bench. Using the micropipette, 50 µL of stirred yeast solution, was placed into the 36 clear cuvettes. Each cuvette was then placed in the SpectroVis analysis machine in order to test initial cell density. The initial cell density for each cuvette was recorded. This is due to the fact that each initial cell density of *Saccharomyces cerevisiae* may be different. The yeast mixture in each cuvette was then transferred back into its respective test tube. Then, the CBD or EGCG was exposed to the yeast cells. In the case of the CBD, the yeast was transferred to the water soluble CBD solution along with maltose needed for the fermentation process. The test tube was then covered in order to force anaerobic respiration. Additionally, in the case of EGCG, the respective amounts of capsules were cut and the contents were poured into the test tubes and mixed in with the yeast. The water will then be heated to 95°F using a hot water bath and ice to maintain the temperature. After 20 minutes, each trial, starting with the test tube that was first exposed to either the CBD or EGCG, will be mixed together and transferred into the clear cuvettes to get another reading of cell density.

Experimental Design Matrix

Title of the Experiment

Assessing intervals of epigallocatechin gallate (EGCG) and cannabidiol (CBD) on *Saccharomyces cerevisiae* cell reproduction as a possible application on cancer cell spreading

Hypothesis
If *Saccharomyces cerevisiae* are exposed to epigallocatechin gallate or cannabidiol, then the rate of cell production after they are exposed will decrease.

Independent Variable
The levels of EGCG and cannabidiol

	EGCG Supplement			Cannabidiol Supplement	
Levels of Independent Variable	Low: 1.4 mg/mL	Medium: 2.1 mg/mL	High: 4.2 mg/mL	Low: 3.6 mg/mL	Medium: 7.2 mg/mL
Number of Repeated Trials	30 trials	30 trials	30 trials	30 trials	30 trials

Dependent Variable
The absorbance at 430 nm of the *Saccharomyces cerevisiae* after being exposed to the supplement. (cell density)

Constants
- Location of Test
- Temperature
- Sugar solution
- Amount of *Saccharomyces cerevisiae* per trial
- Type of CBD and EGCG used

Control
The group of *Saccharomyces cerevisiae* with no supplements given.

Results

After the experimentation process, data was collected to test the original hypothesis. It was then categorized into different graphs and tables. The raw data table was used to collect and organize the data of the initial versus final absorbances of yeast can be found in Appendix A. After the data was collected and organized, two one-way ANOVAs (one for CBD and one for EGCG) were run to compare the mean differences between the groups (low, medium, and high). Both the CBD and EGCG had an f-value that was greater than the f critical value. In addition to that, p-value for CBD was <0.00001 and the p-value for EGCG was 0.003. Two one-way ANOVAs were run between subjects to compare the effect of concentration of CBD and EGCG on the absorbance of yeast in standard conditions.

Since they were both less than the α value of 0.05 (at a confidence interval of 95%), it can be supported that the null hypothesis was rejected. In other words, the increase in concentration of CBD and/or EGCG caused the absorbance (cell density) of the yeast to decrease. This means that there was statistical importance between the changing concentrations of CBD and EGCG and the growth of the yeast.

Table 1. Averages differences between initial and final absorbances of CBD. This table shows the averages of the differences between the initial and final absorbances of yeast at a wavelength of 430 nm for the control, low, medium, and high treatments.

	Averages
Control	0.0183
Low Concentration	0.00667
Medium Concentration	0.0141
High Concentration	0.0028

Table 2. One-Way ANOVA: Effect of CBD on yeast growth. Since α=0.05 and the difference between all three concentration groups for CBD had a p-value<0.0001, the null hypothesis is rejected. It was run to compare the mean differences between groups that have been split into three concentrations: low, medium,

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.0044334	3	0.0014778	10.790618	<0.00001	2.6828095
Within Groups	0.0158865	116	0.0001370			
Total	0.02031989	119				

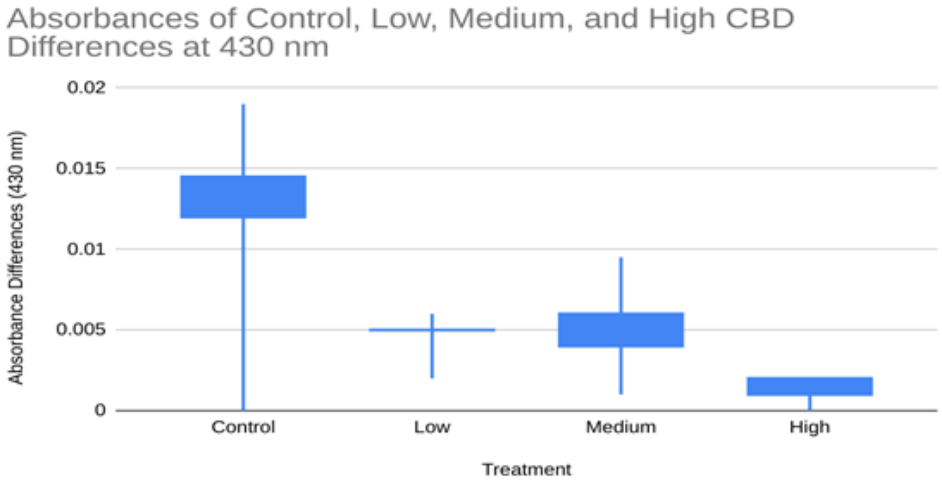

Figure 1. Control, Low, Medium, and High CBD Initial vs Final Absorbances. This graph shows the absorbances of yeast at a wavelength of 430 nm for all concentrations of CBD.

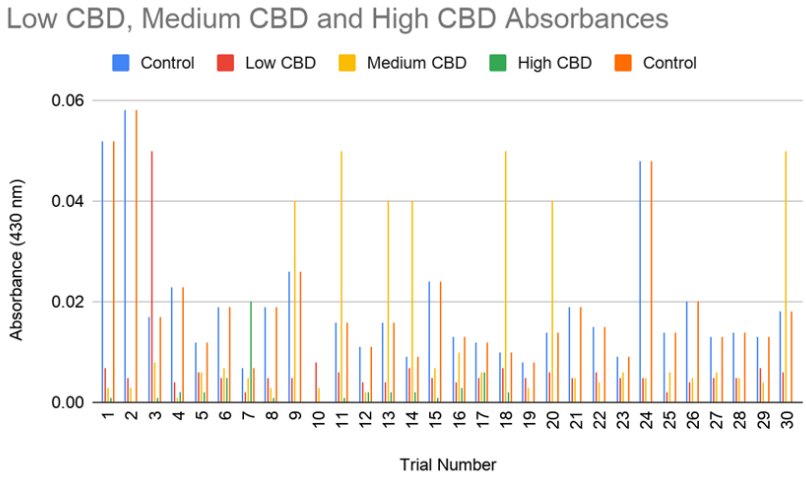

Figure 2. Comparison between control, low, medium, and high concentrations of CBD absorbances. This graph shows the difference in the initial and final absorbances (at 430 nm) of the control (no treatment), low, medium, and high concentrations of CBD medium in which the yeast was grown.

Table 3. Averages differences between initial and final absorbances of EGCG. This table shows the averages of the differences between the initial and final absorbances of yeast at a wavelength of 430 nm for the control, low, medium,

	Averages
Control	0.0183
Low Concentration	0.081267
Medium Concentration	0.017533
High Concentration	0.0062

Table 4. One-Way ANOVA: Effect of EGCG on Yeast growth. Since □=0.05 and the difference between all three concentration groups for CBD had a p-value<0.0001, the null hypothesis is rejected. It was run to compare the mean differences between groups that have been split into three concentrations: low, medium, and high.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.1045289	3	0.0348430	4.7105037	0.0038620	2.6828094
Within Groups	0.8580364	116	0.0073969			
Total	0.9625653	119				

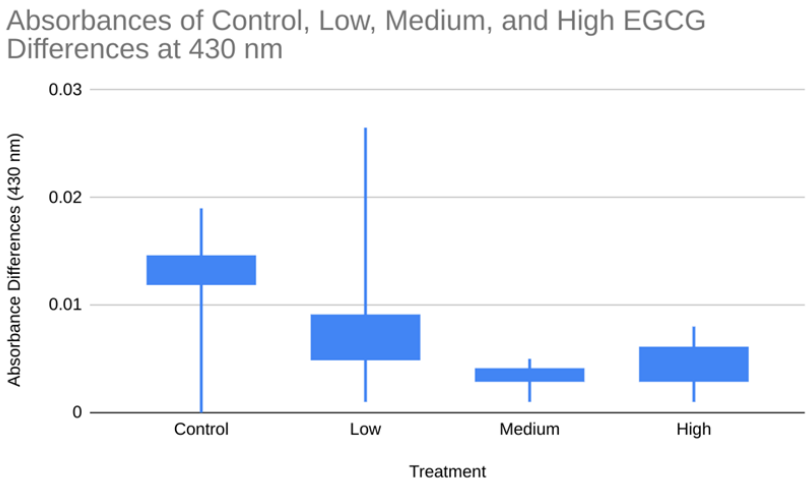

Figure 3. Control, Low, Medium, and High EGCG Initial vs Final Absorbances. This graph shows the absorbances of yeast at a wavelength of 430 nm for all concentrations of CBD.

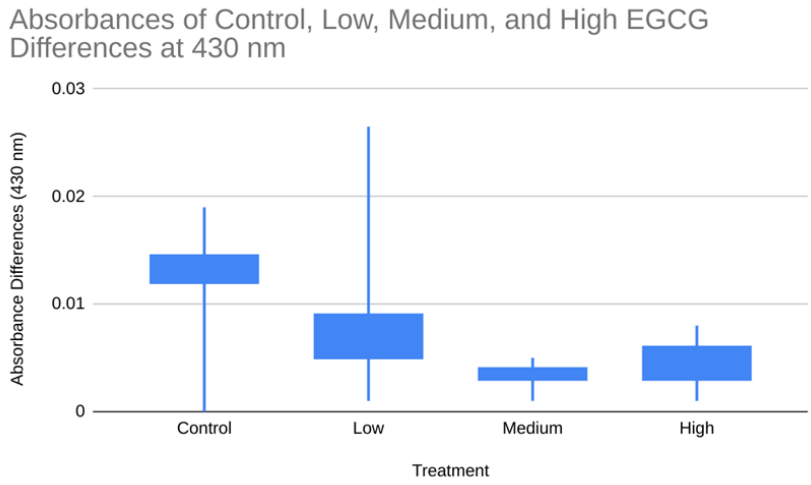

Figure 4. Comparison between control, low, medium, and high concentrations of EGCG absorbances. This graph shows the difference in the initial and final absorbances (at 430 nm) of the control (no treatment), low, medium, and high concentrations of EGCG medium in which the yeast was grown.

Discussion

The purpose of this study was to test whether there was a correlation between increased concentrations of CBD and increased concentrations of EGCG on the growth of *Saccharomyces cerevisiae* (yeast). Since yeast is an organism that can be used as a model for human cancer cells, this study could be applied to current cancer treatments as a supplement to help aid the process. It was hypothesized that if *Saccharomyces cerevisiae* were exposed to epigallocatechin gallate (EGCG) or cannabidiol (CBD), then the amount of regeneration after they are exposed would decrease. In this case, the hypothesis was supported because as the concentration of CBD and EGCG began to increase, the rate of cell reproduction of the yeast cells decreased. When compared to the control group (no treatment), both the CBD and EGCG showed significant differences in their means indicating that the CBD and EGCG acting on the yeast caused a decrease in the rate of cell reproduction.

Since there was an initial and final absorbance being observed for each concentration in CBD and EGCG, to find the difference between the two, an equation was applied. Another equation was applied to each of the initial and final groups to compare their differences between each other; because of the structure of the experiment, two one-way ANOVAs were run; one for CBD (Table 1) and one for EGCG (Table 2). From the ANOVAs, many box plots were created to show the comparison between the control, low, medium, and high absorbances. Figure 1 shows a comparison between the control, low, medium, and high growth medium concentrations of CBD for the yeast. It can be seen in this graph that there is a general trend of the control group (no treatment) having the greatest difference in the initial and final concentrations and as the concentration of

CBD and EGCG increases the difference in the initial and final concentrations starts to decrease. This is because the CBD and EGCG are affecting the rate of reproduction of the yeast.

The two one-way ANOVAs that were run showed that both of the p-values were significant, meaning that the hypothesis itself was statistically supported. This also showed that there was enough evidence to support the hypothesis. The null hypothesis, that there was no significant difference between the three trials, was not supported since there was significantly enough evidence to support the claim. Since there was a statistical significance in CBD, with a p-value of <0.00001 at an alpha value of 0.05, it means that the increasing concentration of CBD caused the rate of growth in yeast to decrease. Similarly, because of the statistical significance in EGCG, with a p-value of 0.003, the increasing concentration of EGCG also caused the rate of growth in yeast to decrease.

Guillermo Velasco, Sonia Hernández-Tiedra, David Dávila, & Mar Lorente (2016) conducted a study in which they gathered that cannabinoids show a very remarkable anticancer ability in preclinical models of cancer. This study also discusses, if taken correctly at the right dosages, it could provide maximum coverage for the patient with cancer. Velasco, Hernández-Tiedra, Dávila, & Lorente (2016) conclude that because there is solid scientific evidence supporting the fact that cannabinoids show a very strong anticancer activity in preclinical models of cancer.

Due to sources of error, some data may not have been completely accurate. An example of this may have been when transferring the CBD or EGCG solution from the cuvette after the initial reading in the spectrophotometer to the test tube. When pouring the solution out of the cuvette into the test tube, some of the yeast cells may have remained in the cuvette causing the final absorbance of a certain sample to read lower than it was. Another error may have been the amount of time that each of the samples had to ferment. Each sample was systematically tested for its initial absorbance and then placed into a test tube. After 24 hours had passed, they were each tested for their final concentrations in the same order that they were initially tested, however, due to one sample taking a minute or two longer to test, another sample may have had longer to ferment and therefore a higher growth. Finally, the distribution of cells when the sample was tested could have been low or high in a certain area that the light from the spectrophotometer was shining through. This could also have been affected by how much the cuvette was disrupted when being handled. Each cuvette was shaken before being placed in the spectrophotometer to allow for a generally even distribution of yeast cells, however, it is possible that one cuvette was shaken either too much or too little. This would most likely explain the outliers in the data. An example of this can be seen in Figure 1 during trial 3. This sample shows an extremely high final absorbance compared to every other trial where its initial absorbance looks average.

In the future, an expandable aspect of this research study would be to increase the number of concentration intervals of CBD and EGCG to find out exactly where the amount of yeast begins to completely stop growing. When doing this, isolating the yeast and keeping them exposed to oxygen as little as possible would ensure that the yeast truly kept to its fermentation process to its maximum.

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