

# The effects of *Helianthus Annuus* on Amyotrophic Lateral Sclerosis using *Drosophila Melanogaster*

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## SUMMARY

Amyotrophic lateral sclerosis (ALS) affects nearly 200,000 people worldwide. The main cause of ALS is unknown as 90% of people diagnosed do not have genetic mutations or a family history of ALS. Currently, there is no cure for patients with ALS, and doctors recommend that patients do physical therapy to retain muscle strength and control. The purpose of the study was to determine if *Helianthus annuus* seeds helped reduce nerve degeneration and increase locomotion. *H. annuus*, also known as the sunflower, has antioxidant properties which can help to protect the nerves. *H. annuus* is also rich in magnesium, which helps regulate muscle and nerve conduction. In this experiment, we used *Drosophila melanogaster* as the model organism. We hypothesized that incorporating *H. annuus* into the diet of *D. melanogaster* would decrease nerve degeneration, leading to an increase in locomotion and a decrease in the disorderliness of the ommatidia in the eye. Through this experiment, we found a general trend suggesting that *H. annuus* helped increase the mobility of the *D. melanogaster*. Thus, with further experimentation, *H. annuus* could become a viable supplement for patients with ALS to help with the control of muscles and prevent the progression of the disease.

## INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurological disease that affects the motor neurons in the brain and the spinal cord (1). Some of the common genes identified to trigger ALS include *SOD1*, *TDP43*, and *FUS* (2). ALS currently affects more than 200,000 people worldwide, and 90% of these cases occur without a family history of ALS or known disease-causing genetic mutations. Only 5% of cases have a known family history. Some common symptoms of ALS include weakening of the muscles and difficulty in swallowing, talking, and controlling the movements of limbs (1). As ALS progresses, 80% of patients face spasticity in their arms or legs, causing a loss of control of the affected limb (2). On the other hand, 20% of patients face difficulty in controlling speech and swallowing (2). ALS may also lead to difficulty breathing, which may eventually lead to respiratory failure.

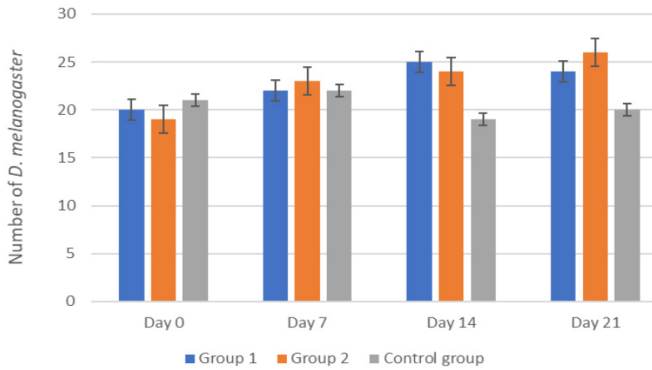
Currently, there is no cure or viable treatment for ALS other than physical therapy to retain the strength of the muscles, palliative care, and surgeries to help with mobility

(1). There are also a couple of drugs available that have been shown to increase life expectancy by a couple of months. One such drug is Radicava (edaravone), which reduces the amount of oxidative stress in the body (3). Oxidative stress is an imbalance in the amount of oxygen-reactive species in the body, which leads to the death of nerve cells. However, many patients do not prefer Radicava due to the inconvenience of the administration, time commitment, and cost involved. The cost of a single administration costs \$1,000 and it takes 1 hour for each infusion and patients take them for up to one year (5).

*Helianthus annuus*, a plant in the Asteraceae family, is rich in vitamin E and vitamin B and has antioxidant properties that help to protect the nerves and strengthen the nerve signals from the brain and spinal cord. *H. annuus* is also rich in mineral elements and phytochemicals like manganese,  $\alpha$ -tocopherol, glutathione reductase, flavonoids, phenolic acids, carotenoids, and many more (4). These compounds have been shown to help lower blood pressure, increase diabetic control, and lower cholesterol, which decreases the risk of developing neurodegenerative diseases (4). Furthermore, *H. annuus* is rich in choline and selenium, which help with brain function and memory retention (7). *Drosophila melanogaster*, also known as the fruit fly, was used for this study as they are easy to experiment on, cheap, and have similar genes to those of humans.

In our experiment, we wanted to test the effects of *H. annuus* consumption on locomotion and the disorderliness of ommatidia in *D. melanogaster*. The disorderliness of the ommatidia was checked because the ommatidia shows the degeneration in the nerves. In our experiment, we wanted to verify whether adding *H. annuus* to *D. melanogaster* diet would decrease nerve degeneration, leading to an increase in locomotion and a decrease in the disorderliness of the ommatidia in the eye. We hypothesized the *D. melanogaster* provided with higher concentrations of *H. annuus* would have a greater increase in locomotion and decrease in disorderliness in the eyes.

We found that *H. annuus* did have a significant effect on the locomotion and ommatidium disorderliness in the *Drosophila* model, but the effects of *H. annuus* on *D. melanogaster* did not increase with dosage. Thus, further testing will need to be performed to strengthen the connection between an increase in the intake of *H. annuus* and a decrease in nerve degeneration. *H. annuus* has shown promise in improving

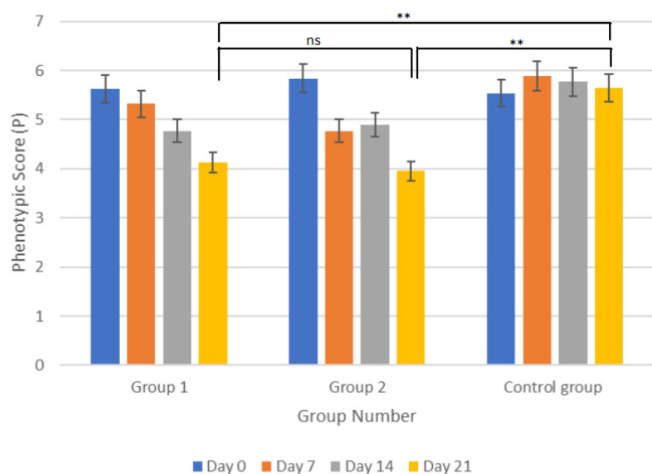


**Figure 1: The climbing assay measures the number *D. melanogaster* that crossed the 10 cm mark in a 15-second duration.** The graph shows the average number of flies in each group to cross the target mark after each of the 4 tests conducted on day 0, 7, 14, and 21. Group 1 received 0.5 g of *H. annuus*, group 2 received 1 g of *H. annuus*, and the control group received no *H. annuus*. The climbing assay was performed on all four days for each group.

ALS-like symptoms in *D. melanogaster* and thus should be investigated further as a candidate for medications to help people with ALS.

## RESULTS

At the start of the experiment, lead acetate was given to the *D. melanogaster* in all the groups to weaken their nerves. After 48 hrs, group 1 received 0.5 g of *H. annuus*, group 2 received 1 g of *H. annuus*, and the control group received no *H. annuus*. Two treatment amounts were given to see whether an increase in *H. annuus* plays a role in the locomotion and



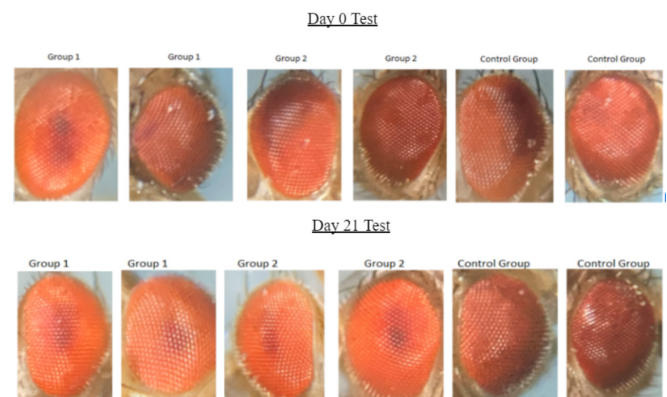
**Figure 2: The phenotypic score for ommatidial disorderliness in *D. melanogaster* as reported by Flyntyper.** The graph shows the phenotypic score for each group after each test. This value was calculated based on the distance and angle ommatidial disorderliness indices by the software. The lower the phenotypic score the more symmetric the ommatidia. The error bars represent the standard deviation. When the phenotypic score of groups 1 and 2 was compared they were not statistically significant, but they were statistically significant when compared to the control group. \*\* =  $p < 0.01$ .

disorderliness of the ommatidia. After every seven days, the ommatidia of the *Drosophila* were viewed under the microscope and a climbing assay was performed. The test was performed 4 times, on day 0, day 7, day 14, and day 21. For the climbing assay, the flies were transferred to another vial, and a timer was started for 15 seconds to see the number of flies that crossed the target mark, which was 10 cm. On average the climbing assay results improved by 25% when compared with the control group (Figure 1). For examining the eye, the flies were viewed under a Stereo Light Microscope and a photo was taken and uploaded into the software. The phenotypic score of the disorderliness of the ommatidia decreased by 26% from the control group. The data from day 21 for groups 1 and 2 were statistically significant when compared to the initial test results for groups 1 and 2 for the climbing assay and the ommatidial disorderliness (ANOVA,  $p < 0.01$ ). The ommatidial disorderliness for groups 1 and 2 had decreased and the ommatidia were more symmetric and evenly distributed when viewed under a microscope (Figure 2, 3). This supports our hypothesis that *H. annuus* could help decrease ommatidia disorderliness caused by damage in neurons and increase locomotion, suggesting that *H. annuus* helps with ALS-like symptoms in *D. melanogaster*.

## DISCUSSION

From our experiment, we found that the disorderliness in the ommatidia of the eyes had reduced and the locomotion had increased of the *D. melanogaster* in groups 1 and 2 after the final test. The decrease in disorderliness showed that *H. annuus* helps control the degeneration of the nerves in *D. melanogaster*. These findings suggest that further and repetitive testing could help strengthen the connection and viability of *H. annuus* as additive treatment of ALS-like symptoms.

In our experiment, we had to transfer the adult flies to new vials every few days to ensure that we did not have larvae in the experimental vials. This helped us obtain accurate results,



**Figure 3: *D. melanogaster* eyes examination at day 0 and day 21.** The images show the eye of two *Drosophila* from each group. Day 0 images were taken after 48 hours of exposure to lead and before *H. annuus* was provided. Day 21 images were taken after groups 1 and 2 received *H. annuus* for 3 weeks.

as the larvae did not receive the lead acetate treatment.

We also had to maintain a consistent temperature and moisture level in the vials, as these variables can affect the lifespan and mobility of *D. melanogaster*. Furthermore, the amount of *H. annuus* that was actually consumed by each fly was immeasurable, which could have affected the results. To minimize error, the average values of the disorderliness of the eyes were taken from each group. The microscope could have also affected the results, as a microscope with higher resolution could have produced images with higher clarity. Higher resolution images could have improved the accuracy with which the software reported the ommatidial disorderliness.

Although there were some limitations in the experiment, we conclude that *H. annuus* may help improve disorderliness caused by damage in the neurons and increase locomotion, thus may help with ALS-like symptoms in *D. melanogaster*.

In the future, we would like to experiment with different invertebrates, such as *Caenorhabditis Elegans*, *Danio Rerio*, and *Eisenia Fetida*, and use different quantities of *H. annuus* to investigate the effects on their nerves. These invertebrates are good model organisms because of their similarities to the human nervous system. In addition, we would like to test with herbal plants like valerian root, kava, and turmeric, which have been reported to have similar medicinal and nutritional properties to *H. annuus*. Eventually, we would also like to perform clinical tests to determine the benefits and the correct dosages of *H. annuus*. This will aid in creating medications using *H. annuus*. The medications may help to prolong the number of years a patient lives after being diagnosed with ALS. In addition, it can help them to continue their daily activities like eating and walking with ease. This can really help enhance the lives of patients with ALS, as there is currently no cure for ALS on the market. A drug is really necessary, and our idea is a promising option.

## MATERIALS AND METHODS

In the experiment, we had 3 groups: group 1, group 2, and the control group. Each group had around 30 *Drosophila melanogaster*. On day 1, 0.05 g of lead acetate was mixed in with the *Drosophila* instant food mix for all the groups. Next, 0.05 g of lead acetate was mixed in with 3 grams of *Drosophila* instant food mix to dilute the concentration. The *D. melanogaster* were placed in vials containing lead acetate food for 48 hrs and then transferred to new vials. Group 1 and group 2 were given 0.5 g and 1 g of *H. annuus*, respectively. The climbing assay and the examination of the eye were performed on day 1 to serve as a baseline with which to compare our results at the end of the 3 weeks. Both the tests were repeated every 7 days for a duration of 3 weeks.

### Food:

For the food source, we used the Formula 4-24 Instant *Drosophila* Medium (Carolina Biological). To make the *Drosophila* diet, we mixed 250 mL of Formula 4-24 and 250

mL of water. When the medium started to gel, we added 0.5 g of *H. annuus* for group 1 and 1 g for group 2. Then the food mix was transferred to the vials.

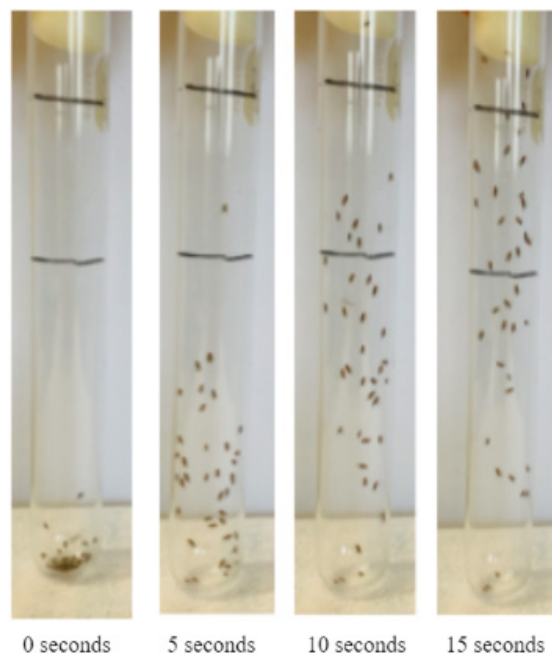
### Climbing assay:

The climbing assay measured the locomotion of the flies. This test was performed on the flies without any anesthetic to not impair the organism's climbing abilities. Before starting the experiment, a marking was made on the testing vial at 10 cm and 15 cm from the opening. Then, around 25 flies at random from one of the groups were transferred from their vials to the testing vial. This process was repeated for all three groups.

The tube was firmly taped on a flat surface which caused the flies to fall to the bottom of the tubes. A recording was started to examine the locomotion of the flies with the camera focused at the 10 cm mark. Immediately, a timer was started. After 15 s, the recording and timer were stopped. Later, each video was paused every 5 s to count the number of flies that crossed the target mark (**Figure 4**). This process was repeated three times for each group to confirm that the results were consistent. The three recordings for each of the three groups were averaged to get the data points. During the test, the flies were not exposed to sunlight or other light stimuli, as it might affect their direction of locomotion.

### Assessment of Eye Phenotypes:

The flies were anesthetized using FlyNap for immobilization while their eyes were examined. Five *D.*



**Figure 4. The number of *Drosophila* that crossed the target mark after 15 seconds.** An image was taken every 5 seconds to capture the locomotion of the flies for 15 seconds. The two marks on the vial are the 10 cm and 15 cm marks. Every 5 seconds, the number of flies that crossed the target mark was recorded.

*melanogaster* at random from each group were examined every 7 days under a stereomicroscope. To quantify the differences in the phenotypes, the images of the eyes were uploaded to the Flyntyper software, which was used to examine each ommatidium in the eye and report a phenotypic score of the amount of disorderliness in the arrangement of the ommatidia (6). The software reported the distance ommatidial disorderliness index which is the distance from one ommatidium to the surrounding six ommatidia, the angle ommatidial disorderliness index which determines the angle formed by two adjacent ommatidia, and the total ommatidial disorderliness index which is the sum of the distance and angle ommatidial disorderliness indices. The last column is the phenotypic score which is calculated using the number of detected ommatidia and total ommatidial disorderliness. The phenotypic score is calculated as  $1/\log Z$ , which represents the number of ommatidia detected by the software, all of which are multiplied by the function of the total ommatidial disorderliness. The numeric values helped us find the amount of degeneration in the eye due to the damage to the neurons.

#### Statistical Analysis:

A one-way ANOVA statistical test with a  $p < 0.01$  was used to compare the results from the climbing assay of groups 1 and 2 with the control group. We also compared the phenotypic score that was reported from the software of groups 1 and 2 with the control group.

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