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The Effects of GlyNAC on the Regeneration Rate and Movement of Dugesia tigrina

Abstract

This study aims to investigate the effects of GlyNAC on planaria tissue regeneration and behavioral changes. There has been a focus on anti-aging in the older population due to the adverse effects that come with it such as muscle fatigue, a decrease in tissue regeneration, etc. As a result, there was an increase in research on finding an effective supplement to combat health issues and aging. GlyNAC, a combination of Glycine and N-acetylcysteine, has been shown to reverse and improve health by increasing glutathione levels. Glutathione is an antioxidant with multiple health benefits such as protecting against oxidative stress, a main factor in causing cell death. This experiment was conducted using planarians because they have a similar central nervous system, tissue regeneration, and glutathione production to humans. There were 1 control group and 3 experimental groups. The control group was fed regular ground beef while the experimental group was fed ground beef with GlyNAC. For tissue regeneration, each group would be measured and observed before and after the ground beef or GlyNAC-mixed ground beef. The planarians' motility was recorded for 20 minutes with grid paper underneath their petri dishes. The results indicate that GlyNAC treated groups had increased regeneration and movement. With this support, our hypothesis of GlyNAC increases the regeneration and movement in planarians. Future Research can be done by having bigger experimental groups for more accurate data.

Introduction

GlyNAC

There has been an interest in anti-aging in the modern day due to one's fear of aging and the many symptoms that come with it, such as muscle fatigue, inflammation, a decrease in tissue

regeneration, and oxidative damage(Kumar, et al. 2023). Oxidative stress occurs when there's an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify them (Pizzino et al,.2017). Excessive oxidative stress leads to aging and cell death, while low oxidative stress supports cell regeneration (Lee et al.,2018).

There is limited research on why older people face these symptoms and the lack of effective treatments to treat these symptoms. Despite this, many try to preserve their health and prevent these symptoms from happening through various means such as dieting, supplements, exercise, and antioxidants. Previous experiments have shown that both glycine and N-acetylcysteine, are antioxidants that can protect against oxidative stress (Ruiz-Ramírez, 2014; Kerksick, et al. 2005). These components are precursors to glutathione(GSH), an important antioxidant that protects against oxidative stress, increased energy, cell proliferation, etc. Therefore, GlyNAC is expected to boost glutathione production by combining Glycine and N-acetylcysteine (Kumar, et al. 2023).

As one age, there is a deficiency of GSH due to the lack of glycine and cysteine needed to synthesize the GSH. With this deficiency of GSH, fewer antioxidant defenses are protecting against oxidative stress that can damage cells that promote a healthy body. Therefore, GlyNAC demonstrates that it has the potential and ability to benefit those who are aging.

Planarian

Planaria, specifically *Dugesia tigrina*, are flatworms known for their remarkable tissue regeneration abilities and nervous system. This makes them ideal for studying tissue regeneration and the nervous system, which helps provide insights into human tissue regeneration and the nervous system since both planaria and humans use stem cells for this process and have similar nervous systems. Planaria are famously used to research tissue regeneration, toxicology, and other pharmacology studies. Planarians can regenerate their tissue by using stem cells which are

unspecialized cells that can develop into various types of cells, such as skin and nerve cells (Reddien, et al 2018). Additionally, planaria have glutathione in their redox system and glutathione is an essential component in balancing the redox system (Bijnens, et al.2021). With this planaria can create glutathione in balancing their redox system.

Materials and Method:

Preparation of GlyNAC treatment

Glycine and N-acetylcysteine) were purchased on Amazon (Nutricost). Glycine and N-acetylcysteine were mixed with a 1:1 ratio to make GlyNAC. The GlyNAC was blended into the ground beef with their desired mass concentrations of 0%, 1%, 5%, and 10% (Table 1.)

Table 1. The concentration of GlyNAC in Ground Beef.

Group	Percentage of GlyNAC in Beef (g/g)	Amount of GlyNAC used (g)	Amount of Glycine (g)	Amount of N-acetylcysteine(g)	Amount of Ground beef (g)	Number of planaria
A	0	0	0	0	20	10
B	1%	0.2	0.1	0.1	19.8	10
C	5%	1.0	0.5	0.5	19	10
D	10%	2.0	1.0	1.0	18	10

Table 2. The concentration of GlyNAC in Ground Beef.

Group	Percentage of GlyNAC in Beef (g/g)	Amount of GlyNAC used (g)	Amount of Glycine (g)	Amount of N-acetylcysteine(g)	Amount of Ground beef (g)	Number of planaria
A	0	0	0	0	20	10
B	0.5%	0.1	0.05	0.05	19.9	10
C	1%	0.2	0.1	0.1	19.8	10
D	5%	1.0	0.5	0.5	19	10

★ The treatments were changed since in trial 1 majority of group D's planarians had died. So, Group D's treatment was changed to 5% of GlyNAC.

Preparation of Planarians

40 planarians have been ordered from the website, Carolina.com, and the catalog number is 132954. The planarians were kept in loosely sealed jars of Poland spring water. They were then separated into 4 groups of 10. Which were each used for different concentrations of GlyNAC. Afterward, the planarian jars were stored in the cabinet with a gap. The planarians were fed ground beef for a week.

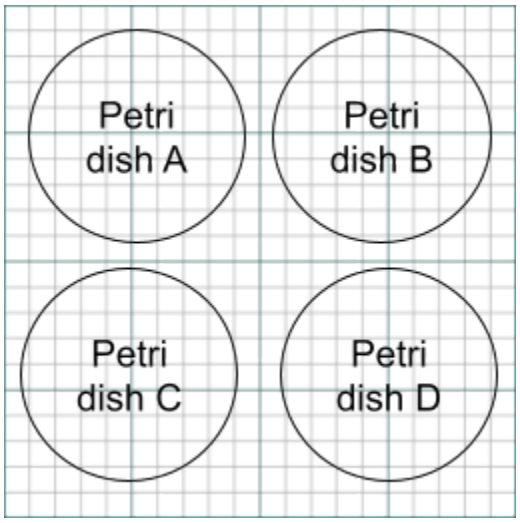
Treating Planarian

Before the planarians are used, they must be nurtured. When working with them, safety equipment was worn and materials used in the experiment, as well as the workspace, were disinfected beforehand. The groups were treated with 20g of correspondent food according to Table 1. This was done a maximum of 3 times a week. After feeding, leftover beef was removed from the container using a pipette. Additionally, dirtied water was replaced with clean Poland spring water using a pipette, avoiding taking any planarians along with the dirtied water. The planarians were treated for 7 days before beginning the experiment.

Mobility Measurement

Before adding the treatment, the behaviors of planarians were measured. To do this, safety gear was worn, and all materials, gear, and workspace were disinfected. Next, 30 mL of Poland spring water was poured into 4 petri dishes. Then, the planarians were put into the petri dishes based on their respective groups. Afterwards, graph paper "(Figure 1) was placed below the petri dishes, and a camera was used to record the planarian groups for 30 minutes.

Figure 1. Experimental setup for movement



Planarian Cutting

Before cutting the planarians, the scalpel was disinfected, which was done by wetting a paper towel with ethanol. Next, group A planarians were put onto ice for one minute and all the planarians were cut individually with the disinfected scalpel before the pharynx. The scalpel was disinfected every 3 cuts and repeated the process for groups B, C, and D. Then, a small jar was used and labeled as "AH" and another jar was labeled as "AT". The tails of group A planarian were put into the "AT" jar and the heads of group A planarian were put into the "AH". This process was repeated for the other groups but the letter of the labels was changed to represent the appropriate group. After that, the planarians were measured for the average mass of the planaria groups, AH, BH, CH, DH, AT, BT, CT, and DT to record the measurements.

Tissue regeneration

Every Monday, Wednesday, and Friday, the planarians' eyespot and length would be recorded and observed. The amputated planarian tail groups were observed under a microscope to see the time when the planarian's eye spot appeared; this process was repeated for other groups. Then, the planarians' lengths were measured in millimeters and the lengths were recorded in an Excel sheet of all planarians in group A and then the process was repeated for other groups. After the trials, the planarians were given to the supervisor to be autoclaved.

Data analysis

The planarian motility was collected by observing the number of line tiles crossed by each planarian under a grid paper and was recorded on an Excel sheet before and after the treatment for 2 weeks. The means and standard deviation were done by Excel standard functions.

Then created a scatter plot line graph. The data's significance was calculated by using ANOVA (Analysis of Variance) followed by Tukey HSD (from https://www.socscistatistics.com/tests/anova/default2.aspx) P-values under 0.05 were considered significant. The graphs were a scatter plot of each concentration tile crossed per day.

The planarian regeneration was done by comparing how fast the eye spots grow between the tail groups. The length was measured between both the heads and tails. The means and standard deviation were done by Excel standard functions. The data's significance was calculated by using ANOVA (Analysis of Variance) followed by Tukey HSD (from https://www.socscistatistics.com/tests/anova/default2.aspx) P-values under 0.05 were considered significant. The graphs made were bar graphs showing the difference in growth from day 0 to day 11 and the mean scoring of the eyespot regeneration plotted as a line graph.

Results

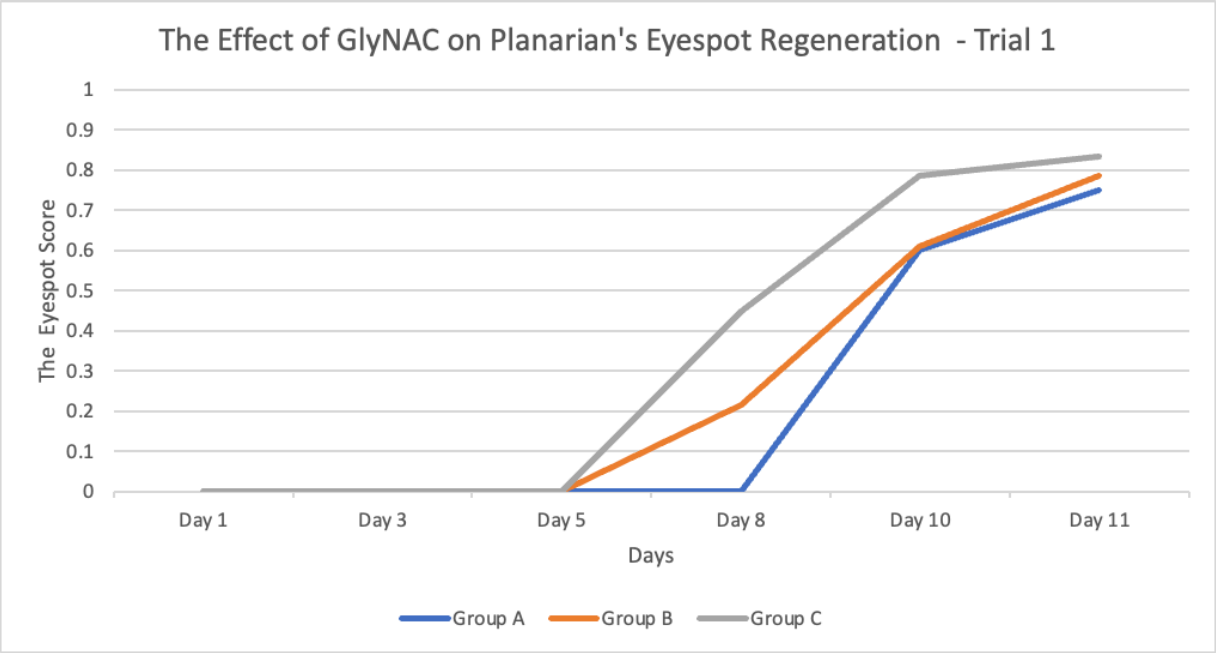


Figure 2. The Effects of GlyNAC on Planarian's Eyespot Regeneration - Trial 1. Each line represents the different concentrations of GlyNAC (A: 0% of GlyNAC, B: 1% of GlyNAC, C: 5% of GlyNAC, and D: 10% of GlyNAC). There were 10 planarians in each group. The data was not significantly different. The trend of this graph reveals that groups C and B had a faster rate of regenerating eyespots in group A.

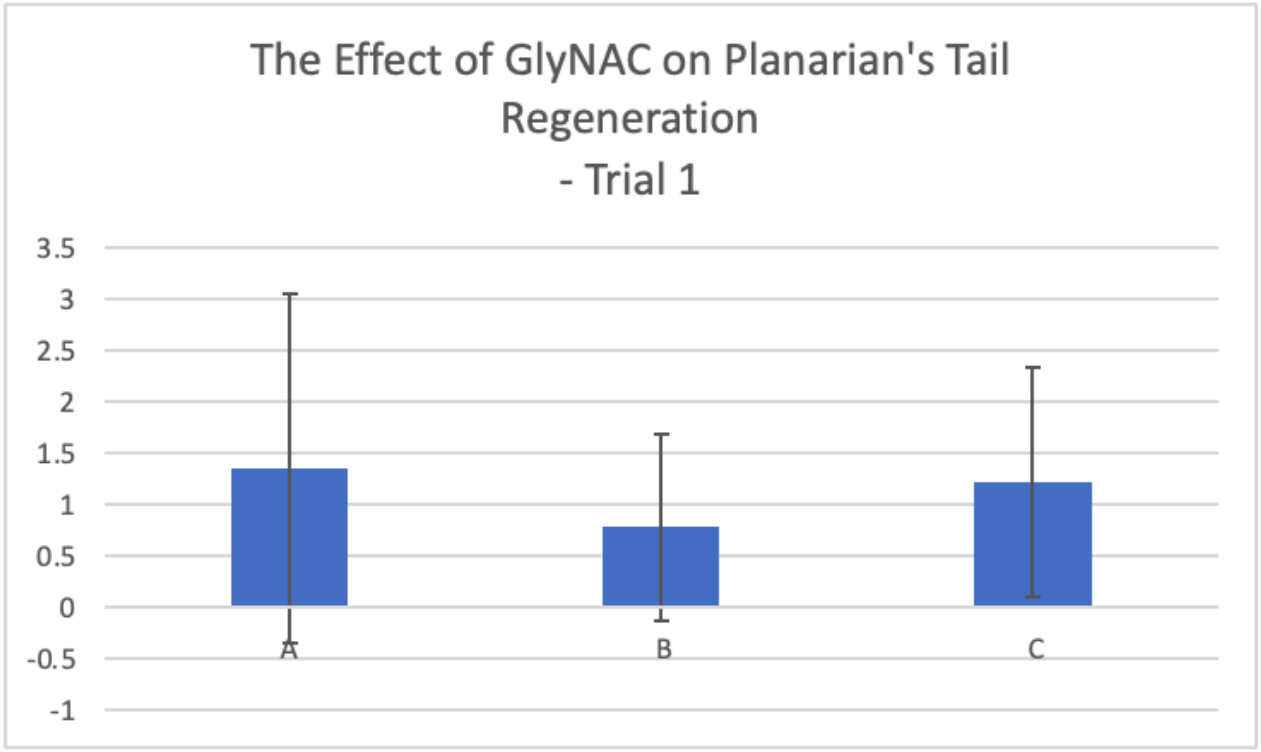


Figure 3. The Effect of GlyNAC on Planarian's Tail Regeneration-Trial 1. Each bar represents the planarian's tail length difference from day 1 to day 11 in their respective treatment. There were 10 planarians in each group. The error bars represent the standard deviation. The trend of this graph shows that GlyNAC groups had delayed regeneration. ANOVA was used to determine the significance difference. * represents the p values < 0.05, and ** represents p value <0.01. The p-value is .927452 therefore the result is not significant.

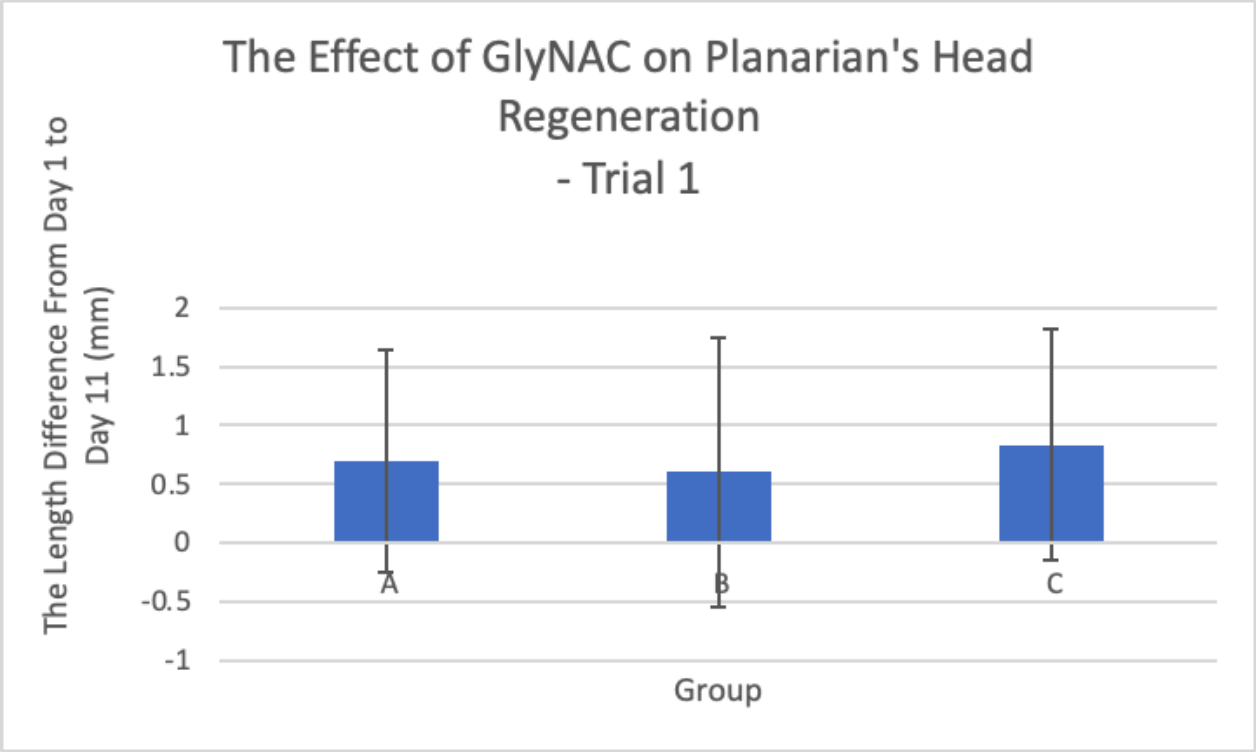


Figure 4. The Effect of GlyNAC on Planarian's Head Regeneration-Trial 1. Each bar represents the planarian's head length difference from day 1 to day 11 in their respective treatment. There were 10 planarians in each group. The error bars represent the standard deviation. The trend of this graph shows that the highest concentration of GlyNAC had a higher regeneration rate difference than the control group. ANOVA was used to determine the significance difference. * represents the p values < 0.05, and ** represents p value <0.01. The p-value is .684276 therefore the result is not significant.

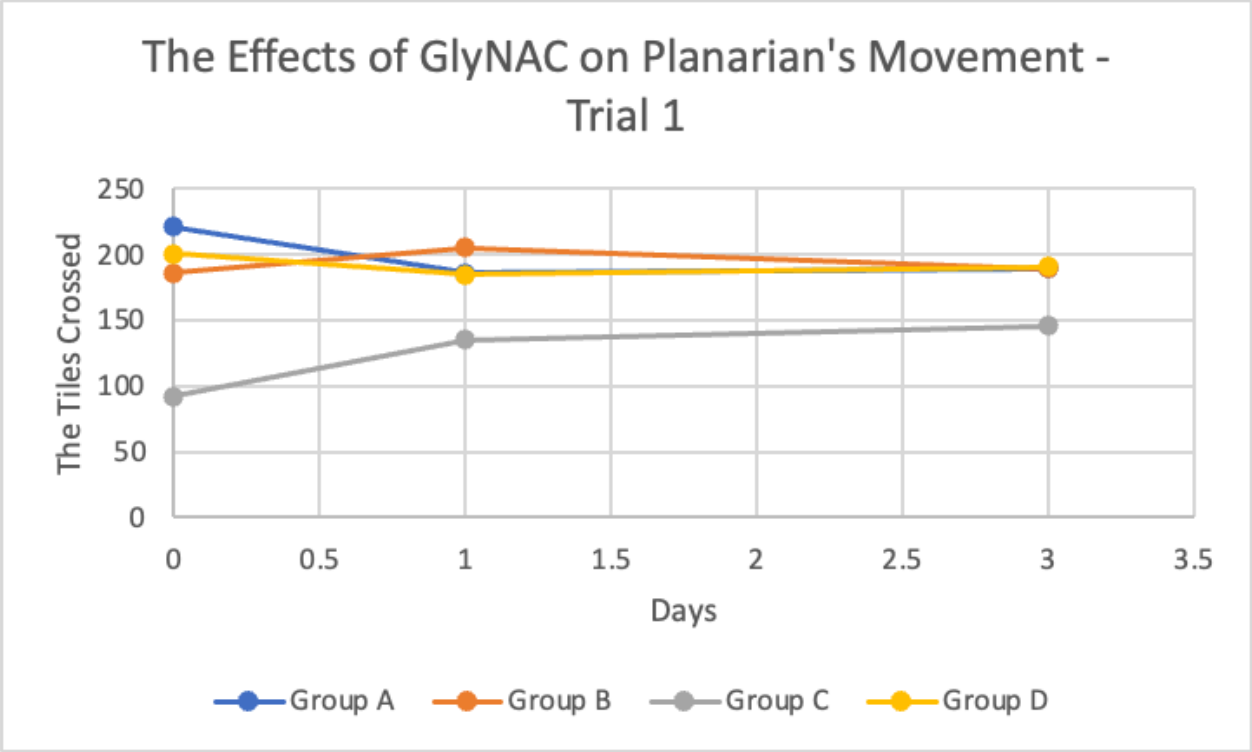


Figure 5. The Effect of GlyNAC on Planarians Movement-Trial 1. Each line represents the relationship between the days and the number of gridlines crossed per planarian in their respective treatment. There were 10 planarians in each group. The error bars were not displayed for it not being significant. The trend shows that there is a positive effect of 5% GlyNAC on planarian's movement. On the other hand, there was minimal effect on the planarian's movement when giving 1% and 10% GlyNAC. And over time the movement of the control group decreases.

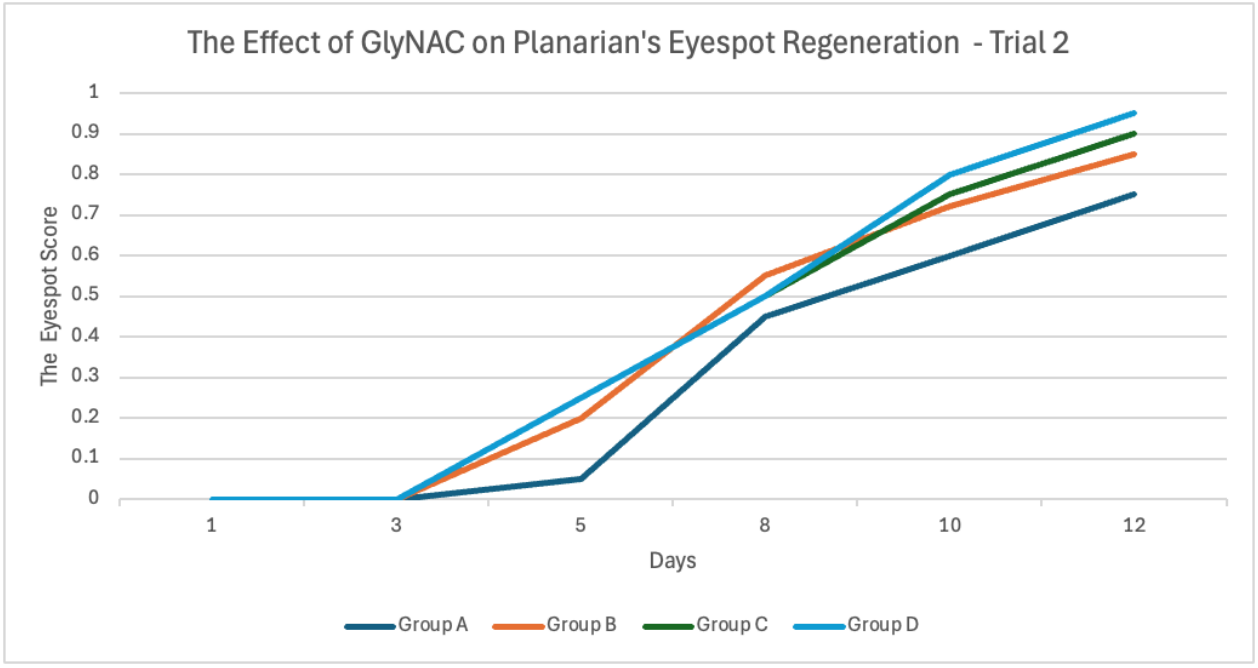


Figure 6.. The Effect of GlyNAC on Planarian's Eyespot Regeneration-Trial 2. Each line represents the relationship between the days and the eyespot score per planarian in their respective treatment. There were 10 planarians in each group. The error bars were not displayed for it not being significant. The trend shows that there is a positive effect of GlyNAC on planarians eyespot regeneration but there is no statistical significance.

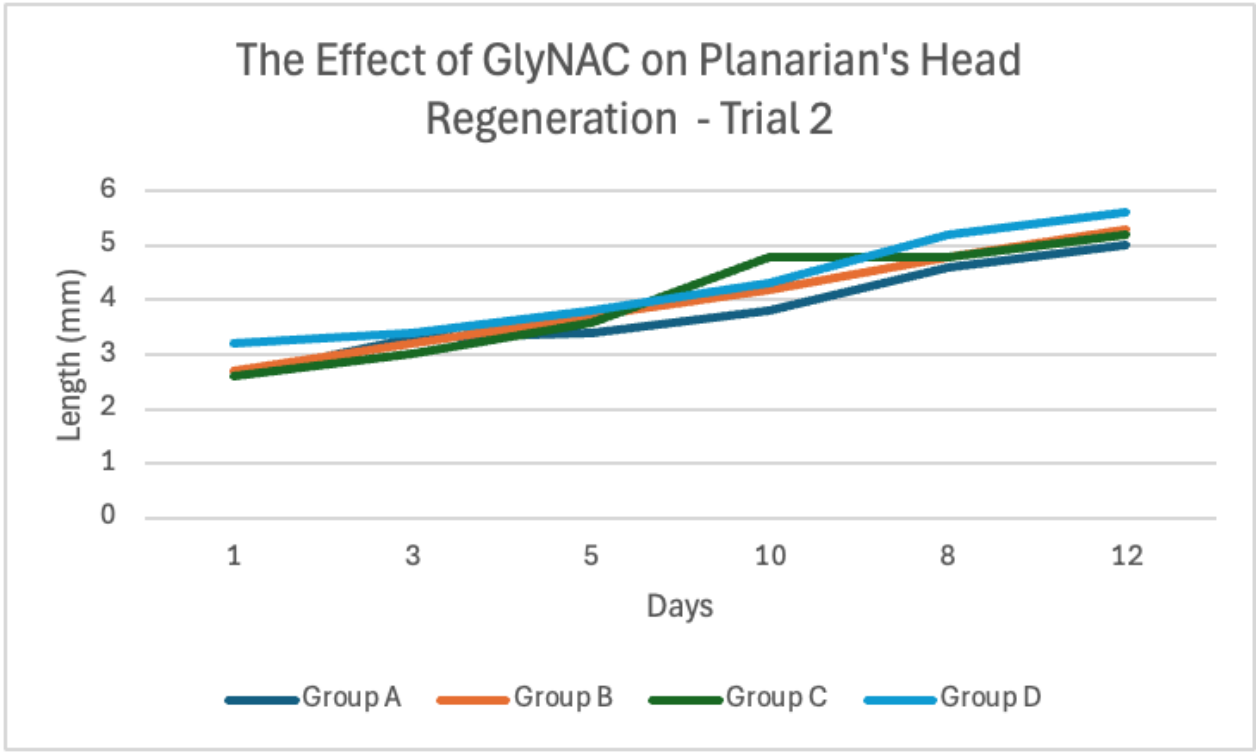


Figure 7. The Effect of GlyNAC on Planarian's Head Regeneration - Trial 2. Each line represents the growth of the planarian's head length in their respective treatments. There were 10 planarians in each group. The error bars were not displayed for it not being significant. The trend shows that there is a positive effect of GlyNAC on planarian's head regeneration but there is no statistical significance.

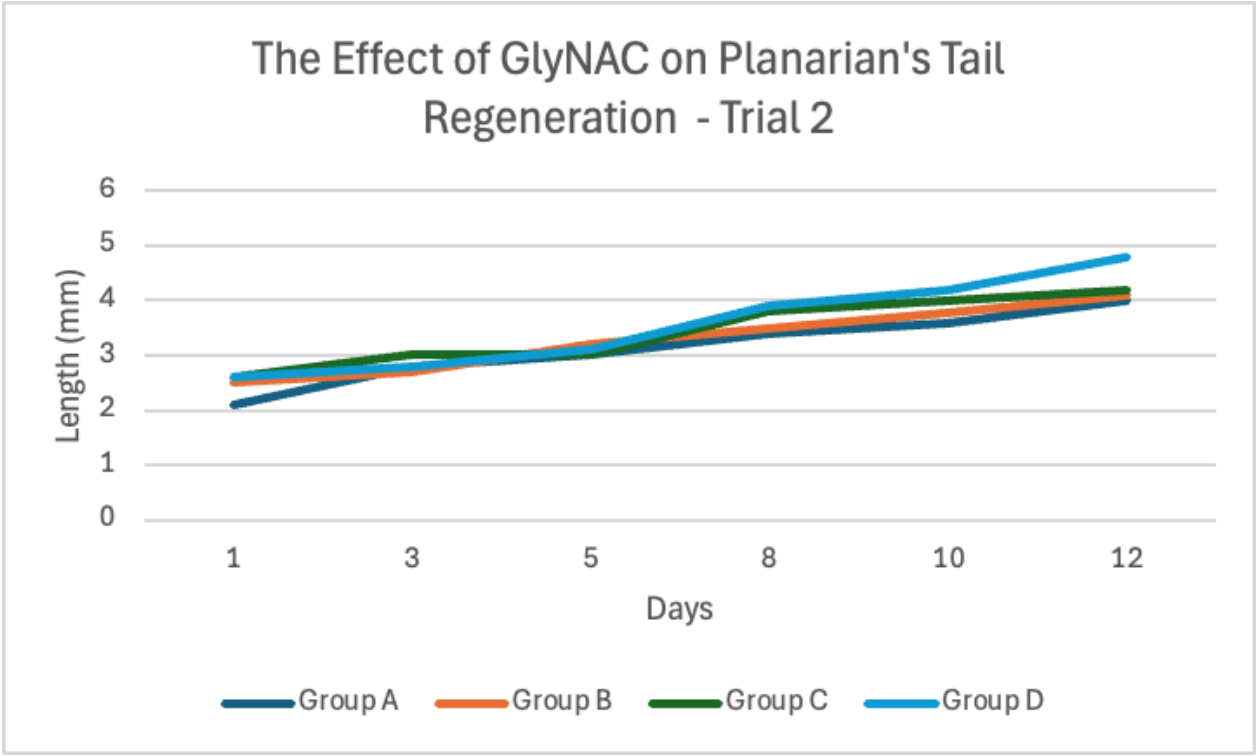


Figure 8. The Effect of GlyNAC on Planarian's Tail Regeneration - Trial 2. Each line represents the growth of the planarian's head length in their respective treatments. There were 10 planarians in each group. The error bars were not displayed for it not being significant. The trend shows that there is a positive effect of GlyNAC on planarian's tail regeneration but there is no statistical significance.

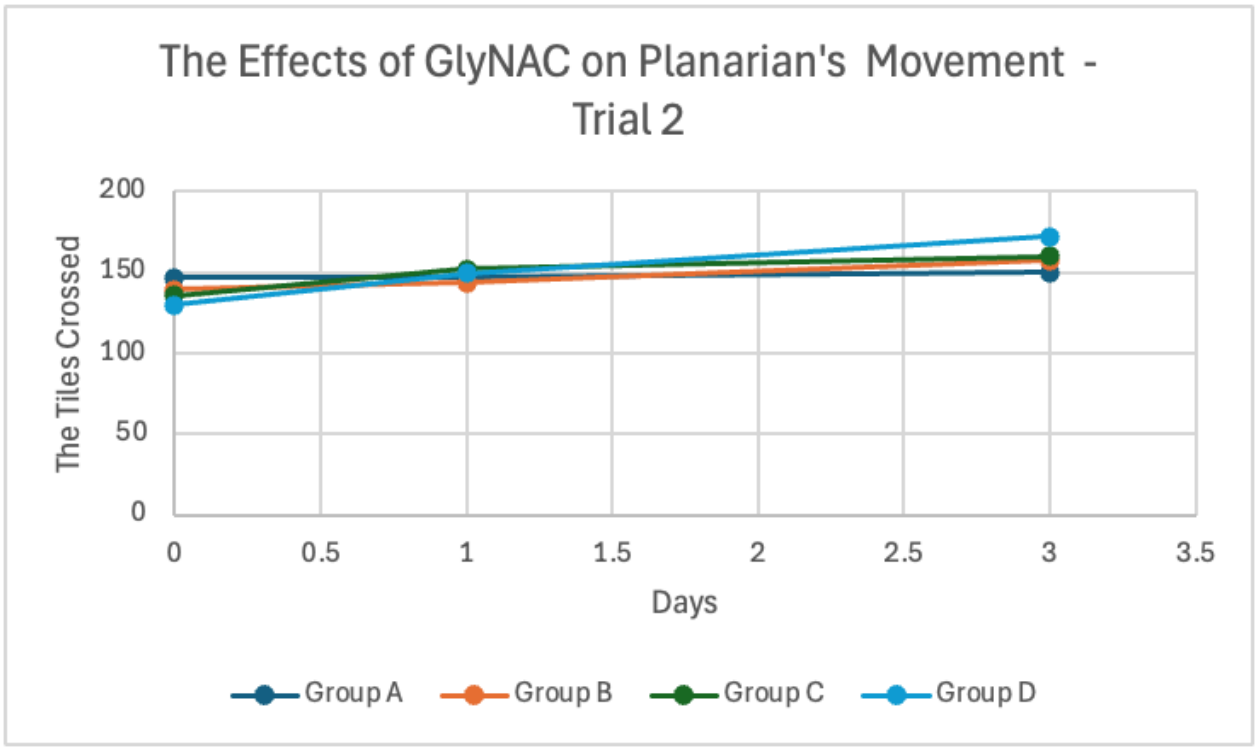


Figure 9. The Effect of GlyNAC on Planarians Movement-Trial 2. Each line represents the relationship between the days and the number of gridlines crossed per planarian in their respective treatment. There were 10 planarians in each group. The error bars were not displayed for it not being significant. The trend shows that there is minimal positive effect of GlyNAC on planarian's movement, but there is no statistical significance.

Discussion/Conclusion

The data reveals that the GlyNAC had increased the regeneration of eyespots and length. This supports the hypothesis that GlyNAC has a positive effect on the regeneration of a planarian. This suggests that GlyNAC may also have a positive effect on tissue regeneration in humans. This is because since there is increased regeneration in planarians then these effects will show up in the human regeneration of cells.

The limitation of this experiment is that it can cause errors within this experiment. During the data collection, the researchers were manually counting the number of lines crossed by the planarians; this can lead to human error and time-consuming. This issue was solved by having the video recording shortened. This allows the researchers to be more time-efficient. On the contrary, this did not solve the problem of human error. Another limitation is holidays, where data cannot be collected. This can make comparing data from different trials hard. Trials were also not long enough to see the full extent of GlyNAC's impact. Due to the time constraints, this was not resolved. In trial 1 the planarians treated with 10% had died. The researchers had resolved the issue by changing the Group D treatment to be 5% of GlyNAC. This resolution had guided the researchers to seeing the benefits of GlyNAC.

Future Research

This is because our substance (GlyNAC) is most effective in older patients because of their deficiency of glutathione. Future research should use a thermal camera or an artificial intelligence to track the movements of the planarian better because when counting it manually it can be more inaccurate. Additionally, future research should focus on a better way of treating the planarians. Such as soaking the planarian in a GlyNAC solution. Lastly, future researchers should extend the time giving planarians GlyNAC and increase the sample size.

Acknowledgments

Dr. D. Marmor, Mrs. N. Jaipershad, Dr. L. Wang, Ms. Zhu, Ms. Khemlani, Dr. J. Cohen, Dr. S. Lin, Mr. Z. Liang, Ms. DePietro

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