Evaluating the effects of Mojave yucca on Drosophilae with Huntington's Disease

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2/26/24

Problem:

Huntington's disease takes the lives of millions every single year. With no apparent cure, rates of HD are only on the rise. This problem is in need of addressing due to its spiking rates. The implementation of Mojave yucca could provide a potential cure for HD and needs to be tested in a standard research environment.

Hypothesis:

It is hypothesized that if the Mojave yucca herb is implemented on drosophila melanogaster with early-stage Huntington's disease, then the herb will provide positive results and will slow down HD greatly. This is because of the neuroprotective abilities Mojave yucca holds, as well as its antioxidant and anxiety-reducing abilities.

Variables and constants:

The independent variable of this research would be the implementation of the herb and its different dosages. The implementation of Mojave yucca dosages is being varied throughout the research. The dependent variable of this research would be the effects of the herb on HD. Some of these effects would include motor skills, cognitive strength, overall progression, and more. The control for this experiment would be the drosophila that have HD, but no cure has been administered. This is the benchmark that is being measured with the data from the other groups with herb implementation and dosages. There are many constants in this research. Some main constants would include the living conditions of the flies, the food of the flies, the herb that is

being implemented on the flies, the implementation of Huntington's disease, the time period where the flies are being tested, and the route of herb administration.

Groups:

1. Normal Fly
2. Normal Fly with Mojave Yucca
3. Fly with HD
4. Fly with HD and Mojave Yucca Dosage

Repeated Trials:

In this experiment, when conducting assays, each assay will vary greatly. The climbing assay will consist of four trials lasting around 10 minutes each. The aversive phototaxic assay will have a large number of trials, around 16. This is due to the fact that flies do not display any phototaxic behavior in the first 4-6 trials, so many trials need to be conducted to achieve accurate results.

Materials:

Toxicity Assay	**Climbing Assay**	**Aversive Phototaxic Assay**	**Fly Maintenance**	**Huntington's Disease Related**	**Misc.**
Jazz- Mix (Fisher Scientific #AS153)	Fly strains to be tested of either sex, aged 3-4 days	T-maze apparatus:	Fly food	(P{w[+mC]=GAL 4-elav.L}2/CyO). **GAL-4**	
Water (from lab purification system)	Ice	Quinine solution	Water	w[*]; P{w[+mC]=UAS-HTT.ex1.Q93}4F1 **UAS**	

Bulk polystyrene fly vials (Genesee Scientific, #AS 520)	Cold sorting machine	Filter Paper	Drosophila tubes		
Vial plugs (Flystuff.com # 32-116BF)	Metal forceps	Fly Food	Vials		
Cheese cloth (Flystuff.com # 53–100)	Empty vials to transfer anesthetized drosophilae	Water	Plugs		
Stirring hot plate			Cages		
Thermometer			Filters		
Cool water bath			Sponges		
2 nd stir plate			Anesthetic		
2 L glass beaker			Yeast		
100 ml glass beaker Digital scale (1 kg range)			Sucrose		
Stir bar			Agar		
Standard microwave oven			Propionic Acid		
25 mL serological pipettes					
Micropipette filler/dispenser					
Mojave Yucca Herb					

Procedure:

Obtain a lab environment that can be used for research

Fly Food

1. Put out different vials for drosophila to perform fly tapping and create fly food

a. Fly Food: (procedure adapted from *https://cshprotocols.cshlp.org/*)

b. 1 batch of fly food

i. Add 6.75 grams of yeast, 2.9g soy flour, 28.5 yellow cornmeal, and 2.25g agarose by measuring with a digital scale

ii. Take 390mL of distilled water and add to beaker and gently stir up until mixed

iii. Measure out and add 30mL of corn syrup into beaker with ingredients

iv. Microwave for 3 minutes and with hot hands, take out beaker and mix every minute to prevent overflow of the beaker inside the microwave

v. Place a cheesecloth and let cool for roughly 1 hour until the food is lukewarm

vi. Add 1.88mL propionic acid to harden the fly food

vii. Immediately, carefully and equally pour out the food into roughly 30 vials and place in the fridge to cool

Drosophilae Models (adapted from Satvika Aruva)

2. Order drosophilae for research

a. Stock standard drosophila need to be ordered

b. (P{w[+mC]=GAL4-elav.L}2/CyO).

i. This is the GAL-4 Drosophila

c. w[*]; P{w[+mC]=UAS-HTT.ex1.Q93}4F1

i. *This is the UAS system*

Fly Tapping and Separation

3. Once drosophila are obtained, immediately tap them into new vials

4. Gender separates flies and identifies virgin males.
    a. For this lab, cold sorting is optimal for dealing with cognitive studies of the drosophilae
    b. Turn on the cold sorter and take a batch of flies
        i. Place a paper sheet in between the cold sorter and the flies and place them in
        ii. The cold sorter will put them to sleep allowing for easy identification of flies
            1. Male drosophila are smaller, have a darker and rounder abdomen, along with less lines across abdomen as compared to females
        iii. Once separated based on gender, the flies can be transferred back, the cold sorter should be turned off, and ethanol should be sprayed to clean the area

Toxicity Assay

5. The first assay to be conducted will be the toxicity assay
    a. Develop different dosages and find at least 8 dosages
    b. Administer these dosages to fly food
        i. Measure weight of fly food vial and add a specific dosage ratio based on weight
        ii. Gently stir with a glass stirring rod and place in fridge
    c. Tap drosophila into herb vials and observe effects for 2 weeks
        i. At the end, record, larvae production, fly movement, reactions, and # of flies dead at the bottom of the vial

Climbing Assay

6. Steps adapted from *("Quantitative Analysis of Climbing Defects in a Drosophila Model of Neurodegenerative Disorders")*
7. After constant culturing of flies for a period of time, the climbing assay must next be conducted.
    a. Take 20 flies and place them in a cylinder of 250 mL and mark the cylinder height and more to keep constant throughout the assay
    b. Make sure that the experiment is done in a natural setting where there is no sources of error for drosophila
    c. Close the top of the cylinder and set up a camera focused at the 190mL line of the cylinder
        i. This is focused on the finish line of the climbing assay
    d. Start the assay and time them for a certain amount of time
        i. Count the dead flies at the bottom this is the death rate of the flies within your assay
        ii. Throughout the experiment, make sure to tap the vial to displace the flies so that their geotaxis can be properly measured
    e. Measure for two minutes and record data every 10 seconds of the experiment. Make sure to accurately measure data to minimize error
    f. Keep redoing experiment for all trials for all the different groups of flies
        i. Once done, clean and spray 90% ethanol into the fly vial
    g. (Madabattula et al., 2023).

Aversive Phototaxic Assay

8. Next, the Aversive Phototaxic assay needs to be conducted.
    a. Drosophila are placed in a bright vial with a quinine drenched flug for 1:30 minutes.
        i. This allows flies to associate the bright vial with quinine taste
    b. Next, drosophila are placed in between a bright and dark vial and are given the opportunity to choose between vials
        i. Number of drosophila on each side will be counted and this will be tested with different flies for 2 more trials
    c. Once all the assays are conducted, flies need to be disposed of properly and data needs to be collected, and the next step is statistical analysis.

Lab Safety:

There are many lab safety protocols and safety issues that need to be addressed. The first main concern is that since many flammable materials, technology, and more are being used, lab coats, goggles, closed-toed shoes, and hair should be tied back. Next, glassware is being used, so glasses should be used to prevent damage to the eyes in the event of glass shattering. When working with hot substances, tongs and hot gloves should be utilized. Next, when working with flies, if they are released, it can cause some problems with contamination. To avoid this, flies should be asleep when sorting, they should be transferred properly, and they should be stored properly with all lids closed as well. When working with Mojave Yucca, it is known to cause rashes and more when coming into contact with human eyes. To avoid this issue, gloves should be used when handling the herb, and all contact with the herb should be minimized.

References:

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Statistics

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1. The main goal of this research project is to study the effects of Mojave Yucca on drosophila with HD. With the implementation of the climbing assay and the Aversive Phototaxis Assay, the motor skills of drosophila and cognitive skills of the drosophila are what is being measured throughout the project. Two main statistical analysis tests are being conducted for this assay. The three-way ANOVA test is being conducted to study the results and provide a result whether the herb is effective with motor skills in the climbing assay. To assess the Aversive Phototaxic Assay, the Mann Whitney U test will be utilized to measure whether the herb is effective with improving cognitive strength in flies.

2. For both the Mann Whitney U test and the three-way ANOVA test, there are four main conditions that will be compared with each other. The first group is the fly with no HD and no herb. The second group is the fly with HD and no herb. The third group is the fly with HD and the herb. The fourth group consists of the fly with no HD and the herb. This is why a 3-way ANOVA test is used to study all four conditions and compare them with each other.

3. There are two main control groups of flies that will be used for this experiment. The first control group is the flies with HD but no administration of herb. This is the benchmark used to compare the flies with the herb and HD to because it provides insight on whether the herb was effective on HD. The next control used is the flies with no HD and no herb.

This can be used as a second benchmark to compare the abilities of flies with the herb and No HD.

4. There are 2 main replicates for this research project. For the climbing assay, 4 trials that are 10 minutes each will be conducted. This will allow for the most optimal collection of data. The main limiting factor for the number of trials for the climbing assay would be the time that is given. Due to the large workload that is needed for this project, only 4 trials would be able to be conducted for the climbing assay. Still, this is more than enough to ensure accurate data. For the Aversive Phototaxic Assay, around 10 trials should be conducted on three different times. This large number of trials is due to the fact that the first 4 trials usually are meant for the flies to register information, and because the first 4 will not provide fully accurate results. This trial is limited to only 10 for 3 days due to the resources that are not available. Firstly, the assay model is 3D printed and this leaves for issues with the apparatus. Following this, after every 10 trials, the apparatus needs to be reset and a new one should be used. With these trials alone, 3 different Aversive Phototaxic Apparatuses need to be used.
5. Data will be randomized as random flies within each of the 4 groups will be used for the assays. Along with this, flies will constantly be disposed and new ones will be used throughout the experiment

Three-Way ANOVA Test Data Table				
	Group A	Group B	Group C	Group D
	HD Fly No Herb	HD Fly with Herb	Normal Fly with Herb	Normal Fly No Herb
Time Taken				
Mortality				
df				

Mann Whitney U Data Table				
	Group A	Group B	Group C	Group D
	HD Fly No Herb	HD Fly with Herb	Normal Fly with Herb	Normal Fly No Herb
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
df				

6. The study will use the three-way ANOVA test to compare the climbing ability of flies between all four groups. This statistical analysis is a parametric test. The nonparametric version of the test (Kruskal-Wallis) is effective for comparing the medians of three or more groups. The main issue is that it studies medians and not means. This leads to issues, as it is more susceptible to skewed data with one or two outliers. The three-way

ANOVA is more powerful than the Kruskal-Wallis and therefore is the more optimal test to study the climbing assay.

For the aversive phototaxic assay, the Mann-Whitney U test will be used to compare the data of the four groups. The Mann-Whitney test is a nonparametric test and is used to compare the medians of the two groups. This is a strong suit for the aversive phototaxic assay, as it rather samples and focuses on the number of flies that follow their taxis as opposed to specific values. The parametric version of this test is the two-sample T test. This test is more sensitive to outliers and can lead to a skewed data distribution. This is why the Mann-Whitney U test is most effective for studying the aversive phototaxic assay.