The Effects of Fucoidan on Depression Using a *Drosophila melanogaster* Model

Grant Proposal

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Abstract

Depression is a common mental illness that affects many people. Recently, studies have shown the gut:brain axis has a great impact on mental illness. Brown seaweed has been believed to have many pharmaceutical benefits for centuries. Fucoidan, a polysaccharide found in brown seaweed, has the potential to cause antidepressant effects when included in a patient's diet. To test the effects of fucoidan on depression, a *Drosophila melanogaster* model will be induced with depression using two different methods. Depression will be induced through administering L-Dopa through their diets and using chronic unpredictable mild stressors to induce “learned helplessness”. Different dosages of fucoidan into the diet of the *Drosophila* will be tested for its effects on locomotion, aggression, and feeding preferences. The expected result is that specific dosages of fucoidan will decrease the number of behaviors symptomatic of depression present in the flies. The decrease in depressive symptoms corresponding to dosages of fucoidan show fucoidan has antidepressant capabilities.

The effects of fucoidan on depression using a *Drosophila melanogaster* model

Depression is a prevalent issue in our society. In 2020, the prescription of antidepressants was 20% higher than it was during the time period of 2015 to 2019 (Pazzagli et al., 2022). While there are current antidepressant medications, they have many side effects that can prevent a patient from taking them; including drug interactions with other medications, w. (Selective serotonin reuptake inhibitors(SSRIs), 2023). A safer treatment for depression is needed, and fucoidan may be the answer. Fucoidan is a sulfated polysaccharide and is primarily found in brown seaweeds (Meinita et al., 2021). Previous studies have shown fucoidan can stabilize the surface α-amino-3-hydroxy-5- methyl-4-isoxazolepropionic acid receptors (AMPARs), which regulates neuronal plasticity. Increases in neuronal plasticity have been

shown to help treat depression; henceforth, fucoidan has the possible ability to act as an natural anti-depressant (Li, et al., 2020; Albert, 2019). Hizikia *fusiformis* is a type of brown seaweed; in which, polysaccharides, like fucoidan, account for 40-50% of the seaweed cell wall's dry matter (Meinita et al., 2021). H. *fusiformis* was shown to regulate serotonin and dopamine levels of in vitro mouse cerebral cortical neurons (Jung et al., 2023). Fucoidan should present antidepressant effects in the *Drosophila* model. Two types of depression-induced *Drosophila* will be tested. Control groups will be measured against groups of *Drosophila* with the treatment diet. Depression symptoms will be monitored through three different assays: locomotive, aggressive behavior, and feeding habits.

Depression

Major Depressive Disorder(MDD) is a severe form of depression, characterized by persistent feelings of depression. Currently, therapies and medications are the two main ways MDD is treated. The current antidepressant medications have many issues that could cause inability or for a patient to take them, including many side effects; a few of which are drug interactions with other medications and worsened feelings of depression (Selective serotonin reuptake inhibitors(SSRIs), 2023). It can be linked to many behavioral assays including aggression, anhedonia, and a decrease in movement.

Fucoidan

Fucoidan is a sulfated polysaccharide. It can be found in many brown seaweeds, including *Hizikia fusiformis. H. fusiformis* is a brown seaweed that has been used by East Asian countries for traditional cuisine and medicine. *H. fusiformis* extract was found to have possible regulatory effects on serotonin and dopamine (Jung et al., 2023). It is known that polysaccharides, like fucoidan, account for 40-50% of the seaweed cell wall's dry matter (Meinita et al., 2021). Fucoidan was shown to have

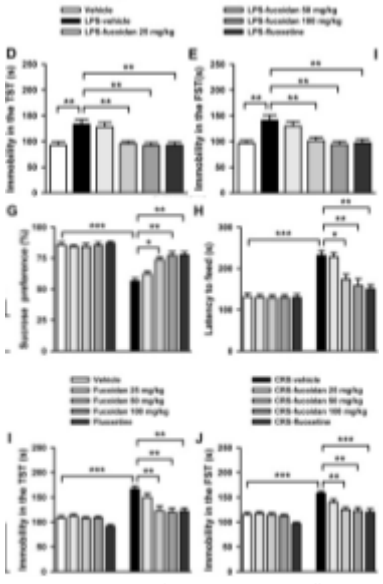

Figure 1: Results from study on the effects of fucoidan on depression (Li et al., 2020).

antidepressant effects in mice with lipopolysaccharide (LPS) and Chronic Restraint Stress (CRS) induced-depression (Li et al., 2020). The graph (Figure 1) provides convincing evidence that fucoidan has an effect on LPS and CRS induced-depression in mice. Further studies into the effects of fucoidan on depression using other depressive models should be conducted to provide further evidence.

Drosophila melanogaster as a model organism for depression

Drosophila melanogaster uses many of the same neurotransmitters as mammals, but are easier to study as they are less expensive, and have faster breeding and testing. Henceforth, they are the preferred model for many neurological studies (Martin and Krantz, 2014). In this study, *Drosophila* will be induced with depression through the inclusion of levodopa, also called L-DOPA, in their diets and through chronic unpredictable mild stress (CUMS) inducement. Including levodopa into the diet of *Drosophila* has been proven to increase depression symptoms and increase biochemical biomarkers related to depression (Jiang et al., 2017). The chronic unpredictable mild stressors (CUMS) protocol has also been proven to increase depression symptoms and decrease biogenic amines in *Drosophila* (Araujo et al., 2018).

Section II: Specific Aims

This proposal's objective is to test the effects fucoidan has on depression. Our long term goal is to test if including fucoidan in the diet could be used as an antidepressant treatment. The rationale is that fucoidan was shown to exert antidepressant effects on two models of depression in mice (Li et al., 2020). The work we propose here will provide further evidence to the antidepressant qualities of fucoidan. To assess the antidepressant qualities of fucoidan, the following specific aims must be met:

Specific Aim 1: Test the effects of fucoidan dosages on aggression in 2 depression models of *Drosophila*.

Specific Aim 2: Test the effects of fucoidan dosages on locomotion in 2 depression models of *Drosophila.*

Specific Aim 3: Test the effects of fucoidan dosages using the sucrose preference test in 2 depression models of *Drosophila*.

The expected outcome of this work is that the fucoidan treatment groups will show lower behaviors linked to depression as compared to the control groups. The explanation of what this would present as in each of the specific aims is in Section III: Project Goals and Methodology.

Section III: Project Goals and Methodology

Relevance/Significance

Depression is a very prevalent issue in today's society. Approximately 280 million people worldwide suffer from depression (Depressive disorder(depression), 2023). With such a common issue, an easily accessible, effective treatment is necessary. Fucoidan is commonly found in man brown seaweeds and is found in many supplements.

Specific Aim #1:

Determine the aggression levels of L-DOPA and CUMS depression induced *Drosophila* when treated with different fucoidan. The objective is to measure depression in *Drosophila* through their aggressive behavior. Our approach is to monitor aggressive encounters between two Drosophila, more specifically monitoring the aggressive attacks made, type of attacks, and time it takes before initiating an aggressive encounter.

Methodology

To assay aggression, two same-sex flies will be forced into close proximity with little space. Their interaction will be recorded with the laboratory's camera. An adjustment period will be allocated, where this period will be the same amount of time for all pairs. Time taken from the end of adjustment period till the first attack will be recorded. The number of aggressive attacks in a specified time period will be recorded.

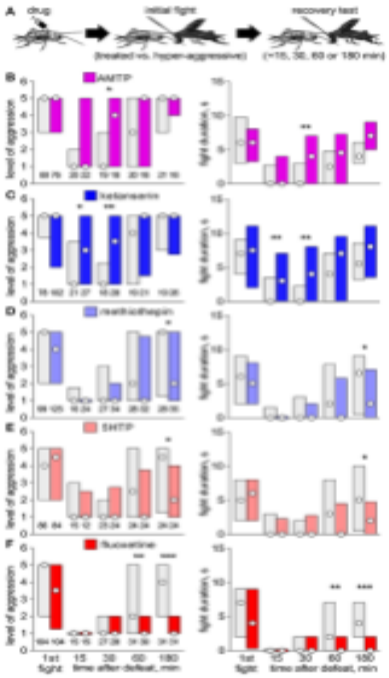

Figure 2: Graph of the level of aggression and fight durations of crickets treated with different substances that affect depression. (Rillich & Stevenson, 2018)

Justification and Feasibility

Our justification is that aggression is a sign of a depressive-like state in *Drosophila*; henceforth, monitoring aggressive attacks between *Drosophila* is a quantitative way to measure the extremity of their depressive state. Depression has consistently been linked to an increase in aggressive behaviors. Aggression has been shown to have a direct correlation with neurotransmitters in the brain (Rillich & Stevenson, 2018). Figure 2 shows results from this study.

Expected Outcomes

The expected outcome is that dose-specific treatments of fucoidan will decrease the prevalence of aggression in *Drosophila.*

Specific Aim #2:

Determine the average locomotion of *Drosophila* depressive models (L-DOPA and CUMS induced) for different fucoidan dosages. The objective is to measure depression in *Drosophila* by measuring locomotion.

Methodology

Our approach is to test negative geotaxis of flies during a 5 second time period. To do so 24 hours before experimentation flies will be anesthetized with FlyNap, and their wings clipped.

Groups of three to six flies are placed in an empty graduated cylinder and allocated a two minute adjustment period. The graduated cylinder is sharply tapped against a table three times so flies will fall to the bottom of the container. The height each fly has reached after five seconds will be noted. Each group of flies will be tested up to six times, between tests a one minute period will be given to let the flies readjust. After all data has been collected these flies will be disposed of. The method used is adjusted from the RING assay described by Nichols et al., 2012. It has been adjusted to take specific heights and have a five second time period. Due to taking specific heights, less flies are examined per trial group.

Justification and Feasibility

Flies with induced-depression have presented less negative geotaxis than flies(Moulin et al., 2021). Henceforth, monitoring the average negative geotaxis of *Drosophila* that has been induced with depression and treated fucoidan and comparing it to those not treated with fucoidan shows a change in depressive symptoms present in the organism due to fucoidan.

Expected Outcomes

Dose-specific treatments of fucoidan will create an increase in negative geotaxis as compared to the depression-induced groups not treated with fucoidan.

Specific Aim #3:

Determine the feeding preferences of depression-induced *Drosophila* that have been treated with different dosages of fucoidan. The objective is to measure anhedonia through a quantitative test that measures the prevalence of a specific feeding preferences of *Drosophila*. Anhedonia is a symptom of depression that causes a loss in interest of activities that one would normally have interest in.

Methodology

Our approach is by following the sucrose preference feeding (SPT) test protocol. The SPT is a commonly used practice (Yin et al., 2021). In our study sucrose preference will be tested by separating flies into groups of approximately five. Each will be put in an empty vial that has four evenly spaced out holes in the lid. Four capillaries will be put through these holes and set in place with modified micropipette tips. two of the capillaries will be filled with 10 µg sucrose solution each and the other two will be filled with 10 µg of water each. After 24 hours, the amount of sucrose solution and water left in the capillaries will be measured. The preference of sucrose solution as compared to the water will be calculated.

Justification and Feasibility

Our rationale for this approach is that the SPT measures the anhedonia present in the model organism. This test is one of the only ways to monitor anhedonia in model organisms. Anhedonia is a major symptom of depression and should be monitored (Yin et al., 2021).

Expected Outcomes

The groups treated with fucoidan will show less anhedonia as compared to the untreated, depression-induced control groups.

Preliminary Data to be Collected

The preliminary data for this project will consist of the results of the three chosen assays with flies that have not been induced with depression. The preliminary data will show a baseline of what a “healthy” fly should be. There will be a control group of types of flies in the actual experiment. Obtaining this preliminary data will provide a healthy baseline to compare to the project's control group of “healthy” flies. It will also provide lab practice in measuring the assays used in the experiment.

Section IV: Resources/Equipment

All lab work will be completed in the lab at Mass Academy of Math and Science at WPI.

Materials needed:

- *Drosophila Melanogaster*
- Food for *D. Melanogaster*
- Fucoidan
- Levodopa
- Lamp
- Heater
- Refrigerator or other tool for storage at a low temperature
- Thermometer
- Box to contain *Drosophila* while under light conditions
- incubator
- Sucrose solution
- Water solution
- Vials
- capillaries
- Test tubes
- Cotton balls
- Arena chamber
- Camera(will use cell phone)
- Timer (will use cell phone)
- FlyNap
- Funnel

Section V: Timeline

The project in total should span around 5 to 6 months, this length goes from brainstorming to finishing the final paper. The total project should be completed and ready to be presented by February 15th.

Preliminary data

Collection of preliminary data will start November 23. After that date, weekly or twice weekly lab days will start and go until preliminary data collection is completed. Preliminary data collection should be completed by December 15th. On December 12th, preliminary data collected to that point and the project idea will be presented to Mass Academy Alumnus, teachers and my fellow students. The poster and presentation will be created in the weeks leading up to December 15th.

Data Collection

The experiment will be conducted during January and early February. This will also happen at weekly or twice weekly lab days, as schedules allow.

Data Analysis:

Once data collection is complete, the analysis and conclusion will be written up with figures and tables representing collected data. The write up should be completed before February 15th. On February 15, the project will be presented at a STEM fair.

Section VI: References

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