Project Proposal

Project Title: The Effects of Ethanol Metabolism on the Reproductive System of *D. melanogaster*

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Project Definition:

In this project, I will research how the metabolic processes of ethanol in *D. melanogaster* affect the ability of the reproductive system to produce offspring. The hypothesis is that if the rate of the metabolism of ethanol increases, then the number of healthy offspring produced will decrease because the toxic molecules and free radicals produced when ethanol is broken down will lead to oxidative stress which is known to lower the functionality of the body and raise the risk of disease. By testing on *Drosophila melanogaster,* the repercussions of alcohol consumption on the reproductive system can be studied.

Background:

Importance:

It is a well-known fact that the consumption of alcohol can do great damage to the human body. Many studies have been done to show that those who have a higher intake of alcohol are more susceptible to malfunctions throughout the body. Some of these effects are specific types of cancer, epilepsy, and heart disease. Other risks may have other attributing factors but have an association with drinking. (Rehm, 2011). In the last two decades, scientists have begun to look at the deeper causes of these diseases, and have studied the way that ethanol affects the body at the molecular level.

Topic Explanation:

When alcohol is taken into the body, it is absorbed into the bloodstream, mainly through the stomach and the small intestine. The ethanol in the blood gets delivered to many places around the body. In these places, primarily the liver, alcohol dehydrogenase, catalase, and P4502E1 work to break down the ethanol into acetaldehyde. Acetaldehyde is an extremely toxic substance that can cause many diseases or malfunctions of the body. A chemical reaction with aldehyde dehydrogenase in mitochondria eliminates the acetaldehyde and produces acetate. When a surplus of ethanol is in the body, the function of alcohol dehydrogenase increases to accommodate. However, the rate at which aldehyde dehydrogenase works stays the same, so the result is an excess amount of acetaldehyde which remains in the body for hours until it is broken down. When this occurs often, it becomes especially dangerous for the body as acetaldehyde is unsafe (NIAAA Publications, n.d.).

In addition to this, the function of alcohol dehydrogenase and aldehyde dehydrogenase produce free radicals such as 1-hydroxyethyl and hydroxyl (Comporti et al., 2010). Free radicals are a byproduct of a metabolic process and are chemically unstable molecules, meaning that they are very reactive. The body balances these toxins out with antioxidants, but when there are too many free radicals, the ratio of the toxins to antioxidants becomes too high. In this way, the reactive oxygen species can damage parts of cells. This situation, called oxidative stress, occurs when too much alcohol is consumed. When the body breaks down the large amounts of ethanol, it reaches a point where the free radical to antioxidant ratio becomes too high, increasing the risk for disease (Zima et al., 2001).

Past Studies:

Recently, scientists have worked to understand how the metabolism of ethanol affects different areas of the body or the chances of contracting a certain disease. One study found that the consumption of ethanol led to lowered fertility rates in male rats. This was because of the accumulation of acetaldehyde and 1-hydroxyethyl in testes where ethanol metabolism occurred damaged the organ's ability to produce sperm (Quintans et al., 2005). Female fertility was also found to be lowered by ethanol when scientists found structural change and malfunctions to the ovaries of female rats (Faut et al., 2009). In another study, researchers discovered that the metabolism of ethanol in the uterine horn of female rats caused damage to the uterus for similar reasons (Buthet et al., 2011). The breaking down of ethanol even in small amounts can change the function of many organs in the body (not just the liver where it is primarily metabolized), particularly the reproductive system.

Similar effects have also been found with *Drosophila melanogaster.* For example, it was discovered that ethanol intake during the gestation period affects the lipid regulation of offspring, which can be used to model fetal alcohol syndrome in humans (Logan-Garbisch et al., 2014). Using *D. melanogaster* as a model organism, I can research the effects of ethanol on the reproductive system. Using the knowledge above about how ethanol affects the body, I hypothesize that the reproductive system of *D. melanogaster* will produce healthier offspring in greater numbers if less alcohol is consumed.

Experimental Design/Research Plan Goals:

Experimental Design Factors:

Independent variable: the amount of ethanol consumed

- concentration ranging from 0% to 10%, increasing by 2% for each group

Dependent variable: the functionality of the reproductive system (number of offspring produced), the health of the offspring and parent

Controls: amount and type of food, moisture in habitat, the temperature of habitat, number of males/females exposed to

Groups:

- Females receiving alcohol only during gestation (several groups with varying amount of ethanol in diet)
- Females receiving alcohol only during mating (several groups with varying amounts of ethanol in diet)
- Females receiving alcohol during gestation and mating (several groups with varying amounts of ethanol in diet)
- Males receiving alcohol during mating (several groups with varying amounts of ethanol in diet)
- Control (no ethanol in diet)

Iterations: 2-3 times, modifying procedure as necessary throughout the process

Materials:

- Several cylindrical, plastic vials (height: 4 in., diameter, 1.25 in.) for environment and toppers
- Instant *Drosophila* medium
- Distilled water
- Yeast
- Ethyl alcohol
- *Drosophila melanogaster*
- Fly anesthetic
- Microscope

Procedure Outline:

1. Prepare environments for *D. melanogaster,* adding a 1:1 ratio of *Drosophila* medium and water, adding alcohol for experimental groups.
2. Anesthetize the flies and move 8 females and 3 males into each vial with varying amounts of ethanol.
3. Anesthetize a group of older flies that have already mated (check through microscope) and move five females carrying offspring into each vial with varying amounts of ethanol.
4. Observe the offspring in each experimental group using a microscope.

Risk/Safety Concerns:

- The experiment will be run at home
- Wearing eye and skin protection (safety goggles, rubber gloves, lab coat) and washing hands before and after handling flies to prevent contact with chemicals in the fly food and anesthetic
- Wiping down area before and after handling flies
- Being careful with the anesthetic and informing those around to not inhale it

Data Analysis:

- Observe the number of offspring in each vial and the differences between the experimental groups
- Observe the differences between the experimental groups for the health of the offspring and the parent
    - Includes: ability for offspring to survive to expected lifespan, length of life from giving birth to death for mother, any abnormalities found in anatomy (using a microscope)

Potential Roadblocks:

- Handling *Drosophila melanogaster* without affecting the outcome of the experiment
    - Solutions: handling them very carefully while moving them and anesthetizing them, creating environments with a proper amount of water and yeast, adding ethanol in appropriate concentrations (do mathematical calculations, look at what amounts other researchers added)
- The tools or methods used are not effective in measuring a difference between experimental groups
    - Solutions: change the tools and/or modify the methods, look for different dependent variables that can be measured more easily
- The sample size is not large enough to proceed with the experiment or create an analysis
    - Solutions: buy or culture more flies to run experiment on

Timeline:

Research:

11/03 - 11/15 - Prepare for testing, preliminary tests
11/16 - 11/24 Set up the experiment, gather data
11/25 - 11/29 Thanksgiving break, gather data
11/30-12/04 Finish first testing
12/12 - 12/18 Begin second testing
12/19 - 01/03 Winter break, finish testing
01/04 - 01/31 Finish up any testing (a third testing?)

December Fair 12/11/2020:

12/05 - 12/10 - Prepare presentation

February Fair 02/11/2020:

01/04 - 01/16 - Analysis
01/16 - 02/10 - Prepare write-up and presentation

References

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