
ABSTRACT

The Illinois Junior Academy of Science

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CATEGORY	<u>Health Science</u>	STATE REGION #	<u>6</u>
SCHOOL	<u>Barrington High School</u>	IJAS SCHOOL #	<u>6049</u>
CITY/ZIP	<u>Barrington/60010</u>	SCHOOL PHONE #	<u>(847) 381-1440</u>
SPONSOR	<u>Mrs. Polly Foley</u>		

MARK ONE: EXPERIMENTAL INVESTIGATION ☒ DESIGN INVESTIGATION ☐

NAME OF SCIENTIST*	<u>Om Gandhi</u>	GRADE	<u>9th</u>
NAME OF SCIENTIST	<u></u>	GRADE	<u></u>
NAME OF SCIENTIST	<u></u>	GRADE	<u></u>
NAME OF SCIENTIST	<u></u>	GRADE	<u></u>

* If this project is awarded a monetary prize, the check will be written in this scientist's name, and it will be his/her responsibility to distribute the prize money equally among all participating scientists.

PROJECT TITLE The Effects of Antioxidants on the Survival Rate of *S. cerevisiae* Exposed to Ultraviolet Radiation

Purpose: To determine the effectiveness of antioxidant protection against UVB radiation and to determine which antioxidants out of Ascorbic Acid, Zinc Oxide, and Vitamin E most effectively prevent UVB radiation from damaging cells as well as to design an in-vitro model that can help test the ultraviolet absorbing capabilities of different natural antioxidants.

Procedure: *Saccharomyces cerevisiae* was used as a model organism in this project as they have a similar anatomy and physiology compared to human skin cells. They were dissolved in sugar water with different antioxidants, incubated, and placed under UVB radiation. Viability rates of the cells were calculated.

Conclusion: The experimental data partially supported the first hypothesis. The results showed, based on the viability rates calculated, that Zinc Oxide was indeed significantly more effective at protecting the cells against radiation compared to Vitamin E; however, Zinc Oxide and Ascorbic Acid are not significantly different from each other in their protective capabilities against UVB radiation. The second hypothesis of this project was supported. All the cultures that are tested with antioxidants indeed did have higher viability rates in some degree compared to the positive control and had lower viability rates compared to the negative control.

SAFETY SHEET

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Directions: The student is asked to read these introductions carefully and fill out the bottom of this sheet. The science teacher and/or advisor must sign in the indicated space. By signing this sheet, the sponsor assumes all responsibilities related to this project.

Safety and the Student: Experimentation or design may involve an element of risk or injury to the student, test subjects and to others. Recognition of such hazards and provision for adequate control measures are joint responsibilities of the student and the sponsor. Some of the more common risks encountered in research is those of electrical shock, infection from pathogenic organisms, uncontrolled reactions of incompatible chemicals, eye injury from materials or procedures, and fire in apparatus or work area. Countering these hazards and others with suitable safety practices is an integral part of good scientific research. In the **chart** below, list the principal hazards associated with your project, if any, and what specific precautions you have used as safeguards. Be sure to read the entire section in the *Policy and Procedure Manual of the Illinois Junior Academy of Science* entitled "Safety Guidelines for Experimentation" before completing this form.

Possible hazards	Precautions taken to deal with each hazard
<ul style="list-style-type: none">• UV radiation can potentially cause harm to skin and eyes.• Chemicals and cultures can potentially irritate skin when skin comes in contact with them.• Hand/finger could be burned in the making of sugar water.	<ul style="list-style-type: none">• Wear safety goggles, lab coat, and gloves.• Use mittens so no one gets burned.• Perform experiment in a laboratory• Perform experiment near clean, running water in case of contact with chemicals.• Perform experiment under adult supervision.• Destroy culture using a suitable NaClO (bleach) solution before disposal.

Specific safety practices related to materials requiring endorsement sheets should be detailed on the specific endorsement sheet and not included on this safety sheet.

Please check off any other possible endorsements needed. Include these documents in your paper and on your board.

_____ Humans as Test Subjects –for any projects involving humans including survey administration;

☒ Microorganisms-for any projects involving bacteria, viruses, yeasts, fungi or protozoa;

_____ Non-Human Vertebrates -for any projects involving fish, amphibians, reptiles, birds or mammals;

_____ Tissue Culture-for any projects involving growing eukaryotic tissues or cell cultures;

_____ Letter from institution where research was done or IJAS SRC, if an exception to the IJAS rules has been granted...

SIGNED _____

Student Exhibitor(s)

SIGNED _____

Sponsor *

*As a sponsor, I assume all responsibilities related to this project.

Microorganism Endorsement

The Illinois Junior Academy of Science

These rules will be strictly enforced for the State Science Exposition.

No region should send a project to the State Exposition that does not meet these regulations.

Students and sponsors doing a microorganism project must complete this form. The signature of the student or students and the sponsor indicates that the project was done within these rules and regulations. Failure to comply with these rules will mean the disqualification of the project at the state level. This form must follow the Safety Sheet in the project paper and on the project board.

1. This area of science may involve many dangers and hazards while experimenting. It is the sole responsibility of all teacher(s)/sponsor(s) to teach students proper safety methods and sterile techniques.
2. The Illinois Junior Academy of Science prohibits the use of primary or secondary cultures taken from humans or other vertebrate animals in any project because of the danger from unknown viruses or other disease-causing agents that may be present. Pure cultures of microorganisms known to inhabit vertebrate animals may be obtained from reputable suppliers and used in proper settings.
3. Microorganism experiments must be conducted in a laboratory such as science classroom or research facility.
4. Projects involving viruses and recombinant DNA should be done with the help of a professional and should comply with the National Institutes of Health (NIH) Guidelines unless the project is limited to a kit obtained from a legitimate supply house.
5. All cultures should be destroyed by methods such as autoclaving or with a suitable NaClO (bleach) solution before disposal.

Complete all boxes of the following chart.

Genus and species of organism(s) being used.	<i>Saccharomyces cerevisiae</i>
Name of the reputable source of the organism(s) being used.	RED STAR ®
Method of disposal of the organism(s) being used.	NaClO (bleach) method of disposal
List the location where the lab work was conducted.	Barrington High School
Describe the use of microorganisms in this project.	<i>Saccharomyces cerevisiae</i> was used as a model organism in this project as they have a similar anatomy and physiology compared to human skin cells. They were dissolved into sugar water with different antioxidants, incubated, and placed under UV radiation. Viability rates of the cells were calculated.
Other precautions taken to ensure microorganisms are used safely in this investigation.	Student wore gloves and wore goggles at all times while performing the experimentation. All materials to come in contact with the microorganisms were sterilized and thoroughly cleaned before and after the experimentation.

The signatures of the student or students and sponsor below indicate that the project conforms to the above rules of the Illinois Junior Academy of Science.

(Sponsor)*

(Student)

(Date)

(Student)

*As a sponsor, I assume all responsibilities related to this project.

The Effect of Antioxidants on the Survival Rate of *S. cerevisiae* Exposed to Ultraviolet Radiation

Om Gandhi | Barrington High School | 9th Grade

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Purpose

The purpose of this project is as follows:

- To determine the effectiveness of antioxidant protection against UV radiation.
- To determine which antioxidants out of Ascorbic Acid, Zinc Oxide, and Vitamin E most effectively prevent UV radiation from damaging cells.
- To design an in-vitro model that can help test the ultraviolet absorbing capabilities of different natural antioxidants.

Hypotheses

The hypotheses for this project are:

- The culture with Zinc Oxide will have higher viability rates compared to Vitamin E, but Zinc Oxide will have lower viability rates when compared to Ascorbic Acid.
- All the cultures that are tested with antioxidants will have higher viability rates in some degree compared to the positive control but will have lower viability rates compared to the negative control.

Review of Literature

INTRODUCTION

Oxygen is an essential element of life. The oxidative property of oxygen plays a vital role in various biological phenomena; being essential for life, oxygen can also create the damage within the cell by oxidative events. Oxygen is used by the cell to generate energy, and free radicals are formed as a consequence of ATP (adenosine triphosphate) production by the mitochondria. “Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the by-products resulting from the cellular redox process,” (Sen and Chakraborty, 2011, [Online]). These reactive species play a dual role in human health as both toxic and beneficial compounds. The delicate balance between their two opposite effects is undoubtedly a key aspect of life. At low or moderate levels, reactive species exert beneficial effects on cellular redox signaling and immune function, but at high concentrations, they produce oxidative stress, a harmful process that can damage cell function and structures (Sen and Chakraborty, 2011).

FREE RADICALS AND ANTIOXIDANTS

Free radicals are defined as molecules or molecular fragments containing one or more unpaired electrons. “This unpaired electron(s) are unstable and usually gives a significant degree of reactivity to the free radical,” (Sen and Chakraborty, 2011, [Online]). Oxygen and nitrogen free radicals can be converted to other non-radical reactive species, which are also dangerous for health. Free radicals can be produced from both endogenous and exogenous substances. The immune system cells, metabolic processes, inflammation, and stress are all endogenous

substances from which free radicals can be produced. Pollution, radiation, dietary factors, toxins and drugs are all exogenous substances from free radicals can be produced (Bauer, 2005).

The relation between free radicals and molecular damage can be described by the concept of 'oxidative stress'. The harmful effect of free radicals causing potential biological injury is termed *oxidative stress* and *nitrosative stress*. The term oxidative stress describes a harmful condition caused by the excess ROS (reactive oxygen species) production and/or a decrease in antioxidant levels. Oxidative stress has been implicated in various diseases, like cardiovascular diseases, neurological disorders, diabetes, and aging (Sen and Chakraborty, 2011).

Similarly, overproduction of reactive nitrogen species is called *nirosative stress*. Prolonged exposure to free radicals, even at a low concentration, may be responsible for the damage of biologically important molecules and potentially lead to tissue injury. Every biological molecule present in the body is at risk of damage by free radicals. Such damaged cell molecules can impair cell function or can lead to cell death, ultimately resulting in disease states (Sen and Chakraborty, 2011).

Antioxidants are a class of molecules that are capable of inhibiting the oxidation of another molecule. The human body produces some antioxidants. In addition, the body's natural antioxidant production can decline as one ages. Antioxidants are nature's way of providing the body's cells with an adequate defense against attack by reactive oxygen species. To counter free radicals, antioxidants bind to these free radicals before they have the chance to cling onto the cells. And once a radical is paired with the antioxidant, it can no longer cause cellular/tissue damage (Sharma and Clark, 1998). Antioxidants can help prevent and/or slow progressions of various diseases, such as Arthritis, Alzheimer's disease, Parkinson's disease and heart disease, in our body. At the point when our body needs to set up its best protection, antioxidants are vital to

our health and well-being. Mother Nature gives us a large number of diverse antioxidants from different sources such as Vitamin C, Vitamin E, Beta Carotene, Quercetin, Zinc, Selenium, and Alpha-lipoic acid (ALA) (Axe, 2017). From these sources, Ascorbic Acid, Zinc Oxide, and Vitamin E are most commonly used and most effective.

- **Zinc Oxide:** Zinc oxide reacts slowly with fatty acids in oils to produce the corresponding carboxylates, such as oleate or stearate. ZnO forms cement-like products when mixed with a strong aqueous solution of zinc chloride and these are best described as zinc hydroxy chlorides. Regarding natural sources, pomegranates are among the richest in Zinc Oxide, with about 1 milligram of zinc in one fresh pomegranate. Avocados are also relatively high in zinc, with about 1.3 milligrams in one medium fruit. (Axe, 2017).
- **Ascorbic Acid:** Ascorbic acid is a chemical compound ($C_6H_8O_6$) that is commonly found in nature and can be used as an antioxidant food additive. Ascorbic acid can be used in a variety of forms, including salts and esters. In these forms, it will appear on ingredient lists under different names, such as sodium ascorbate, calcium ascorbate, potassium ascorbate, ascorbyl palmitate, or ascorbyl stearate. Ascorbic Acid is also found in many foods, mostly fruits and veggies, such as: oranges, green peppers, and watermelon. (Moncel, 2017).
- **Vitamin E:** Vitamin E helps treats and prevent diseases of the heart and blood vessels, such as chest pains, high blood pressure, and blocked or hardened arteries. Vitamin E is found only in plant foods, including certain oils, nuts, grains, fruits and wheat germ. It's also available as a supplement (Axe, 2017).

SKIN CANCER AND RADIATION

Skin cancer is the most common of all human cancers. Cancer occurs when normal cells undergo a transformation and grow and multiply without normal controls. As the cells multiply, they form a mass called a tumor. Tumors are cancerous only if they are malignant, which means that they encroach on and invade neighboring tissues (especially lymph nodes) because of their uncontrolled growth. Tumors may also travel to remote organs via the bloodstream. This process of invading and spreading to other organs is called metastasis. Tumors overwhelm surrounding tissues by invading their space and taking the oxygen and nutrients they need to survive and function. There are three major types of skin cancers: basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma. The first two skin cancers are grouped together as non-melanoma skin cancers. Other unusual types of skin cancer include Merkel cell tumors and dermatofibrosarcoma protuberans (Kenneaster, 2005).

The incidence of skin cancer has been increasing throughout the United States and much of the world. Although the mortality rate of nonmelanoma skin cancer is low, that type of skin cancer accounts for considerable morbidity, including cosmetic and functional impairment. Melanoma, although less common, is a life-threatening malignancy if not detected and treated early. Skin cancer also significantly contributes to the rising costs of health care in the United States. "Prevention, early diagnosis, and treatment is critical in helping to reduce the incidence, morbidity, and mortality associated with skin cancer," (Kenneaster, 2005, [Online]). Treatment for skin cancer depends on the type and stage of the disease, the size and place of the tumor, and your general health and medical history. Surgery is the usual treatment for people with skin cancer. In some cases, the doctor may suggest chemotherapy, photodynamic therapy, or radiation

therapy. People with melanoma may also have biological therapy. There are many types of surgery used to treat skin cancer.

- **Excisional skin surgery:** This is a common treatment to remove any type of skin cancer. After numbing the area of skin, the surgeon removes the growth (tumor) with a scalpel. The surgeon also removes a border (a margin) of normal skin around the growth. The margin of skin is examined under a microscope to be certain that all the cancer cells have been removed. The thickness of the margin depends on the size of the tumor (Lee, 2016).
- **Mohs surgery** (also called Mohs micrographic surgery): This method is often used for basal cell and squamous cell skin cancers. After numbing the area of skin, a specially trained surgeon shaves away thin layers of the tumor. Each layer is examined under a microscope. The surgeon continues to shave away tissue until no cancer cells can be seen under the microscope. In this way, the surgeon can remove all the cancer and only a small bit of healthy tissue. Some people will have radiation therapy after Mohs surgery to make sure all of the cancer cells are destroyed (Lee, 2016).
- **Electrodesiccation and curettage:** This method is often used to remove a small basal cell or squamous cell skin cancer. After the doctor numbs the area to be treated, the cancer is removed with a sharp tool shaped like a spoon (called a curette). The doctor then uses a needle-shaped electrode to send an electric current into the treated area to control bleeding and kill any cancer cells that may be left. This method is usually fast and simple. It may be performed up to three times to remove all of the cancer (Lee, 2016).
- **Cryosurgery:** This method is an option for an early stage or a very thin basal cell or squamous cell skin cancer. Cryosurgery is often used for people who are not able to have other types of surgery. The doctor applies liquid nitrogen (which is extremely cold)

directly to the skin growth to freeze and kill the cancer cells. This treatment may cause swelling. It also may damage nerves, which can cause a loss of feeling in the damaged area (Lee, 2016).

One of the biggest causes of skin cancer is radiation. “Ultraviolet radiation (UVR) is the major etiologic agent in the development of skin cancers. UVR causes DNA damage and genetic mutations, which subsequently lead to skin cancer,” (Narayanan, Saladi, and Fox, 2010, [Online]). A clearer understanding of UVR is crucial in the prevention of skin cancer. Several factors influence the amount of UVR reaching the earth's surface, including ozone depletion, UV light elevation, latitude, altitude, and weather conditions. The current treatment modalities utilizing UVR (i.e. phototherapy) can also predispose to skin cancers. Unnecessary exposure to the sun and artificial UVR (tanning lamps) are important personal attributable risks (Narayanan, Saladi, and Fox, 2010, [Online]). There are three different main types of radiation: UVA, UVB, and UVC. UVB radiation is the biggest cause of skin cancer. UVB radiation comes from invisible rays that are part of the energy that comes from the sun. UVB radiation causes sunburn, darkening and thickening of the outer layer of the skin, and melanoma and other types of skin cancer. It may also cause problems with the eyes and the immune system. In medicine, UVB radiation also comes from special lamps or a laser and is used to treat certain skin conditions such as psoriasis, vitiligo, and skin tumors of cutaneous T-cell lymphoma (Cunha, n.d.).

SACCHAROMYCES CEREVISIAE: MODEL ORGANISMS

Saccharomyces cerevisiae is a species of yeast that is commonly used for brewing and baking.

They are eukaryotic microorganisms classified in the kingdom Fungi. Yeast cells can grow in

two forms: diploid or haploid (Mucka et al. 2010). *S. cerevisiae* usually reproduce asexually by an asymmetric division process called budding; however, under stressful conditions, the diploid cells of yeast (the preferred form) can pursue sporulation and produce haploid spores via meiosis. *S. cerevisiae* can grow aerobically on glucose and maltose but fail to grow on lactose (Yu et al. 2011). *Saccharomyces cerevisiae* “is one of the simplest eukaryotic organisms but many essential cellular processes are the same in yeast and humans. It is therefore an important organism to study to understand basic molecular processes in humans.” An important feature of these yeasts that makes them such useful organisms for studying biological processes in humans, is that their cells, like human skin cells, have a nucleus containing DNA packaged into chromosomes. Most metabolic and cellular pathways thought to occur in humans, can be studied in yeast. For example, studying signaling proteins in yeast has advanced the understanding of brain and nervous system development. Yeast cells divide in a similar manner to human cells. It has been found that many of the genes that work to regulate cell division in yeast, have equivalents that control cell division in higher organisms, including humans. At least 20 percent of human genes known to have a role in disease have functional equivalents in *S. cerevisiae*. This has demonstrated that many human diseases result from the disruption of very basic cellular processes, such as DNA repair, cell division, the control of gene expression and the interaction between genes and the environment. Due to *S. cerevisiae* having various similarities with human skin cells, they are being used as a model organism in the project (Yu et al. 2011).

SUMMARY OF REVIEW

Antioxidants are natural molecules found in certain foods that help neutralize free radicals in our bodies. Free radicals are byproducts of metabolism and the environment which cause damage to

cells all over the body. UV exposure leads to an increase in free radical production and can cause skin cancer. Antioxidants serve as protection against the cell damage and may play a role in preventing skin cancer. Ascorbic Acid, Zinc Oxide, and Vitamin E are the most effective and most widely used sources of antioxidants provided by Mother Nature.

Variables

- The independent variable is the antioxidant, either Ascorbic Acid, Zinc Oxide, or Vitamin E, that was dissolved into the sugar solution with the culture.
- The dependent variable is the viability rate of the *S. cerevisiae* after being exposed to UVB radiation.
- The constant variables include time and environment that the experimentation took place in. Each flask of culture was exposed to UVB radiation for the same amount of time and in the same environment.
- The controls are the negative control and the positive control. The negative control will contain the sugar solution and the yeast cells but will neither contain antioxidants nor be exposed to the UVB radiation. The positive control will also contain the sugar solution and the yeast cells but will be exposed to the UVB radiation. The positive control will also not contain antioxidants.

Materials

The materials (for one trial) used for this project are:

- | | | |
|------------------------------------------|------------------------|--------------------|
| - 1750 ml of Distilled Water | - An Incubator | - Paper Towels |
| - 120g of Granulated Sugar | - 50 Eppendorf Tubes | - Stirring Rod |
| - 20g of <i>Saccharomyces cerevisiae</i> | - A microscope | - A Sink |
| - 15g of Zinc Oxide | - 2 UVB Light Bulbs | - A Stove |
| - 15g of Ascorbic Acid | - A Pot | - Cloths |
| - 15g of Vitamin E | - A Graduated Cylinder | - Camera |
| - 91% Isopropyl Alcohol | - A Measuring Scale | - Timer |
| - Methylene Blue | - A Plant-Grow Table | - Plastic Pipettes |
| - A 1-liter Flask | - Bleach | - Safety Goggles |
| - A Hemocytometer | - Rubber Gloves | - A Lab Coat |
| - Twenty-five 20-mL Small Flasks | - Calculator | - Rubber Gloves |
| - Twenty-five 5ml Serological Pipettes | | |

Procedure

Part 1: Preparing the Cultures

1. To prepare the cultures, first measure 800 mL of distilled water using a graduated cylinder. Then, using a measuring scale, measure 120 grams of granulated sugar.
2. Pour the measured distilled water and the measured granulated sugar into a pot. Set the pot on the stove and boil the water. Stir often to help the sugar dissolve.
3. As soon as the water begins to boil, reduce the heat and bring the water down to a simmer. Wait about three minutes for the sugar to dissolve.
4. Take the pot off the stove and let it cool. Set it down onto a heat-resistant surface and let the mixture return to room temperature.
5. Carefully transfer the mixture from the pot to a one-liter flask. Add 20 grams of *Saccharomyces cerevisiae* to the mixture in the one-liter flask. Gently swirl the flask to ensure that the cells are evenly distributed.
6. Obtain twenty-five 20-mL small flasks. Carefully pour 15 mL of the cultured solution from the stock solution in Step 5 into each of the twenty-five smaller flasks. Gently swirl all flasks.
7. Arrange the flasks into five groups of five. Label each of the five flasks in the first group as “Ascorbic Acid”. Label each of the five flasks in the second group as “Zinc Oxide”. Label each of the five flasks in the third group as “Vitamin E”. Label each of the five flasks in the fourth group as “negative control”. Label each of the five flasks in the last group as “positive control”. These 20-ml flasks will serve as samples for each of the trials.

8. Add 3 grams of Zinc Oxide into each of the five flasks labeled as “Zinc Oxide”. Add 3 grams of Ascorbic Acid into each of the five flasks labeled as “Ascorbic Acid”. Add 3 grams of Vitamin E into each of the five flasks labeled as “Vitamin E”.
9. Gently swirl all the flasks to ensure that the antioxidants are evenly distributed. The negative control will contain the sugar solution and the yeast cells but will neither contain antioxidants nor be exposed to the UVB radiation. The positive control will also contain the sugar solution and the yeast cells but will be exposed to the UVB radiation. The positive control will also not contain antioxidants.

Part 2: Incubating and Testing the Cultures against UVB Radiation

1. Before incubating the cultures, clean the incubator to minimize error. Carefully wipe off any visible dust or debris from all the surfaces of the incubator. Then, wipe down all surfaces with a sponge dipped in a diluted bleach solution, which is twenty drops of bleach mixed with 0.95 liters of distilled water. Use gloves to protect hands from the bleach.
2. Place all twenty-five small flasks into the incubator. Set the temperature to 30 degrees C and set the humidity to 50%. Incubate the culture for 24 hours.
3. After incubation is complete, take out and gently swirl all the flasks to ensure that the cells are evenly distributed. Then, repeat Step 1 from Part 2.
4. To test the incubated cultures, obtain a plant-grow table and carefully insert light bulbs which release UVB radiation.
5. Place all of the flasks except the ones that are labeled as “negative control” into the plant-grow table.

6. Turn the UVB bulb on. Make sure no other light is in contact with the cultures and keep the cultures in contact with the UVB radiation for 30 minutes.
7. Take the flasks out and sterilize the plant-grow table with diluted bleach.

Part 3: Counting Cells and Calculating Viability Rates

1. Clean the hemocytometer before use each time with 91% isopropyl alcohol. Moisten the coverslip with water and affix to the hemocytometer.
2. Gently swirl the flask to ensure the cells are evenly distributed.
3. Before the cells have a chance to settle, take out 0.5 mL of cell suspension using a 5-mL serological pipette and place it in an Eppendorf tube.
4. Take 100 μ L of cells into a new Eppendorf tube and add 400 μ L of Methylene Blue. Mix gently.
5. Using a pipette, take 100 μ L of Methylene Blue-treated cell suspension and apply to the hemocytometer. Very gently fill both chambers underneath the coverslip, allowing the cell suspension to be drawn out by capillary action.
6. Using a microscope, focus on the gridlines of the hemocytometer with a 40X objective.
7. Count and record the live, unstained cells in one set of 16 squares. When counting, employ a system whereby cells are only counted when they are set within a square or on the right-hand or bottom boundary line. Following the same guidelines, count and record the dead cells stained with Methylene Blue.
8. Move the hemocytometer to the next set of 16 corner squares and carry on counting until all 4 sets of 16 corners are counted and recorded.

9. To calculate the viability rate, first add together the live and dead cell count to obtain a total cell count. Then, divide the live cell count by the total cell count and multiply the result by 100 to calculate the percentage viability.
10. Repeat “Part 3” for all twenty-five samples.

Part 4: Disposing the Culture

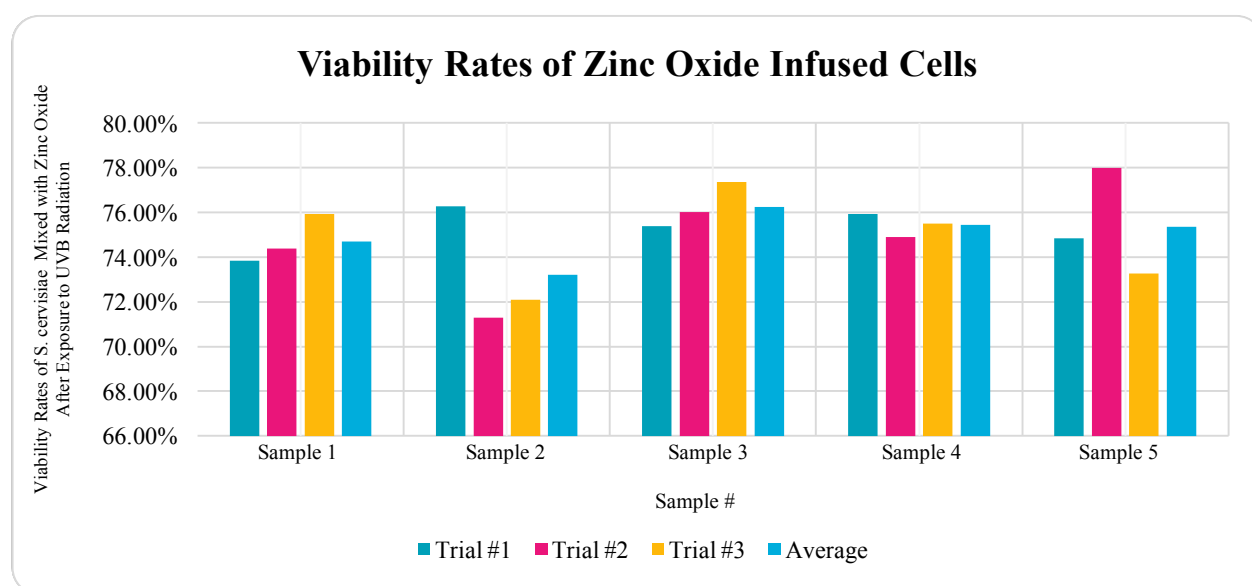
1. Firstly, protect hands from the bleach by wearing rubber gloves and protect eyes with safety goggles.
2. Carefully pour a small amount of bleach into all the flasks used, holding the flasks over a sink.
3. Take pictures and repeat all steps from Part 1 Step 1 to Part 4 Step 2 twice to serve as trials and conclude final results.

Results

Table 1- Viability Rates of Zinc Oxide Infused Cells

Viability Rates of Zinc Oxide Infused Cells					
Trial #	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
Trial #1	73.85%	76.27%	75.37%	75.93%	74.83%
Trial #2	74.38%	71.29%	76.02%	74.91%	77.99%
Trial #3	75.92%	72.08%	77.36%	75.50%	73.27%
Average	74.72%	73.21%	76.25%	75.45%	75.36%

Figure 1- Viability Rates of Zinc Oxide Infused Cells



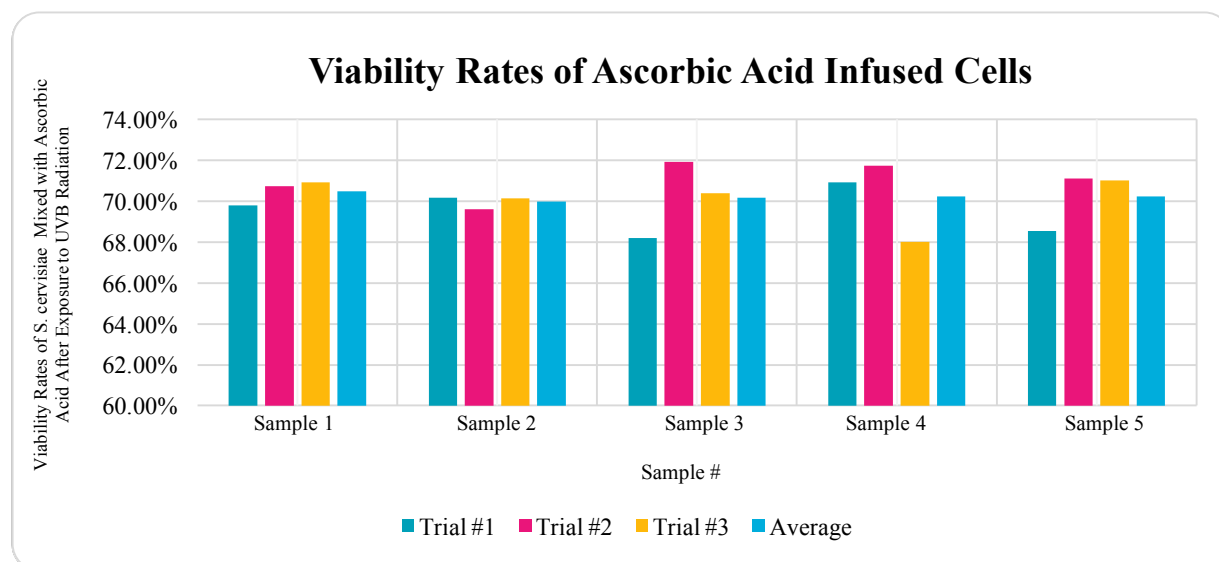
Data Analysis: The *Saccharomyces cerevisiae* cells are model organisms to represent human skin cells. The higher the viability rates of the cells after exposure to UVB radiation, the more of a protective effect the antioxidants had, thus simulating the effect of one's skin cells having absorbed or is in contact with the specific antioxidants under sunlight radiation. According to the experimental data outcomes, the average viability rate of the *S. cerevisiae* cells infused with Zinc Oxide was at 75.00%, which is a greater viability rate than any other antioxidant infused cell viability rate. Even though Samples 2 and 5 have slightly inconsistent results throughout trials, the average standard deviation all samples is approximately 1.14% and the average standard

error of all samples is approximately 0.51%, assuring that the data is completely relevant and has minimal error.

Table 2- Viability Rates of Ascorbic Acid Infused Cells

Viability Rates of Ascorbic Acid Infused Cells					
Trial #	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
Trial #1	69.80%	70.17%	68.19%	70.93%	68.55%
Trial #2	70.75%	69.60%	71.91%	71.74%	71.11%
Trial #3	70.92%	70.15%	70.39%	68.01%	71.03%
Average	70.49%	69.97%	70.16%	70.23%	70.23%

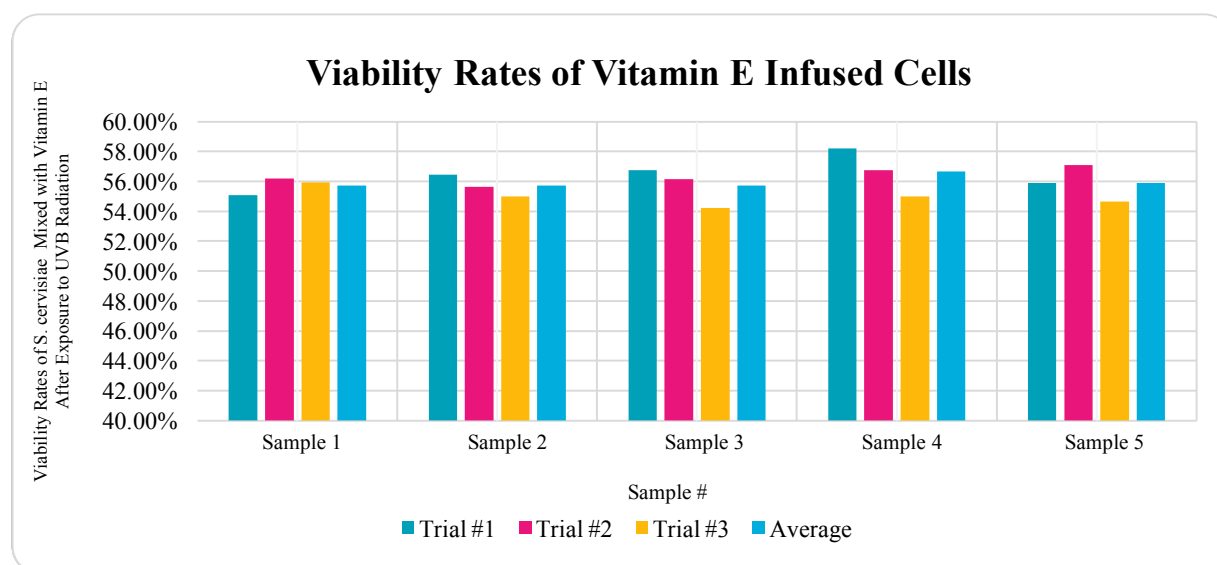
Figure 2- Viability Rates of Ascorbic Acid Infused Cells



Data Analysis: While not being as protective compared to Zinc Oxide, Ascorbic Acid infused cells had an average viability rate of 70.22%, still showing to be effective against UVB radiation. Although there is some deviation present, the averages of each of the samples are relatively similar, having less than a percent of error.

Table 3- Viability Rates of Vitamin E Infused Cells

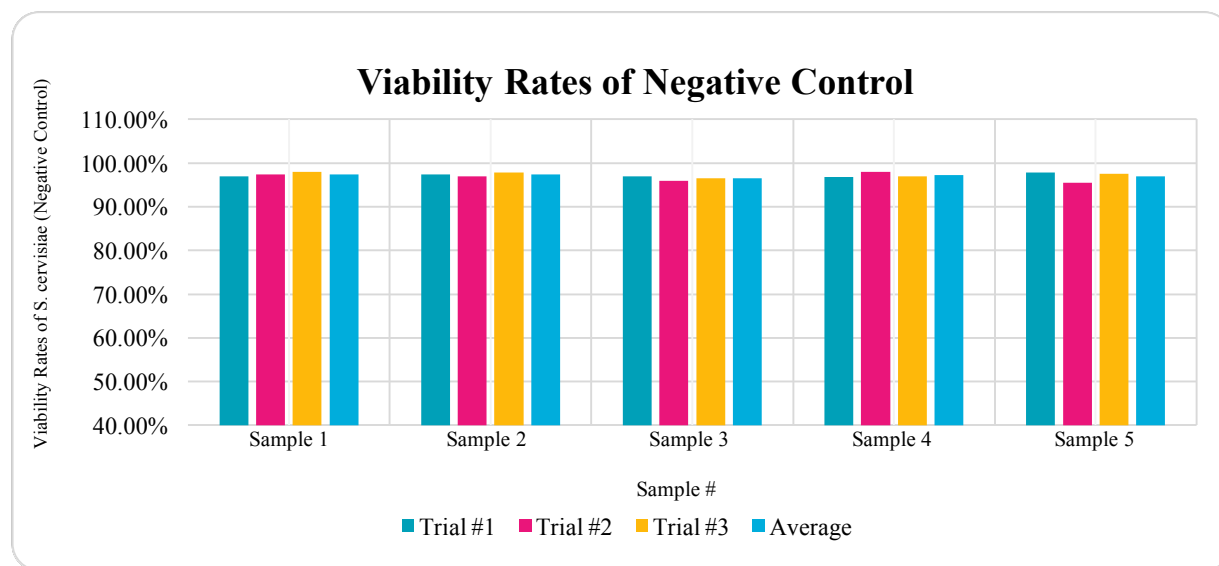
Viability Rates of Vitamin E Infused Cells					
Trial #	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
Trial #1	55.10%	56.47%	56.74%	58.21%	55.90%
Trial #2	56.18%	55.65%	56.15%	56.74%	57.08%
Trial #3	55.92%	54.99%	54.23%	55.01%	54.66%
Average	55.73%	55.70%	55.71%	56.65%	55.88%

Figure 3- Viability Rates of Vitamin E Infused Cells

Data Analysis: The average viability rate of Vitamin E infused cells was 55.93%, which is a lower rate than any other antioxidant infused cells. In regards to observations, most of the cells infused with Vitamin E were smaller in size than compared to other antioxidant infused cells.

Table 4- Viability Rates of Negative Control

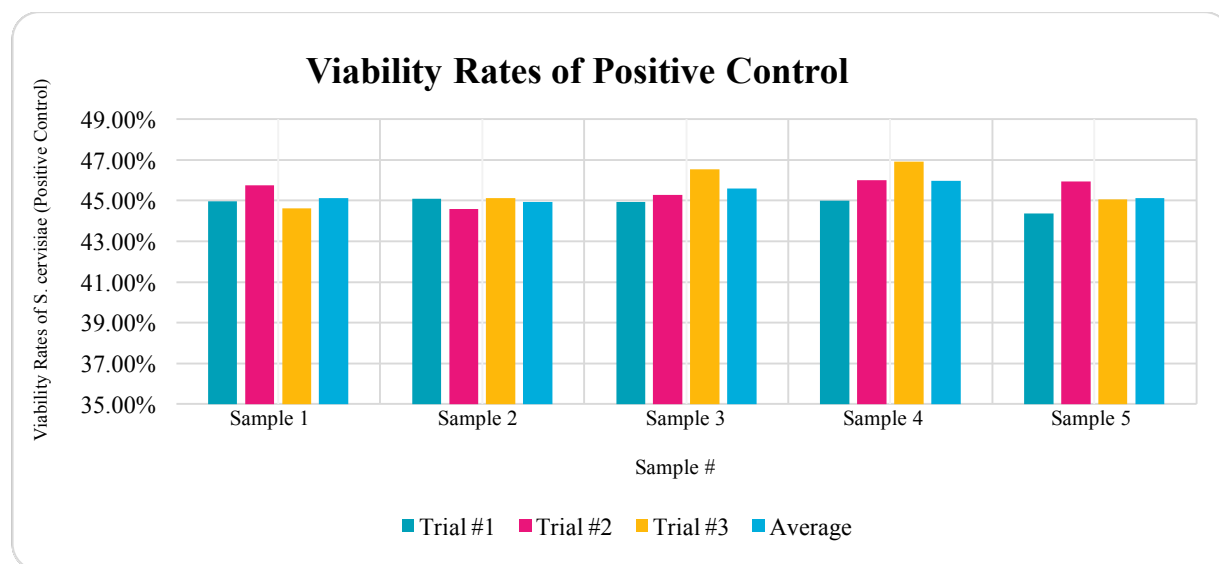
Viability Rates of Negative Control					
Trial #	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
Trial #1	96.97%	97.34%	97.01%	96.78%	97.77%
Trial #2	97.36%	96.98%	95.88%	98.04%	95.54%
Trial #3	98.00%	97.86%	96.48%	96.93%	97.55%
Average	97.44%	97.39%	96.46%	97.25%	96.95%

Figure 4- Viability Rates of Negative Control

Data Analysis: The negative control contained the sugar solution and the yeast cells but neither contained antioxidants nor was exposed to the UVB radiation, therefore it has the best viability rates compared to any other solution or control as there was no major factor that had the potential to decrease the population of the cells drastically.

Table 5- Viability Rates of Positive Control

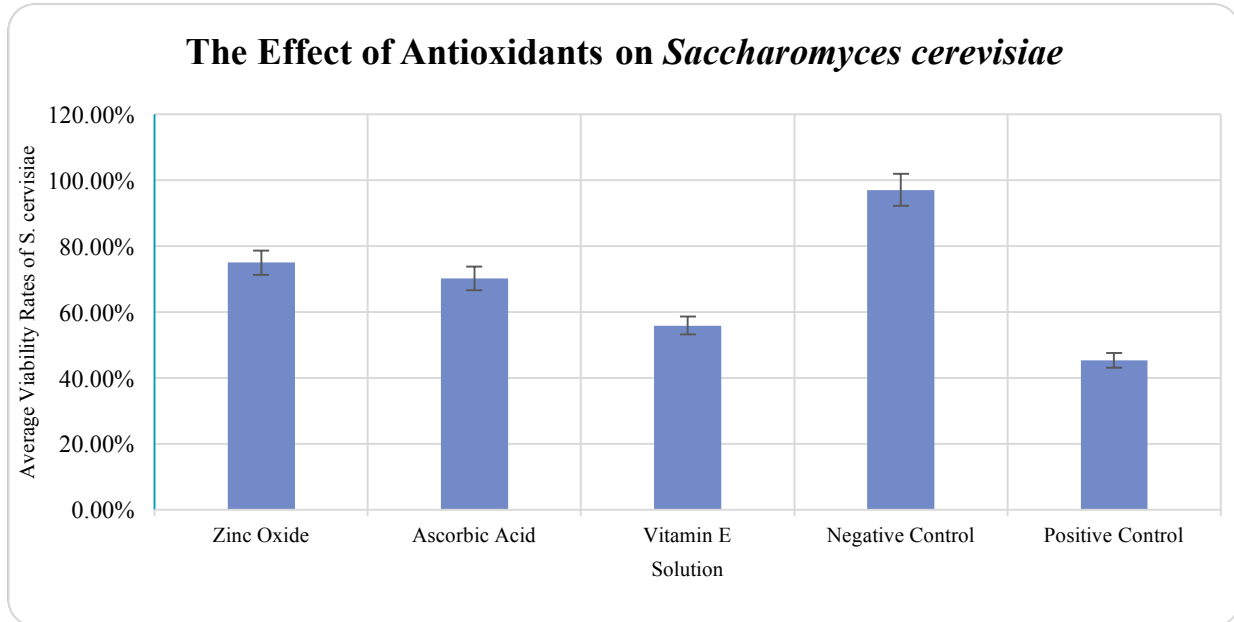
Viability Rates of Positive Control					
Trial #	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
Trial #1	44.96%	45.10%	44.93%	45.01%	44.38%
Trial #2	45.75%	44.60%	45.29%	46.00%	45.94%
Trial #3	44.62%	45.11%	46.54%	46.92%	45.07%
Average	45.11%	44.94%	45.59%	45.98%	45.13%

Figure 5- Viability Rates of Positive Control

Data Analysis: The positive control contained the sugar solution and the yeast cells but was exposed to the UVB radiation. The positive control also did not contain antioxidants. This control has the lowest viability rates compared to any other tested solution or control as there were no antioxidants to protect the cells in some way from the UVB radiation, which decreased the population of the cells to an average of 45.35%.

Table 6- Viability Rates: Summary Data and Statistical Analysis

The Effects of Antioxidants on <i>Saccharomyces cerevisiae</i>									
Solutions	S1	S2	S3	S4	S5	Average	SD	SE	95%CI
<u>Zinc Oxide</u>	74.72%	73.21%	76.25%	75.45%	75.36%	74.50%	1.14%	0.51%	1.08%
<u>Ascorbic Acid</u>	70.49%	69.97%	70.16%	70.23%	70.23%	70.21%	0.19%	0.083%	0.18%
<u>Vitamin E</u>	55.73%	55.70%	55.71%	56.65%	55.88%	55.93%	0.41%	0.18%	0.39%
<u>Negative Control</u>	97.44%	97.39%	96.46%	97.25%	96.95%	97.10%	0.40%	0.18%	0.39%
<u>Positive Control</u>	45.11%	44.94%	45.59%	45.98%	45.13%	45.35%	0.43%	0.19%	0.41%

Figure 6- Viability Rates: Summary Data and Statistical Analysis

Data Analysis: Firstly, the negative control was not exposed to UVB radiation whereas the positive control was exposed to UVB radiation; neither were in contact with antioxidants. The negative control and the positive control have significantly different viability rates, the negative with an average of 97.10% and the positive with an average of 45.35%. This data is vital to this experiment as it shows that the exposure of cells to UVB radiation does in fact significantly damage the cells and reduces the viability of cells. Secondly, in regards to the antioxidant infused cells, Zinc Oxide had the greatest viability rate, followed by Ascorbic Acid, followed by Vitamin E. They all had significantly higher viability rates compared than the positive control, showing that they all had protective properties. However, since the error bars, which were set at $\pm 5\%$, between Zinc Oxide and Ascorbic Acid overlap, they are not significantly different from each other in their protective capabilities against UVB radiation. Lastly, standard deviation, standard error, and 95% confidence interval were calculated on the averages for each sample solution. All of these numbers are very low showing that the data was consistent and is valid.

Experimental Errors

- Potential experimental errors are possible. One possible error is the variability of the exposure of the cells to other elements, such as oxygen, which could also affect viability rates. Another possible error is the possibility of the color-tainting of the solutions to absorb radiation.

Practical Application

This project helps to identify which natural antioxidants are the best protection for one's skin in order to assess which foods have optimal health benefits. Since skin cancer is caused by mostly UVB damage and since yeast cells have a similar cellular anatomy to human skin cells, this experiment also identifies the antioxidant capabilities in regards to the reduction of the chances of skin cancer. This experiment also practically allows one to measure and quantify the degree of defense capabilities against radiation of these natural substances.

This methodology also defines a new model of studying ultraviolet-related damage to skin cells and how this experimentation is able to test natural or chemical compounds that may be able to protect them from such damage.

Conclusion

As stated in the first hypothesis, the culture with Zinc Oxide will have higher viability rates compared to Vitamin E, but Zinc Oxide will have lower viability rates when compared to Ascorbic Acid. The experimental data partially supported this hypothesis. The results showed, based on the viability rates calculated, that Zinc Oxide was indeed significantly more effective at protecting the cells against radiation compared to Vitamin E; however, Zinc Oxide and Ascorbic Acid are not significantly different from each other in their protective capabilities against UVB radiation.

The second hypothesis of this project was supported. All the cultures that are tested with antioxidants indeed did have higher viability rates to some degree compared to the positive control and had lower viability rates compared to the negative control.

According to this experiment and its results, consuming foods or applying ointment onto the skin that are rich in Zinc Oxide or Ascorbic Acid provides the most amount of protection from radiation which is beneficial in reducing chances of skin cell damage caused by radiation.

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