Effects of Benadryl on chloroplast bleaching in *Euglena gracilis*

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Abstract

Benadryl was used to temporarily bleach the chloroplast of the *Euglena gracilis* at varying concentrations of low concentration, high concentration, and no Benadryl. Three trials of *Euglena* were left in mediums with different concentrations and incubated for 24 hours. After 24 hours, the cells were fixed and the number of bleached chloroplasts were counted using 40x and 100x microscopy. It was found that a higher concentration of Benadryl will result in a higher number of bleach chloroplasts, while the control group did not show any bleaching. Using ANOVA and Post Hoc Tukey test to analyze the data, the p-value was less than 0.0001 and the results are significant.

Introduction

The genus, *Euglena*, consists of single-celled eukaryotic organisms that are found worldwide in stagnant freshwater areas and moist soil and they are able to feed like a heterotroph and autotroph (Shao et al., 2019). *Euglena* contains fully developed chloroplasts that allow it to be autotrophic under light (Shao et al., 2019). There have been theories that the chloroplast of the *Euglena* was originally an endosymbiotic green algae and is supported by the presence of the third membrane (Gibbs, 2011). In addition, the chloroplast of the *Euglena* has low stability and can be lost under stress (Shao et al., 2019). Antibiotics and other experimental factors can induce loss of the chloroplast genome, resulting in a bleached chloroplast (Gibbs, 2011; Shao et al., 2019). Without the genes needed for photosynthesis, *Euglena* is able to act like a heterotroph by absorbing organic matter from its surroundings (Shao et al., 2019). This is important as *Euglena* contributes to producing oxygen gas and reducing greenhouse gas.

A previous study has been conducted on *Euglena gracilis,* the model species for *Euglena*, and bleaching of its chloroplast with various types of antihistamines and antibiotics (Zahalsky et al., 1962). The purpose of this study is to test the effectiveness of permanent bleachers of both

antihistamines and antibiotics and possible damage to the 8th cranial nerve (Zahalsky et al., 1962). Zahalsky et al. used several types of antihistamines and found that some caused either permanent or temporary bleaching (1962). They also found some antihistamines and antibiotics that did not cause any bleaching, even when tested up to the inhibitory concentration (Zahalsky et al., 1962). This study is crucial for our experiment as it gives evidence that varying concentration of antihistamine will result in bleaching or no bleaching of the chloroplast. However, it does not state the amount of bleaching caused by the varying concentration. Thus, this will be the focus of our paper.

This experiment will be using the model species, *E. gracilis,* which will be immersed in a medium mixed with Benadryl at high and low concentrations or just pure medium in order to examine the number of bleached chloroplasts. The model species is chosen for this experiment because some strains of *E. gracilis* are highly sensitive to bleaching (Zahalsky et al., 1962). Diphenhydramine is the primary ingredient used to make Benadryl and this antihistamine is used and will induce temporary bleaching in chloroplasts (Boren, 2015; Zahalsky et al., 1962). According to the data from Zahalsky et al. study, concentration less than 10% mg of Diphenhydramine does not cause bleaching but anything above 30% mg shows bleaching (1962). It is predicted that there will be a higher number of chloroplasts bleached in the treatment with a higher Benadryl concentration compared to the lower concentration.

Methods:

Our initial preparation took place on November 3rd, 2021. The sterilized *E. gracilis* stock we used was prepared by the Biology 342 lab technician Mindy Chow a few weeks prior to our experimental setup. The Benadryl source we used was an unopened bottle of Children's Liquid

Benadryl. It contained 6.25 mg of diphenhydramine hydrochloride per 5 mL, resulting in a concentration of 0.00428 M. We tested 3 treatments of Benadryl concentrations: a control group (0M of Benadryl), low concentration (0.0001M Benadryl), and high concentration (0.001M Benadryl), with 3 replicates per treatment. Using a sterile technique, we pipetted 2.5mL of Euglena sample into nine 6mL test tubes, 1.25 mL of 2x medium, and added distilled water and Benadryl based on our desired Benadryl concentration which is outlined in figure 1. After adding the Benadryl into each sample, we swirled the test tubes to ensure adequate mixing of the solution. We incubated our resulting solutions for 24 hours at 20°C in a VWR light incubator.

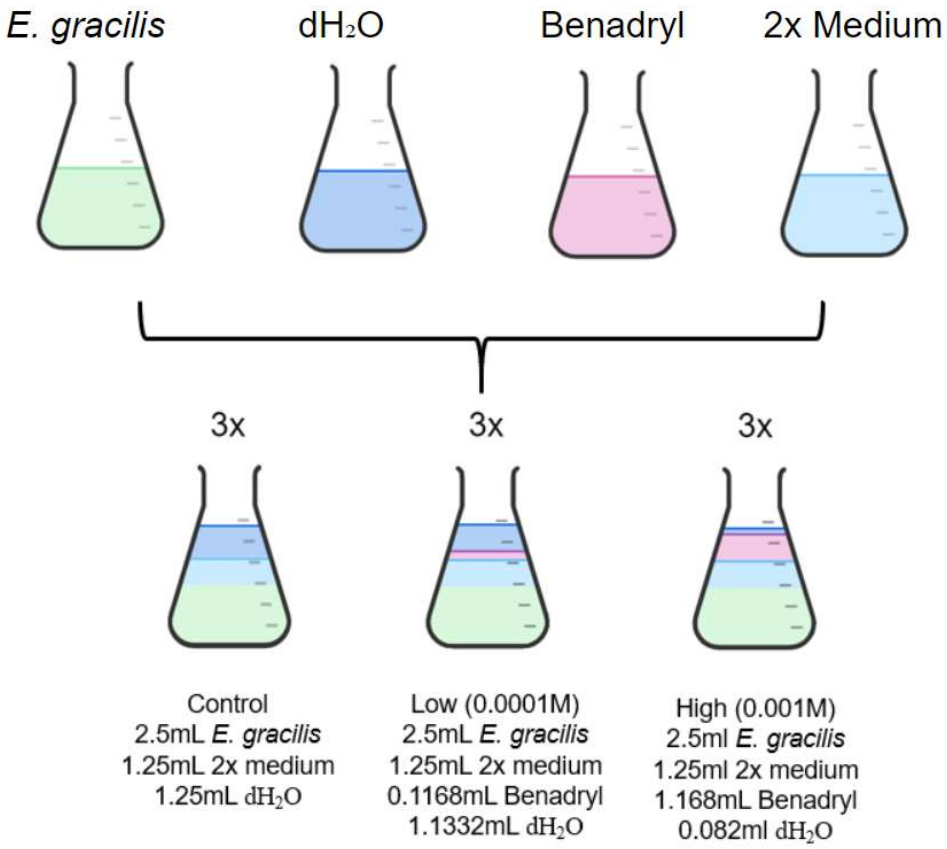

Figure 1: Ingredient amounts used to create replicates. Each replicate was incubated at 20 °C for 24 hours after Benadryl was pipetted.

Counting Chloroplasts:

After the incubation period, on November 4th, 2021, we removed the samples from the incubator and pipetted 100ul of each sample into individual Eppendorf tubes and 10ul of fixative to each Eppendorf tube. The microscope we used was a Zeiss Axio compound microscope using the 40x and 100x objective lens settings. We calibrated the microscope before use and attached a mountable camera to take photos of a cell. After fixing each sample, we transferred 20uL of a sample onto a slide and placed it under the microscope at 40x magnification. After identifying the *E. gracilis* cell we wanted to investigate, we added a drop of immersion oil to our slide cover and shifted the magnification to 100x. We took a photo using the mountable camera and recorded the number of bleached and unbleached chloroplasts we found in a single cell. We repeated this process until we counted the chloroplasts in 10 cells for a replicate. After 10 cells have been counted in one sample, we cleaned the lens on the microscope using a kimwipe and lens cleaning solution, and repeated the counting process for all our other remaining replicates.

We were unable to complete counting within a single day, so we came into the lab the following day on November 5th, 2021, to complete the counting of the remaining replicates. We lost all our photos we took on the previous day as the lab computer in which the photos were saved was experiencing technical difficulties. Furthermore, the cell walls of *E. gracilis* for 2 of our high concentration replicates erupted, making it impossible to count the chloroplasts for those two samples.

Data Analysis:

We conducted an ANOVA and Post Hoc Tukey test on both the counted bleached chloroplasts and unbleached chloroplasts between all 3 replicates to identify if there was significant difference in the populations. These calculations were carried out on excel.

Results

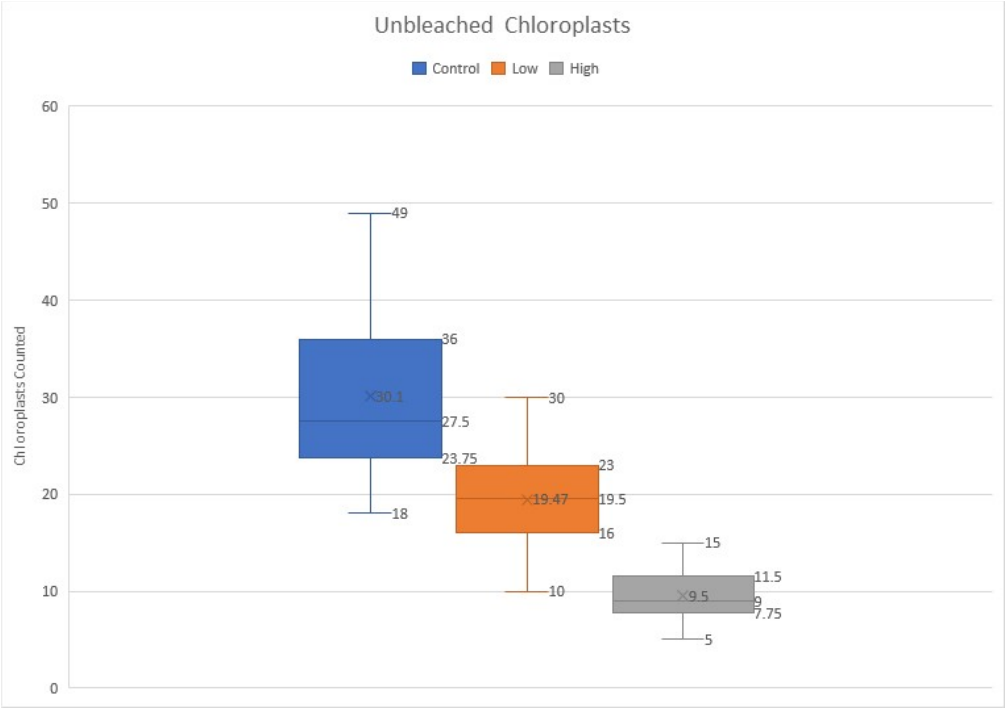

Figure 2: A box-and-whisker plot comparing unbleached chloroplasts counted for each treatment, our control had an average of 30.1, our low concentration treatment average was 19.47, and our high concentration treatment average was 9.5, with N=30 for the control and low and N=10 for the high.

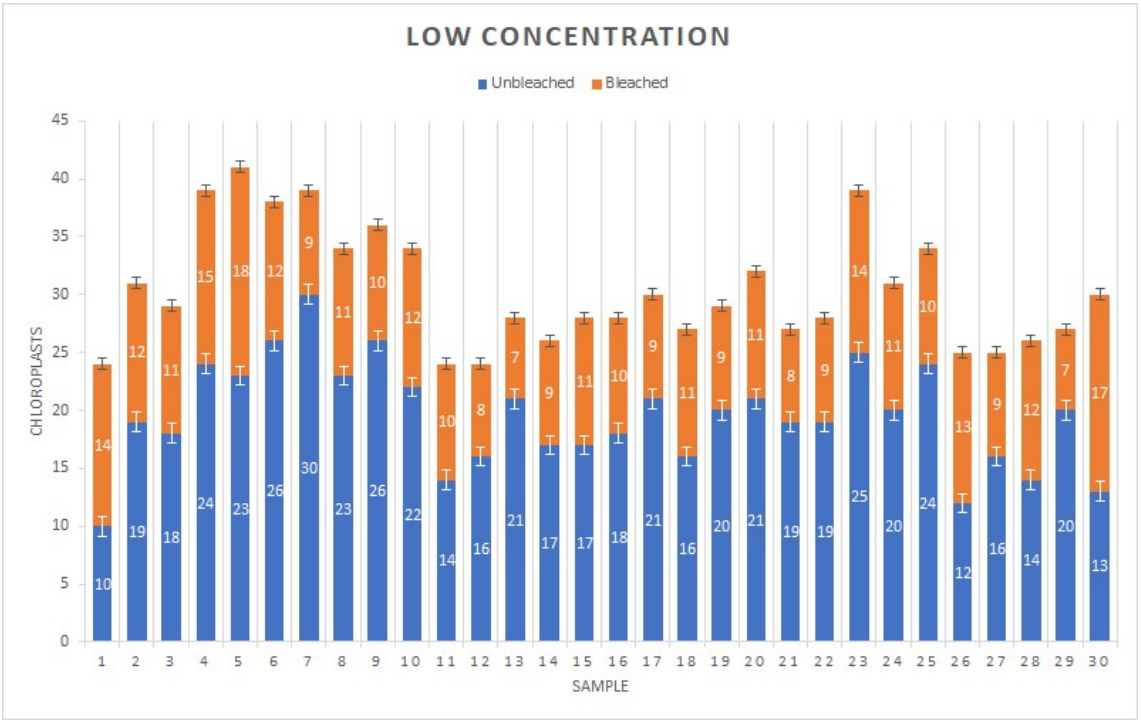

Figure 3: A stacked bar graph for the low concentration samples, error bars calculated using standard error (0.84 and 0.49 for Unbleached and Bleached respectively), Unbleached had a mean of 19.47, Bleached had a mean of 10.97.

Our data analysis showed a significant difference between our three treatments, as seen in figure 1, with higher concentrations of Benadryl resulting in more bleached chloroplasts. There were differences in standard deviation between replicates, with the largest gap being within the control group, while the low concentration replicates stayed fairly consistent. Figures 2 and 3 show how much of each cell sample's chloroplasts bleached and clearly show the difference in effect. There was no observable bleaching in our control group.

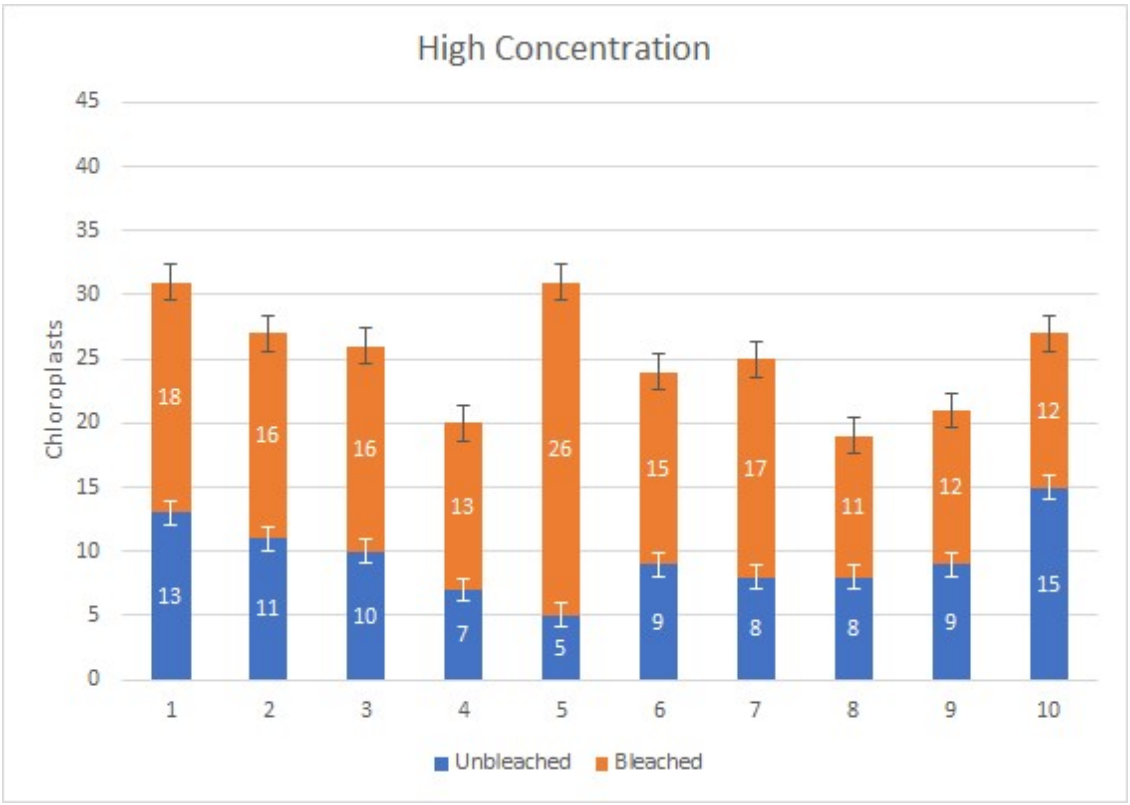

Figure 4: A stacked bar graph of our high concentration sample, error bars were calculated using standard error (0.92 and 1.38 for unbleached and bleached respectively), the average count of unbleached chloroplasts was 9.5 and the average of bleached chloroplasts was 15.6.

Discussion

The hypothesis states that treating *E. gracilis* with varying amounts of Benadryl would result in different numbers of bleached chloroplasts. Parallel to the hypothesis, it was apparent from observations that the presence of bleached chloroplasts for each *E. gracilis* was directly proportional to the concentration of the Benadryl treatments. When Benadryl was absent for the control group, the chloroplasts for each algae were well-defined in terms of their distinctive green colour, membranes and disc-like shapes. As concentration of Benadryl gradually increased for the low and high treatment groups, the chloroplasts began disintegrating and the green colour

faded away; rather, each chloroplast gradually became more transparent. Such a general trend between the concentration of Benadryl and the resulting magnitude of chloroplast bleaching is well-depicted in Figure 1. As the varying treatments possess different bleaching capabilities, the control group without the Benadryl treatment resulted in the most amount of unbleached chloroplasts present, an average of 30.1 for each algae, while the high treatment group had the majority of their chloroplasts bleached upon observation with only 9.5 in average present for each cell. Furthermore, the decreasing average number of unbleached chloroplasts with the increasing concentration of Benadryl treatments comply and reinforce the initial observations. The minimal standard deviation apparent from the low and high treatment groups indicate that the concentration of treatment and its effect had been adequately consistent for these groups.

In order to determine whether such differences in the average number of unbleached chloroplasts for the three experimental groups were statistically significant, ANOVA and Post Hoc Tukey tests were conducted. For ANOVA test, the p-value for each group was calculated with the significance level of 0.05; since the p-value for the control, low and high treatment groups were close to zero and far lower than the significance level, we concluded that the resulting difference in the average number of unbleached chloroplasts was statistically significant. However, over the course of preparing and observing *E. gracilis* samples, a couple of the algae samples had been damaged, which made them unable to be observed. Consequently, the sample size had not been kept constant between all experimental groups, which violated ANOVA test's statistical assumption that sample sizes are all equal for the groups in interest. With the resulting sample sizes not deviating too much from one another and the p-value being kept minimal across all groups, the ANOVA test had successfully confirmed the statistical significance in the obtained data. Furthermore, upon confirming statistical significance from the

ANOVA test, the Post Hoc Tukey test was conducted, which indicates specific and detailed statistical significance between each pair in comparison, rather than all the groups as a whole. The resulting p-values for each pair of groups compared had also been minimal, close to zero. Thus, through the series of statistical analysis conducted, the null hypothesis was rejected and the results obtained from the data had been statistically significant and different from one another.

Several obstacles were encountered throughout the study, which may have resulted in unexpected errors. The observations on the chloroplasts had been limited to the 2D image while the chloroplasts themselves had been arrayed on a 3D plane. Thus, it was difficult to accurately count the number of chloroplasts that were stacked on top of others. The presence of standard deviation for all groups, although it was small, indicates that there was a difference away from the average value to a certain extent. With everything else kept equal, such as, the targeted concentration of the treatments and the same species of algae, we suspect that the underlying difficulty in counting chloroplasts interfered with the counting accuracy, resulting in slight deviation from the average for each group. For the future study, a single cell could be isolated and observed from its top and bottom side to account for their 3D nature and improve the accuracy in counting chloroplasts.

Conclusion

The experiment tested varying concentrations of antihistamines from Benadryl on the formation of bleached chloroplasts, as the chloroplasts may lose functionality when under stress and in the presence of antibiotics. We hypothesized that increasing the concentration of Benadryl results in more bleached chloroplasts. It was found that the control, low concentration and high concentration of Benadryl had a significantly different amount of bleached chloroplasts, with

higher concentrations of Benadryl resulting in more bleached chloroplasts. Similarly, the amount of unbleached chloroplasts were significantly higher in lower concentrations of Benadryl. The results were in agreement with multiple other studies. Although these results were significant, the experimental procedure could be more robust with an increased number of replicates for each concentration to account for the loss of data from experimental conditions.

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Literature Cited

Boren, H. G. (2015). Studying the Effects of Theraflu on the Growth of Selenastrum Capricornutum. Honors Theses. 173.

Gibbs, S. B. (2011) The chloroplasts of *Euglena* may have evolved from symbiotic green algae. *Canadian Journal of Botany*. *56*(22): 2883-2889. DOI:10.1139/b78-345

Shao, Q., Hu, L., Qin, H., Liu, Y., Tang, X., Lei, A., et al. (2019) Metabolomic response of *Euglena gracilis* and its bleached mutant strain to light. *PLoS ONE 14*(11): e0224926. https://doi.org/10.1371/journal.pone.0224926

Zahalsky, A.C., Hutner, S.H., Keane, M. & Burger, R. (1962) Bleaching euglena gracilis with antihistamines and streptomycin-type antibiotics. *Archiv für Mikrobiologie, 42*, 46–55. https://doi.org/10.1007/BF00425189