Final Paper

This study aims to investigate the effects of GlyNAC on planarian tissue regeneration. Planarian and organism regeneration are needed to maintain a healthy body. However, as one ages, their regeneration declines. 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 to find 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. To conduct this experiment, planarians are going to be used because they have a similar central nervous system, tissue regeneration, and glutathione production like humans. There will be 1 control group and 3 experimental groups. The control group will be fed regular ground beef while the experimental group will get ground beef with GlyNAC mixed into it. For tissue regeneration, each group would be weighed and measured before and after the ground beef or GlyNAC-mixed ground beef. The behaviors that are going to be observed are the positive behavior, negative behavior, and the planarians' motility by recording them for 30 minutes with grid paper underneath their petri dishes.

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, 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, 2017). Excessive oxidative stress leads to aging and cell death, while low oxidative stress supports cell regeneration (Lee, 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, which are antioxidants that can protect against oxidative stress (Ruiz-Ramírez, 2014; Kerksick, 2005). These components are precursors to glutathione(GSH), an important antioxidant that protects against oxidative stress, increased energy, cell proliferation, balancing oxidative stress, etc. Therefore, GlyNAC is expected to boost glutathione production by combining Glycine and N-acetylcysteine (Kumar, 2023).

As one ages, 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, causing an increase in oxidative stress that can damage cells that promote a healthy body. GlyNAC, having the components of Glycine and N-acetylcysteine, helps supply the needed glycine and cysteine to synthesize GSH. 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 that are unspecialized cells that can develop into various types of cells, such as skin and nerve cells (Reddien, 2018).

Materials and Method:

Preparation of GlyNAC treatment

The GlyNAC treatment was made by purchasing individual Glycine and N-acetylcysteine on Amazon to ensure the 1:1 ratio needed for GlyNAC. The GlyNAC was embedded into the ground beef with their desired concentrations (0%, 1%, 5%, and 10%). The concentrations of the GlyNAC were done by mixing a certain amount of ground beef with the 1:1 of Glycine and N-acetylcysteine.

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	5	10
B	1%	0.05	0.025	0.025	4.95	10
C	5%	0.25	0.125	0.125	4.75	10
D	10%	0.50	0.25	0.25	4.5	10

Preparation of Planarians

40 planarians have been ordered from the website, carolina.com. The planarians were kept in sealed jars of Poland spring water. They were then separated into 4 groups of 10, which were each used for different concentrations of GlyNAC. This was done to ensure that the planarians were not exposed to other planarians which were fed different concentrations of

GlyNAC. Afterwards, the planarian jars were stored in incubators. This ensured that the planarians had a good environment, were safe while they were unsupervised, and were not used in experiments yet.

Nurturing Planarian

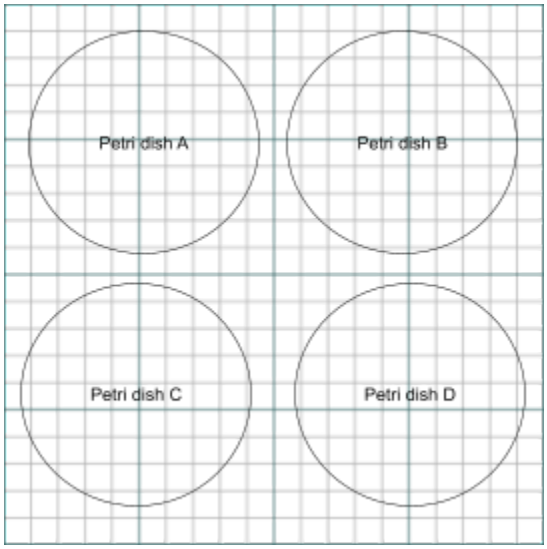
Before the planarians are used, they must be nurtured. When working with them, safety equipment was worn and materials used in the experiment, as well as the workspace, was disinfected beforehand. The control group was fed with 5g of regular ground beef. For the experimental group B, planarians were fed with 4.95g of ground beef and 0.05g of GlyNAC. For the experimental group, C, planarians were fed 4.75mg of ground beef and 0.25g of GlyNAC. For the experimental group, D, planarians were fed with 4.5g of ground beef and 0.50g of GlyNAC. This was done a maximum of 3 times a week. 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 nurtured for 7 days before beginning the experiment.

Observing Planarians

Before adding the treatment, the planarians were observed. To do this, safety gear was worn, and all materials, gear, and workspace was 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 was placed below the petri dishes, and a phone or camera was used to record the planarian groups for 30 minutes. Finally, the negative and positive behaviors and their mobility were recorded based on the video that was taken. Positive planarian behaviors were identified by frequent moving their heads and tails left to right, frequent swimming from end to the end of the container, keeping the body straight lined,

frequent eating, and frequent turns. The negative planarian behaviors were identified by curling up into C-shape, contractions, fraying of the body, less frequent movement, minimal eating, and minimal turns.

Figure 1. Experimental setup for behaviors and movement



Planarian Cutting

The length and mass of each planarian were taken before as well as after the planarian was treated with the GlyNAC. Length and mass of the planarian were used as important information of how much the planarians regenerate and the rate of their regeneration. When cutting the planarian, safety gear like a lab coat, long pants, shoes that do not expose feet, gloves, and goggles were used. Equipment and materials were disinfected before use. Then, the tabletop was bleached and wiped with a 10% bleach solution. After the preparation was done, 25mL of

Poland Spring water was poured into a weighing boat and weighed on a balance. Next, the average mass was recorded as m1. Group A was then transferred to a weighing boat with a pipette. The planarians were then weighed and their mass was recorded as m2. Afterwards, the equation (m2-m1)/# of planaria was used to calculate the average weight of group A before amputating the planaria mass. This process was repeated for groups B, C, and D. The length of planarians of groups A, B, C, and D were recorded. After that, planarians of group A were placed on the ice until they lengthened. Then, the planarians' lengths were measured in millimeters and the lengths were average of all planarians in group A and then the process was repeated for other groups. 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. Next, a weighing boat was used to pour 25 mL of Poland Spring water. Then recorded the average mass of the water as m1. Group AH planaria was transferred onto the weighing boat with a pipette. Then, the planarians mass was weighed and recorded as m2. After obtaining all the data needed, the equation (m2-m1)/# of planaria was used to calculate the average weight of group AH before the planarians were amputated the planarians' mass. Finally, this process was repeated for groups BH, CH, DH, AT, BT, CT and DT.

Tissue regeneration

Each group's planarians were not fed until the pharynx was fully regenerated. Every day, the planarians' average mass and length would be recorded and observed. The amputated planarian tails were observed to see the time when the planarians eye spot appeared. GlyNAC was given to the planaria when the planarians were fully dead or regenerated. After the trials, the planarians were given to the supervisor to be autoclaved.

Data analysis

The data for behaviors was collected by observing the negative and positive behaviors of the planarian before and after the treatment for 2 weeks. The amount of positive and negative behaviors was recorded on an Excel spreadsheet. Then, the average amount of the positive and negative behaviors were inputted into a bar graph.

The planarians' motility was collected by observing the planarian's movement on a grid paper sheet before and after the treatment for 2 weeks. The amount of movement was recorded on an Excel sheet and used to create a bar graph.

The data for tissue regeneration was collected by measuring the average lengths, mass, and eyespot of the planarian before and after the treatments for 2 weeks. The measured masses and lengths were recorded on an Excel sheet. Planaria's average mass were calculated by the equation (m2-m1)/# of planaria was used before and after the GlyNAC treatment. Planaria's average length was calculated by the planaria's lengthening size was measured by millimeters and then it was averaged in each group before and after the GlyNAC treatment. The planarian's eye spots were calculated by observing the time that the planarian's tail fully regenerated the

eyespots before and after the GlyNAC treatment. The data collected was then created into a bar graph to show the different groups.

References:

Bijnens, K., Jaenen, V., Wouters, A., Leynen, N., Pirotte, N., Artois, T., & Smeets, K. (2021). A spatiotemporal characterization of redox molecules in planarians, with a focus on the role of glutathione during regeneration. Biomolecules, 11(5), 714.

Jeong, E. M., Yoon, J. H., Lim, J., Shin, J. W., Cho, A. Y., Heo, J., ... & Kim, I. G. (2018). Real-time monitoring of glutathione in living cells reveals that high glutathione levels are required to maintain stem cell function. *Stem cell reports*, *10*(2), 600-614.

Pawelec, Graham, David Goldeck, and Evelyna Derhovanessian. "Inflammation, aging, and chronic disease." *Current opinion in immunology* 29 (2014): 23-28.

Kawai, Nobuhiro, et al. "The sleep-promoting and hypothermic effects of glycine are mediated by NMDA receptors in the suprachiasmatic nucleus." *Neuropsychopharmacology* 40.6 (2015): 1405-1416.

Kumar, P., Liu, C., Hsu, J. W., Chacko, S., Minard, C., Jahoor, F., & Sekhar, R. V. (2021). Glycine and N‐acetylcysteine (GlyNAC) supplementation in older adults improves glutathione deficiency, oxidative stress, mitochondrial dysfunction, inflammation, insulin resistance, endothelial dysfunction, genotoxicity, muscle strength, and cognition: results of a pilot clinical trial. *Clinical and translational medicine*, *11*(3), e372.

Lee, J., Cho, Y. S., Jung, H., & Choi, I. (2018). Pharmacological regulation of oxidative stress in stem cells. *Oxidative Medicine and Cellular Longevity*, *2018*.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., ... & Bitto, A. (2017). Oxidative stress: harms and benefits for human health. *Oxidative medicine and cellular longevity*, *2017*.

Pompella, A., Visvikis, A., Paolicchi, A., De Tata, V., & Casini, A. F. (2003). The changing faces of glutathione, a cellular protagonist. *Biochemical pharmacology*, *66*(8), 1499-1503.

Reddien, P. W. (2018). The cellular and molecular basis for planarian regeneration. *Cell*, *175*(2), 327-345.

Robaczewska, J., Kedziora-Kornatowska, K., Kozakiewicz, M., Zary-Sikorska, E., Pawluk, H., Pawliszak, W., & Kedziora, J. (2016). Role of glutathione metabolism and glutathione-related antioxidant defense systems in hypertension. *J Physiol Pharmacol*, *67*(3), 331-337.

Ruiz-Ramírez, A., Ortiz-Balderas, E., Cardozo-Saldaña, G., Diaz-Diaz, E., & El-Hafidi, M. (2014). Glycine restores glutathione and protects against oxidative stress in vascular tissue from sucrose-fed rats. *Clinical Science*, *126*(1), 19-