Curcumin Mitigates the Detrimental Effects of Nicotine in a *Drosophila melanogaster* Model of Diabetes

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ABSTRACT

The total estimated cost of diagnosed diabetes was $327 billion in 2017 or more than 1 in 4 health care dollars. Vaping has been promoted as a safer alternative to smoking, but diabetics are at risk of complications. Nicotine triggers changes in metabolism, as well as high blood sugar levels in diabetics, which can lead to diabetes complications. However, minimal research has focused on mitigating the effects of nicotine on diabetic patients. A *Drosophila melanogaster* diabetic model was utilized to confirm the negative effects of nicotine, as well as to determine the plausibility of curcumin as a treatment due to its antioxidant properties. Drosophila was tested for locomotive ability, foraging behavior, and respirometry to quantify their metabolism.

All nicotine treated groups experienced a significant decline in their mobility (HS+N3 vs CHS+N3: -0.603 vs -0.248). Foraging behavior was significantly suppressed by Day 7 in all nicotine treated groups, especially those exposed to a higher concentration of nicotine within the food-seeking assays (N1 vs N3: 0.675 vs .56). Curcumin treatment significantly restored mobility and foraging behavior in all groups, although not to control levels. Metabolic function was immediately significantly suppressed only in diabetic fruit flies when treated with nicotine, and remained suppressed the entire trial; however, the application of curcumin completely mitigated the negative effects of nicotine and restored metabolism to control levels (HS+N1 vs CHS+N1: 360.606 vs 750.060).

The impact of nicotine on a diabetic Drosophila model bears similarities to the risks that diabetics face when they consume nicotine. The observed positive effects of curcumin on the diabetic model demonstrate the potential applicability of curcumin treatment in mammals.

BACKGROUND AND REVIEW OF LITERATURE

The usage of electronic cigarettes has steadily increased, particularly among the youth and young adults. Many e-cigarette users begin vaping to quit smoking as they believe it is a safer alternative. However, both cigarettes and e-cigarettes contain nicotine, which is an addictive substance that causes inflammation and oxidative stress when consumed. Recent research has discussed nicotine and its link to causing and progressing Type II diabetes. Diabetes is a group of diseases that causes blood sugar levels to rise and puts the human body at risk. Over 25 million individuals in the US are currently diagnosed with diabetes and the total estimated cost for treatment in 2017 was $327 million, which is equivalent to 1 in 4 health care dollars. Nicotine consumption leads to complications for diabetics as it triggers changes in the metabolism and damages cells, causing them to inefficiently respond to insulin and raise blood sugar levels.

A high conservation in basic biological, physiological and neurological properties exists between *Drosophila melanogaster* and mammals. It has been estimated that between 65% and 75% of human disease-causing genes are present in *Drosophila melanogaster* (Morris, 2018). The fruit fly has several discrete organ systems paralleling those in mammals that play key roles as metabolic regulators (Rajan, 2013). *Drosophila melanogaster* has also been seen to display a series of motor behaviors that are similar to those observed in mammals when exposed to addictive substances such as cocaine, nicotine, and ethanol (Kaun, 2012). A recent study used *Drosophila melanogaster* as a model organism to identify genes involved in adult nicotine sensitivity and molecular mechanisms that regulate responses to drugs of abuse

(Velazquez-Ulloa, 2017). Therefore, the usage of *Drosophila melanogaster* in metabolic and drug-induced research will provide accurate data in regards to how metabolic rate changes when flies are exposed to nicotine. Previous research has established the use of a high sugar diet to induce diabetes in *Drosophila melanogaster*.

The research behind nicotine and its relationship to diabetes is relatively new. Minimal studies have been conducted to mitigate the detrimental effects of nicotine on diabetics. Curcumin was used as a viable treatment as it is an antioxidant found in turmeric and contains alleviating properties. Previous research has found curcumin to increase longevity and provide health benefits in two different *Drosophila melanogaster* strains.

The purpose of this research is to establish the detrimental effects of nicotine on diabetic fruit flies, which will be achieved by observing their behavior and changes in their metabolism. In addition, the novel use of curcumin as a treatment and its ability to alleviate the effects of nicotine on a *Drosophila melanogaster* diabetic model will be investigated.

MATERIALS AND METHODOLOGY

Multiple procedures and experimental groups were utilized to generate optimal findings. Fruit flies were separated into 16 experimental groups based on the concentration of nicotine exposed, whether they were induced with diabetes through a high sugar diet, and whether they were given curcumin as a treatment. Each vial contained 4 flies in total and 20 grams of food media. Concentrations of nicotine included 0.01%, 0.02% and 0.03%, which were mixed into the food media. Eight of the 16 vials contained fruit flies fed on a high sugar diet while the other 8 contained flies fed on a regular diet. Four control groups were also accounted for and were separated based on sex and diet only. In total, 96 flies were exposed to nicotine, 24 flies consuming regular food media and the other 24 consuming a high sugar diet. Sixteen flies were not exposed to nicotine to represent the control group but were separated based on diet and treatment. The fruit flies were placed into their respective vials one day prior to experimentation.

A single fruit fly assay was performed on Day 1 and Day 7 to test for foraging behavior. Flies were starved for 24 hours in advance before being placed one by one in a Petri dish containing a single drop of apple cider vinegar in the middle. Each singular fly was recorded for 3 minutes and if the fly landed on the apple cider vinegar drop for more than 3 seconds, it was considered that they had found the food. A startle-induced negative geotaxis assay was also performed from Day 2-6 to account for fly activity and locomotive capacities. Each flies distance traveled was recorded in centimeters.

The metabolic rates of the flies were also measured using a respirometer. The respirometer was made by gluing a pipette tip to a glass capillary tube, before inserting a sponge, a small amount of soda lime, and another sponge before placing the flies into the pipette tip and closing the open top with modeling clay. The respirometers were suspended into a fish tank with distilled water mixed with a visible dye. The tank was then closed and monitored for 2 hours. Pictures were taken before and after the 2 hours to display height differences in the respirometers to indicate CO_2 levels.

RESULTS

The data revealed that nicotine treated groups experienced a significant decline in their mobility compared to the slopes of the curcumin-treated groups which displayed restoration during the negative geotaxis assays. For the females, group CN2 had a slope of $-0.205x + 7.86$, which was significant when compared to group N2 which had a slope of -0.402x + 7.75. CN3 had a slope of -0.25x + 7.77, which was significant when compared to group N3 which had a slope of -0.483x + 7.96. CHSN3 had a slope of -0.248x + 7.53, which was significant when compared to HS+N3 which had a slope of -0.603x + 7.84. As for the males, group CN1 had a slope of -0.0875x + 7.73 which was significant when compared to N1 which had a slope of -0.295x + 7.97. Group CN3 had a slope of -0.0825x + 7.83 which was significant when compared to N3 which had a slope of -0.282x + 7.89. Group CHSN2 had a slope of -0.11x + 7.69 which was significant when compared to HS+N2 which had a slope of -0.428x + 7.99. When combining the data of both genders, it was found that on Day 6 groups CN2, CN3, CHSN1, CHSN2, and CHSN3 significantly flew higher when compared to nicotine-only treated groups. Therefore, curcumin significantly restored mobility to an extent.

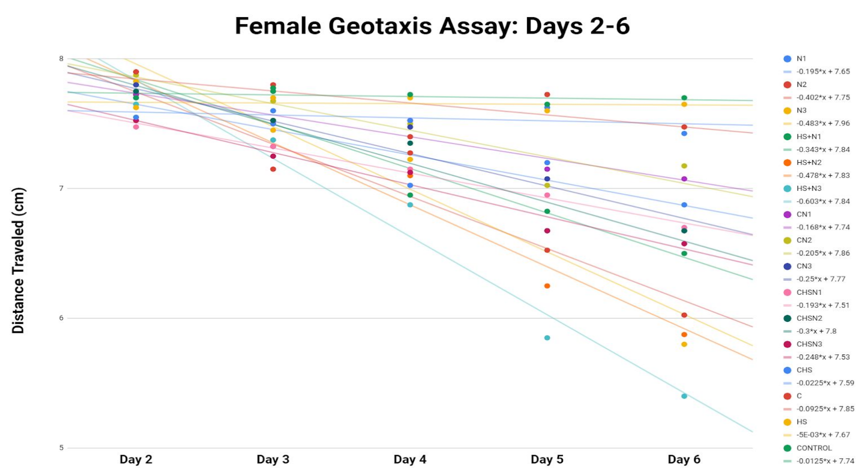

Figure 1: The locomotive abilities of the female fruit flies within the negative geotaxis assay from Days 2-6 recorded in centimeters. * indicates a significant difference ($p<0.05$) as compared to slope of untreated

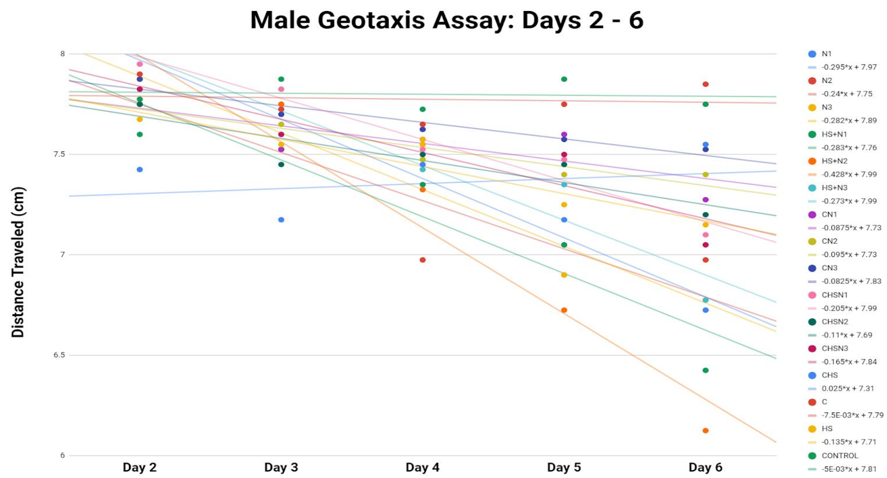

Figure 2: The locomotive abilities of the male fruit flies within the negative geotaxis assay from Days 2-6 recorded in centimeters. * indicates a significant difference (p<0.05) as compared to the slope of the untreated

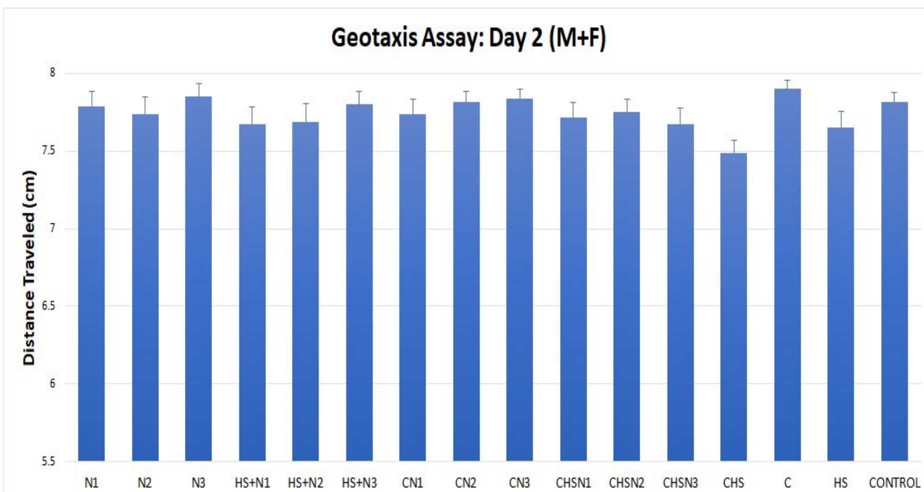

Figure 3: The locomotive abilities of all the fruit flies within the negative geotaxis assay on Day 2 recorded in centimeters.

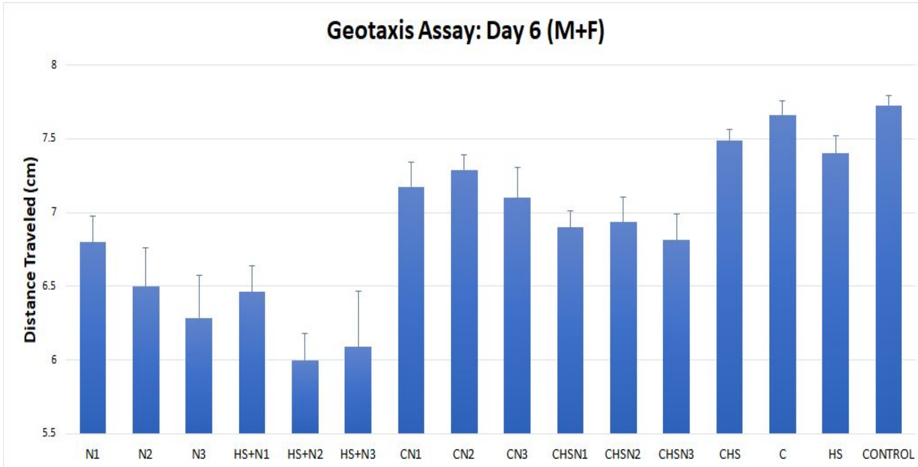

Figure 4: The locomotive abilities of all the fruit flies within the negative geotaxis assay on Day 6 recorded in centimeters. * indicates a significant difference ($p<0.05$) compared to fruit flies without the curcumin treatment.

Foraging behavior was significantly suppressed by Day 7 in all nicotine treated groups, especially those exposed to a higher concentration of nicotine within the food-seeking assays. Group N3 (0.56%) took significantly more time to locate the ACV when compared to group N1 (0.675%). Group CHSN1 (0.674%) took significantly less time to locate the AVC when compared to group HS+N1 (0.624%).

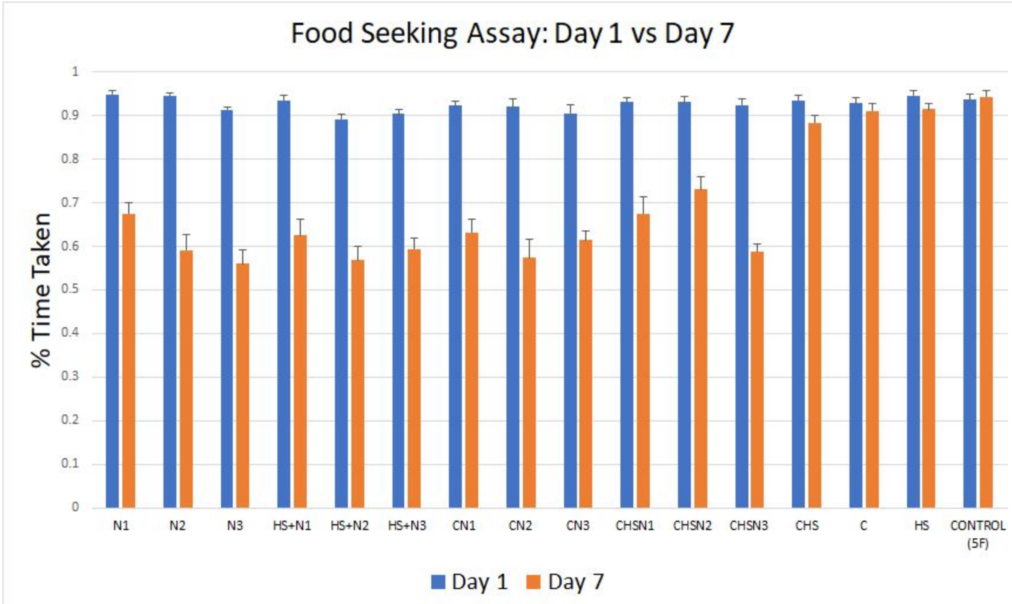

Figure 5: The foraging behavior of the fruit flies were recorded on Day 1 and Day 7. * indicates a significant difference (p<0.05) compared to the same fruit flies from Day 1.

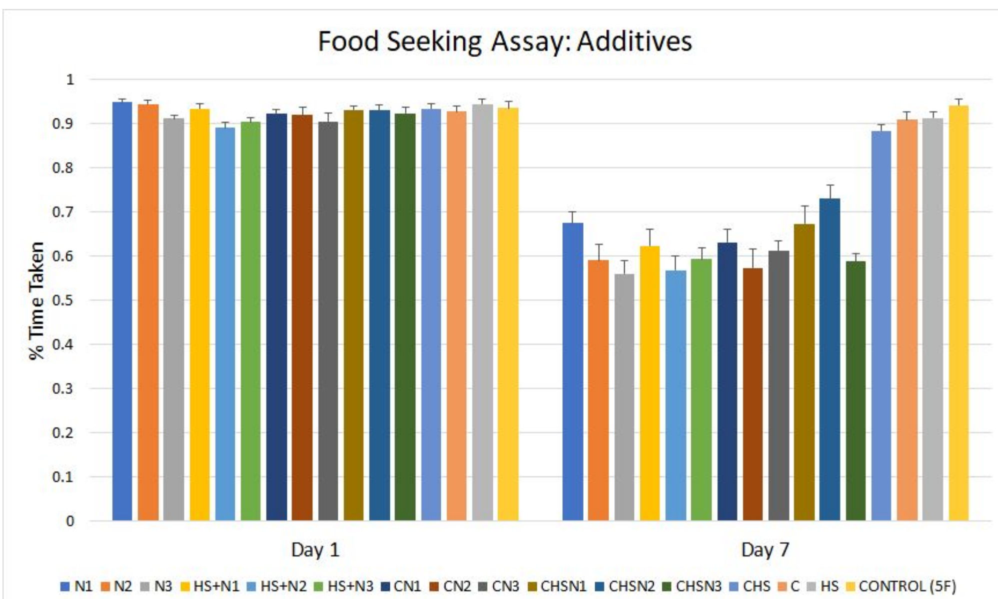

Figure 6: The foraging behavior of the fruit flies within the food seeking assays recorded on Day 1 and Day 7. * indicates significance as p<0.05.

Diabetic fruit flies experienced a lower metabolism while curcumin prevented a loss of metabolic function when calculating metabolic rates. On Day 2, groups HS+N1 (360.606 µL/hr/fly), HS+N2 (389.454 µL/hr/fly), and HS+N3 (403.878 µL/hr/fly) had significantly lower metabolic rates when compared to groups N1 (663.514 µL/hr/fly), N2 (699.575 µL/hr/fly), and N3 (764.484 µL/hr/fly). However, groups CHSN1 (750.060 µL/hr/fly), CHSN2 (836.605 µL/hr/fly), and CHSN3 (764.484 µL/hr/fly) did not experience a decline in their metabolism, which was significant when compared to groups HS+N1 (360.606 µL/hr/fly), HS+N2 (389.454 µL/hr/fly), and HS+N3 (403.878 µL/hr/fly) that did. Similar results appeared on Day 6, where groups HS+N1 (230.788 µL/hr/fly), HS+N2 (346.181 µL/hr/fly), and HS+N3 (403.878 µL/hr/fly) also had significantly lower metabolic rates when compared to groups N1 (713.999 µL/hr/fly), N2 (497.636 µL/hr/fly), and N3 (656.302 µL/hr/fly). Correspondingly, groups CHSN1 (656.302 µL/hr/fly), CHSN2 (677.939 µL/hr/fly), and CHSN3 (735.636 µL/hr/fly) did not experience a decline in their metabolism, which was significant when compared to groups HS+N1 (230.788 µL/hr/fly), HS+N2 (389.454 µL/hr/fly), and HS+N3 (403.878 µL/hr/fly) that did.

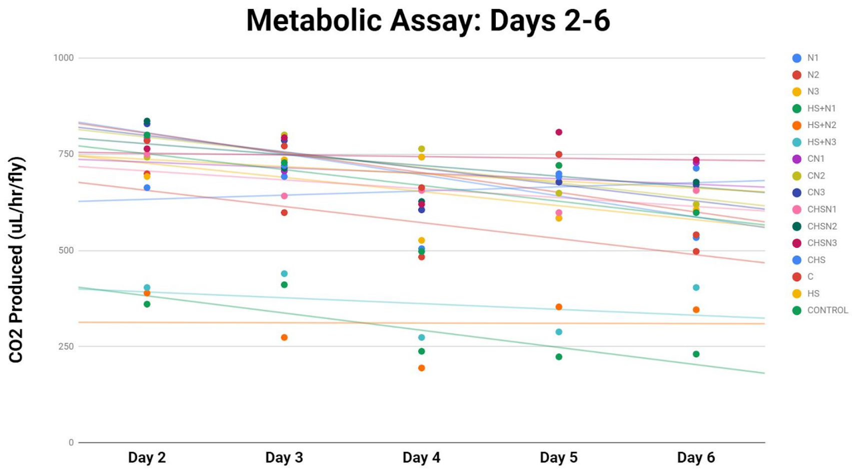

Figure 7: The metabolic rates of the fruit flies from Days 2-6 was recorded in terms of CO_2 production.

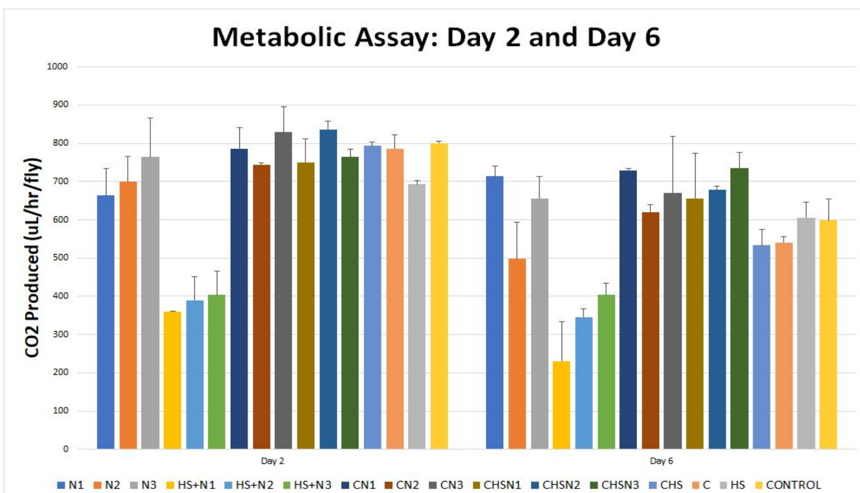

Figure 8: The metabolic rates of the fruit flies on Day 2 and Day 6 and recorded in CO_2 production. * indicates significance as $p<0.05$

DISCUSSION

The data from the negative geotaxis assay revealed that the nicotine exposed fruit flies experienced a significant decrease in their mobility over the course of 5 days when compared to the slopes of the control groups. The diabetic fruit flies had a greater decrease in mobility compared to the non-diabetic flies, which was slightly significant. The curcumin treated fruit flies declined less and had significantly improved mobility by Days 5 and 6.

All of the experimental groups exposed to nicotine exhibited significant suppressed foraging behavior by Day 7. It was found that as the concentration of nicotine increased, foraging behavior significantly decreased as fruit flies took much longer to locate the ACV drop. However, curcumin-treated groups such as CN3 and CHSN2 significantly took less time when compared to the untreated groups.

Metabolic rates generally remained constant from Days 2-6 when calculating the metabolism. Diabetic fruit flies exposed to nicotine had a significant decline in their metabolism when compared to the non-diabetic fruit flies. Fortunately, the curcumin treatment acted as a protective barrier as curcumin-treated fruit flies did not experience a loss in metabolic function.

CONCLUSION

The *Drosophila melanogaster* model of diabetes was severely impacted when exposed to nicotine. These results bear similarities to diabetics who consume nicotine by vaping e-cigarettes. Curcumin was deemed a viable treatment as it mitigated the loss of function caused by the nicotine in the diabetic model. Limitations of this study include the inability to confirm diabetes in the fruit flies as insulin and biomarker levels were not measured. This study should also be repeated in a mammalian or marine model of diabetes to further validate the results. Future research would mainly focus on a later application of curcumin and whether it still provides protective benefits against nicotine. Furthermore, more research is needed to assess other antioxidants and their plausibility to mitigate the negative effects of nicotine on diabetics.

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