CALIFORNIA LUTHERAN UNIVERSITY/DEPARTMENT OF BIOLOGY

The Effect of Hesperidin on a Rotenone-induced model of Parkinson's Disease in *Lymnaea stagnalis*

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ABSTRACT

The objective of this study is to determine whether the antioxidant hesperidin counteracts the effects of rotenone in the rotenone-induced Parkinson's Disease (PD) model of the freshwater pond snail, *Lymnaea stagnalis*. Thirty-two snails were treated with either rotenone, hesperidin, or a combination of both. For twelve days, locomotion and feeding (lettuce consumption) was measured daily to determine neuroprotective effects. Separate acute exposure tests measured locomotion and feeding in eighty-eight snails. Snails were pretreated with 0, 2.5, 5, or 10 µM hesperidin for four hours followed by exposure to 5 µM rotenone. After 24 and 48 hours, locomotion and feeding were measured. While there was a trend in pilot tests suggesting that hesperidin reduced the effect of rotenone, subsequent tests, did not produce statistically significant results (p=0.091). Additional trials and modified tests are needed to determine whether hesperidin can block the effects of rotenone.

INTRODUCTION

Parkinson's Disease (PD) is the 2nd most common neurodegenerative disease that affects about 6 million people worldwide.[1] Symptoms include loss of motor function, mental problems, and tremors. PD is characterized by a selective loss of dopaminergic neurons in the substantia nigra of the midbrain (Figure 1)[2].

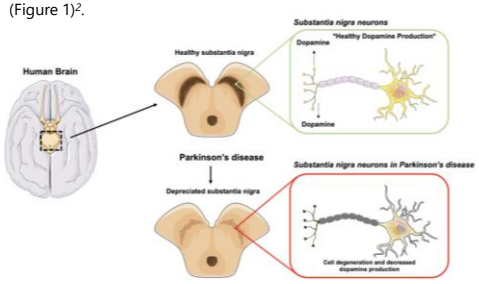


Figure 1: Substantia nigra of a healthy individual versus a person with Parkinson's Disease [2]

Studies on tissue samples taken from PD patients revealed that complex I of the electron transport chain has decreased activity[3] (*Figure 2*). As a result, there is a build-up of free radicals, which have an unpaired electron that makes them highly reactive. This can cause oxidative stress and decreased ATP synthesis, which is harmful and ultimately leads to cell death.[3] The complex I inhibitor, rotenone, has been used to create animal models of Parkinson's Disease such as *Lymnaea stagnalis*[7]. Rotenone acts by blocking electron transfer between the Fe-S center N2 and ubiquinone by blocking ubiquinone binding sites.[9]

AIM

In the present study, we aimed to determine if hesperidin has a neuroprotective effect on a rotenone-induced PD model in *Lymnaea stagnalis*. Feeding and locomotion was recorded in both chronic and acute exposure tests.

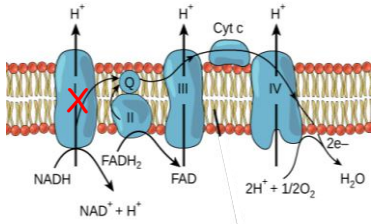


Figure 2 : In PD, complex I (red X) of the electron transport chain has decreased activity. [4]

Antioxidants are substances that can donate an extra electron without becoming a free radical themselves. Through this, the free radical is stabilized and is inhibited from causing further damage.[3] Studies using the antioxidant hesperidin on PD mouse models have shown that the addition of hesperidin prevented loss of mobility and cognitive defects, as well as decreased dopamine loss.[5] Apart from neutralizing free radicals, hesperidin increases antioxidant cellular defenses by upregulating certain genes.

ACUTE EXPOSURE TEST

Four groups of 22 snails each were tested. Feeding was measured by calculating feeding rate calculated from the surface area of lettuce disks consumed. Locomotion was measured for 15 minutes for each snail. Snails were then pretreated in one of the following concentrations for four hours:

1. 0 µM Hesperidin (Control Group)
2. 2.5 µM Hesperidin
3. 5 µM Hesperidin
4. 10 µM Hesperidin

After pretreatment, snails were placed in a tank containing 5 µM rotenone solution. After 24 and 48 hours, locomotion and feeding was measured.

CHRONIC EXPOSURE TEST

36 snails were divided into 4 groups and placed into tubs containing the following four solutions:

1. .05µM Rotenone
2. .05 µM Rotenone and .05 µM Hesperidin
3. .05 µM Hesperidin
4. Control – containing just Artificial Pond Water

Solutions were replaced daily over 12 days. Locomotion and feeding tests were measured daily as previously described.

RESULTS

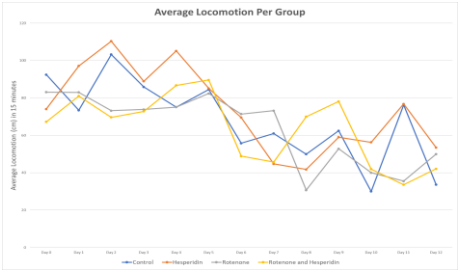
Acute Exposure Test Results:

- Results for feeding were insignificant (p=0.933 and 0.771 after 24 and 48 hours)
- Results for locomotion were insignificant after 24 hours in solution (p=0.111)
- After 48 hours, there was a significant difference in locomotion between groups (p=0.046). Post-hoc tests showed a significant difference between the 2.5 µM and 5 µM pretreatment group (p=0.038).

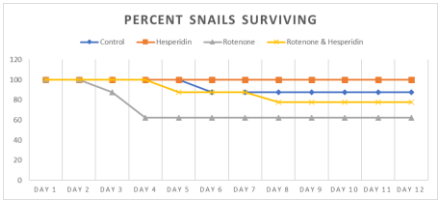
Chronic Exposure Test Results:

- Pilot tests showed a greater amount of feeding in 20 snails after 3 days in hesperidin and rotenone (left) versus 20 snails after 3 days in rotenone (right).

- Subsequent tests did not appear to show a difference in feeding between the rotenone group and rotenone & hesperidin group over time. Further tests with modifications are required to form a conclusion.
- Locomotion was insignificant between the rotenone and rotenone & hesperidin group on all days (p=0.273)



- On day 2, there was a significant difference in locomotion between the hesperidin and rotenone group (p=0.041) and between the hesperidin and rotenone & hesperidin group (p=0.022).
- On day 11, there was a significant difference in locomotion between the hesperidin and rotenone & hesperidin group (p=0.044).



- Percent of snails surviving in the rotenone group appeared to decline more rapidly initially than in other groups (n=8 in each group), however, past day 5, deaths in other groups were observed. Further tests with larger sample sizes. are required.

DISCUSSION

Methods were developed and tested to measure the neuroprotective effects of hesperidin on *Lymnaea stagnalis*. The current study did not present with any statistically significant results that indicate a neuroprotective effect of hesperidin; however, preliminary data suggests snails treated with hesperidin and rotenone had better feeding results over 3 days compared to groups treated only with rotenone.

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A complete list of references is available upon request.

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