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The Effects of *Momordica Charantia* on the Regeneration of Dugesia Tigrina

Research Article

Miriam Yushavaiev[1]*, Areej Amir[1], Felicity Liu[1]

1 Francis Lewis High School, 58-20 Utopia Pkwy, Fresh Meadows, NY 11365

Abstract: *Momordica Charantia*, also known as bitter melon, is a unique fruit found in subtropical and tropical regions worldwide. The plant is a long vine that belongs to the *Cucurbitaceae* family, and it also has a characteristically bitter taste. Bitter melon is rich in nutrients such as vitamin A, vitamin C, iron, and phosphorous, as well as multiple active chemicals, phenolic acids, and flavonoids. All of these traits make bitter melon useful in medicine as an anti-diabetic, an anti-inflammatory agent and as a wound-healing agent; however, its effects on regeneration have not been explored. Planaria are freshwater flatworms that are known for its ability to regenerate. Their stem cells are unique because they have adult stem cells that express pluripotency. This experiment can further test the potential of the bitter melon extract on stem cell regeneration so that they can be better-cultured outside of the body in large numbers. The planaria were fed for a week, then were amputated, and divided into five groups. Each was treated with different concentrations of bitter melon. The length and weight were measured every other day and the data was put into excel. In the first trial, the results came out insignificant because there were a lot of errors in the trial and three of the groups died. This happened due to improper adherence to the method. In trial 2, Group C heads and tails were significantly smaller than the other groups; because all of the planaria in the Group C heads died in the middle of the data collection process possibly due to an inconsistent feeding schedule or anxiety from exposure to the light. In trial 3, all of the length and weight data in this trial was insignificant, except for in day 5, where Group A appeared significantly larger than Group B. In this trial the percentage of regeneration over time was measured and it appeared that for the tails, Group B, D, and E all reached 100% regeneration before the control, and for the heads, Group C showed complete regenerated planaria the earlier than all of the other groups In conclusion, bitter melon does not have an effect on the efficiency of regeneration as seen by the insignificant results of length and weight in trials 1-3; however, it does have a positive effect on the rate of regeneration according to the data found in trial 3. In the future, more consistent trials, with consistent feeding times and measuring times should be done to have more consistent data with fewer sources of error. The sample size of the planaria can also be increased to gain more reliable results, and the planaria can be exposed to light less often to reduce their anxiety.

Keywords: Bitter melon • Regeneration • Stem Cell • Planaria

* *E-mail: mby8459@gmail.com*

1. Introduction

1.1. Planaria

Planaria are flat and soft-bodied, free-living worms in the phylum Platyhelminthes, otherwise known as flat worms, in the order tricladida. The word "planaria" is a colloquial term referring to any organism in the tricladida class. Planaria, especially freshwater species are inexpensive and easy to keep and maintain in labs [8]. They are also a model organism for bacterial resistance because of their ability to resist certain bacterial strains such as Staphylococcus aureus and Mycobacterium tuberculosis [1]. Additionally, they are commonly used as a model system to study tissue homeostasis and stem cell biology due to their unique ability to regenerate using pluripotent stem cells. In fact, many studies were done to map out the planarian genome to see how much of a part genetics contributes to the ability of regeneration [2]. Planaria regenerate due to their abundance of adult stem cells, which are called neoblasts. When these neoblasts are transplanted into another worm originally lacking in adult stem cells, the transplantation would be successful, and even rescue the worm from a fatal injury [10]. This shows that these neoblasts are pluripotent, meaning that these adult stem cells differentiate into all types of cells, similar to embryonic stem cells. They are also likely totipotent, which means they most likely have the ability to differentiate into all cell types including extraembryonic tissues [8]. When a part of a planaria is amputated, the neoblasts will proliferate, and form a blastema, that will eventually differentiate into the different cell types that the planaria had previously lost. The pluripotent stem cell regeneration ability that planaria exhibit has always fascinated scientists, especially when the stem cells that adult humans possess only have limited potential, with the exception of embryonic stem cells [8].

1.2. Stem Cells

There are two types of stem cells found in humans, adult stem cells and embryonic stem cells. These two types of stem cells each have their own benefits and drawbacks. For example, embryonic stem cells are pluripotent, and the process of growing them outside of the body is fairly simple and efficient. Adult stem cells have limited potential, meaning that they can only differentiate into certain cells, depending on where the adult stem cells are found. They are also found in much smaller numbers, as opposed to embryonic stem cells; however, the use of embryonic stem cells has caused ethical controversy regarding its use. This has led some to focus more on adult stem cell research and more advancements in this specific field [4].

Embryonic stem cells can be cultured in Petri dishes and grown in a culture medium. Culturing embryonic stem cells can be difficult, as they can form embryonic bodies and begin to differentiate spontaneously and uncontrollably. This problem is resolved by coating the inner surface of the Petri dish with a feeder layer, so they are still able to divide and multiply while remaining unspecialized. Once one Petri dish has acquired enough cells, the excess cells are gently transferred to other Petri dishes, where they can also divide and multiply. When a cell is required to differentiate into a specific type of cell, they would change the composition of the culture medium

in order to stimulate specialization. It was through many years of experimentation, that scientists are now able to have a direct influence on the differentiation of embryonic stem cells [4].

Adult stem cells are found among specialized cells in the human body. They are quiescent, meaning that they do not divide unless neighboring tissue that may have died, disease, or tissue injury triggers them. They are also thought to occupy a specific stem cell niche in the organs and tissues that they are found in. These places include the brain, bone marrow, liver, skeletal muscles, and many more. Scientists are constantly finding new places in the body where stem cells can be found. However, once these stem cells are removed from the body, it can be very difficult to manipulate its specialization because of its very small number, and its capacity to divide is limited. This makes adult stem cells very difficult to culture, unlike embryonic stem cells [4].

The limited capacity for adult stem cells to divide, once outside the body, makes further research into the potential of adult stem cells limited. In this experiment, the adult stem cells of planaria are used as a model to see how the bitter melon extract can affect its regeneration rate. This practice could potentially be used on human adult stem cells, so that they can divide once removed from the body, and be better cultured [4].

1.3. Bitter Melon

Momordica Charantia also known as bitter melon, bitter gourd, balsam, bitter apple, and Carilla, is a unique fruit found in subtropical and tropical regions of South America, Asia, the Caribbean, and some parts of Africa [5]. The plant is a long vine that belongs to the Cucurbitaceae family. The fruit-vegetable is an oblong cucumber shaped plant. Over a dozen species of bitter melon are known and each species has a different texture and appearance. Some bitter melons are tart and sour while others are strongly bitter. All parts of the bitter melon plant can be used for medicinal purposes, such as the leaves and vines; however, it is the fruit that is the most commonly used part because it is rich in nutrients such as vitamin A, vitamin C, iron, and phosphorous. The fruit/vegetable of the bitter melon is edible and, as the name suggests, it has a bitter taste. It is either cooked or drank in a liquid form. Due to its antiviral properties, bitter melon can also be used to treat herpes and HIV. It also possesses contraceptive and laxative properties. Studies show that bitter melon is able to help regulate blood glucose levels in patients with type 2 diabetes. Due to this ability, it can also help reduce weight, as seen in an animal study [5]. Bitter melon also has potential immunosuppressive effects such as decreasing the inflammation of cells and inhibiting cell growth in cancer cells [7].

At least 32 active chemicals are in bitter melon. Studies show that certain phenolic acids and flavonoid composition in bitter melon give it anti-diabetic and anti-cancerous properties. These compounds include acids such as Gallic acid, tannic acid, catechin, caffeic acid, p-coumaric, gentisic acid, chlorogenic acid, and epicatechin. Bitter melon also helps in treating skin conditions such as eczema, scabies, and psoriasis. It also helps in getting rid of kidney stones and gout [6]. Bitter melon even has potential in wound healing. For example, a study done on the effects of bitter melon extract on wound healing in rabbit skin shows that the extract is able to speed up the wound healing process by stimulating proliferation of fibroblasts and epithelization [6]. The potential in

wound healing that bitter melon shows could also mean that it has a potential in increasing the regeneration rate of stem cells. Since in wound healing, the bitter melon stimulated proliferation of fibroblasts and other connective tissues, the bitter melon could also possibly stimulate the proliferation of blastemas in planarian stem cells, and even maybe human adult stem cells.

1.4. Wound Healing vs.Regeneration

Wound healing is a process in which missing tissue or cell structures are replaced, similar to how planaria use regeneration to regain missing tissues or even essential body parts. In adult humans, uninterrupted wound healing is a complex process involving 4 broad stages: hemostasis, inflammatory, proliferative, and maturation phase. Within these stages there are even more stages involving processes such as epithelization and angiogenesis. Other processes such as fibroblast migration, blood clots and the wound epithelium formation are also seen in the regeneration process. All of these are key mechanisms, which are found in both regeneration and wound healing. However, there are differences such as blastema formation in regeneration and scarring in wound healing. As well as the types of cells that goes through each process. In regeneration, stem cells perform blastema formation and stem cells are unspecialized. While in wound healing, the cells are specialized, and can only divide and develop into one type of cell [3].

1.5. Previous Published Studies

Since this project has never been published in a journal before, it is hard to find a study that could help predict the outcome of our results. However, there are studies that link bitter melon and its contents to wound healing, a process related to regeneration. [6]tested the effects of Momordica charantia on the wound healing of rabbit skin. In this experiment, the rabbits are given an excision wound under anesthesia. Then the experimental group received either topical creams such as nitrofurazone, or a bitter melon olive oil mixture. At the end of the 28 days, the rabbits whose wounds were treated were macroscopically and microscopically analyzed. The data was also histopathologically scored. In the macroscopical analysis, the bitter melon group had the highest area of re-epithelialization and had the lowest non-epithelialized area. The microscopic analysis reveals that the bitter melon groups also have a higher epidermis than papillary dermis. The fraction of epidermis was also greater than the other groups. The bitter melon group also displayed a higher mean density of fibroblasts and a higher fraction of fibroblasts to reticular dermis. Lastly, the histopathological scores determined that the bitter melon group had the least amount of acute inflammation and granulation tissue, as well as the most fibroblast maturation, collagen deposition, and neovascularization. Overall, these results indicate that the bitter melon is the most successful in the wound healing.

The experiment done by [9] tested the effects of Vitamin A on impaired wound healing. Two groups of rats were injected with streptozotocin to induce diabetes. The control group was injected with a saline solution. The first group of rats was the control group. The second group was experimental with no vitamin A and the third

group was the experimental group with Vitamin A. Then the control group and second group were fed laboratory made chow whereas, the third group was fed with a vitamin A rich diet. The rats were then given incision wounds on the 29th day. The vitamin A group displayed a quicker time frame compared to the diabetic group and was less prone to abrasion or cuts making the rat more impervious against wounds. This experiment is relevant because bitter melon does have vitamin A in it and since vitamin A has wound healing potential it could mean that the vitamin could attribute to bitter melons wound healing potential. This experiment further supports the potential bitter melon has in wound healing, and makes the possibility that it has potential in regeneration rate also stronger.

Another experiment was done by [11], which tested vitamin C on stem cell sheet formation and tissue regeneration. First, the experiment called for a collection of human, and guinea pig molars/canines. Then the scientists collected human bone marrow from the spine and umbilical cords. From the teeth, the scientists extracted periodontal ligament stem cells and from the umbilical cords, human umbilical cord mesenchymal stem cells were extracted. Finally, from the bone marrow, human bone marrow mesenchymal stem cells were extracted. From these extracted cells, stem cell sheets were created and used in different tests. The stem cells were subjected to different assays where they were treated with vitamin C. Telomerase activity was also measured in the stem cells with vitamin C. Some stem cell sheets were placed inside live swine and mice after being subjected to vitamin C to test the effectiveness of sheet formation. The results from the live animal test reveal that periodontal ligament stem cells treated with vitamin C had a higher bone/cementum-like matrix. This means that the tissue regeneration was much faster than the regular stem cells. After a histopathological analysis, vitamin C not only helped tissue regeneration but bone regeneration as well. The telomerase activity is shown to be very high in periodontal ligament stem cells. Therefore the study speculates that if vitamin C does have a faster regeneration rate then the telomerase action could be the reason why. The study determined that for all the stem cell sheets the ideal concentration of vitamin C is 20 g/mL. Overall, the vitamin C induced sheets had a faster and more efficient regeneration than the control. The study concludes that vitamin C has a faster time rate in stem cell regeneration in periodontal ligament stem cells because of its increased telomerase activity. Bitter melon is a good source of vitamin C. These results could then help correlate bitter melon to a regenerative quality and could result in positive results for our experiment. Furthermore, if this experiment is effective in speeding up the rate of regeneration of stem cells, then the bitter melon could also be tested to see if the certain components of vitamin c present in the extract attribute to the quality of regeneration.

Since Bitter melon has the potential to accelerate wound healing, it may have positive effects on regeneration. However, no one has expanded on its regenerative properties, especially not on planaria. This study will fill the gap. The results could also be used to solve the problem of the scarcity of adult stem cells in the human body. The application of bitter melon extract could help human adult stem cells cultivate outside of the body and multiply. The method is unique to this experiment since there are no experiments that utilize planaria and Bitter melon power. The method was created and refined through multiple trial and errors before and during the experiment

including the ratio for the bitter melon stock solutions and the incorporation of bitter melon solutions in the water of the planaria. Additionally, the care for the planaria was found in the guide that came with the planaria and the amputation was the standard planaria amputation found in most experiments.

2. Method

2.1. Preparation before Experiment

Before staring the experiment safety gear such as aprons, masks, and gloves were worn and hands were washed thoroughly. The counter was wiped down with 10 vol% bleach solution and then with water. Each Petri dish was filled with 25 mL of water, which was then filled with 30 planaria (10 per Petri dish) using a pipette. The planaria were fed three times a week with 0.4 g of ground beef for 30 minutes. After feeding, the planaria were transferred into a new Petri dish that was filled with 25 mL of fresh Poland Spring water. Each time the water was changed the old water was thrown into the sink and the old Petri dishes were thrown out. The process of feeding, changing water, and transferring was repeated every other day for two weeks.

2.2. Bitter Melon Treatment

The bitter melon stock solutions were made in various concentrations that are listed in Tab. 1. Stock solution B, was made by first dissolving 0.1g of bitter melon extract in 100 mL of spring water. This made 1 mg/mL stock solution. Then, this stock solution was diluted to 1 g/mL stock solution by adding 0.1 mL of stock solution 1 to 99.9 mL of spring water. This process was repeated for stock solutions A-E listed in Tab. 1.

2.3. Planaria Amputation

First, 10 plates were prepared and labeled (2/group) with the group number according to Tab. 1. In each group, one of the plates was used to store the heads, and the other plate was used to store the tails. Then, 20mL of the stock solution was poured into each plate. Stock solution A was poured into the plates labeled A, and likewise for the rest of the groups. After that, planaria were placed onto a separate Petri dish with 5 mL of spring water with a pipette. This Petri dish had ice underneath it so that the planaria would stay still during the amputation. The planaria were amputated by cutting laterally across just below the head with a scalpel,

Table 1. **Solutions Set-up**

Groups	Extract (μg/mL)	Vol of 1 μg/mL Stock Solution 2 (mL)	Vol of Water (mL)
A	0.0	0	40
B	0.1	4	36
C	0.2	8	32
D	0.3	12	28
E	0.4	16	24

which separated the planarians head and tail (Figure 1). Each group had six planaria, so when six planaria were amputated, a weighing boat was placed on a scale and the scale was tared. The six heads were placed onto the boat with a pipette, and the weight was divided by 6 in order to find the weight for each head. The heads were then placed into one of the labeled plates. The same was done to the six tails. This was repeated for each group. The length of each head and tail were recorded, and after 30 minutes, the planaria were transferred into 25mL of clean spring water in 10 new plates, all labeled properly. The process of treating the planaria for 30 minutes and measuring them was repeated every day for two weeks. The cut for the amputation can be seen in Fig. 1.

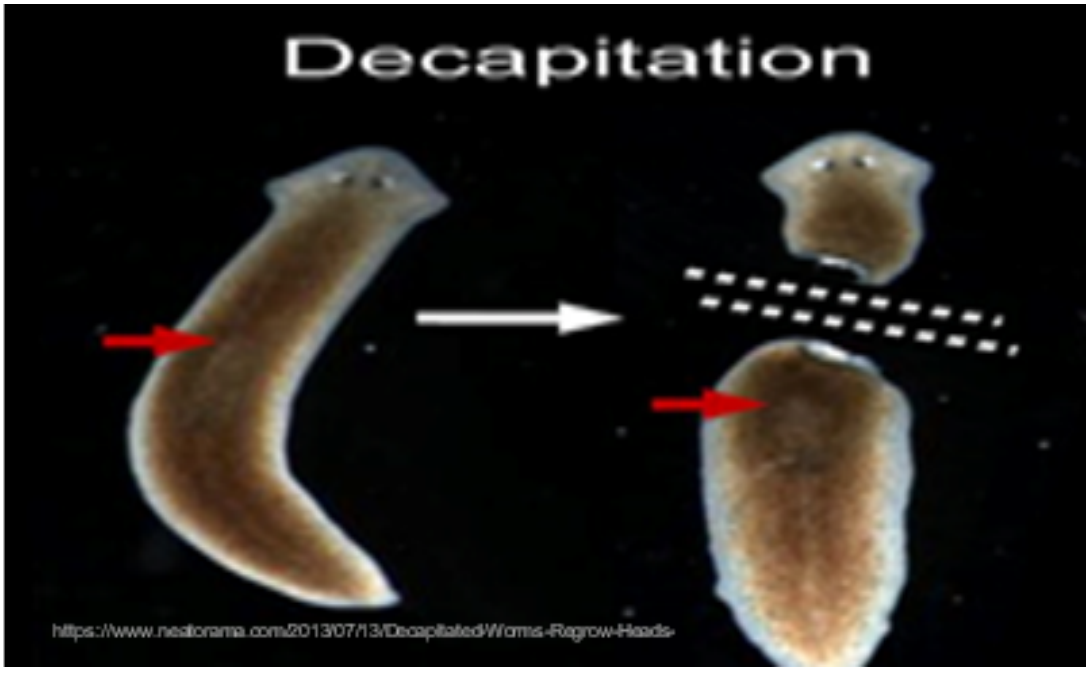

Figure 1. **Planaria Amputation: A transverse cut across the region below the head was done on the planaria**

2.4. Data Analysis

The data was collected by measuring the length and weight of the planaria's head and tail by using a ruler and electronic scale every day after treatment and was stored in Microsoft Excel. The data was plotted on a scatter plot with an x and y axis. The x-axis was labeled as the time that passed since the amputation, and the y-axis was labeled as the number of centimeters grown. Another scatter plot had the y-axis as the number of grams gained, and the x-axis as the time passed since the amputation. The data was also plotted on a bar graph. In the bar graph, the five different groups were labeled on the x-axis, and the average number of centimeters grown since the amputation was on the y-axis. Another bar graph was plotted with the same x-axis as the aforementioned bar graph, except its y-axis was the number of grams gained since the amputation. These bar graphs compare the effect of each group on the regeneration rate of planaria. An ANOVA test was done to compare the data from multiple groups, each with a different concentration of extract. The webpage

`http://astatsa.com/OneWay_Anova_with_TukeyHSD` was used to calculate the significant differences.

3. Results

Throughout the first week of feeding, in Trial 1, there were many errors. The ground beef spoiled, causing the planaria to miss a feeding day, this resulted in a very limited amount of planaria surviving and being able to take part in the study. Not only that, but a majority of groups died in Trial 1 because the method was not followed closely enough, and the stock solution was not diluted enough. The results of Trial 1 can be seen in Figs. 2-7. The long exposure to the strong concentrations of bitter melon extract, caused groups C, D, and E to die. No significant data came from this trial. More trials were necessary to make sufficient conclusions.

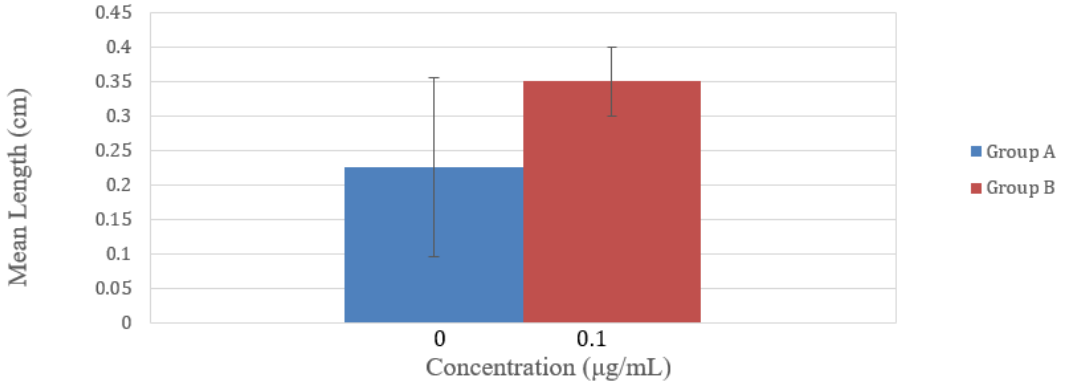

Figure 2. **Trial 1. Mean Length(cm) vs. Concentration($\mu g/mL$), Bar Graph. The values represent the mean length of the heads on the last day. Error bars represent standard deviation. Group A was the control group treated with 0$\mu g/mL$ concentration and Group B was treated with 0.1 $\mu g/mL$.**

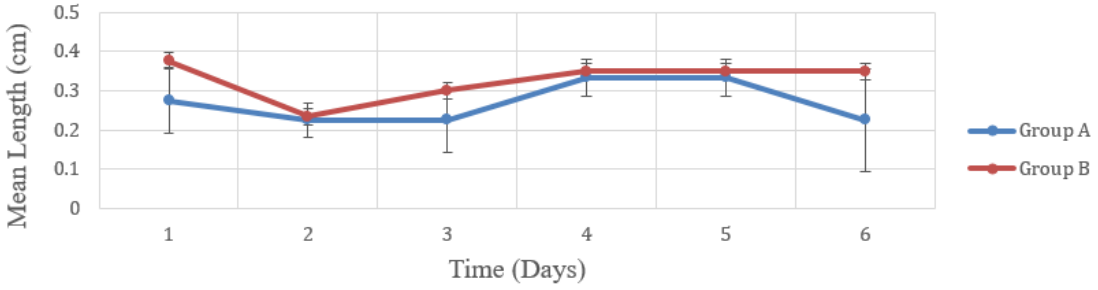

Figure 3. **Trial 1. Mean Length (cm) versus Time (days), Heads Scatter Plot. The values represent the means of the data. Error bars represent standard deviation. Group A was the control group treated with 0 $\mu g/mL$ concentration and Group B was treated with 0.1 $\mu g/mL$.**

For Trial 2, the results can be seen in Figs. 8-13. The heads group for Group C died on day three most likely due to the anxiety of being measured and weighed in the light and feeding was reduced to one week instead of two weeks because from the previous trial, it was determined that one week of feeding was sufficient. The shorter feeding time also could have helped reduce the chance of the food spoiling because the food would be kept and

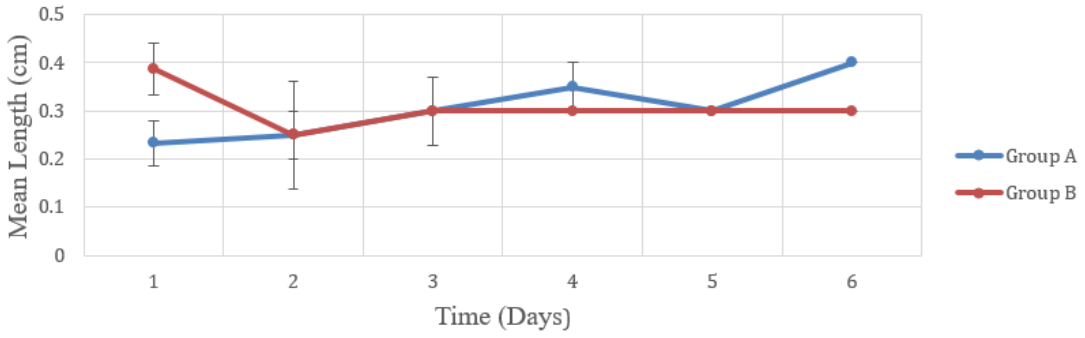

Figure 4. **Trial 1. Mean Length (cm) versus Time (days), Tails Scatter Plot. The values represent the means of the data. Error bars represent standard deviation. Group A was the control group treated with 0 $\mu g/mL$ concentration and Group B was treated with 0.1 $\mu g/mL$.**

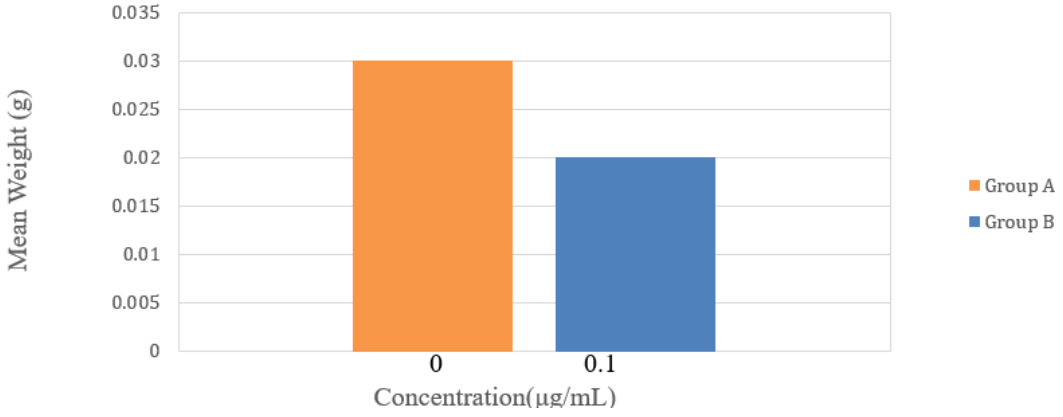

Figure 5. **Trial 1. Mean Weight (g) versus Concentration ($\mu g/mL$), Bar Graph.The values represent the mean weight of the heads on the last day. The weight was calculated for each group by taking the weight of the group and dividing it by the number of planaria weighed. Group A was the control group treated with 0 $\mu g/mL$ concentration and Group B was treated with 0.1 $\mu g/mL$.**

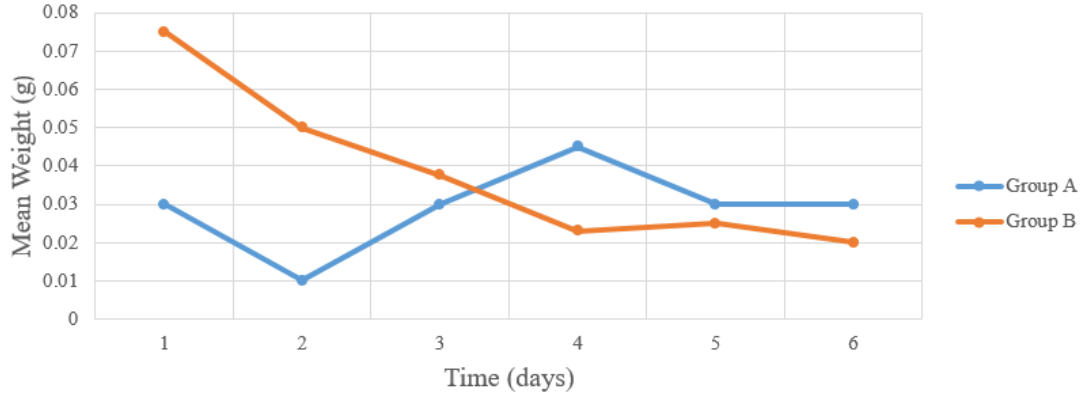

Figure 6. **Trial 1. Mean Weight (g) versus Time (days), Heads Scatter Plot.The values represent the mean weight. The weight was calculated for each group by taking the weight of the group and dividing it by the number of planaria weighed. Group A was the control group treated with 0 $\mu g/mL$ concentration and Group B was treated with 0.1 $\mu g/mL$.**

stored for a shorter period of time. The weight did not show a clear trend or correlation in the XY scatter plots and this could have happened because the weights were not measured accurately enough. Since Group C heads

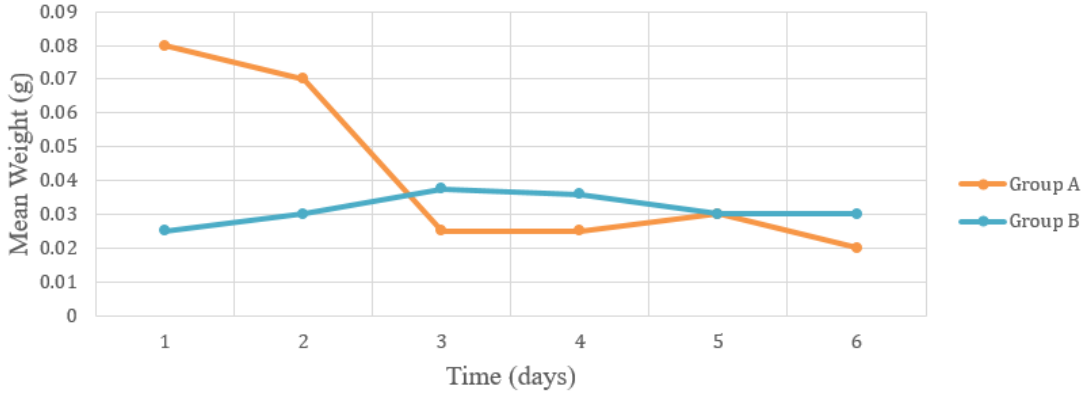

Figure 7. **Trial 1. Mean Weight (g) versus Time (days),Tails Scatter Plot.The values represent the mean weight. The weight was calculated for each group by taking the weight of the group and dividing it by the number of planaria weighed. Group A was the control group treated with 0 $\mu g/mL$ concentration and Group B was treated with 0.1 $\mu g/mL$.**

died by the time day 3 of measuring started, all of the other head groups grew significantly larger than Group C heads from day 3 onward. With the tails, Group A grew significantly larger than Groups B, D, and E on day 2 of measuring, and Group E on day 5. Since Group A is the control group, this suggests that the treatment was ineffective, and a third trial was done to confirm these findings.

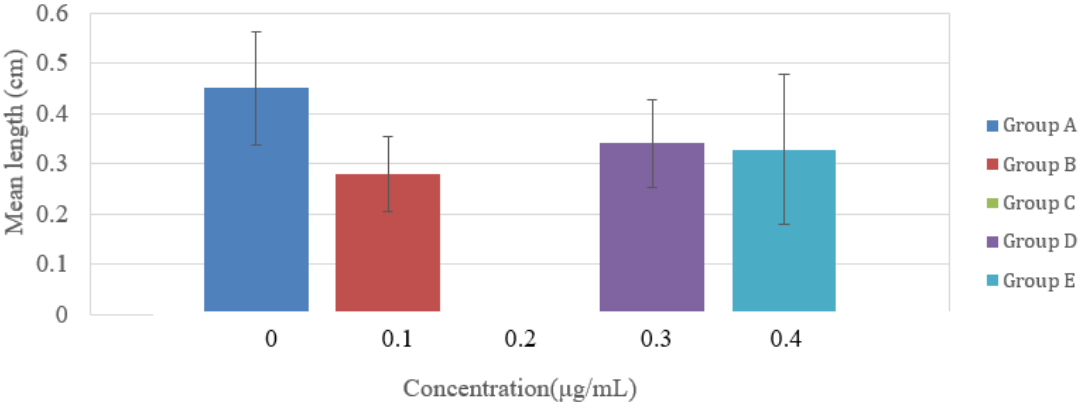

Figure 8. **Trial 2. Mean Length (cm) vs. Concentration ($\mu g/mL$), Bar Graph. The values represent the mean length of the heads on the last day. The error bars were calculated using standard deviation. Group A was the control group treated with 0 $\mu g/mL$ concentration, Group B was with 0.1 $\mu g/mL$, Group C was with 0.2 $\mu g/mL$ concentration, Group D was with 0.3 $\mu g/mL$ and Group E was with 0.4 $\mu g/mL$. From days 3 to 5 Group C was significantly smaller than Groups A, B, D, E, **=p $<$ 0.01.**

For Trial 3, the results can be seen in Figs. 14-21. The number of tails in all Groups except for Group B increased on day 4. One theory on why this could have occurred is accidental fragments of planaria could have gone into the water during planaria amputation. But this would not explain why only the tails group exhibited this change. Another theory is that the bitter melon encouraged reproduction among the planaria. There was also the death of 1 planaria in both Group D and Group E, which is four less than the last trial, meaning that the right precautions when taking care of the planaria were taken. The weight still showed no clear trend or correlation. All of the data in this trial was also insignificant, except for in day 5, Group A was significantly

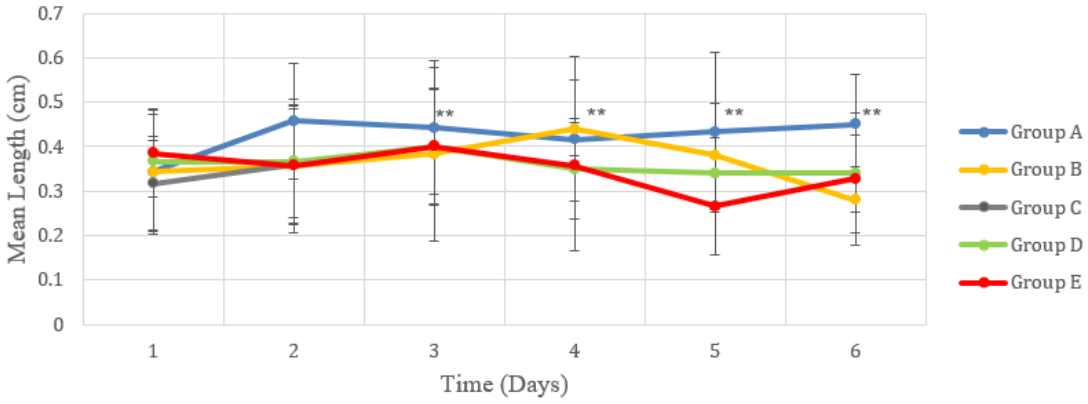

Figure 9. **Trial 2. Mean Length (cm) vs. Time (days), Heads Scatter Plot. The values represent the mean length. The error bars were calculated using standard deviation. Group A was the control group treated with 0 $\mu g/mL$ concentration, Group B was with 0.1 $\mu g/mL$, Group C was with 0.2 $\mu g/mL$ concentration, Group D was with 0.3 $\mu g/mL$ and Group E was with 0.4 $\mu g/mL$. From days 3 to 5 Group C was significantly smaller than Groups A, B, D, E, **=p $<$ 0.01.**

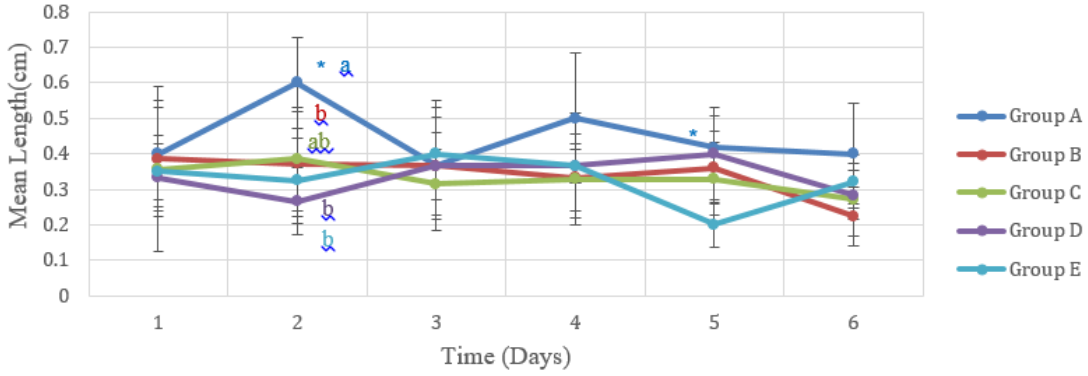

Figure 10. **Trial 2. Mean Length (cm) vs. Time (days), Tails Scatter Plot. The values represent the mean length. The error bars were calculated using standard deviation. Group A was the control group treated with 0$\mu g/mL$ concentration, Group B was with 0.1 $\mu g/mL$, Group C was with 0.2 $\mu g/mL$ concentration, Group D was with 0.3 $\mu g/mL$ and Group E was with 0.4 $\mu g/mL$. On day 2 Group A was significantly larger than Groups B, D, and E and on day 5 Group A was significantly larger than Group E, *=p $<$ 0.05.**

larger than Group B. This would support the results from the previous trial demonstrating that bitter melon does not have an effect on the regeneration of stem cells. However, all of the experimental groups' regeneration rates were higher than the control's. The rate of regeneration measures the percent of planaria in the group that has fully regenerated. For the tails, Group B, D, and E all reached 100 before the control, and for the heads, Group C showed completely regenerated planaria the fastest out of all of the other groups. Group E heads also had a high regeneration rate because it was the first to completely regenerate.

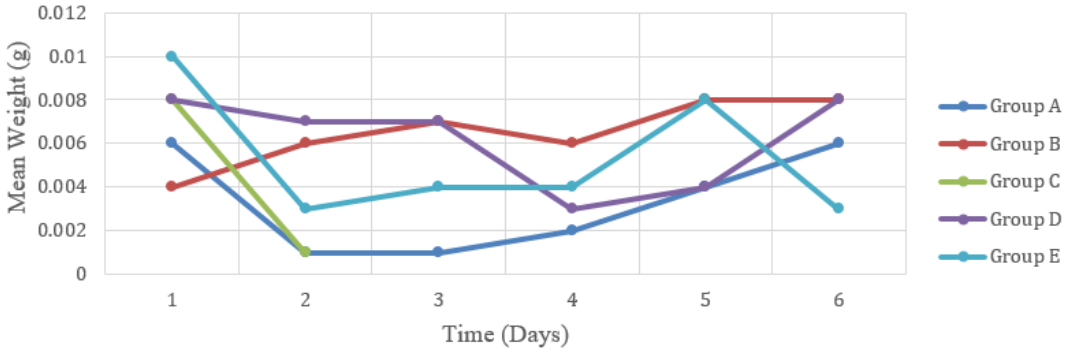

Figure 11. **Trial 2. Mean Weight (g) vs. Time (days), Heads Scatter Plot. The values represent the mean weight. Group A was the control group treated with 0 $\mu g/mL$ concentration, Group B was with 0.1 $\mu g/mL$, Group C was with 0.2 $\mu g/mL$ concentration, Group D was with 0.3 $\mu g/mL$ and Group E was with 0.4 $\mu g/mL$. The weight was calculated for each group by taking the weight of the group and dividing it by the number of planaria weighed.**

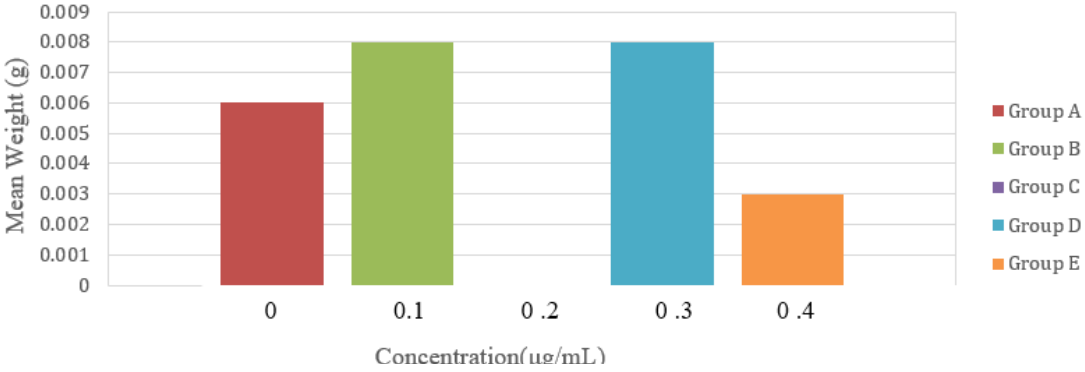

Figure 12. **Trial 2 .Mean Weight (g) vs. Concentration ($\mu g/mL$), Heads Bar Graph.The values represent the mean weight. Group A was the control group treated with 0 $\mu g/mL$ concentration, Group B was with 0.1 $\mu g/mL$, Group C was with 0.2 $\mu g/mL$ concentration, Group D was with 0.3 $\mu g/mL$ and Group E was with 0.4 $\mu g/mL$. The weight was calculated for each group by taking the weight of the group and dividing it by the number of planaria weighed.**

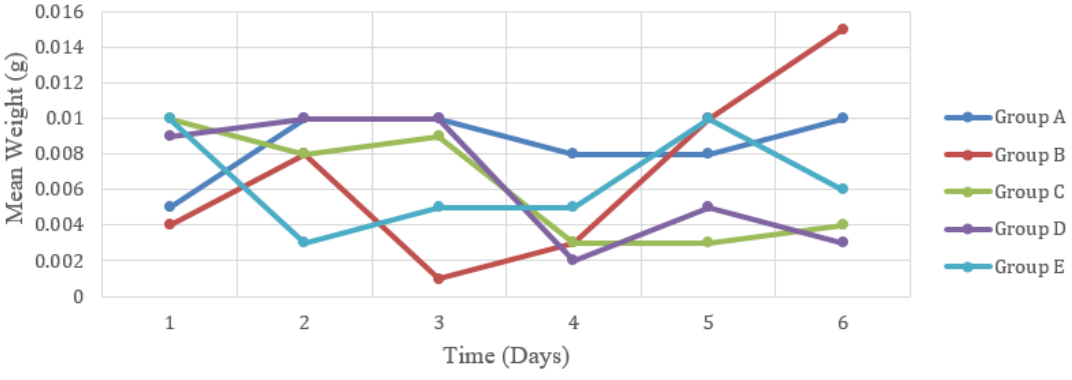

Figure 13. **Trial 2. Mean Weight (g) vs.Time (days), Tails Scatter Plot.The values represent the mean weight. Group A was the control group treated with 0 $\mu g/mL$ concentration, Group B was with 0.1 $\mu g/mL$, Group C was with 0.2 $\mu g/mL$ concentration, Group D was with 0.3 $\mu g/mL$ and Group E was with 0.4 $\mu g/mL$. The weight was calculated for each group by taking the weight of the group and dividing it by the number of planaria weighed.**

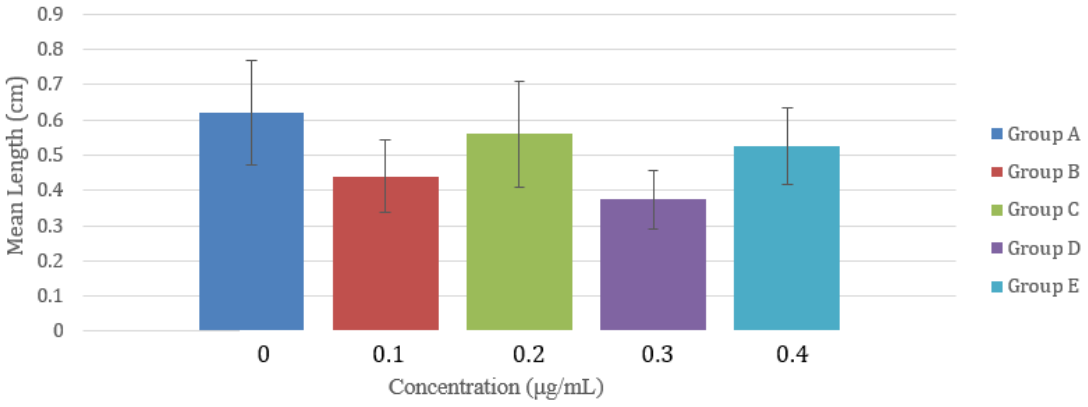

Figure 14. **Trial 3. Mean Length (cm) vs. Time (days), Bar Graph.The values represent the mean length of the heads on the last day.The error bars were calculated using standard deviation. Group A was the control group treated with 0$\mu g/mL$ concentration, Group B was with 0.1 $\mu g/mL$, Group C was with 0.2 $\mu g/mL$ concentration, Group D was with 0.3 $\mu g/mL$ and Group E was with 0.4 $\mu g/mL$.**

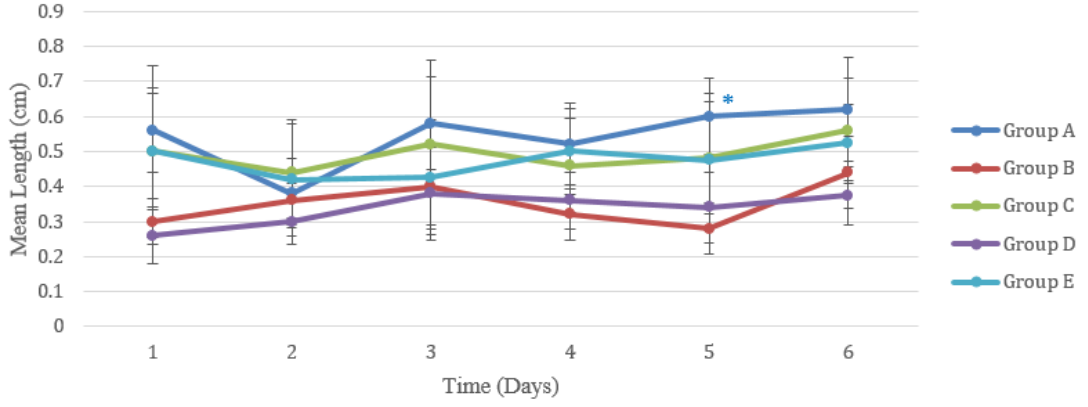

Figure 15. **Trial 3. Mean Length (cm) vs. Time (days), Heads Scatter Plot.The values represent the mean length. The error bars were calculated using standard deviation. Group A was the control group treated with 0 $\mu g/mL$ concentration, Group B was with 0.1 $\mu g/mL$, Group C was with 0.2 $\mu g/mL$ concentration, Group D was with 0.3 $\mu g/mL$ and Group E was with 0.4 $\mu g/mL$.On day 5 Group A was significantly larger than group B, *=p < 0.05.**

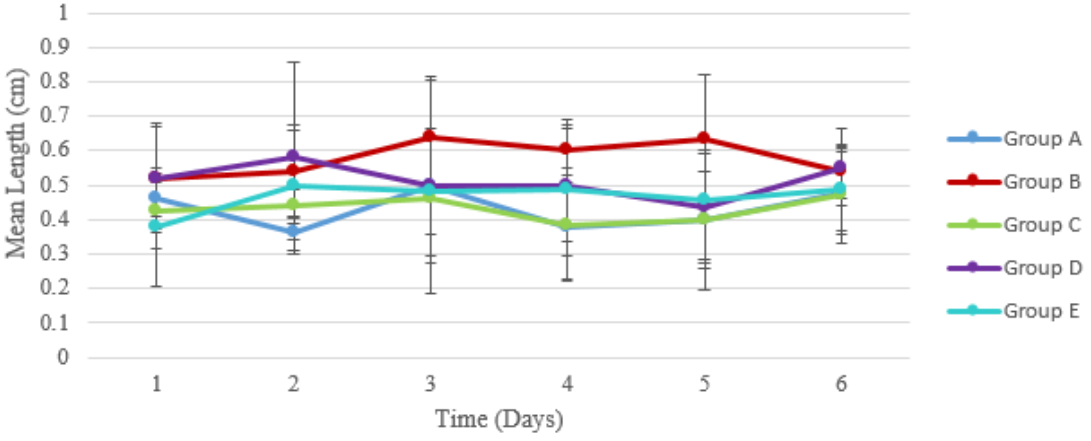

Figure 16. **Trial 3. Mean Length (cm) vs. Time (days), Tails Scatter Plot.The values represent the mean length. The error bars were calculated using standard deviation. Group A was the control group treated with 0$\mu g/mL$ concentration, Group B was with 0.1 $\mu g/mL$, Group C was with 0.2 $\mu g/mL$ concentration, Group D was with 0.3 $\mu g/mL$ and Group E was with 0.4 $\mu g/mL$.**

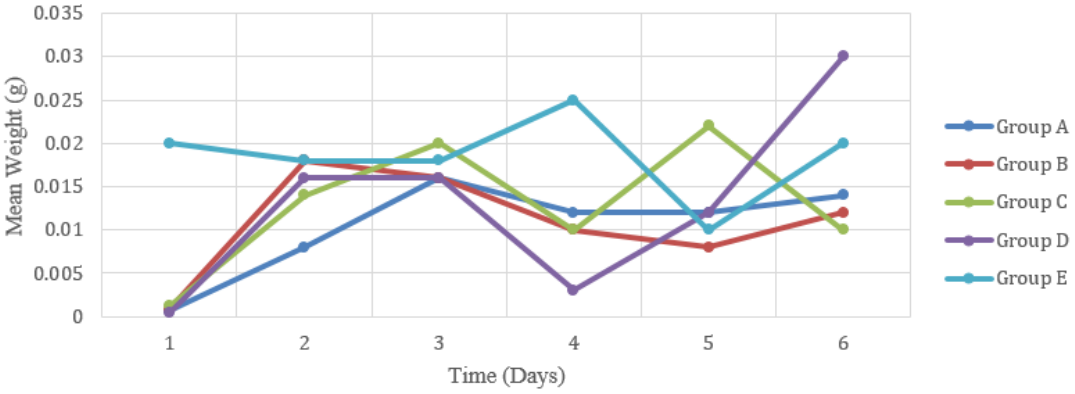

Figure 17. **Trial 3. Mean Weight (g) vs. Time (days), Heads Scatter Plot. The values represent the mean weight. Group A was the control group treated with 0$\mu g/mL$ concentration, Group B was with 0.1 $\mu g/mL$, Group C was with 0.2 $\mu g/mL$ concentration, Group D was with 0.3 $\mu g/mL$ and Group E was with 0.4 $\mu g/mL$.The weight was calculated for each group by taking the weight of the group and dividing it by the number of planaria weighed.**

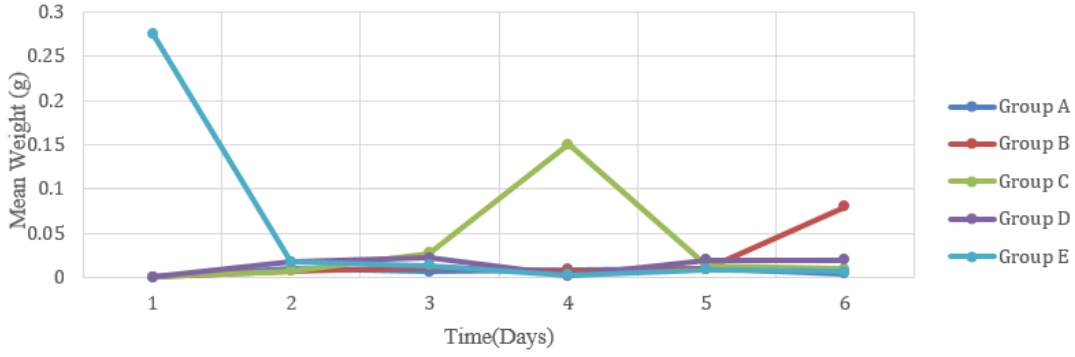

Figure 18. **Trial 3. Mean Weight (g) vs. Time (days), Tails Scatter Plot. The values represent the mean weight. Group A was the control group treated with 0$\mu g/mL$ concentration, Group B was with 0.1 $\mu g/mL$, Group C was with 0.2 $\mu g/mL$ concentration, Group D was with 0.3 $\mu g/mL$ and Group E was with 0.4 $\mu g/mL$.The weight was calculated for each group by taking the weight of the group and dividing it by the number of planaria weighed.**

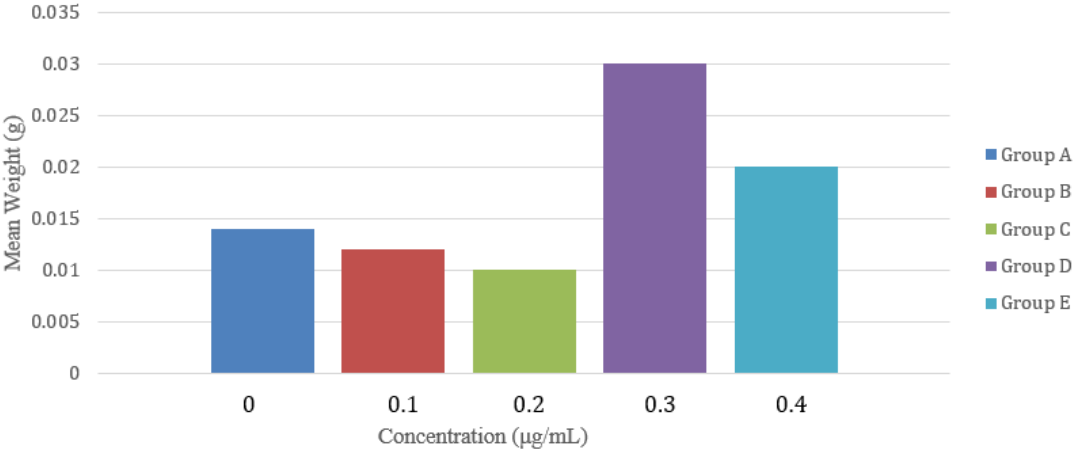

Figure 19. **Trial 3. Mean Weight (g) vs. Concentration ($\mu g/mL$), Bar Graph. The values represent the mean weight. Group A was the control group treated with 0$\mu g/mL$ concentration, Group B was with 0.1 $\mu g/mL$, Group C was with 0.2 $\mu g/mL$ concentration, Group D was with 0.3 $\mu g/mL$ and Group E was with 0.4 $\mu g/mL$.The weight was calculated for each group by taking the weight of the group and dividing it by the number of planaria weighed.**

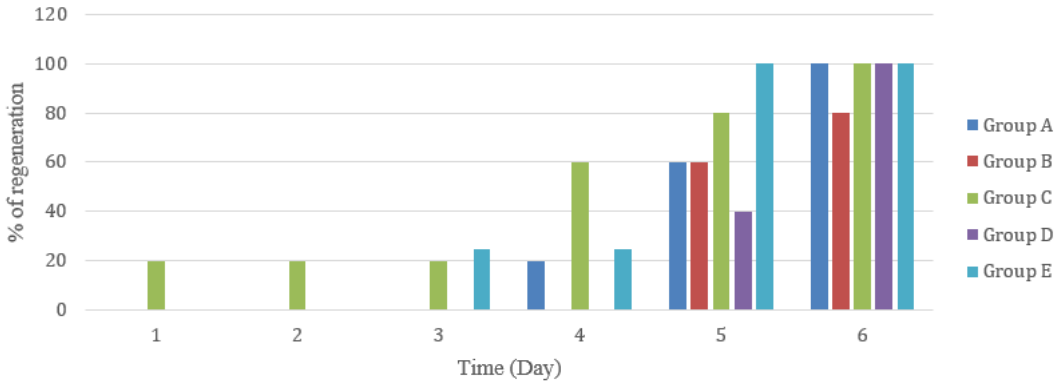

Figure 20. **Trial 3. Percent of Regeneration (%) vs. Time (days), Heads Bar Graph. The percentage was calculated by dividing the amount of regenerated planaria by the total amount of planaria each day for each group. The values represent the speed of which the planaria groups regenerated.**

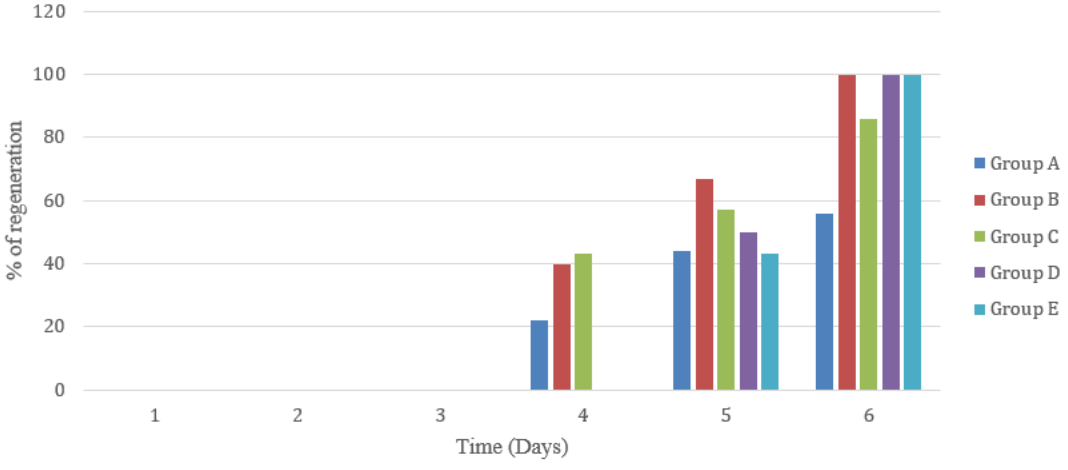

Figure 21. **Trial 3. Percent of Regeneration (%) vs. Time (days), Tails Bar Graph. The percentage was calculated by dividing the amount of regenerated planaria by the total amount of planaria each day for each group. The values represent the speed of which the planaria groups regenerated.**

4. Conclusion

While conducting the experiment for Trial 1, there were some misunderstandings while reading the methods, which lead to the death of planaria in groups C, D, and E. All of these problems were resolved in the later trials. In the next two trials, no significantly larger length or weight was yielded by the experimental groups. In Trial 2, the only significance was between Group C heads and all of the other head groups because Group C died in day 3 of measuring, so all of the other groups were significantly larger than Group C. In the tails group, only Group A grew significantly larger than other groups, which means that the treatment did not have a large effect on the regeneration of stem cells. The third trial confirmed this because there weren't one or more groups that grew significantly larger than the others. However, in Trial 3 the percentage of growth over time was measured and the results show that Group B, D, and E have all reached 100% before the control. To conclude, the bitter melon did not affect the efficiency of the regeneration; however, it did increase the rate of regeneration producing earlier 100% regeneration and faster regeneration than the control.

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Conflict of Interest

Authors of this article declare that they have no conflict of interest.

Human Studies/Informed Consent

No human studies were carried out by the authors for this article.

Animal Studies

No animal studies were carried out by the authors for this article.

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