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Article

Effect of Curcumin on Motor Behaviors in *Drosophila melanogaster* PINK1 Mutant Parkinson's Model

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SUMMARY

Curcumin is a polyphenol and an active component of turmeric (*Curcuma longa*), a dietary spice commonly used in Indian cuisine and medicine. We hypothesized that an increase in curcumin fed to fruit flies (*Drosophila melanogaster*) would increase dopamine levels in the brain and mitigate symptoms of Parkinson's disease. We chose fruit flies as they have been used as a genetic model for Parkinson's disease in the past due to their similar exhibition of proteins and genes. We acquired flies with loss-of function mutations in the PINK-1 gene to induce Parkinsonism in the flies. Both mutated flies and regular flies were fed diets with no curcumin (control) or diets containing 1.0 milligram of curcumin per gram of diet. Through a climbing assay, it was discovered that on average, fruit flies with Parkinson's disease that were fed a curcumin-supplemented diet climbed 3 cm more than flies on the control diet, showing that motor skills were significantly improved in flies with Parkinson's disease after curcumin was fed. Thus, we were able to demonstrate that curcumin has the potential to improve motor skills in Parkinson's disease.

INTRODUCTION

Parkinson's disease (PD) affects more than 10 million people in the United States, with doctors diagnosing 60,000 new cases every year (1). PD is a neurodegenerative disorder that heavily affects movement. It is known to affect predominantly dopamine-producing neurons in a specific portion of the brain called substantia nigra (1, 2). The substantia nigra is a nucleus in the midbrain that is considered part of the basal ganglia (3).

PINK-1 is a mitochondrial gene that translates into a protein called PTEN induced putative kinase 1 (PTEN) (5). Within cells, this protein is generally found in the mitochondria, the organelle that is generally responsible for cellular respiration and to provide energy for cellular activities. Even though the direct function of PTEN is not fully understood, the protein is essential as it helps protect mitochondria from malfunctioning during cellular stress (5). Loss-of-function mutations in the PINK-1 gene have been identified to cause early-onset PD, showing to disrupt delivery of PTEN to the mitochondria (18). With reduced or absent PTEN, mitochondria may malfunction with high energy demand, causing autophagy of mitochondria (18). The PINK-1 and Parkin mutations in the genes are not found in the same amino acids as found in humans, but mutations in those genes still produce the same effect (18). However, it is not fully understood why mutations in the PINK-1 gene cause selective death of neurons in the substantia nigra that characterizes early-onset PD (18).

As PD symptoms are linked with the degeneration of dopamine (DA) neurons, treatments for the disease are primarily developed to treat symptoms by compensating for depleted levels of DA in the brain (6). Curcumin is a polyphenol and an active component of turmeric (*Curcuma longa*), a dietary spice widely used in Indian cuisine and medicine (17). Curcumin exhibits antioxidant, anti-inflammatory and anti-cancer properties, crosses the blood-brain barrier and is neuroprotective in neurological disorders (17). Several studies in different experimental models of PD strongly support the clinical application of curcumin in PD (17). With India's apparent resistance to PD and the PD clinical applications done with a spice Indians use almost daily, curcumin becomes a logical treatment in PD cases (16).

To model the impacts of curcumin on PD, we chose to use *Drosophila melanogaster*, because they are commonly used as a genetic model for several human diseases including central nervous system disorders such as PD and Alzheimer's diseases. As Drosophila are manageable and easy to breed, many scientists use them in experimentation (6). Drosophila PINK-1 loss-of-function mutants exhibit a set of phenotypes similar to Parkinson's disease, such as impaired locomotor activity, reduced longevity, mitochondrial abnormalities, and DA neuron degeneration (10, 11, 12, 13, 14). The PINK-1 and Parkin proteins are conserved between humans and flies (18).

Overall, this study explores the therapeutic potential of curcumin in PD, but as curcumin has not been thoroughly clinically tested yet, there is still not enough evidence to support its usage in PD treatments. The goal of this study is to evaluate the effect of curcumin on PD symptoms thereby determining its potential use in the treatment of neurodegenerative disorders. Thus, the hypothesis was that flies with PD that were fed curcumin would exhibit mitigated PD symptoms when compared to PD flies in the control group. Data from the assay pointed towards curcumin significantly increasing motor abilities and decreasing PD symptoms in *Drosophila melanogaster*.

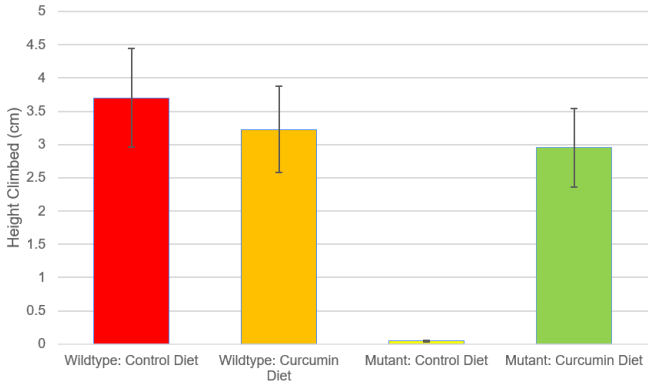

Figure 1: The average height climbed in cm of flight for *Drosophila melanogaster* and the 95% CI of the mean calculated for the trials. Control diet fed fruit flies with PD symptoms climbed a significantly lower distance than the other groups, further evidenced by how there was no overlap between the SEM bar of the mutant flies with the control diet with the other SEM bars. The * shows a significance of $p<0.5$ between the two groups.

RESULTS

We tested the effect of curcumin on PD in *Drosophila melanogaster* through a climbing assay. We compared the Wildtype flies to flies with PD symptoms that contained a mutation in the PINK-1 gene. We utilized two groups of flies with PD and two groups of wildtype flies, with one subgroup in each group being fed a curcumin-supplemented based diet. On average, the height climbed by fruit flies with PD was significantly higher when fed curcumin than flies that were not. The mutant flies fed with a control diet climbed 0.045 cm on average, while the mutant flies fed a curcumin-supplemented diet climbed an average of 2.95 cm. Meanwhile, the wildtype flies fed the curcumin and regular diet climbed on average 3.23 and 4.05 cm. Additionally, wildtype flies climbed almost the same height as PD flies with curcumin (**Table 1**). We further examined all results using a 95% confidence interval (CI) of the mean to validate the results (**Figure 1** and **Table 1**). In the mutated flies group, there was no overlap in the SEM bars, showing that there is a significant difference between height climbed (in cm) between the groups. However, there was overlap in the SEM bars in the wildtype flies group, which showed that height climbed by wildtype flies was not influenced by curcumin. Finally, we examined the results through a Two-Way ANOVA, as shown in **Table 2**. The null hypothesis used was that there is no statistically significant difference in the height climbed in the four groups, while the alternate hypothesis was that there is a presence of a statistically significant difference between the groups. The ANOVA resulted in a *p*-value of 0.00966 for the interaction between the groups, which is significantly less than the alpha value of 0.05 (**Table 2**). The *p*-value between the normal and curcumin-supplemented groups was 0.135, which was greater than the alpha value (**Table 2**). This showed that curcumin does not have a tangible effect compared to the normal diet on some of the fly groups. Also, the *p*-value between the wild-type and mutated fly groups was 0.00339, which showed that the height climbed was not similar between the two groups. To further examine the difference between the groups, we conducted a Tukey-Kramer post-hoc test (**Table 3**). The post-hoc test revealed how groups only have a statistically significant difference with the mutant flies with a control diet. The first three comparisons in **Table 3** show the significant comparisons as they have a significant difference between the groups. There was no significant difference between the other groups, shown by **Table 3**. **Figure 1** further provides evidence that flies with PD symptoms climbed a significantly less height than mutant flies fed curcumin or wildtype flies. The Two-Way ANOVA and Tukey-Kramer Post-Hoc tests showed that when curcumin was supplemented, the mutated flies climbed a significantly higher distance.

DISCUSSION

We concluded that the addition of curcumin in the diets of *Drosophila melanogaster* with PD improved the motor abilities of those fruit flies. As the *p*-value of 0.0000297 was less than the alpha value of 0.05, we reject the null hypothesis that there is no statistically significant difference in the height climbed in the four groups. Additionally, the mean of the height the mutant flies with regular diet climbed in the assay was 0.033 cm and the mean of the height for flies with the curcumin diet is 2.955 cm. Direct comparison with the wildtype flies shows that curcumin does not have an impact

	Wildtype: Control Diet	Wildtype: Curcumin Diet	Mutant: Control Diet	Mutant: Curcumin Diet
Mean (cm)	3.625	3.192	0.033	2.954
Standard Deviation (cm)	2.280	2.840	0.129	2.659
Range (cm)	8	6	0.5	7
95%CI of mean (cm)	2.013 - 5.237	1.617 - 4.768	-0.033 - 0.100	1.351 - 4.558

Table 1: Summary of climbing assay statistics. Either PINK1 mutant or WT flies were fed a diet supplemented with curcumin or control for 2 weeks, followed by a climbing assay. The mean, standard deviation (std. deviation), range, and 95% confidence interval (CI) of the mean were calculated for each condition.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Normal vs Curcumin Diets	12.02272727	1	12.02272727	2.324763788	0.135197935	4.084745733
Wildtype vs Normal Flies	50.20454545	1	50.20454545	9.707756537	0.003388926	4.084745733
Interaction	38.20454545	1	38.20454545	7.387387387	0.009662622	4.084745733
Within	206.8636364	40	5.171590909			
Total	307.2954545	43				

Table 2: Summary of Two Way ANOVA. The table shows the overall results of the ANOVA. The Sum of Squares (SS), degrees of freedom (df), Mean Square (MS), F-statistic, *p*-value and F-critical value were calculated for each condition. The test was conducted using the Analysis ToolPak.

on the wildtype flies and has a drastic effect on motor skills in mutant flies. Further studies are recommended to see the effects of curcumin on different doses and gender differences can be studied. It is likely that curcumin does decrease PD phenotypes in *Drosophila melanogaster*. With further studies and analysis, it may be possible to test whether curcumin also decreases PD's effect in humans as well. The differences between the heights climbed between the two wildtype fly groups could be attributed to purely chance, especially as there is no statistically significant difference between the two groups. The difference in sample size in each group could have also contributed to slight differences in results.

As the error bars are equally as wide on the wildtype flies fed a standard diet, it shows that the climbing assay itself is the main source of variability in the dataset. Further research can examine the quantitative difference of dopamine in the brain with differing amounts of curcumin. As we know with this experiment that curcumin does have an impact on PD symptoms, we can conduct further experimentation to see which amount of curcumin in diets have an optimal impact of curcumin in the brain.

MATERIALS AND METHODS

To conduct our experiment, 2 sets of diets were given to mutated flies and wild-type flies: one diet with curcumin and a diet without, in order to compare the effectiveness of one over the other. The normal diet was made of yeast (5 mg), Drosophila diet mix (6 g) and distilled water (10 mL for every 3 g of diet used). One mg of curcumin was added for every 6 mg of normal diet added for the vials with the curcumin diet. Thus, the curcumin supplemented diets had more food by weight. Five grams of diet were also added for all the flies. This allowed a more even distribution of curcumin throughout the diet. The diet and yeast were obtained from the Bloomington Drosophila Stock Center. The wildtype and mutated flies were from Bloomington Drosophila Stock Center Stock #6321 and #51469 respectively. The flies were approximately 2 weeks old when used in experimentation. After breeding and distributing the diets to the flies for 2 weeks, the flies were tested with a climbing assay test. This climbing assay tested the locomotion and motor abilities of the flies, being directly proportional to the dopamine levels of the flies (17). We transferred 30 random flies to a graduated cylinder from the individual vials by pushing the flies in a graduated cylinder and sealing the top before the flies could escape. The vial was also sharply tapped down on the ground three times, ensuring that the tap was hard enough to knock down all the flies to the bottom of the vials. Using a scale, the height of the flies climbed was calculated in a graduated cylinder for 5 seconds and compared the individual datasets. The same test was performed for all four sets of flies. After completing the assay, the images were procured during the assay to record the height climbed by each fly. Then, the average height climbed for each vial was calculated and recorded. A two-way ANOVA was conducted using the Analysis ToolPak to analyze the data. To check for further comparisons, a Tukey-Kramer post-hoc test was conducted.

		Difference	n (Group 1)	n (Group 2)	SE	q
Mutant: Control Diet	Wildtype: Control Diet	3.159	15	13	0.591	5.348
Mutant: Control Diet	Wildtype: Curcumin Diet	3.592	15	8	0.682	5.263
Mutant: Control Diet	Mutant: Curcumin Diet	2.921	11	15	0.619	4.721
Mutant: Curcumin Diet	Wildtype: Control Diet	0.238	11	13	0.639	0.372
Mutant: Curcumin Diet	Wildtype: Curcumin Diet	0.670	11	8	0.724	0.926
Wildtype: Curcumin Diet	Wildtype: Control Diet	0.433	8	13	0.700	0.618

Table 3: Summary of Tukey-Kramer Post-Hoc test statistics. The standard error of the mean (SE), number of flies (n) and the q-value were calculated for each comparison.

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