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Utilizing The Zebrafish species, *Danio rerio*, to assess the effect of acute treatment with St. John's Wort Supplements on Anxiety as measured by the Novel Dive Tank Test

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Abstract

Background: The zebrafish species, *Danio rerio*, is a widely utilized model organism with well-characterized behaviors including anxiety-like behavior when placed into a novel environment. In addition, the fish can be treated with substances by immersion.

St. John's Wort has been suggested as a plant-based treatment for depression and anxiety because it contains compounds that inhibit the serotonin transporter (SERT) among other effects. A number of St. John's Wort supplements are commercially available.

The National Institute of Mental Health (NIMH) estimates that about 19% of US adults suffer an anxiety disorder each year and about 31% will experience an anxiety disorder in their lifetime. Clearly effective, inexpensive, and readily available treatments for anxiety represent an important area of research.

Objectives: Assess the ability of acute treatment with St. John's Wort to reduce anxiety-like behavior in zebrafish.

Method: Zebrafish will be treated by immersion in a solution of St. John's Wort supplements and assessed for anxiety in the Novel Dive Tank (NDT) Test.

Results: Acute treatment with St. John's Wort supplements increased anxiety as measured using the NDT.

Conclusions: Acute treatment with the water-soluble constituents of St. John's Wort supplements tested did not reduce apparent anxiety in the fish. Future studies will utilize a longer treatment time and more hydrophobic solvents.

Introduction

There are a wide range of anxiety disorders that affect adults in the United States. While they differ in the circumstances that cause them, they share excessive anxiety and behavioral changes that can affect daily activities, work performance and interpersonal relationships. The National Institute of Mental Health (NIMH) estimates that about 19% of US adults suffer an anxiety disorder each year and about 31% will experience an anxiety disorder in their lifetime.

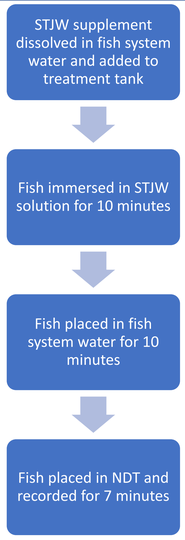
The plant, *Hypericum perforatum*, commonly called St. John's Wort (STJW), contains compounds that inhibit the serotonin transporter (SERT) among other effects and has been suggested as a plant-based treatment for depression and anxiety. A number of St. John's Wort preparations are commercially available.

The zebrafish species, *Danio rerio*, is a widely utilized model organism since it offers several advantages: well characterized behaviors, the ability to absorb treatments from their environment, and significant homology to humans.

A novel dive tank test (NDT) is an established method for measuring anxiety in the zebrafish species, *Danio rerio*. The test fish is placed into a 1- to 2-liter free-standing tank, typically trapezoid-shaped. In this new environment, the fish will swim down to the bottom in order to hide from predators. As the fish becomes more comfortable, it will begin to explore the tank, looking for food and other fish. Food, in particular, is most often located at the surface of the water. As a result, the time that elapses before the fish approaches the upper portion of the novel tank is a measure of anxiety. More anxious fish take longer to approach the surface, while less anxious fish approach the surface sooner.

This research project is designed to assess the ability of acute treatment with St. John's Wort to reduce anxiety-like behavior in zebrafish by utilizing commercially available STJW supplements and the NDT paradigm.

Methods



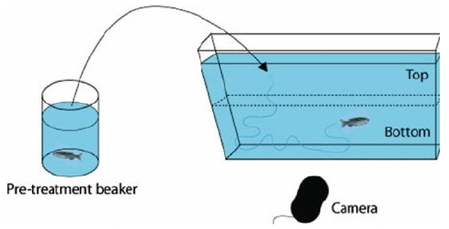


Figure 1: The novel dive tank (NDT) Image courtesy of Cachat et al. Noldus Ethovision XT software is used to analyze the video, providing distance traveled, average velocity, time spent in each zone, and other parameters.

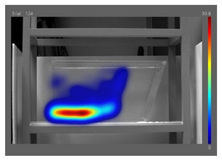


Figure 2: Heat map image collected by the NoldusEthovisionsoftware of a zebrafish in the novel dive tank test.

Results

Figure 3: Average total distance traveled during the NDT for control and St. John's Wort treated fish. N = 27 for each treatment. Control fish moved more (1063 cm) than STJW treated fish (685 cm).

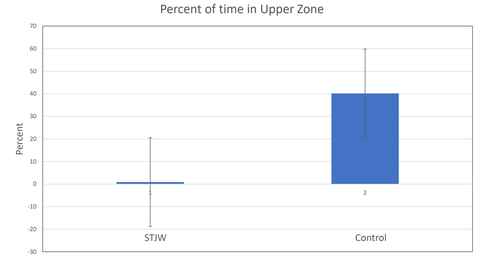


Figure 4: Average percentage of time spent in the upper zone during the NDT for control and St. John's Wort treated fish. N = 27 for each treatment. Control fish spent significantly more time in the upper zone (40%) compared to STJW treated fish (1%).

Discussion

Discussion: Fish treated acutely with STJW moved less on average than control fish did in the novel dive tank test. In addition, the STJW treated fish spent a significantly lower proportion of their time in the upper zone. These data suggest that the STJW treated fish were more anxious than the control fish, contradictory to our predictions. STJW is purported to contain inhibitors of several neurotransmitter transporters including serotonin, norepinephrine, and dopamine. Inhibitors of these neurotransmitter transporters are used to treat anxiety disorders but require weeks of daily treatment in order to see the benefit.

Limitations: Since the zebrafish were treated by immersion, we cannot be certain of the dose of STJW that each fish absorbed. Furthermore, the fish were only exposed to water-soluble constituents of the STJW supplement and the exposure was acute. In addition, we did not analyze data from male fish and female fish separately, so there may be sex-dependent differences in response to the treatment. Finally, while the Danios share significant homology to humans, they are not identical, so results in humans may vary from findings in this model organism.

Future Studies: Future studies will utilize a longer treatment time, including chronic treatment to determine whether the effect of STJW, like that of SSRIs, requires weeks of treatment to develop. Additionally, STJW supplements will be dissolved in oil and applied to food in order to incorporate lipid-soluble components likely to penetrate the blood-brain barrier. The components of the STJW supplements will be separated and identified using HPTLC to determine which constituents are present. Finally, additional testing will include analyzing male and female zebrafish separately to identify any sex-based differences in response.

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