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ARTICLE

The Role of Geroprotectors on Cellular Senescence in Ribbon Plants

By: Maitri Shah, Edited by: Alena McFaul Freeman, Designed by: Ahmed Nadeem

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I. INTRODUCTION

1.1. Biological Aging and Age-Related Disorders

Biological aging is measured by the amount of changes that have occurred at a cellular level in the body, making a person more susceptible to diseases and infections (Frothingham, 2019). This form of aging is dependent on an organism's development and the accumulation of senescent cells in vital tissues of the body (Jeyapalan et al., 2007). As senescent cells are cells that do not perform basic cellular functions and instead promote inflammation and deterioration, they present in the form of numerous external and internal complications (i.e., hair loss and compromised immunity).

Age-related disorders are those that can be seen with an increasing frequency when senescent cells begin to accumulate in the body (Kaur & Farr, 2020). Though every human ages, not all will experience the same age-related disorders due to different biological aging processes.

1.2. Cellular Senescence

Cellular senescence is the process by which somatic cells enter a state in which they are unable to function correctly, resulting in negative effects throughout the body (Campisi & Fagagna, 2007). During DNA replication in the stage before mitosis, the entirety of the DNA strand is unable to be replicated and bits of both ends are lost in the process. Due to this, DNA strands have telomeres (repeated nucleotide sequences that act as protective caps during replication) at the ends, which are lost during replication instead of the important coding segments of DNA. However, after a certain number of mitotic divisions (dependent on the cell type and organism), the telomeres become too short and any further division will result in coding segments of DNA being lost or damaged (Deursen, 2014). At this point, it is said that the cell has reached a stage of senescence. Not only are senescent cells unable to divide for the growth of organs and tissues, but they also release proinflammatory cytokines into healthy tissues and are known to cause the initial stage of many age-related disorders (Kreshin, 2020). Senescent cells are ultimately the primary cause of aging and age-related disorders due to their accumulation in certain organs and tissues which prevents proper bodily functioning.

1.3. Geroscience and Geroprotectors

As humans age, their bodies begin to deteriorate at an increasing rate. While aging itself is not a disease, it does cause most diseases to have significantly more harmful effects on the body (Adams, 2004). Geroscience is the study of biological aging and age-related diseases. Its goal is to determine methods of slowing down the process of deterioration due to aging ("Geroscience:", n.d.). Geroprotectors are agents that can either help delay the body's aging process or increase its resistance towards damage due to aging (Moskalev et al., 2017). While the mechanisms behind geroprotectors are not entirely understood, it is likely that they lengthen telomeres or act in anti-inflammatory methods, essentially delaying the formation of senescent cells, to increase healthspan (Kreshin, 2020).

1.4. Objectives

The purpose of this project is to determine whether various potential geroprotectors, especially those that are natural, are successful in delaying plant cellular senescence. Groups of plants were given mixtures with aspirin, spinach, and turmeric, all of which are potential geroprotectors, and forcefully deteriorated. Through this, the best geroprotector for delaying the aging of cells in ribbon plants was to be determined; a greater amount of deterioration in plants signifies increased cellular senescence, thus indicating that the geroprotector in question was unable to effectively delay senescence.

II. HYPOTHESIS

Based on previous research, it is believed that geroprotectors will delay cellular senescence (Kim & Kim, 2019). Therefore, when plants are deliberately stressed and mitosis is occurring more frequently to repair damage and carry out regular cellular functions, the presence of geroprotectors should delay the rate at which the mitotically dividing cells reach senescence.

Specifically, it was hypothesized that plants given aspirin will deteriorate the least. This is because aspirin is a proven geroprotector that has been shown in numerous studies to delay cellular senescence and increase the longevity of organisms (Lushchak et al., 2021). Moreover, it was expected that plants not given any geroprotectors will deteriorate the most because the lack of geroprotector presence would result in cells reaching senescence without interference (since a deliberate stress is placed on the plants, the rate of mitosis, and therefore senescence, is increased). It was believed that turmeric would cause the second least amount of deterioration in plants (after aspirin) because of the presence of curcumin, which is considered to geroprotective qualities, along with anti-oxidative and anti-inflammatory properties (Gunnars, 2021). Spinach contains magnesium, nitrate, and niacin, which, though are not known to have the added properties of curcumin, are believed to contain qualities of geroprotectors; it was therefore hypothesized that plants given spinach will deteriorate more than those given turmeric, but less than those in the control group.

III. MATERIALS AND METHODS

3.1. The Usage of Plants

Plants were used as a model of humans in this study, as their cells have highly similar structures and processes of reaching senescence to those of humans. Since both cell types are eukaryotic and the major differences between the two are not relevant in this case (main relevance for senescence is the presence of a nucleus, DNA, and telomeres), both are comparable. Plant cells have also been found to reach a stage of cellular senescence in a process similar to that of human mitotic cells (both cell types must divide via mitosis for organism development). As this study focuses solely on the ability of cells to reach senescence, plants can be considered effective models for testing.

More specifically, ribbon plants were used in this study due to their simplistic structure and asexual reproductive nature. Ribbon plants reproduce through the process of asexual reproduction, in which their runners are replanted to grow into identical plants; thus, they are primarily made up of cells that undergo mitosis (as opposed to having additional cells that undergo meiosis for sexual reproduction). The primarily mitotic cells of the ribbon plants can be directly correlated to the mitotic cells of humans that undergo cellular senescence.

3.2. Materials

For this project, 12 ribbon plants that were planted on the same day and grown under the same conditions were obtained. The three potential geroprotectors tested were turmeric, spinach, and aspirin. Every four days, one 325mg tablet of aspirin and 25mL of tap water were required for each plant in group D, 25mL spinach paste (½ spinach and ½ tap water) were needed for each plant in group C, approximately 25mL turmeric paste (25mL tap water and ½ teaspoon turmeric) was required for each plant in group B, and 25mL of tap water was used for each plant in group A. A spoon to stir each solution (three spoons every four days) and a fork in order to poke holes in the soil (four forks every four days) were also required. Additionally, a ruler was used to measure height.

3.3. Deliberate Stress on Plants

Since the study was based on the deterioration of the plants, the ribbon plants were deliberately stressed throughout the entirety of the experiment. To elaborate, they were kept at a slightly colder temperature than ideal, watered every four days instead of every two to three days, and provided an insufficient amount of light. This was done because the project was completed on a short time-scale and it was required for the plants to reach a visible level of senescence faster than normal for the study. Deliberately stressing the plants causes cells to die and thus, other cells are more likely to proliferate and replace them faster (Burton, 2008). This would increase the speed at which the cells reach senescence because they are proliferating at a greater rate.

3.4. Procedure

Twelve one-month-old ribbon plants grown on the same day and under the same conditions were obtained and divided into four groups of three (groups A, B, C, and D). All plants were placed in the same location: an environment with a mediocre natural light source and room temperature conditions. Each group of plants was watered with a specific solution: those in group A were watered with 25mL of tap water each; those in group B were given a solution of 25mL tap water and ½ teaspoon turmeric; those in group C were given 25mL of spinach paste with the proportions of ½ spinach to ½ tap water; and those in group D were given a solution of 25mL tap water with one crushed 325mg tablet of aspirin. Before watering, a fork was used to gently poke holes at the surface of the soil of each plant to allow more nutrients to reach the roots.

Plants were watered every four days. After watering, the height**, condition of soil, colour of plant, and number of leaves for each plant were recorded; a picture of each group was also taken to compare qualitatively. This routine lasted for approximately two months (17 days of watering and measurement), after which observations were analyzed for conclusions.

Figures 1-4 show exact values of the heights of each plant in their respective groups over time (measured every four days). The trendline for individual plants represents the trend of deterioration in each group; the slope of that trendline shows the rate of change of height. The average of the slopes for plants in group A (control; given plain water) was -4.73cm, the average for group B (turmeric) was -2.07cm, the average slope for group C (spinach) was -3.40cm, and the average for group D (aspirin) was -6.03cm.

Figure 5 represents the percentage of change in average height of each group of plants over time. Values at each point were determined by calculating the mean height in a group on a single day, after which the percentage change of that height compared to the starting mean (100%) was determined. Such a method was utilized to ensure that each of the plants would be compared from the same baseline. The plants in group A (control) ended with approximately 73.51% of their original height, those in group B (turmeric) were at 89.07% of their original height, those in group C (spinach) were at 81.52% of their original height, and the plants in group D (aspirin) ended at 67.21% of their original height.

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**Note: For the measurements of height, the height of the plant was measured beginning at soil level and up to the highest point on the leaves as is (no manipulation to stretch leaves).

IV. RESULTS

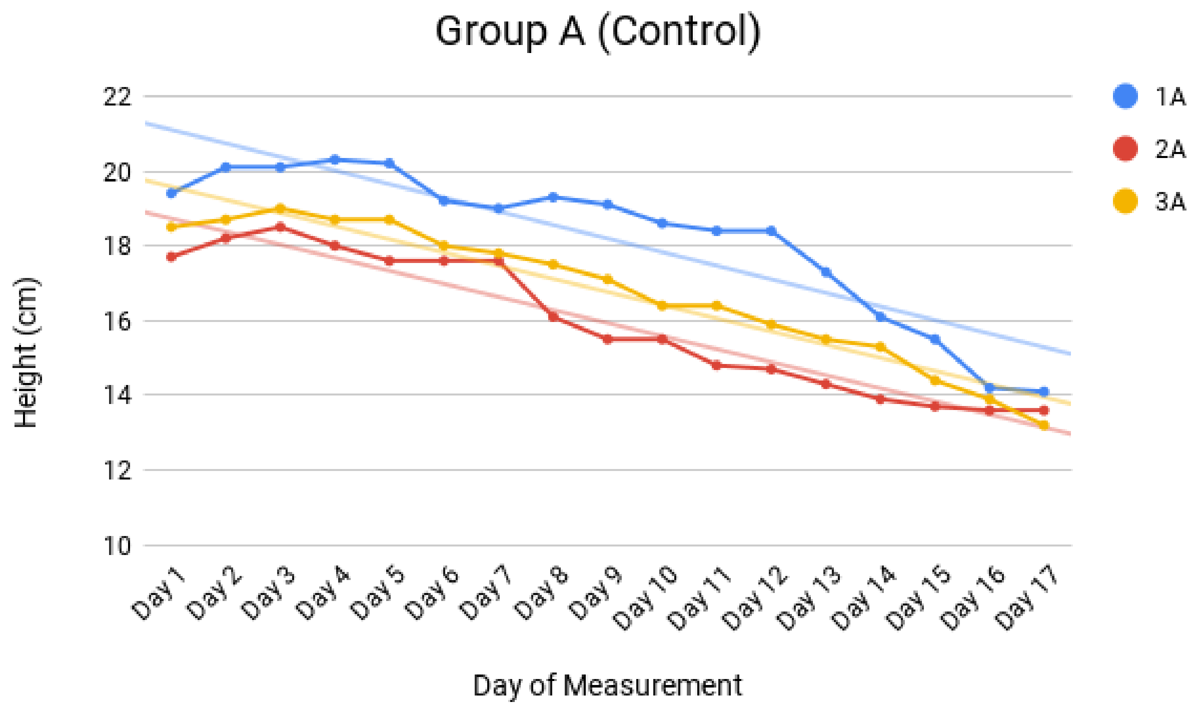

Figure 1: Heights of plants in group A (control) over time

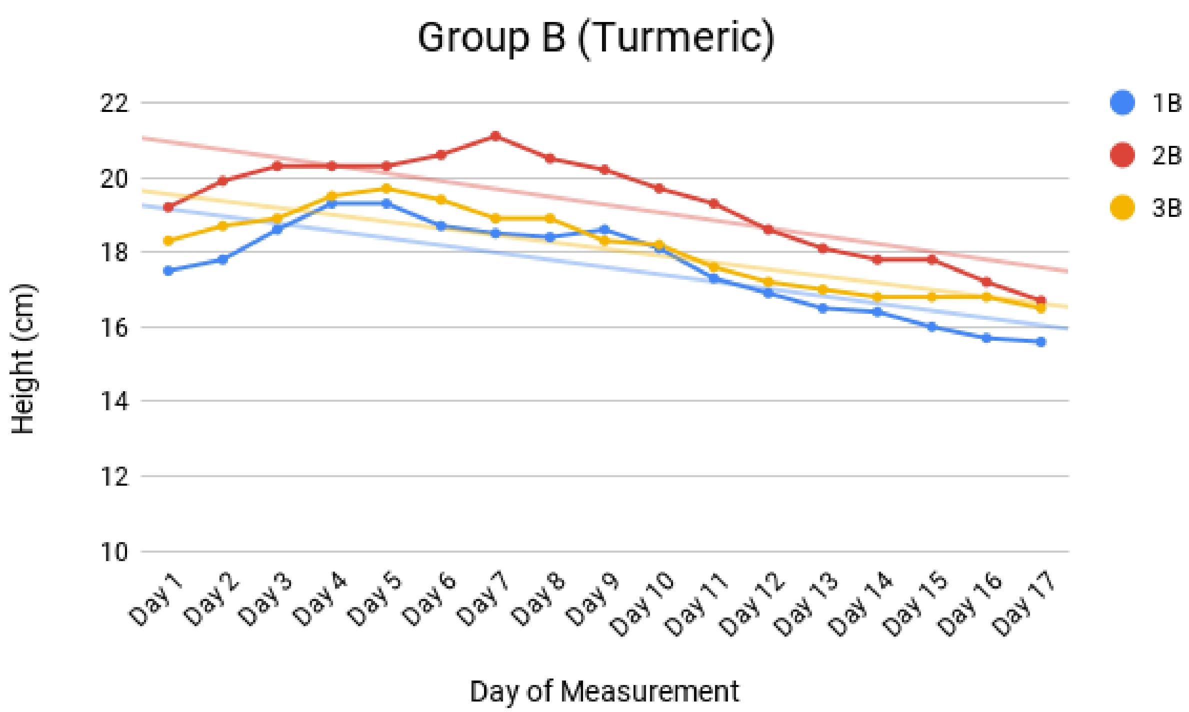

Figure 2: Heights of plants in group B (turmeric) over time

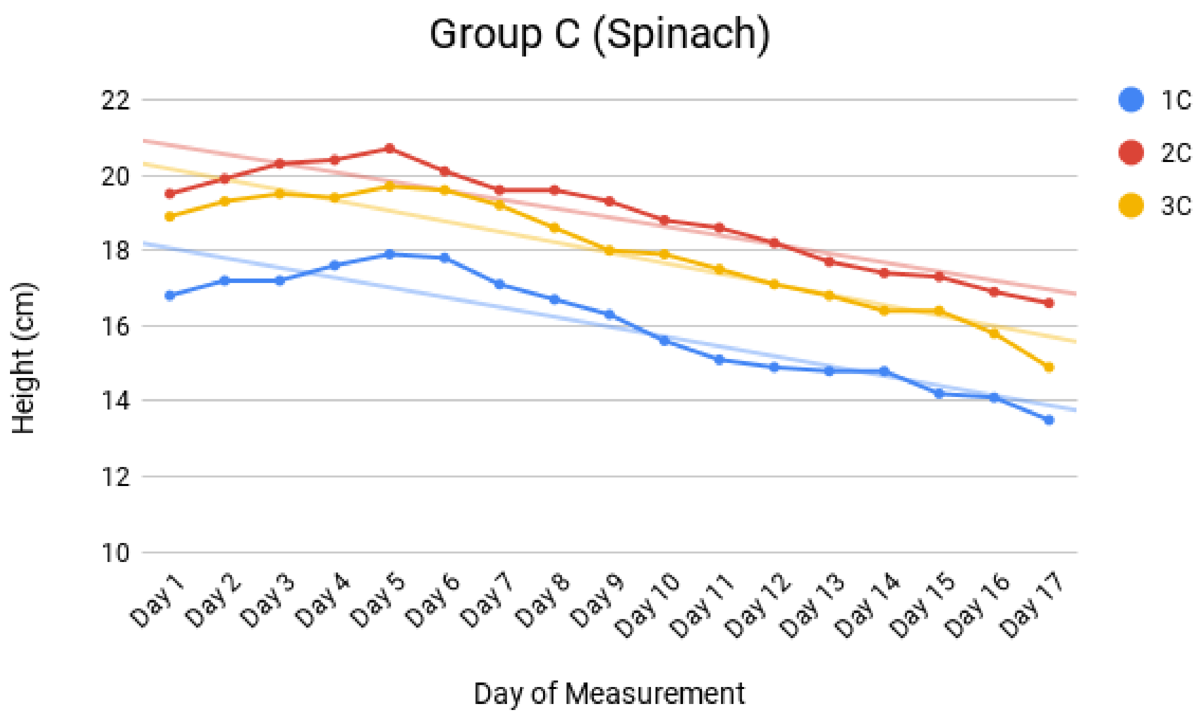

Figure 3: Heights of plants in group C (spinach) over time

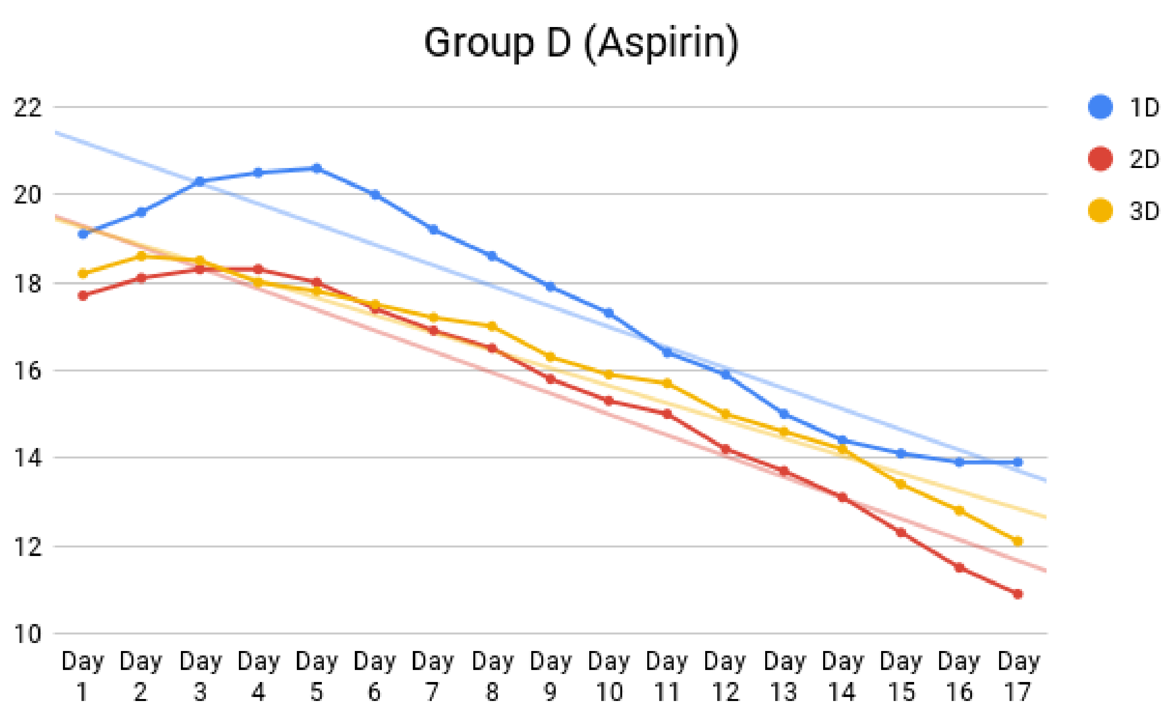

Figure 4: Heights of plants in group D (aspirin) over time

respective groups over time (measured every four days). The trendline for individual plants represents the trend of deterioration in each group; the slope of that trendline shows the rate of change of height. The average of the slopes for plants in group A (control; given plain water) was -4.73cm, the average for group B (turmeric) was -2.07cm, the average slope for group C (spinach) was -3.40cm, and the average for group D (aspirin) was -6.03cm.

Figure 5 represents the percentage of change in average height of each group of plants over time. Values at each point were determined by calculating the mean height in a group on a single day, after which the percentage change of that height compared to the starting mean (100%) was determined. Such a method was utilized to ensure that each of the plants would be compared from the same baseline. The plants in group A (control) ended with approximately 73.51% of their original height, those in group B (turmeric) were at 89.07% of their original height, those in group C (spinach) were at 81.52% of their original height, and the plants in group D (aspirin) ended at 67.21% of their original height.

V. DISCUSSION

5.1. Analysis of Figures

The average slopes of plants, as shown in Figures 1-4, demonstrate that the greatest decrease in height (and therefore, greatest deterioration) was experienced by plants in group D (aspirin). The average rate of change of height can be used to order the groups from least to greatest deterioration: group B (turmeric), group C (spinach), group A (control), group D (aspirin). As the success of a geroprotector in delaying cellular senescence would result in less deterioration, these results indicate that turmeric is likely the strongest geroprotector from those studied, with spinach following it.

The results from Figure 5 confirm that deterioration occurred from least to greatest in the aforementioned order. The lack of deterioration in group B (turmeric) indicates that it is likely the strongest geroprotector out of the three studied, and since it faced less deterioration than the control, it can also be seen that the geroprotective properties of turmeric (and spinach) are effective in delaying cellular senescence in ribbon plants.

5.2. Applications

The usage of geroprotectors, or any other agents that have the potential to increase human lifespan, is a highly relevant field of research. This is because the increasing prevalence of age-related disorders in the population is not only detrimental to a large group of people, but also places a huge burden on the healthcare system (Glossmann & Lutz, 2019).

The results of this study can be utilized to promote easily available substances as geroprotectors because of their ability to delay cellular senescence. As deterioration in plants, especially in regards to tissue and leaf decay, is caused by an increase in cell senescence, plants that have deteriorated less in this study would have experienced a decreased amount of senescence due to the effects of the added geroprotectors (Khan et al., 2014).

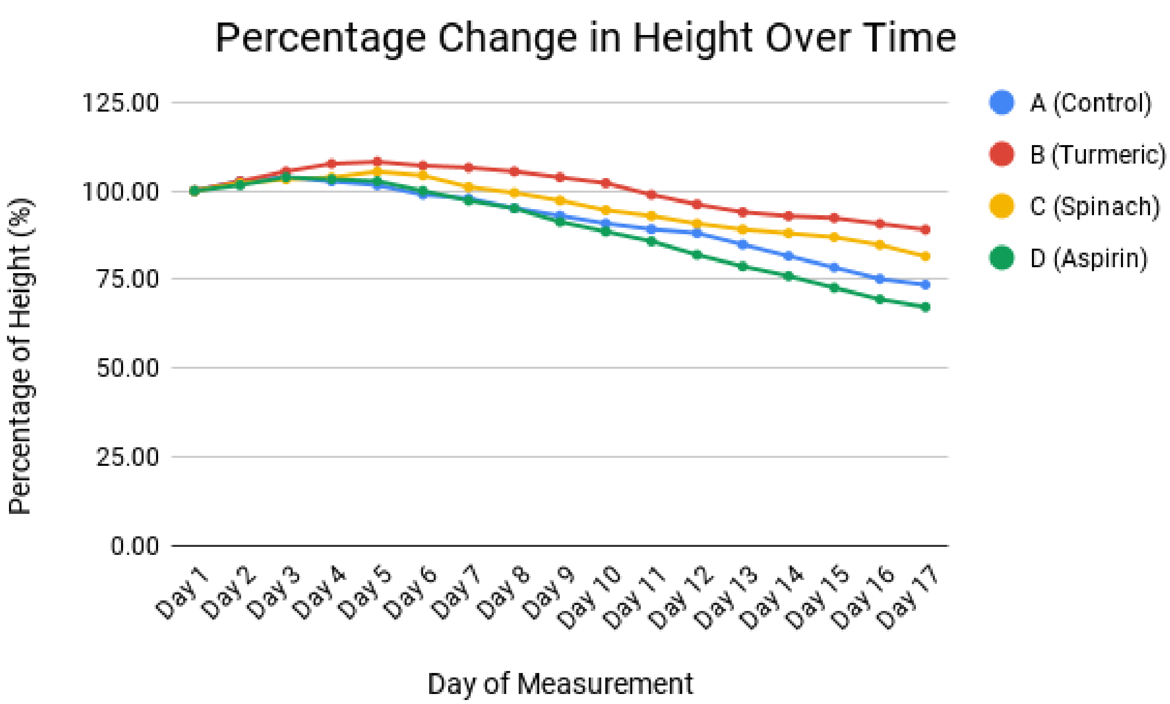

Figure 5: Percentage of change over time of average height in each group from initial

It was found that turmeric caused the least deterioration, as the average height in group B (turmeric) deteriorated least, indicating that this substance is likely best for delaying cellular senescence. Therefore, it is highly advised to consume increased amounts of turmeric. Furthermore, the primary agent in turmeric, curcumin, has a wide variety of other health benefits; it is an antioxidizing, immunomodulatory, and cardio-protective agent with properties that protect the body in numerous ways (Vaiserman et al., 2020). After turmeric, spinach was found to be the next best at reducing deterioration in ribbon plants, likely by delaying cellular senescence. It is thus advised to also increase portions of spinach in the diet. Along with this property, spinach is beneficial because it contains nitrates, which help prevent insulin resistance; it also contains beta-carotene and the antioxidant Vitamin C, both of which protect against the formation of cancer cells (Li et al., 2016; "Cut", 2021).

On the contrary, it seems that aspirin sped up cellular senescence in the plants, as they deteriorated at a rate faster than those in the control group. While it is common knowledge that the pharmaceutical has benefits in certain cases, it is likely that too much aspirin is harmful and causes detrimental effects in the plants. Therefore, it is advised for humans to be wary of the amount of aspirin they are taking (Janssens & Houtkooper, 2020).

The results of this study provide the basis for the idea that delaying the process of senescence in plants can increase their longevity. As ribbon plants and humans both have eukaryotic cells with similar methods of reaching cellular senescence, the results can be carried over to humans. It is thus advised that humans eat foods rich in turmeric and spinach for the benefits of protection against cellular senescence and a lengthened healthspan.

5.3. Sources of Error

There are a few factors that limit the conclusions drawn by this study. Confounding variables, such as the exact amount of light and moisture received by each plant, likely exist and would impact the accuracy of results.

Sources of error may have decreased the accuracy of results. A major source of error is the amount of aspirin given to each of the plants in group D. Since aspirin is a painkiller, it can be very concentrated and also has detrimental effects (i.e., in humans it can cause nausea and acidity) that may be unknown in plants. Therefore, even if the pharmaceutical has geroprotective qualities, the plants would have experienced overall deterioration because of the overpowering harmful effects of such a high concentration of aspirin. In the future, it would likely be best to attempt this aspect of the experiment again with a lower dosage of aspirin given to the plants every four days (i.e., instead of one 325mg tablet, ¼ of the tablet can be used). Another source of error that must be considered is the possible effect on plants that deliberate deterioration may have caused. For instance, stressing the plants in regards to light, water, and temperature of their environment impacts their cellular cycles; this may have altered the level at which they reach senescence or of their speed undergoing mitosis, both of which could affect the results.

VI. FUTURE STEPS

If the study were to be done again, the sources of error outlined above would be controlled as best as possible. In addition, it would be interesting to study other potential natural and artificial geroprotectors; they can also be compared to the ones studied in this experiment to understand which is best. Furthermore, it would be interesting to test combinations of geroprotectors, such as turmeric and spinach, on plants and discover whether or not they have an increased combined benefit. Along with this, the study should be repeated on more complex organisms, such as mice, that are more closely related to humans. Ribbon plants were used for this experiment because of their simple structure and asexual reproductive system (cells only undergo mitosis); however, human beings have much more complex structures and cells that undergo both mitosis and meiosis, so it would be pertinent to repeat the study with this in mind.

VII. CONCLUSIONS

After the completion of the experiment, one can conclude that natural geroprotectors, such as turmeric and spinach, are more effective in delaying cellular senescence in ribbon plants than pharmaceutical drug-based ones (in this case, aspirin). This is because it is likely that the drug-based agents also have some added toxic effects on the plants when used in excess. On the contrary, natural substances are likely to be much safer, as seen in the results of this study.

It was stated in the hypothesis that the plants given geroprotectors would deteriorate less than the control group (those given plain water) because the geroprotectors would act to delay cellular senescence. Previous research provided the belief that the plants given aspirin will deteriorate the least, then turmeric, then spinach, and that those in the control would deteriorate the most because of the strength of the geroprotectors given. The theory postulated in the hypothesis was proven correct to an extent, but the order of effectiveness of geroprotectors was assumed incorrectly. The results actually showed that plants given turmeric deteriorated the least, then spinach, then plain water, and finally, those given aspirin deteriorated the most.

Overall, results indicate that the usage of natural geroprotectors is highly beneficial and thus, the increase of turmeric and spinach in the human diet should be promoted. Further studies on organisms more closely related to humans and testing an increased variety of potential geroprotectors would allow for this to be proven more accurately.

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ABOUT THE AUTHOR - MAITRI SHAH

Maitri Shah is a grade 12 student at Westmount Charter School in Calgary, Alberta. She has a keen interest in the field of STEM, especially in the health sciences. Maitri demonstrates this interest by competing in numerous science fairs, researching at University of Calgary labs, and participating in science-related youth programs. Her love for inquiry has enabled her to actively ask questions and experiment, qualities which help her succeed in anything to which she sets her mind. Maitri is also passionate about giving back to the community, which is why she has started Work 2 Unify, an international youth philanthropy organization. Outside of academics, she enjoys playing basketball and teaching herself the guitar.